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STUDIES ON THE SUMMER DECLINE OF *CHILOCORUS BIPUSTULATUS* IN CITRUS GROVES OF ISRAEL (*)

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Controlled photoperiodic and temperature regimens, programmed to simulate summer conditions in the coastal plain of Israel, did not adversely affect the fecundity of *Chilocorus bipustulatus* nor its energy reserves. Infection by the fungus *Hesperomyces virescens* (Laboulbeniales) had little effect on the predator's viability. The summer decline of the beetle is tentatively associated with a change in its diet.

Chilocorus bipustulatus (L.) (*Coleoptera* : *Coccinellidae*) is the most important predator of armored scale insects in Israel (AVIDOV & HARPAZ, 1969). It commonly occurs in citrus groves in the coastal plain of Israel, being more abundant in mature plantations than in young ones. The populations of this predators increase their numbers in early summer peak in July and then decline (ROSEN & GERSON, 1965), concurrent with the increase in scale insect populations. This lack of synchronization detracts from the efficiency of *C. bipustulatus* in containing the degree of scale infestation on the developing citrus fruits. BODENHEIMER (1951) attributed the summer decline of *C. bipustulatus* to impaired fecundity resulting from high temperatures, which were also believed to have caused the high mortality observed by HECHT (1936). Lack of sufficient food was alternatively implicated (BODENHEIMER *op. cit.*). KAMBUROV *et al.* (1967) suggested that the summer decline may be due to attack by the fungus *Hesperomyces virescens* THAXTER (Laboulbeniales), for infected field-collected beetles died quicker than healthy ones.

Two other possibilities which might affect the summer decline could be migration of *C. bipustulatus* in preparation for winter hibernation, or summer aestivation in the citrus groves. The purpose of this work was to evaluate several of these possibilities by controlled laboratory investigations concurrent with field observations.

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Methods

The *C. bipustulatus* stock cultures were the progeny of beetles collected in citrus groves and subsequently reared in plastic cages under constant illumination (190 Lux) at 28°C and 70 % R.H. An abundance of California red scale, *Aonidiella aurantii* (MASKELL), reared on potatoes, served as food.

CONTROLLED LABORATORY EXPERIMENTS were conducted with isolated pairs of newly-emerged beetles (0-24 hrs after hatching) placed in half-litre glass jars on single scale - infested potatoes. Strips of white flannel cloth were added to facilitate oviposition. The jars were placed in environmental cabinets (Percival; Boone, Iowa) programmed for both photoperiod (7600 Lux) and thermoperiod. Three photoperiodic and thermoperiodic regimens were assayed as to their comparative effect on oviposition of *C. bipustulatus*. Two of these regimens were chosen to approximate the mean temperature fluctuations prevailing during August in the coastal plain of Israel : a daytime temperature of 32°C and nighttime temperature of 20°C. They differed in the length of the photophase (L) and complementary scotophase (D), which was 13 hrs L and 11 hrs D in regimen I and 15 hrs L, 9 hrs D in regimen II. The photoperiod of regimen I is that prevailing in August in Israel, while regimen II was constructed with a longer photophase in order to ascertain possible effects on various parameters assessed. These two regimens were compared to regimen III under standard conditions (constant illumination, 28°C and 70 % R.H.). Observations were conducted each 1-3 days, and deposited eggs were removed in order to ascertain the percentage of hatching.

RESPIRATION STUDIES were conducted with these same beetles — kept under the various regimens — after the biological observations were completed. At this time they were about 6 weeks old. Oxygen consumption and carbon dioxide release of individual adult beetles were measured by a gas chromatographic micromethod (TADMOR *et al.*, 1970) and respiratory quotients (RQ) were calculated.

ANALYSES OF GLYCOGEN CONTENT AND TOTAL FATTY ACIDS were performed on these same beetles which were deep-frozen immediately after the respiratory measurements.

Lipids were extracted from whole beetles according to DOLE & MEINERZ (1960) and glycogen was determined in the residue by extracting with dimethylsulfoxide (WHISTLER & BE MILLER, 1962) and assayed with the iodine reagent according to KRISMAN (1962), at 380 mμ. Methylation of total fatty acids was carried out in 3 % H_2SO_4 in absolute methanol at room temperature overnight. The methyl esters were determined by gas chromatography on a

column of 15 % diethylene-glycol-succinate on chromosorb W (60/80) (2,44 m × 0,6 cm) at 180°C column temperature with a flame ionization detector.

FIELD POPULATIONS of *C. bipustulatus* were sampled in two mature orange groves located in the central coastal plain of Israel : Givat Brenner (designated henceforth Grove A) and Kfar Warburg (Grove B), during the hot summer months of 1970. Grove B was chosen on the basis of a preliminary survey, conducted in 1965, whose results are incorporated below. The field sampling methods employed by ROSEN & GERSON (1965) for similar purposes were used in the preliminary observations as well as in the present ones. Subsequent to these countings 50 beetles or more were collected at each site from adjacent trees and immediately brought to the laboratory. Some of these beetles were dissected in order to determine ovarial development. Others served for respiratory studies and subsequent determination of glycogen and total fatty acids. The incidence of fungal infection by *Hesperomyces* was determined by direct observations in each sample. Some of these infected beetles were kept under standard conditions in the laboratory (constant illumination, 28°C and 70 % R.H.), to serve as inoculum for infecting newly emerged adult beetles from stock cultures. This was done by placing infected beetles and stock-reared beetles together for two weeks, and subsequently separating them. The laboratory-infected beetles were isolated, kept under standard conditions and observed daily.

Results

No differences were found in respect to oviposition under the conditions of the three regimens (fig. 1), nor in the percentages of the hatching of the eggs deposited during the period of observation : for regimen I — 70.9 %, for regimen II — 62.6 %, and for regimen III — 75.3 %.

No differences were found in regard to respiration or composition of energy reserves in the adult beetles (table 1), except under regimen II, which affords the beetles two additional daily hours of activity at 32°C. Under this regimen fewer fatty acids were detected. Respiration rates were also similar for field-collected males and females (table 2), and did not differ meaningfully from the values obtained under standard laboratory conditions. The RQ values from both groves were much more variable than those obtained in controlled laboratory experiments.

The glycogen content of the field-collected beetles (table 2) was at least as high as that in the laboratory-reared insects (table 1), whereas the total fatty acids content was much lower. This may reflect on the greater activity of beetles in the groves, or on lack

TABLE 1

Respiration and reserve content of adult Chilocorus bipustulatus *under various photoperiodic regimens*

Thermo- and photo-periodic regimen	Number of repli-cates	Average weight (mg)	RESPIRATION µl/mg weight/hr O_2	CO_2	RQ	GLYCOGEN Number of repli-cates	% of body weight	TOTAL FATTY ACIDS Number of repli-cates	% of body weight
			MALES						
I (*) a	7	7.3 ± 0.7(**)	1.1 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	7	13.5 ± 1.6	9	2.0 ± 1.1
I l + a	7	6.8 ± 0.7	1.3 ± 0.3	1.0 ± 0.1	0.8 ± 0.1	8	12.8 ± 1.4	8	3.3 ± 1.6
II a	8	6.6 ± 1.1	1.1 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	8	10.6 ± 3.5	4	0.3 ± 0.1
I-l-II a	8	8.0 ± 0.9	1.1 ± 0.3	1.0 ± 0.3	0.9 ± 0.1	9	9.2 ± 2.2	7	1.1 ± 0.7
III l + a	6	7.3 ± 0.6	1.2 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	7	11.9 ± 2.1	7	2.4 ± 1.8
			FEMALES						
I a	8	9.3 ± 1.0	1.6 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	8	10.6 ± 1.4	8	2.3 ± 0.7
I l + a	6	8.8 ± 0.5	1.7 ± 0.6	1.2 ± 0.4	0.7 ± 0.1	8	9.9 ± 0.9	7	3.5 ± 2.6
II a	9	9.0 ± 0.7	1.5 ± 0.2	1.2 ± 0.3	0.8 ± 0.1	7	9.3 ± 3.0	5	0.2 ± 0.1
I-l-II a	8	10.5 ± 1.1	1.6 ± 0.2	1.2 ± 0.2	0.8 ± 0.1	8	8.5 ± 1.4	7	1.7 ± 0.6
III l + a	7	8.4 + 1.0	1.7 ± 0.2	1.3 ± 0.2	0.8 ± 0.3	7	9.2 ± 1.3	7	2.4 ± 1.5

(*) I = 13 L; 32 °C
11 D; 20 °C
II = 15 L; 32 °C
9 D; 20 °C
III = 24 L; 28 °C

"a" indicates that larvae were reared under standard conditions of III, and adults were subjected to the stated regimen.

"l + a" indicates that larvae were reared under the same regimens at which the adults were subsequently assayed.

(**) The values are given ± standard deviation.

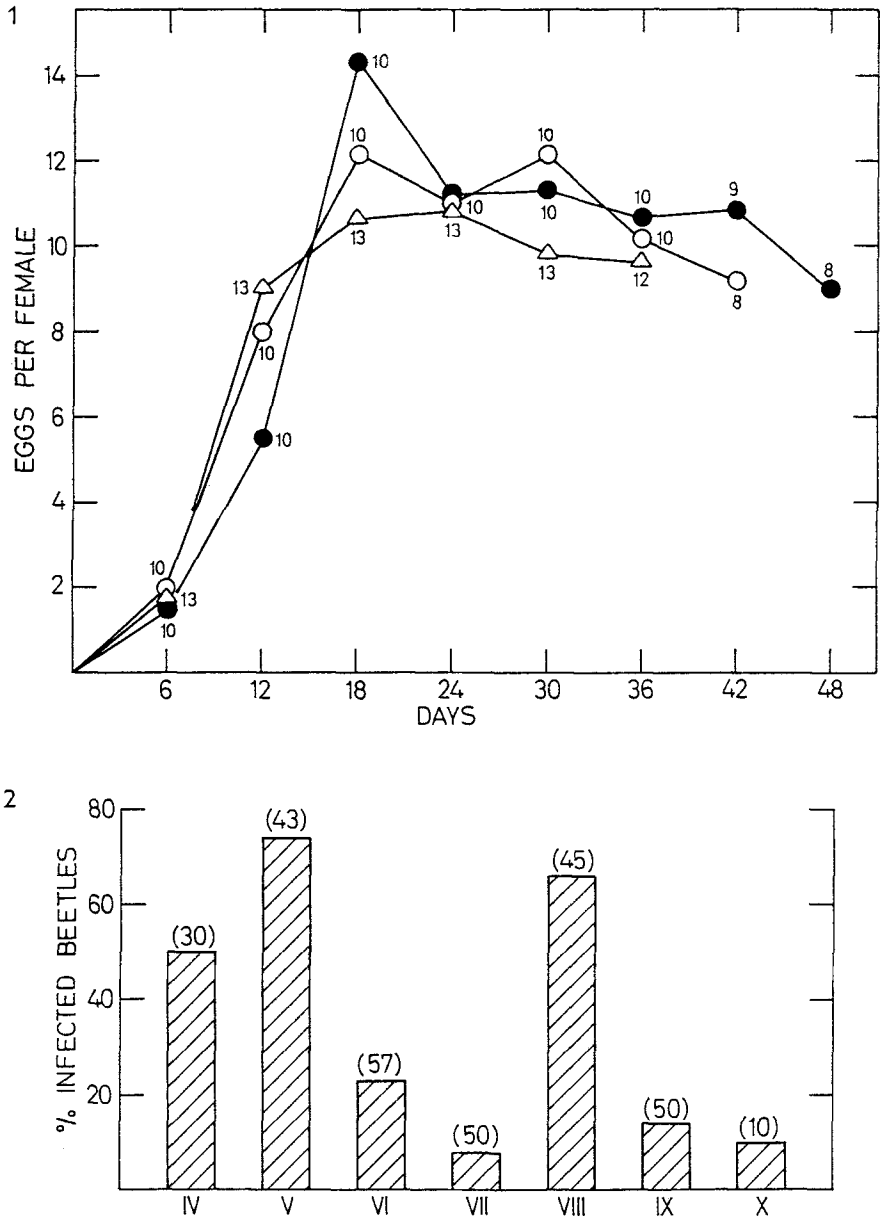

FIG. 1. Effect of photoperiod and thermoperiod on oviposition of *Chilocorus bipustulatus* beetles. (●——● = regimen I; Δ——Δ = regimen II; ○——○ = regimen III). The numbers adjacent to the values indicate the number of surviving females.

FIG. 2. Percentage of *Hesperomyces*-infected *Chilocorus bipustulatus* beetles in grove B (1965). Numbers in parentheses indicate number of adults examined in each sample.

TABLE 2

Respiration and reserve content of adult field-collected Chilocorus bipustulatus (Grove B)

Date of collection	Number of replicates	Average weight	RESPIRATION µl/mg weight/hr			GLYCOGEN		TOTAL FATTY ACIDS	
			O_2	CO_2	RQ	Number of replicates	% of body weight	Number of replicates	% of body weight
			MALES						
15.VI	2	5.3 ± 0.1 (*)	1.8 ± 0.1	1.5 ± 0.1	0.9 ± 0.0	5	14.0 ± 1.9	4	0.6 ± 0.1
29.VI	5	6.1 ± 0.5	1.3 ± 0.5	0.9 ± 0.2	0.7 ± 0.2	7	11.0 ± 1.7	7	1.0 ± 0.7
13.VII	6	5.7 ± 1.0	1.6 ± 0.3	1.3 ± 0.1	0.8 ± 0.1	5	10.5 ± 4.2	6	0.4 ± 0.3
29.VII	6	4.5 ± 0.9	1.5 ± 0.5	1.0 ± 0.2	0.7 ± 0.1	6	13.3 ± 6.5	6	0.3 ± 0.1
10.VIII	6	5.5 ± 0.6	1.6 ± 0.3	1.2 ± 0.2	0.7 ± 0.1	6	11.7 ± 1.8	6	0.2 ± 0.0
			FEMALES						
29.VI	2	6.2 ± 0.3	1.1 ± 0.4	1.0 ± 0.3	1.0 ± 0.1	7	9.7 ± 1.5	7	1.0 ± 0.7
13.VII	5	6.0 ± 1.3	1.4 ± 0.4	1.1 ± 0.2	0.8 ± 0.1	4	9.6 ± 2.5	5	0.2 ± 0.1
29.VII	7	4.8 ± 1.2	1.6 ± 0.3	1.1 ± 0.2	0.7 ± 0.1	7	11.1 ± 4.3	7	0.2 ± 0.1
10.VIII	6	6.3 ± 1.0	1.4 ± 0.4	1.1 ± 0.3	0.8 ± 0.2	6	9.0 ± 1.9	6	0.4 ± 0.3
23.VIII	3	5.2 ± 0.7	1.8 ± 0.3	1.3 ± 0.2	0.7 ± 0.0	3	9.7 ± 4.6	3	0.2 ± 0.0

(*) ± Standard deviation.

of sufficient food to replenish body reserves of fat, these serving as a main source of energy. As the results obtained in these determinations were similar for the beetles collected in the two groves, only the data referring to Grove B are presented in detail (table 2). The percentages of females with developed ovaries (table 3) were always above 50 %, except for a period in late July-early August, in Grove A.

TABLE 3

Percent of females and degree of ovarial development in field-collected samples of Chilocorus bipustulatus

	Date of collection	Number of adults in sample	% females in sample	% females with developed ovaries
Grove A	21.VI	50	44	56
	5.VII	48	40	75
	22.VII	51	43	36
	3.VIII	50	30	38
	17.VIII	19	74	75
Grove B	15.VI	52	50	85
	29.VI	54	37	85
	13.VII	50	38	75
	29.VII	50	38	55
	10.VIII	50	40	86

The preliminary survey for fungus activity in Grove B (fig. 2) showed two peaks of infection, in May and in August, with a distinct ebb during July and in the autumn. During the present stage of our field studies, samples were collected from June through August twice monthly (fig. 3). A comparison of the population decline of *C. bipustulatus* in groves A and B indicates similar trends, while the degree of *Hesperomyces* infection was markedly different in the two groves (fig. 3). In fact, the incidence of the fungus was next to nil during the critical period of *C. bipustulatus* decline in grove A.

The incidence of infection among male beetles was much higher than for females (fig. 4). The fungus appears more commonly on the underside of males and on the dorsum of females and is presumably disseminated by mating.

Table 4 presents respiratory data of *Hesperomyces*-infected beetles. The rate of oxygen consumption and carbon dioxide release, and the calculated RQ values are similar to measurements conducted on non-infected beetles (table 2).

Laboratory infection of newly-emerged adult beetles, attempted by allowing free access of infected individuals to healthy ones, was successful to only a small extent. In the seven beetles which became infected, no mortality was recorded during the first six weeks of adult life, and many viable eggs were deposited. Some mortality was noted at later dates, the last two beetles surviving for almost four months at 28 °C.

TABLE 4

Respiration of Hesperomyces-*infected* Chilocorus bipustulatus *collected in grove B*

Date of collection	Sex	Number of replicates	Average weight (mg)	Respiration µl/mg weight/hr O_2	CO_2	RQ
13.VII	♂♂	5	6.2 ± 0.7 (*)	1.6 ± 0.4	1.2 ± 0.1	0.8 ± 0.1
29.VII	♀♀	5	5.2 ± 0.5	1.4 ± 0.2	0.9 ± 0.1	0.7 ± 0.0
10.VIII	♀♀	6	5.8 ± 1.3	1.7 ± 0.4	1.2 ± 0.2	0.7 ± 0.1
23.VIII	♂♂	5	5.4 ± 1.3	1.6 ± 0.2	1.1 ± 0.2	0.7 ± 0.0
	♀♀	6	4.8 ± 1.4	2.0 ± 0.5	1.3 ± 0.2	0.7 ± 0.1

(*) ± Standard deviation.

Discussion

The purpose of this study, as stated above, was to evaluate the effect of several environmental factors on the summer populations of *C. bipustulatus*. The more obvious seasonal factors, high temperatures and long photoperiods, had no discernible effect on the beetles energy reserves, reproduction or survival. Therefore we conclude that the temperatures and long-day conditions prevalent during the critical July-August period do not directly impair the fecundity and fertility of *C. bipustulatus*. The decline in the field populations of these beetles need thus be attributed to other factors.

The other possible mortality factor studied was infection by the fungus *Hesperomyces*. The similarities in the rate of oxygen consumption, in carbon dioxide release and in the RQ ratio between the healthy and infected field-collected beetles imply that the fungus has no appreciable effect on the metabolsm of *C. bipustulatus*. Further, the laboratory-infected beetles deposited many viable eggs, indicating that oviposition is not curtailed by the disease.

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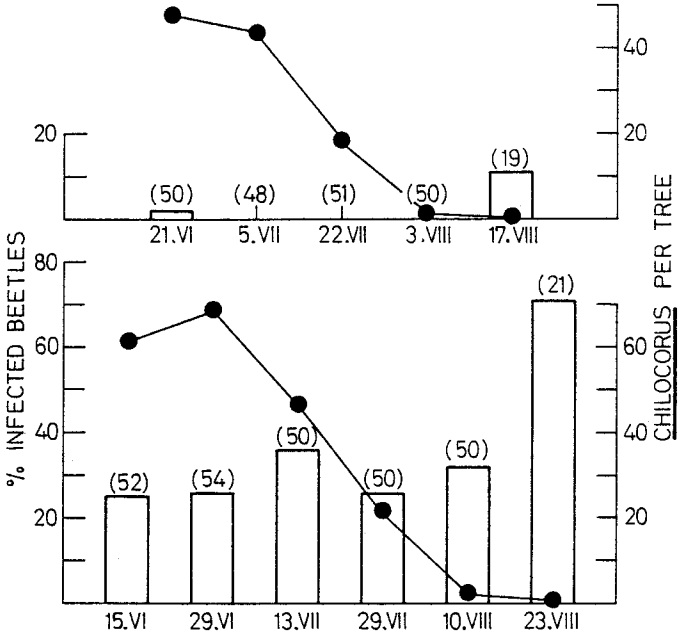

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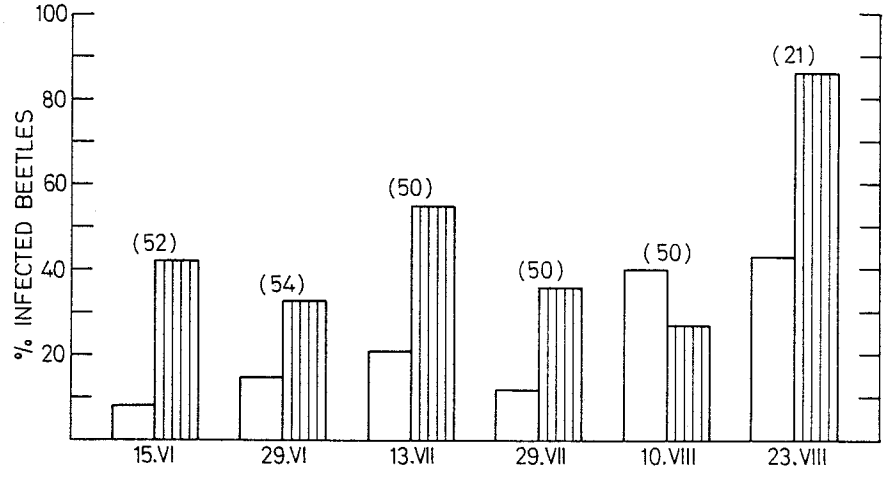

FIG. 3. The population level of *Chilocorus bipustulatus* and of *Hesperomyces*-infection in grove A (upper graph) and grove B (lower graph). The numbers in parentheses indicate the number of adults examined in each sample.

FIG. 4. The incidence of *Hesperomyces*-infection in field-collected *Chilocorus bipustulatus* males and females in grove B. (The white columns indicate females and the hatched columns—males). Numbers in parentheses indicate number of adults examined in each sample.

In the preliminary field studies (fig. 2) the occurrence of the fungus on the beetles coincided with their population fluctuations (ROSEN & GERSON, 1965), suggesting that the prevalence of infection is dependent mostly on the host's age. A marked difference in the number of infected beetles was later found between groves A and B (fig. 3), while the populations of *C. bipustulatus* followed the usual, similar decline pattern in both groves (fig. 3). Our data thus do not support the assumption (KAMBUROV *et al.*, 1967) that the fungus materially contributes to the predator's summer decline. No other member of the Laboulbeniales is known to harm its host (MADELIN, 1966), and it would seem that in the present case *Hesperomyces* is at most a mortality factor of limited importance. A similar conclusion was recently reached by KEHAT *et al.* (1970).

YINON (1969) noted several other diseases of *C. bipustulatus*, all of local character and none apparently of gregarine origin, like those recorded by LAUDÉHO *et al.* (1969). It is hard to postulate a disease which would regularly cause an epizootic during July - August throughout the coastal plain of Israel. Furthermore, our data on the rate of ovarial development in field-collected beetles (table 3) lend no support to any hypothesis concerning fecundity-affecting diseases in the *C. bipustulatus* populations. Thus, notwithstanding the possibility of additional fungal, viral or protozoic diseases affecting the predator, we cannot consider diseases to play a decisive role in the summer decline.

We have no data to either support or refute the possibility of directed migration of *C. bipustulatus*. Several observations on file indicate occasional mass movement of beetles, which did not, however, occur during the critical summer period, and are therefore not relevant to the summer decline.

One additional factor which has to be considered is the possible effect of low relative humidities on the predator during the late summer. Experience in the groves suggests that high humidities actually prevail on the trunk and main limbs of the well-irrigated citrus trees in Israel, which, as they mature, form park-like plantations whose canopies touch. The trunk and the main limbs are the parts of the trees where most of the beetle populations aggregate during summer and where large, live chaff scale (*Parlatoria pergardii* COMSTOCK) populations abound throughout the year (ROSEN & GERSON, 1965). Thus, all active stages of the predator may compensate for any water-losses by increased feeding on their prey, and the larvae may protect themselves during relatively-dry days by hiding amongst the scale-insect incrustations. To sum up, we do not think that humidities are low enough within the citrus trees' canopies to seriously affect the population survival of *C. bipustulatus*.

The effect of inadequate nutrition on the summer decline of *C. bipustulatus*, first suspected by BODENHEIMER (1951), remains to be discussed. The field observations in the two groves during the period under question indicate an apparent preponderance of males in the samples collected. This might be due to insufficient sampling, but could perhaps reflect the lack of adequate nutrition, which would affect females more than males. The relatively low body weights of these field-collected beetles, compared to those reared under optimal laboratory conditions (see table 1) suggest that in fact this may be the case. SMITH (1966) showed that insufficient nutrition decreased the body size of several coccinellids, being more detrimental to females. YINON (1964) showed that females of *C. bipustulatus* required about a third more food than did males. Also, low values of total fatty acids were obtained from the field-collected beetles (table 2) as compared to the laboratory-reared ones (table 1), and there was a consistent decline in these values in the former group of beetles, as summer continued. The source of this nutritional inadequacy may possibly be associated with the aggregation of the beetles on the bark and main limbs of the citrus trees, noted in a former section. In these microhabitats the predator has only one scale-insect prey available in considerable numbers, namely the chaff scale. And it is this diet which may be inadequate, from the nutritional point of view, to promote population increase. Laboratory experiments on the nutrition of this beetle indicate that oviposition is curtailed on suboptimal diets (TADMOR, unpublished data). Taken together, these data implicate lack of adequate nutrition during the critical period as a major factor in the population decline of *C. bipustulatus*. This suggests that the populations of *C. bipustulatus* could be augmented by offering them a supplementary food of a non-viable nature — an artificial diet — during the critical summer period. We are currently exploring this possibility.

RÉSUMÉ

Étude du déclin estival de *Chilocorus bipustulatus* dans les orangeraies d'Israël

La coccinelle coccidiphage *Chilocorus bipustulatus* est un des plus importants prédateurs de cochenilles des *Citrus* en Israël. La cause de la réduction en été des populations de *Chilocorus bipustulatus* a été étudiée par des expériences en laboratoire ainsi que par des observations dans deux orangeraies. Les conditions estivales : températures élevées et photopériode de jours longs n'ont pas réduit la fécondité et la respiration n'a pas été affectée. Le champignon pathogène *Hesperomyces virescens* THAXTER a été retrouvé dans l'une des orangeraies considérées, mais la fécondité, la longévité et la respiration des coccinelles infectées n'étaient pas différentes des normales. Des analyses biochimiques des réserves du corps adipeux et des mesures de la respiration des coccinelles ont été effectuées. Le manque d'une nutrition suffisante dans les mois chauds en été peut être la cause de la diminution des populations de coccinelles.

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