ENTOMOPHAGA 28 (2), 1983, 117-121

EFFECT OF 3 INSECT GROWTH REGULATORS ON LARVAL DEVELOPMENT, FECUNDITY AND EGG VIABILITY OF THE COCCINELID *CHILOCORUS BIPUSTULATUS* [*COL. : COCCINELIDAE*]

BEN AMI PELEG

The Israel Cohen Institute for Biological Control
27 Keren Kayemet Street, Rehovot 76345, Israel

The effect of 3 insect growth regulators – methoprene, diflubenzuron and RO 13-5223, on the coccinelid *Chilocorus bipustulatus* L. was studied in the laboratory. Feeding on *Chrysomphalus aonidum* (L.) or *Aspidiotus hederae* Vallot (*Diaspididae*) treated with the IGRs at the concentration of 0.025 % a.i. revealed the following : diflubenzuron caused a complete mortality of 1st instar larvae ; methoprene and RO 13-5223 did not arrest larval development but inhibited pupation ; fecundity of sexually mature females was not affected by the 3 IGRs but egg hatch was completely inhibited ; egg viability was regained when IGR-exposed females had been transferred to an uncontaminated environment.

The use of insecticides in citrus groves to control insect and mite pests causes disruption of the biological balance and the outbreak of non-target organisms. Insect growth regulators (IGRs) are potentially selective insecticides which have been subjected to studies concerning their impact on non-target pests, as well as on beneficial insect activity (**Westigard**, 1979; **Schroeder** *et al.*, 1980 ; **Madrid & Stewart**, 1981, **Colwell & Schaefer**, 1981). Previous studies demonstrated that 2 IGRs, methoprene and RO 13-5223, had no adverse effect on the development of 4 hymenopterous parasites of scale insects (**Peleg & Gothilf**, 1980 ; **Peleg**, 1982). The present study evaluates the effect of methoprene, diflubenzuron and RO 13-5223 on the coccinelid *Chilocorus bipustulatus* L., an important predator of armoured and soft scales in Israel (**Kehat & Greenberg**, 1970). Therefore it may be worth while to know the effect of potentially IGR insecticides on *C. bipustulatus*, as a representative of *Coccinelidae*, an important and significant group concerning biological control of scale insects.

MATERIALS AND METHODS

The IGRs used were : 1) methoprene [isopropyl (E,E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate] 60 % E.C. (Altoside ^{R} ; Zoecon Corp. U.S.A.) ; 2) diflubenzuron [1- (4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea] 25 % W.P. (Dimilin ^{R} ; Philips-Duphar, Holland) ; 3) RO 13-5223 [ethyl < 2-(p-phenoxyphenoxy) ethyl > carbamate] 12.5 % E.C. (Dr. Maag LTD, Switzerland).

Larvae and adults of *C. bipustulatus* were taken from an one year old laboratory culture. The beetles were fed on Florida red scale, *Chrysomphalus aonidum* (L.) and *Aspidiotus hederae* Vallot (*Diaspididae*) reared on "Butter Nut" squashes. The chemicals were applied by dipping the scale infested squashes in 0.025 % a.i. IGR mixture for 2 s. Laboratory rearing and tests were conducted at 25 ± 1 °C and 55 ± 5 % R.H.

Effect on larval development

Squashes heavily infested with *C. aonidum* at all developmental stages were treated with the various IGRs. Twenty four hours after treatment newly hatched, 1st instar *C. bipustulatus* larvae were transferred from stock culture to the treated squashes with the aid of a fine brush. Larvae were provided with ample of food (10 larvae per infested squash) in order to avoid cannibalism. Observation continued until all immature stages pupated or died.

Effect on oviposition and egg viability

Pairs of sexually mature, 12-14 days old *C. bipustulatus* beetles were collected from stock culture. Ten pairs per replication were caged together in a rectangular plastic box (38 × 32 × 16 cm) covered with a glass plate and 2 cloth windows located on the sides. The beetles were provided with IGR- treated squashes infested with mature, 5 weeks old, *A. hederae*. Each squash was covered with a strip (5 × 20 cm) of white flannel cloth which served as an oviposition site. *C. bipustulatus* female prefers to lay its eggs between the fibers of this material, and the orange-coloured eggs are easily recognized against the white back-ground (**Nadel & Biron**, 1964). Cloth strips and squashes were replaced daily and twice a week, respectively. The majority of the eggs were deposited on the white flannel. The small number of eggs beneath the scales was disregarded. Beetles were allowed to feed on IGR-treated *A. hederae* scales for 3 weeks, and on untreated scale infested squashes for additional 3 weeks. The eggs on the flannel strips were kept for viability observation.

RESULTS AND DISCUSSION

Feeding on diflubenzuron treated *C. aonidum* resulted in a complete mortality of 1st instar *C. bipustulatus* larvae. Development was arrested during the 1st larval ecdysis apparently due to the chitinase blocker activity and none of the diflubenzuron exposed 1st larvae succeeded to transform into the 2nd instar (table 1).

Exposure to either methoprene or RO 13-5223 treated *C. aonidum* scales did not affect larval development of *C. bipustulatus* ; however, all the larvae that fed on the IGR-treated scales failed to pupate (table 1). The 3 larval ecdysis were normal and within 9-15 days all the 1st instar larvae, regardless of treatments, reached the 4th instar. However, while larvae in the cotrols began pupating on the 15th day of feeding, those exposed to IGR-treated scales fed and grew for an additional 2-3 weeks and then stopped feeding and a slow body shrinkage took place until all individuals died. Transferring of methoprene and RO 13-5223 exposed 3rd and 4th instar larvae to an uncontaminated environment did not result in pupation. Such a response to JH-active IGRs is known in many insect species (**Vogel *et al.*,** 1979).

Feeding on *A. hederae* treated with each of the 3 IGRs did not affect the fecundity of sexually mature *C. bipustulatus* females. During 3 weeks of feeding on treated scales, no significant differences were observed between treatments regarding weekly and total number of eggs laid by the beetles (table 2) ; however, none of these eggs hatched. Egg viability was affected immediately following the 1st day of feeding on IGR-treated scales (table 3). Most of the eggs exhibited embryonic development and apparently embryonic ecdysis was inhibited

due to the IGRs activity. This phenomenon was observed in many insect species where embryogenesis and/or embryonic ecdysis are inhibited in eggs exposed to JH-active IGRS in the female's body or after egg deposition (**Staal**, 1975).

TABLE 1

Development of Chilocorus bipustulatus *larvae fed on IGR-treated* Chrysomphalus aonidum *scales*

Treatment	% of 1st instar larvae that reached (a)			
	2nd instar (b)	3rd instar (b)	4th instar (b)	pupa
Methoprene	76.25	72.5	70.0	0
RO 13-5223	77.5	72.5	70.75	0
Diflubenzuron	0	0.	0	0
None	85.0	85.0	77.5	50.0

(a) The results are the averages of 4 replicates, each consisting of 20 1st instar larvae.
(b) The results (except for diflubenzuron) do not differ significantly at the 5 % level (F Test).

TABLE 2

Fecundity of sexually mature Chilocorus bipustulatus *females fed on IGR-treated* Aspidiotus hederae *scales*

Treatment	Weekly number of eggs per female (a, b)		
	Week 1st	2nd	3rd
Methoprene	19.11	28.56	23.17
RO 13-5223	23.94	30.10	24.64
Diflubenzuron	21.07	27.23	16.80
None	23.94	35.63	32.97

(a) The results are the averages of 3 replicates, each consisted of 10 females.
(b) The results do not differ significantly at the 5 % level (F Test).

TABLE 3

Viability of eggs laid by Chilocorus bipustulatus *fed on IGR-treated* Aspidiotus hederae *scales*

Treatment	% of viable eggs (a)					
	Feeding on treated scales (days)		IGR-exposed that were transferred to feeding on untreated scales (days) (b, c)			
	1-3	4-21	1-3	4	10	15
Methoprene	0.3	0	30.3[a]	62.2[a]	60.7[a]	77.5[a]
RO 13-5223	0	0	46.9[a]	71.2[a]	57.2[a]	73.2[a]
Diflubenzuron	0.2	0	40.8[a]	80.0[a]	71.2[a]	71.2[a]
None	73.3	70.0	65.0[b]	80.5[a]	64.7[a]	74.0[a]

(a) The results are the averages of 3 replicates, each consisted of 50-100 eggs laid by 10 females.
(b) Beetles were transferred 3 weeks after being fed on IGR-treated scales.
(c) Numbers within colums followed by the same letter do not differ significantly at the 5 % level (F Test).

After 3 weeks of feeding on IGR-treated *A. hederae* scales the beetles were provided with untreated prey. The transfer to feeding on untreated scales immediately restored egg viability, and after 3 days of exposure to untreated scales no significant differences were observed between treatments as regard to egg hatch (table 3). No mortality of *C. bipustulatus* adults was noticed in all treatments during the 1st 3 weeks of the experiment.

According to **Franz** *et al.* (1980) chemicals are grouped into 4 classes which describe the degree of reduction of beneficial insects performance in comparison to untreated check : < 50 % = harmless (1) ; 50-79 % = slightly harmful (2) ; 80-99 % = moderately harmful (3) ; > 99 % = harmful (4). Diflubenzuron caused almost 100 % mortality of 1st *C. bipustulatus* larvae ; therefore, this chitinase-blocker may be defined as harmful (4) for larval development. The other 2 IGRs tested, methoprene and RO 13-5223, allowed the coccinelid larvae to develop to the last instar, but pupation was completely inhibited. Although IGR-exposed larvae fail to pupate, under field condition they may feed on IGR-treated scales and destroy considerable quantities of the pest. As regards the effect on larval development, methoprene and RO 13-5223, may be considered as moderately harmful (3). All 3 IGRs tested were harmless, with regard to the longevity of *C. bipustulatus* adults. Fecundity was not affected by treatment with these chemicals but egg hatch was completely arrested ; however, when IGR-exposed beetles were transferred to an uncontaminated environment they deposited viable eggs. Thus, under field conditions, *C. bipustulatus* females exposed to IGR-treated scales may regain their capability to lay viable eggs upon feeding on uncontaminated prey. Based on the results obtained in this work the effect of the 3 IGRs tested on fecundity and egg viability may be considered as slightly (2) to moderately (3) harmful.

The overall impact on non-target organisms of the 3 IGRs tested in the laboratory, should be evaluated under field conditions.

ACKNOWLEDGMENTS

The author thanks Dr. **Maag** LTD, Dielsdorf, Switzerland and Palimport LTD, Tel-Aviv, Israel, for providing the IGRs.

RÉSUMÉ

Effet de 4 régulateurs de croissance des insectes sur le développement larvaire, la fécondité et la viabilité des œufs de la coccinelle *Chilocorus bipustulatus* [*Col. : Coccinelidae*]

On a étudié en laboratoire l'effet sur la coccinelle *Chilocorus bipustulatus* L. de 3 régulateurs de croissance des insectes : le méthoprène, le diflubenzuron et le RO 13-5223. L'alimentation de la coccinelle avec *Chrysomphalus aonidum* (L.) ou *Aspidiotus hederae* Vallot (*Diaspididae*) traités avec ces produits à la concentration de 0,025 % a donné les résultats suivants : le diflubenzuron provoque la mortalité totale des larves du 1er stade, le méthoprène et le RO 13-5223 n'arrêtent pas le développement larvaire mais inhibent la nymphose ; la fécondité des femelles mûres n'est pas affectée par les 3 régulateurs de croissance mais l'éclosion des œufs ne se produit pas. La viabilité des œufs est recouvrée lorsque les femelles exposées aux régulateurs de croissance sont transférées dans un milieu non contaminé.

REFERENCES

Colwell, A.E. & Schaefer, C.H. – 1981. Effects of the insect growth regulator Bay SIR 8514 on pest diptera and nontarget aquatic organisms. – *Can. Entomol.*, 113, 185-191.

Franz, J.M., Bogenschutz, H., Hassan, S.A., Huang, P., Naton, E., Suter, H. & Viggiani, G. – 1980. Results of a joint pesticide test programme by the working group : pesticides and beneficial arthropods. – *Entomophaga*, 25, 231-236.

Kehat, M. & Greenberg, S. – 1970. Survey and distribution of lady beetles [*Coccinelidae*] in citrus groves in Israel. – *Entomophaga*, 15, 275-280.

Madrid, F.J. & Stewart, R.K. – 1981. Impact of diflubenzuron spray on gypsy moth parasitoids in the field. – *J. Econ. Entomol.*, 74, 1-2.

Nadel, D.J. & Biron, S. – 1964. Laboratory studies and controlled mass rearing of *Chilocorus bipustulatus* Linn., a citrus scale predator in Israel. – *Rivista Parassitol.*, 25, 195-206.

Peleg, B.A. – 1982. Effect of a new insect growth regulator, RO 13-5223, on the development of two hymenopterous parasites – *Aphytis holoxanthus* DeBach and *Metaphycus bartletti* Annecke & Mynhardt. – *Entomophaga*, 27.

Peleg, B.A. & Gothilf, S. – 1981. Effect of the juvenoid altosid on the development of three hymenopterous parasites. – *Entomophaga*, 25, 323-327.

Schroeder, W.J., Sutton, R.A. & Beavers, J.B. – 1980. *Diaprepes abbreviatus* : fate of diflubenzuron and effect on nontarget pests and beneficial species after application to citrus for weevil control. – *J. Econ. Entomol.*, 73, 637-638.

Staal, G.B. – 1975. Insect growth regulators with juvenile hormone activity. – *Annu. Rev. Entomol.*, 20, 417-460.

Vogel, W., Masner, P., Graf, O. & Dorn, S. – 1979. Types of response of insects on treatment with juvenile hormone active insect growth regulators. – *Experiencia*, 35, 1254-1256.

Westigard, P.H. – 1979. Codling moth : control on pears with diflubenzuron and effects on nontarget pest and beneficial species. – *J. Econ. Entomol.*, 72, 552-554.