Israel Journal of Entomology Vol. I1969

A CONTRIBUTION TO THE BIOLOGY OF PHANEROGAM FLAVITESTACEA FI. A PARASITE OF EC TOM YE LOIS CERATONIAE (ZELL.)

by

*

Shmuel Gothilf

Volcani Institute of Agricultural Research, Beit-Dagan, Israel.

A B S T R A C T

A method for large-scale rearing of Phanerotoma flavitegtacea Fi, on its natural host is described. The parasite is a solitary egg-larval parasite whose immature stage development duration is similar to that of the host, lasting about 30 days at 25 C. Adult life-span is significantly long; females may survive for 8 months at outdoor conditions. Males precede females in emergence and mating takes place shortly after emergence. Progeny of unmated females consists only of males. The weil developed searching ability of the parasite and other attributes described, make it a suitable subject for biological control experiments . Field studies are described in which an increase of carob . moth parasitization by JP. flavitestacea was recorded following field release of the parasite.

Phanerotoma flavitestacea Fi. (Braconidae), an internal egg-larval parasite of the carob moth (Ectomyelois ceratoniae Zell.), is probably indigeneous to the Mediterranean countries. It was first described on the basis of material collected in Yugoslavia (Fischer 1959). Because of the important role this parasite plays in controlling the carob moth population (Gothilf 1969), a study of its biology was initiated. Possible means of increasing its efficiency to control the carob moth through periodic field release of laboratory-reared wasps, were also investigated in the present study.

Large scale rearing

The carob moth, in being the natural host of P. flavitestacea, served as host to the parasite in laboratory rearing experiments. The carob moth was mass-reared by using a method described in a previous study (Gothilf 1968). Essentially, the technique consists of rearing larvae on an artificial diet composed of 43.5% soybean meal, 43.5% sucrose and 13.5% water, in 31 x 21.5 , 7.5 cm deep trays. After larvae had pupated in the food, the trays were transferred to emergence cages. Adult females were collected from the emergence cage and kept individually in plastic cups inverted on rough paper; eggs were laid on this paper.

For large-scale rearing of P. flavitestacea, 1-2 day-old carob moth eggs were exposed to parasitization by adult P. flavitestacea. The wasps were kept in cages measuring 45 x 35, 32 cm high, made of glass and cloth, mounted on a wooden frame (Fig. 1). In each cage containing about 500 wasps, approximately 2000 eggs were placed for a period of 24 hours. The wasps were fed with honey, which was supplied daily by streaking it with a syringe on pieces of folded cardboard placed on the floor of the cage, or on the inside of the glass window. The parasites were active and oviposited mainly during twilight hours. In order to extend wasp activity, a low-intensity, indirect fluorescent light was placed in the room where the cages were kept to give illumination during the nights, thus resulting in almost complete parasitization of the host eggs during one night. Parasitized eggs were transferred to rearing trays - as described in carob moth rearing - except that each tray was covered with a sheet of glass shortly before emergence. Each tray was fitted with two plugged holes, one on each side, which were opened only when adults were removed by sucking. Honey was supplied daily to the emerging parasites. This was done by lifting slightly the glass sheet, inserting a honey-filled syringe and smearing a line of honey on the inside of the glass sheet. A rearing tray with emerging adult parasites is shown in Fig. 2. Each tray yielded on the average 250 adult parasites which were transferred into cages as described above (Fig. 1), and were fed daily with honey. All rearing procedures were conducted in a ventilated room at $25 \pm 0.5^{\circ}C$, and 70 - 75% R.H.; when a stock of adult parasites was kept for a certain

period of time, they were stored in a room at 22 ± 0.5°C. At this temperature the wasps were far less active and lived much longer than at 25°C.

Life History

Methods. Carob moth eggs were exposed to parasite oviposition, as described in the previous chapter. Larvae were reared on artificial diet, usually in trays as described before and, in order to eliminate possible competition, no more than 200 eggs were placed on each tray. When small numbers of larvae were reared, they were kept in 0.5 liter glass jars, half-filled with the artificial medium. The surface of the medium was covered with a sheet of paper and the opening of the jar was closed with a cloth. No more than 30 larvae were kept in each jar. Individual rearing of larvae was carried out in small opaque plastic cups, 3.5 cm in diameter and 1.5 cm high, covered with transparent cellophane paper, so that larval development could be observed from the outside. The cups were checked daily and food was supplied in quantities which did not conceal the larvae; the food was changed three times during the growth period.

Adults tested for their longevity, as well as adults kept for other purposes, were kept in cages, as described before. In the present test no more than 200 wasps were kept in each cage.

Results. P. flavitestacea is a solitary, internal egg-larval parasite; the female lays its eggs inside the host-egg and the larvae continue their development inside the host-larvae. Comparison between P. flavitestacea and its host was made as to the duration of development from egg to adulthood. To test duration of parasite development, carob moth eggs were exposed to the parasites immediately upon being laid. In parallel, a group of eggs from the same batch, but unparasitized, were reared individually on an artificial medium at 23 ± 0.5°C, 80% R.H. Daily checks of the 11 untreated larvae showed that they passed 5 larval instars, spun cocoons and pupated on the 31 to 36th day (including incubation period). Moths emerged on the 42nd - 47th days (45 days average). The 15 parasitized larvae passed 4 larval stages - only a few reached a fifth stage - and spun

cocoons. At this stage, on day 29 - 37, the parasite larvae left the host larvae, devoured the remains of the host, spun their own cocoons and pupated on the 32nd to 43rd day of their life. Wasps emerged on day 42 - 47 (45 days average). Also in other tests we found no significant difference in duration of development up to emergence, between the host, E. ceratoniae and its parasite P. flavitestacea (Table 1).

Emergence of male and female parasites was followed in a group reared from eggs of the same age, which were parasitized on the same day. Rearing was carried out at $25 \pm 0.5^{o}C$, 80% R.H. Emergence under these conditions lasted 13 days. The males, numbering 264, emerged first, mostly on the third day after beginning of emergence, followed by females (190) emerging mostly on the 6th and 7th day (Table 2).

The ratio between males and females in P. flavitestacea depends on the occurrence of mating. From eggs laid by unmated females only males will develop. This was demonstrated in two experiments. In one test 234 carob moth eggs were exposed for 24 hours to 4 three-day old P. flavitestacea females, which had been isolated from males since their emergence. From these eggs 178 larvae hatched, out of which 52 moths and 84 wasps reached adulthood. All wasps were males. In a second test 215 eggs were exposed to 13 isolated females for 48 hours. From these 166 larvae hatched and 140 wasps and 1 moth reached adulthood. Here again, all wasps were males. At the same time 156 eggs were exposed for 2 hours to a large mixed male-female population of the parasite. Of these eggs 105 wasps and 16 moths reached adulthood. The emerging wasps consisted of 48 males and 57 females. The same ratio of about 1:1 males to females was found in all rearings where eggs were exposed to parasitization by a mixed male-female population of P. flavitestacea. The same ratio was encountered among parasites emerging from field collected infested carobs.

Mating usually took place immediately following adult emergence. Upon encountering a female, the male vibrates its wings while circling around the female. The responding female positions its antennae backward, this being followed by copulation, which lasts about 10 seconds. Males mate several times; no observations were made on the number of matings a female goes through. Likewise, no tests were made on the fecundity of the

female parasite. Caltagirone et al. (1964) found that each female lays over 350 eggs. Since the host in their experiments was not the natural host of P. flavitestacea, it is possible that more eggs are being laid when the insect parasitizes the carob moth eggs.*

An interesting point in the life history of P. flavitestacea is the comparatively long life-span of the adults. Longevity was studied in mixed groups of males and females. Longevity of 408 males and 321 females kept at a constant temperature of $25 \pm 0.5^{o}C$, 80% R.H. is shown in Fig. 3a. Male life-span is shorter than female; males reach 50% survival 70 days after emergence, while females reach this stage on the 97th day. Longevity of adults at outdoor conditions was checked during the summer (Fig. 3b) and during the winter (Fig. 3c). The summer group was composed of 59 males and 49 females. Here too, males were shorter-lived than females.

Mortality among males was high at the beginning of June, and this was attributed to the hot and dry weather prevailing at that time. Females, on the other hand, seemed to suffer less from these conditions. The curve of survival of 106 males and 245 females which emerged on October 15, exhibits a significantly long life-span of females during the winter, so that a few females even survived till the following summer. It was observed that adults became motionless at temperatures below $14^{o}C$. Except for a few days during the winter, daily maximum temperatures exceeded $14^{o}C$ for a short period and the wasps were active and feeding during that time. It should be mentioned that in our experiments the wasps were kept in the shade, whereas in nature they are probably exposed to direct sun light, which raises their body temperature, and therefore, a higher activity is expected.

The viability of females surviving the winter (Fig. 3c) was tested. On May 17, 142 carob moth eggs were introduced into the wasp rearing cage for 3 days. From these eggs 115 larvae hatched and consequently 95 wasps emerged - 49 males and 46 females.

---

* The wasps used by Caltagirone et al. were from our stock. The parasite was introduced to California at my suggestion in 1962 and was established there (Caltagirone et al. 1964; Caltagirone 1965).

Discussion. The fact that P. flavitestacea oviposits into the carob moth egg is a clear example of adaptation to the host's behavior. The carob moth egg is the easiest stage to reach from the outside, whereas larvae of the carob moth bore inside the carob fruit, and would thus be out of reach of the adult P. flavitestacea. After being laid inside the host egg the parasite continues to develop internally in the host's larva. Although the term "internal parasite" is most suitable when describing P. flavitestacea, it is not entirely accurate; the parasites feed as ectoparasites for a relatively short period prior to pupation.

Like many other Hymenoptera which reproduce parthenogenetically in the absence of males (Clausen 1940), P. flavitestacea, too, produced only male offsprings when females were kept in isolation. However, natural chances of mating are probably high; a sex ratio of close to 1:1 was observed among the regular laboratory rearings as well as among adults emerging from field-collected samples. Although mating frequency was not examined experimentally, it was observed that mating was common immediately following emergence. Later on males continued courting but females were no longer responsive. It was pointed out that seven month old females parasitized carob moth eggs and their offsprings included females as well. These females had been kept with the males for up to 5 months and then for two months without males. It is therefore obvious that reproducing capacity of females can be preserved for a prolonged period.

While development of the parasite's immature stage spans the period of immature development of the host, the life-span of adult P. flavitestacea is much longer than that of its host. Synchronization with the life cycle of the host is close in egg-larval parasites, where parasite development spans the period of immature development of the host (Hagen 1964). Synchronization with the carob moth life cycle by P. flavitestacea is achieved by the latter through the ability of its adults to lay eggs shortly after emergence and the number of annual generations can thus be equal for both the host and the parasite. On the other hand, adult P. flavitestacea lives much longer than the adult host so that part of its population can survive with fewer generations a year, a fact which might be considered advantageous when adverse conditions for immature development prevail.

Field Release.

Infestation of carob pods by the carob moth depends on the presence of pores or crevices on the fruit, through which the larvae can penetrate. In the spring, with the beginning of carob moth infestation, tha larvae penetrate fruits previously infested by the carob midge (Eumarchalia gennadii Marchal). Later in the season, from late June and onwards infestation is restricted to fruits cracked in the process of ripening. Larvae feeding on such fruits reach adulthood in late July and from then on, until the harvest of carobs, large numbers of emerging moths move to citrus orchards, causing considerable economic damage to grapefruit (Gothilf 1964). Carobs are being harvested in mid-August, and it is obvious that by controlling the carob moth up to this time, considerable damage to citrus can be avoided. The present chapter deals with the possibility of biologically controling the moth during July and August, by field release of its parasite, P. flavitestacea.

Methods. Field releases were made at 4 different locations during the summer of 1962. In a 10-dunam* carob plantation in the coastal plain (A) a first release of 1200 parasites was made on June 29, and 400 more parasites were released on July 4. The releases were made in four places where fruit-cracking was conspicuous. A sample of 400 pods (100 from trees surrounding each point of release) was collected on July 21, few days prior to the expected onset of adult emergence from infested fruits. The sample pods were put in cages, similar to those used for rearing adult parasites, and kept outdoors. Daily checks were made on the number and type of adults emerging. The checks were continued till mid-August. The second plantation (B), also located in the coastal plain, consisted of two rows of carob trees, mostly seedlings, about 1 km long. In the year this study was conducted, not many fruits had cracked and, therefore, only 300 parasites were released in one place on July 4, and a sample of 100 pods was collected on July 24. Emergence started 3 days later, and recordings of moths and parasites were made until mid-August. The third plantation (C), located in the Jerusalem hills, was about the same size as plantation (A). A first release of 2300 parasites was made on June 28; on July 6, 800 more

* 10 dunam = 1 ha.

parasites were released. Releases were made at 4 different locations in the plantation. On July 19 samples of pods were collected from the vicinity of the points of release (a total of 465 pods). First adults began emerging on July 27; emergence was followed up until mid-August. The fourth plantation (D), consisted of about 50 dunams, located in the Jezreel Valley. On July 13, 2000 parasites were released at three locations 40 meters apart. A sample of 300 pods - 100 from each point of release - was collected 18 days later. Adult emergence was followed up until mid-August. The rate of parasitization following the release of parasites was compared to that found in the same plantation in previous years (Gothilf 1969).

Results. The total number of adult carob moths, and its parasites emerging from the samples collected in plantations A to D were 149, 45 , 140 and 349, respectively. The percentage of P. flavitestacea and other parasites from the total number of adults is shown in Table 3. Comparing the occurence of P. flavitestacea in the year of study to its occurence in previous years, it is obvious that following field release, its population increased significantly in plantations A and B, where occurrence of parasites before the release was usually slight. In plantation C P. flavitestacea was also more abundant than in previous years, but other parasites species were not as abundant as in the past. Thus, total parasitism was only slightly higher in the year of the present study. No beneficial effect of the field release was noticed in plantation D, but it should be noted that in this plantation parasites were released late in the season - about 2 - 3 weeks after the onset of carob moth oviposition, and thus many eggs escaped parasitization.

Discussion. In considering the utilization of P. flavitestacea to control the carob moth, attention was focussed on its searching capacity. This quality - most important in determining the effectiveness of the parasite - consists of several factors (Doutt 1964). Some of these factors, e.g. survival, locomotion, host perception, were evaluated in P. flavitestacea.

It was already mentioned that the females of P. flavitestacea can survive for several months, and even survive the winter by using the short periods of elevated temperature for feeding. Males are shorter-lived but sperm cells are preserved in the females for months, and overwintering females are capable of laying fertilized eggs in spring without the presence of males. The wasps searching ability could be followed when starting field releases. Upon being released the wasps do not scatter but tend to remain in the vicinity of the point of release, where they actively search, with the aid of their antennae - for carob moth eggs on the carob fruits. Once a hole or a crevice is detected on the fruit (sites where the host eggs are usually being laid), the wasp inserts its antennae seeking eggs. If an egg is present the wasp inserts its ovipositor into the crevice and parasitizes the egg. This search was followed for a few hours whereupon the fruits visited by the parasite were inspected. It was found that not one egg escaped parasitization by the wasp. Another factor which should be considered when planning field releases is the density of the host. According to available data (Gothilf 1964) it is estimated that about 60 host eggs are being laid each night on a carob tree during the field release season. In view of this data, it was concluded that release of a few hundred wasps should result in a detectable increase in parasitization around the point of release. However, these calculations are mere approximations, to quote Sweetman (1958) "The relationship between a given indigenous insect and its parasites and predators is one involving a complex condition of ecological equilibrium, which is subject to seasonal fluctuation. The component factors which govern these fluctuations together with their individual and collective effects, have not been subject to other than the most elementary analysis. In the absence of fundamental information on the subject the feasibility of the utilization of indigenous parasites and predators can be determined only by actual experiment".

The results of the present study have shown a significant increase in the rate of parasitization in plantations A and B, where rate of parasitization was naturally low. In the two other plantations, located inland and surrounded by wild carob orchards, the parasite was naturally abundant. The higher rate of parasitization by P. flavitestacea during the year of this study in plantation C could be a result of the field releases. On the other hand, the reduced parasitization by other parasites species could be a

result of competition with the released parasites. Considering the ineffectiveness of field releases in plantation D, it should be noted that this plantation was the largest among the experimental plantations and that release was made at a later date. These factors could negatively affect the efficiency of field release in this plantation.

It can be concluded that field releases of P. flavitestacea, although it is an indigeneous parasite, can increase the rate of parasitized carob moths, particularly in areas where natural parasitization is low. Since the number of parasites released in the present study was relatively small, it is quite probable that a release of larger numbers might give better results. The method of rearing P. flavitestacea described in this paper can be used for mass-producing the parasite for periodic large-scale releases. An early spring release, when the first carob-moth eggs are laid on carob-midge infected pods, should also be tried.

REFERENCES

Caltagirone, L. E. 1965 A new Phanerotoma from California. Pan. Pac. Ent. 41, 17-20.

Caltagirone, L. E., Shea, K. P. and G. L. Finney 1964 Parasites to aid control of navel orange worm. Calif. Agric. 18(1), 10-12.

Clausen, C. P. 1940 Entomophagous Insects. McGraw Hill, New York.

Doutt, R. L. 1964 Biological characteristics of entomophagous insects. In: Biological Control of Insect Pests and Weeds. P. De Bach Ed. Chapman & Hall, London.

Fischer, M. 1959 Neue und wenig bekannte Braconiden aus Jugoslavien (Hymenoptera). Acta Musei Macedonici Scientiarum Naturalium 6, 1-25.

Gothilf, S. 1964 Studies on the biology of the carob moth Ectomyelois ceratoniae (Zeller) in Israel. The Volcani Inst. Agric. Res. Bull. No. 76 (Hebrew).

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

Gothilf, S. 1969 Natural enemies of the carob moth Ectomyelois ceratoniae (Zeller). Entomophaga 14, 195-202.

Hagen, K. S. 1964 Developmental stages of parasites. In: Biological Control of Insect Pests and Weeds. P. De Bach Ed. Chapman & Hall, London.

Sweetman, H. L. 1958 The Principles of Biological Control. W. M. C. Brown, Dubuque, Iowa.

Table 1.

Duration of development till adult emergence of

E. ceratoniae and its parasite, P. flavitestacea.

Experimental conditions	No. of adults emerged		Duration of development - days	
	Ectomyelois	Phanerotoma	Ectomyelois mean (min.-max.)	Phanerotoma mean (min.- max.)
$25 \pm 0.5^{o}C$	16	105	30.5 (29-33)	30.3 (27-35)
Outdoor (Nov-June)	430	216	188.7 (172-214)	193.2 (178-220)

Table 2.

Emergence of P. flavitestacea reared at 25 ± 0.5°C. 80% R.H.

Days from onset of emergence	Emergence	
	Males %	Females %
1	2.1	0.0
2	10.0	0.0
3	33.7	1.6
4 - 5	37.5	27.9
6 - 7	11.7	40.0
8 - 9	4.2	22.6
10 - 11	0.8	5.3
12 - 14	0.0	2.6
	100.0	100.0

Table 3.

Effect of field release of P. flavitestacea on rate of parasitization, compared to two previous years when plantations were untreated.

Plantation	Parasitization by P. flavitestacea and other parasites - %					
	1960 - 1961		1961		1962	
	Phanerotoma	Other	Phanerotoma	Other	Phanerotoma	Other
A	0.0	2.0	0.0	0.0	35.6	1.3
B	-	-	3.0	6.2	22.2	2.2
C	13.6	22.8	27.9	20.9	45.0	7.1
D	35.7	1.8	37.7	6.6	32.8	8.0

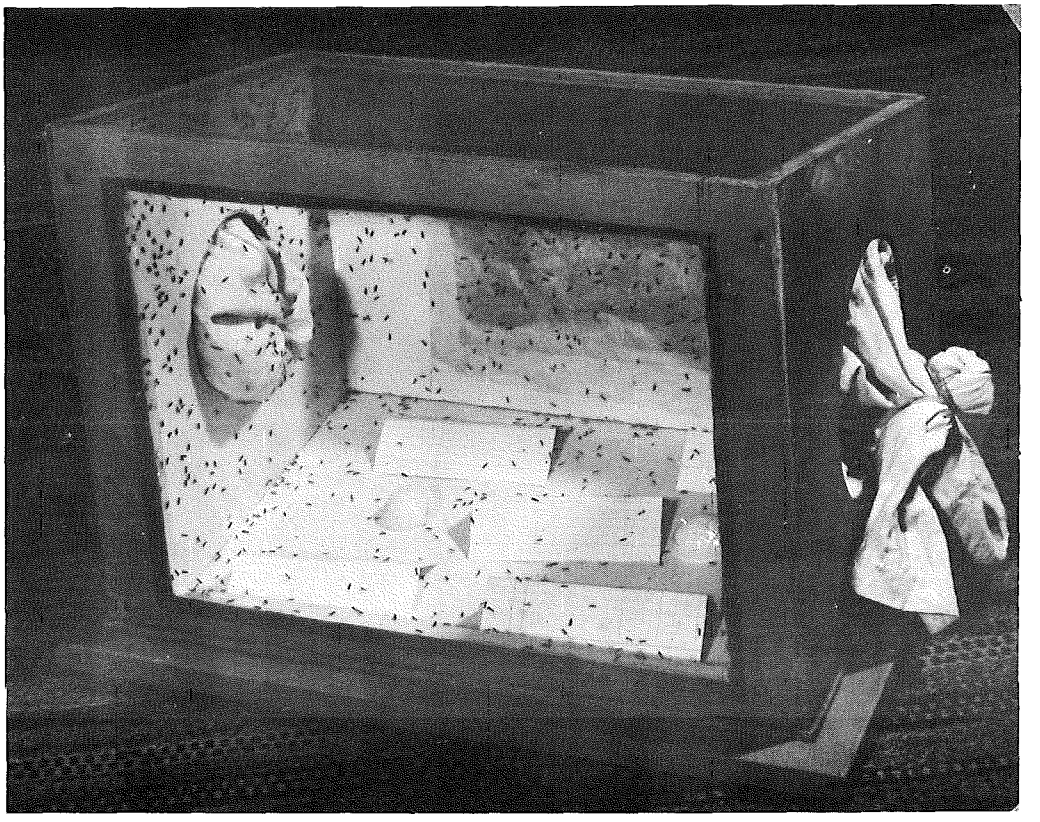

Fig. 1. Cage for rearing adult P. flavitestacea.

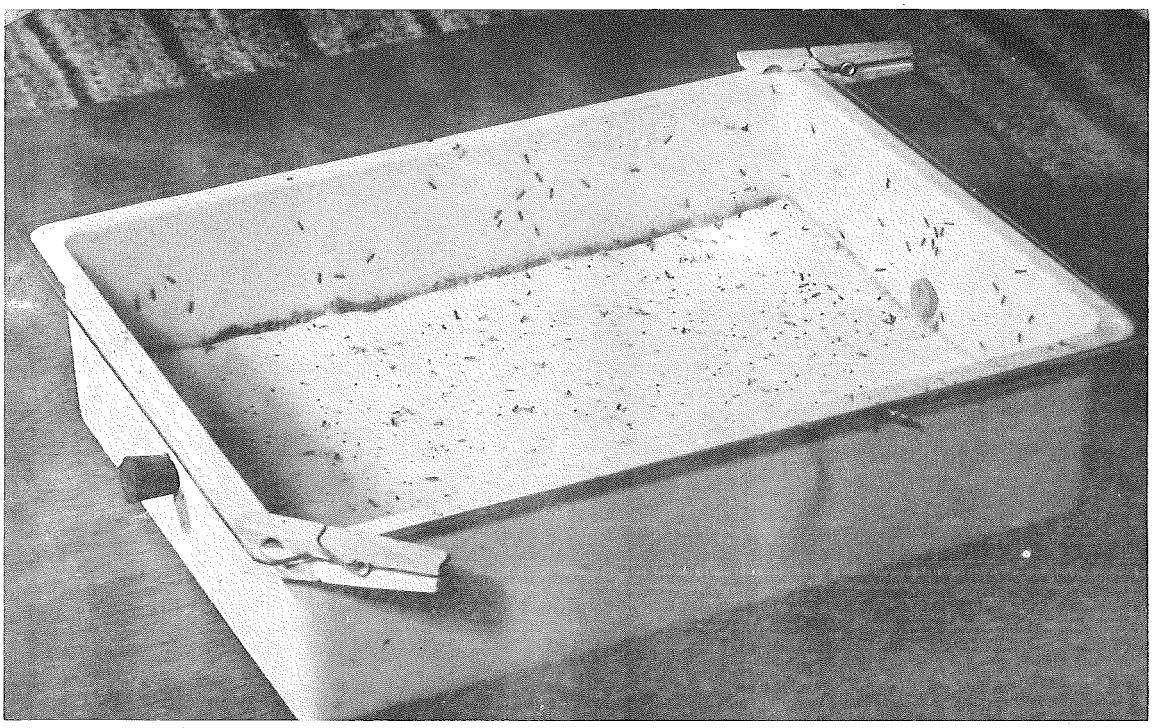

Fig. 2. Tray for rearing carob moth larvae parasitized by P. flavitestacea. New emerging adult parasites are seen.

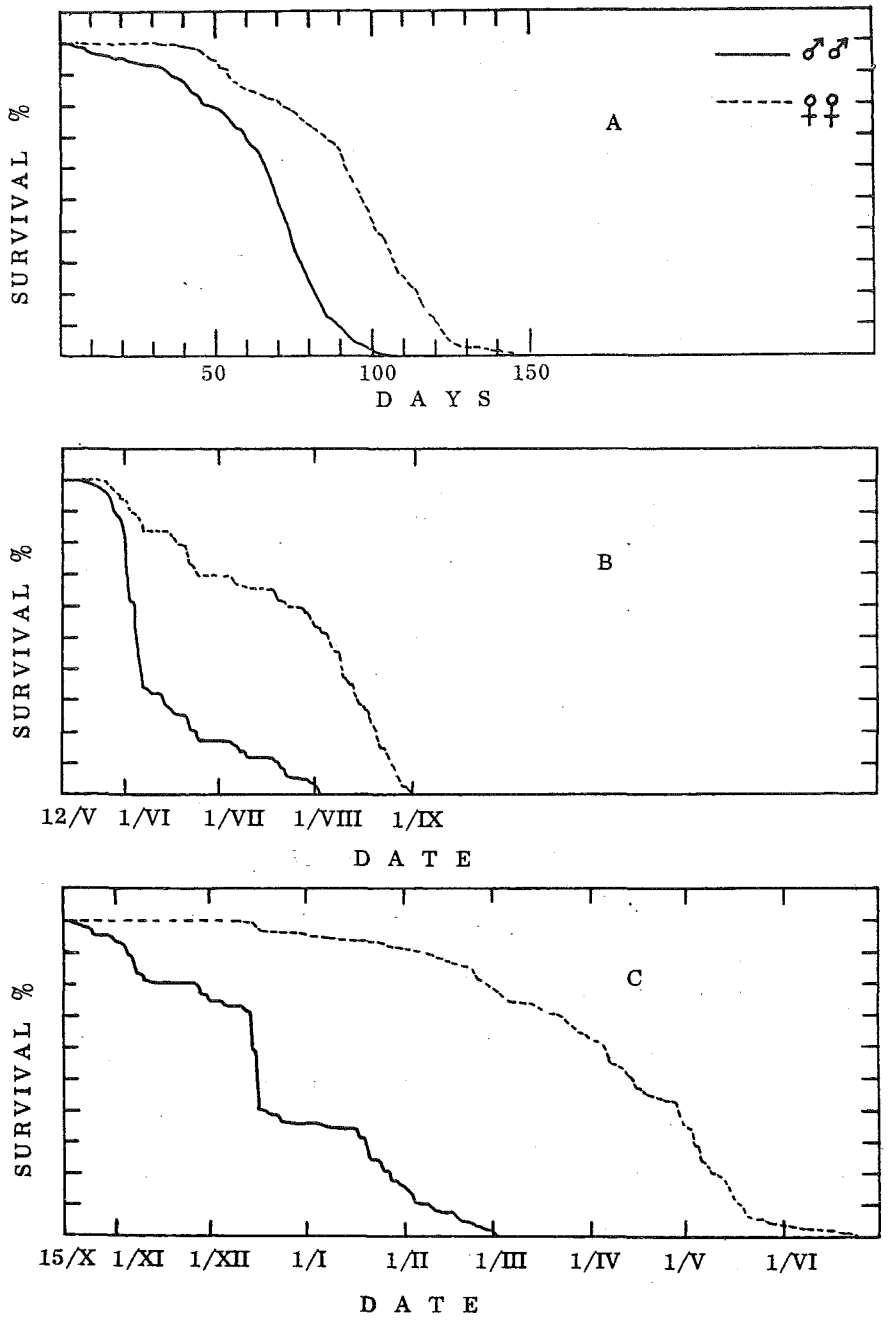

Fig. 3. Survival of adult Phanerotoma flavitestacea at 25 ± 1 °C, 80% R.H. (A) and under outdoor conditions (B, C).