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Effect of Cypermethrin Exposed Hosts on Egg-Adult Development Time, Number of Offspring, Sex Ratio, Longevity, and Size of *Apanteles galleriae* Wilkinson (Hymenoptera : Braconidae)

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ABSTRACT. The effects of sub-lethal doses of cypermethrin onto the larval host *Achoria grisella* Fabr. (Lepidoptera : Pyralidae) were evaluated on egg-adult development time, number of offspring produced, sex ratio, longevity, and size of the larval endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera : Braconidae). Overall time to adult eclosion increased by more than 50% when *Ap. galleriae* was reared on cypermethrin-treated host larvae and the development time of the wasp increased in a dose-dependent manner. Adult longevity and the number of surviving offspring produced decreased with increasing insecticide dose. The number of surviving offspring decreased more than 50% at the lowest dose of the insecticide. Neither sex ratio nor adult body sizes were altered by cypermethrin exposure when compared to untreated wasps. This work suggests that sublethal doses of the insecticide could limit the development, survival, and growth of parasitoid wasps due to possible metabolic, hormonal, and nutritional deficiencies. The potential adverse effects that cypermethrin has on the natural enemy of the pest can impact on the success of IPM programs.

KEY WORDS : *Apanteles galleriae*, cypermethrin, biological control, risk assessment, non-target insect

INTRODUCTION

Insecticides frequently disrupt the balance between a host and its natural enemy (Van Driesche & Bellows, 1996; Xu et al., 2001). Several studies have shown that insecticides applied to insect pests cause various sublethal effects on parasitoids, such as changes in development and emergence rates, and sex ratio (Krespi et al., 1991; Willrich & Boethel, 2001; Saber et al., 2005) either by direct chemical contact or by ingestion of treated prey (Wells et al., 2001). The use of insecticides may have an adverse effect on the life cycle of beneficial nontarget insects, and this may subsequently result in an outbreak of pest numbers (Tomberlin et al., 2002).

Apanteles galleriae Wilkinson (Hymenoptera : Braconidae) is a koinobiont, solitary, larval endoparasitoid of several lepidopterans including the pyralid wax moths, *Galleria mellonella* L., *Achoria grisella* Fabr., *Ac. innotata* Walker, and *Vitula edmandsae* (Packard) (Watanabe, 1987; Shimamori, 1987; Whitfield et al., 2001). Caterpillars of these host species are pests in beehives because they feed on pollen and generally destroy the combs. *Ap. galleriae* adults feed on honey, fruit nectar, and host larvae in nature. Therefore, adult wasps are likely to be exposed to residues of insecticides used against these pests and that accumulate on honeycomb, fruit trees and host larvae.

Pyrethroids are among the most commonly used insecticides worldwide, accounting for more than 30% of global use (Shukla et al., 2002). Cypermethrin (CYP) [α-cyano-3-phenoxybenzyl (1 RS)-cis-, trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] is a synthetic pyrethroid (Shukla et al., 2002) which is used widely in the control of various agricultural pests belonging to the orders Lepidoptera, Coleoptera, Diptera, and Hemiptera (Cox, 1996; Liu et al., 1998; Suh et al., 2000). Like all pyrethroids, cypermethrin kills insects by disrupting normal functioning of the nervous system (Vijverberg & Van Den Bercken, 1990). Even sublethal doses of insecticides can have profound effects on parasitoids (Smilanick et al., 1996), thereby greatly reducing populations of indigenous natural enemies, as well as hindering biological control efforts. Evaluation of the impact of insecticides on non-target insects, like parasitoids, is of great importance for success in biological control applications, and in any integrated pest management program utilizing chemical control. Therefore, this research was undertaken to determine if feeding by a host on a sublethal cypermethrin-treated diet adversely affected emergence time, number, sex ratio, longevity, and size of *Ap. galleriae* offspring.

MATERIALS AND METHODS

Insects. Laboratory colonies of *Ac. grisella* and *Ap. galleriae* were established from adults, which were collected from several beehives located in the vicinity of Ardeşen, Turkey. *Ac. grisella* was reared on honeycomb at 25 ± 1°C, 60 ± 5% RH under a photoperiod of 12 : 12h (L :D). Adults of *Ap. galleriae* were fed a 30% (w/v) honey solution and kept at the same rearing conditions with the host species. For details of the biology and rearing of the parasitoid and the host, see Uçkan & Gülel (2000) and Uçkan & Ergin (2003).

Insecticide Application. Cypermethrin (Imperator, 250g/liter EC, Zeneca Ltd., İzmir, Turkey) was used in all bioassays as water source and prepared in distilled water as parts per million of active ingredient. To ensure survival of host larvae, preliminary tests were carried out on small groups so that an appropriate range of doses (below 50% mortality range) for cypermethrin could be selected. Various doses (10, 20, 50, and 100ppm) of cypermethrin-treated distilled water were incorporated to the synthetic diet of host larvae. Host diet including crumbled honeycomb, bran, honey, glycerin, and distilled water was prepared by the method of BRONSKILL (1961) and SAK et al. (2006).

Bioassays. An individual mating pair of *Ac. grisella* (1- to 2-day-old at 25ºC) was placed in 250ml jars containing 1g honeycomb to provide a mating and oviposition substrate. The adults were removed from the jars on the fifth day. Early instars of one female equivalent host larvae, which is produced by a mated host female in five days (25 to 57 larvae), were exposed to 5g of host diet treated with the selected doses of the cypermethrin in each jar. Host larvae were exposed to parasitization by placing an individual mating pair of adult parasitoids (1- to 2-day-old at 25ºC) in jars two days later. Parasitoid adults were fed a 50% (w/v) honey solution soaked in cotton balls and removed from the jars after five days. The jars were maintained in another rearing room under the same conditions mentioned above for the stock cultures. Control groups were also prepared with the same methodology, but untreated synthetic diet including only distilled water instead of cypermethrin solution was used.

All jars were observed daily for date of adult eclosion. The time required for completion of parasitoid development from egg to adult eclosion was recorded, as was the total number of progeny and the sex of each eclosing adult. Longevity of newly emerged adult female and male wasps was assessed by placing an individual mating pair (n = 5 pairs) in 80ml jars each containing a piece of cotton ball soaked with a 50% (w/v) honey solution. Jars were held under the environmental conditions mentioned above for the stock cultures. Food supplement was replenished at 2d intervals until all parasitoids died. Adult body sizes (length) of cypermethrin-treated wasps and controls were determined by selecting random samples (5 females, 5 males) of wasps for each experimental and control group. However, adult size could only be obtained from a total of 11 females at 100ppm. Adults were measured from the head to the tip of the abdomen using an Olympus S2X 12 stereodissecting microscope with a calibrated eyepiece micrometer. All experiments were repeated three times.

Statistics. Variations due to cypermethrin doses in egg-adult development time, number of viable offspring developing to adulthood, sex ratio, longevity and size were inferred using one-way analysis of variance (ANOVA). Subsequently, means were separated using Tukey's Honestly Significant Difference (HSD) test (SPSS, 1999). Data for adult longevity were also subjected to two-way ANOVA (SPSS 1999) to determine the main effects of cypermethrin dose, sex, and their interaction on adult longevity. An arcsine square-root transformation was performed on percentage values of fecundity before analysis. Results were considered statistically significant when $P<0.05$.

RESULTS

Egg-adult development time of *Ap. galleriae* reared on *Ac. grisella* larvae exposed to different doses of cypermethrin was significantly longer than those parasitoids that developed on untreated hosts (Table 1; $F = 24.594$; df = 4, 10; $P = 0.000$). Wasp development from egg to adult emergence at 25ºC normally requires 27-35d. However, parasitoids reared on hosts exposed to any dose of insecticide tested required 12 to 25d longerthan controls to complete devlopment (Table 1).

TABLE 1

Effect of different sub-lethal doses of cypermethrin-treated *Ac. grisella* larvae on the egg-adult development time of *Ap. galleriae*.

CYP (ppm)	Egg-adult development time (day)	
	Range	($\bar{x}$ ± SE)[a]
C	27 – 35	31.3 ± 2.3a
10	44 – 50	47.0 ± 1.7b
20	49 – 51	50.0 ± 0.6b
50	50 – 60	55.0 ± 2.9b
100	53 – 59	56.0 ± 1.7b

a. Numbers in column followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). CYP : Cypermethrin doses, C : Control group.

Similarly, cypermethrin treatment lowered the number of offspring produced by *Ap. galleriae*. For example, the number of progeny surviving to adulthood by a single parasitoid female throughout its adult life was on average 108 ± 13.2 when the host was fed on an insecticide-free diet. However, when host larvae were treated with cypermethrin, the number of offspring developing to adulthood was significantly lower in all experimental groups in contrast to the controls ($F = 30.615$; df = 4, 10; $P = 0.000$). This decline in total number of offspring was dose-dependent between 10-100ppm cypermethrin (Table 2). Though the number of offspring developing to adulthood was reduced by pyrethroid-treatment, the sex ratio of emerging adults was not disturbed : The sex ratio of adults was always male biased in treated and untreated wasps ($F = 1.505$; df = 4, 10; $P = 0.273$) (Table 2).

The effect of cypermethrin on adult longevity was dose and sex dependent, and the relationship between insecticide dose and adult longevity was not significantly influenced by gender (Table 3). Mean longevity of cypermethrin-treated females and males decreased at all doses of insecticide >10ppm tested with respect to controls ($F = 23.648$; df = 4; $P = 0.000$) (Table 4).

Adult body sizes of male and female parasitoids appeared to decrease with increasing dose of cypermethrin (Table 5), but these differences were not found to be significant for either sex ($F = 2.498$; df = 4, 66; $P = 0.051$ for females and $F = 1.280$; df = 4, 70; $P = 0.286$ for males).

TABLE 2

Effect of different sub-lethal doses of cypermethrin-treated *Ac. grisella* larvae on the number of surviving offspring developing to adulthood and female sex ratio of *Ap. galleriae*.

CYP (ppm)	Number of offspring and sex ratio					
	Female		Male		Total number ($\bar{x}$ ± SEM)[a]	Female sex ratio (%)[a]
	Range	($\bar{x}$ ± SEM)	Range	($\bar{x}$ ± SEM)		
C	19 – 73	40.3 ± 16.6	59 – 84	67.7 ± 8.2	108.0 ± 13.2a	37.6a
10	12 – 13	12.7 ± 0.3	28 – 47	35.3 ± 5.9	48.0 ± 6.0b	26.4a
20	7 – 13	10.3 ± 1.8	16 – 23	19.3 ± 2.0	29.7 ± 3.7bc	34.8a
50	10 – 15	11.7 ± 1.7	12 – 14	13.3 ± 0.7	25.0 ± 2.1bc	46.7a
100	3 – 4	3.7 ± 0.3	4 – 10	7.7 ± 1.9	11.3 ± 2.2c	32.4a

a. Numbers in columns followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). CYP : Cypermethrin doses, C : Control group.

TABLE 3

ANOVA of the effects of cypermethrin dose, sex, and their interaction on adult longevity of *A. galleriae* reared on *Ac. grisella* larvae treated with cypermethrin ($r^2 = 0.451$).

Source	df	MS	F	P
Dose	4	1,234.128	23.648	0.000
Sex	1	395.438	7.577	0.007
Dose*Sex	4	21.143	0.405	0.805
Error	126	52.188		

TABLE 4

Effect of different sub-lethal doses of cypermethrin-treated *Ac. grisella* larvae on the adult longevity (day) of *Ap. galleriae*.

CYP (ppm)	Female		Male		Both sexes
	Range	($\bar{x}$ ± SEM)	Range	($\bar{x}$ ± SEM)	($\bar{x}$ ± SEM)[a]
C	27 – 51	39.1 ± 1.8	31 – 57	40.5 ± 2.0	39.8 ± 1.4ab
10	30 – 50	39.3 ± 1.6	36 – 57	43.9 ± 1.5	41.6 ± 1.2a
20	16 – 44	32.8 ± 2.5	17 – 49	37.6 ± 2.6	35.2 ± 1.8b
50	14 – 37	26.9 ± 1.6	17 – 38	28.6 ± 1.7	27.7 ± 1.1c
100	18 – 29	23.3 ± 1.6	22 – 31	28.3 ± 1.1	25.6 ± 1.1c

a. Numbers in column followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). CYP : Cypermethrin doses, C : Control group.

TABLE 5

Effect of different sub-lethal doses of cypermethrin-treated *Ac. grisella* larvae on the adult size (mm) of *Ap. galleriae*.

CYP (ppm)	Female			Male		
	n	Range	($\bar{x}$ ± SEM)[a]	n	Range	($\bar{x}$ ± SEM)[a]
C	15	2.1 – 2.3	2.8 ± 0.1a	15	2.0 – 2.8	2.4 ± 0.1a
10	15	2.2 – 3.1	2.6 ± 0.1a	15	1.8 – 2.6	2.3 ± 0.1a
20	15	2.2 – 2.8	2.6 ± 0.1a	15	1.8 – 2.8	2.3 ± 0.1a
50	15	2.2 – 2.9	2.5 ± 0.1a	15	1.8 – 2.7	2.2 ± 0.0a
100	11	2.0 – 2.9	2.5 ± 0.1a	15	1.8 – 2.4	2.1 ± 0.0a

a. Numbers in columns followed by the same letter are not significantly different (P>0.05, Tukey's HSD test). CYP : Cypermethrin doses, C : Control group.

DISCUSSION

Our results indicated that the overall time to adult eclosion increased by more than 50% when *Ap. galleriae* was reared on cypermethrin-treated host larvae. This result is in agreement with other reports on the effects of some insecticides on the larval and pupal developmental time of lepidopterous species (GAABOUB et al., 1985; BIDDINGER & HULL, 1999). However, we could find no report on insecticide-dependent delay in adult parasitoid eclosion although a number of studies noted reduced emergence rates as a result of insecticide treatment for various parasitoid species (SCHNEIDER et al., 2003; 2004; SABER et al., 2005). There was a considerable decline in

the number of wasps emerging from the cypermethrin-treated host larvae. Even at the lowest dose (10ppm), the number of adult wasps decreased more than 50% and declined more so at higher doses. The fact that some larvae that were parasitized by *Ap. galleriae* died from cypermethrin should also be taken into consideration for this drastic decrease. However, none of the insecticide doses tested in our study affected the female sex ratio in wasp progeny. The insignificant impact of insecticides on the progeny sex ratio of parasitoids was also previously reported by SUH et al. (2000) and SABER et al. (2005). These results may suggest that insecticides are nonselective toward developing larvae, females and males of parasitoid wasps. Examining the effect of cypermethrin on longevity of adults revealed that insecticide treatment significantly affected longevity of *Ap. galleriae* and the response was both dose- and sex-dependent. Comparisons of the longevity of wasps at four cypermethrin treatments showed that the disruptive effect of cypermethrin on longevity was higher at 50 and 100ppm with respect to 20ppm. Cypermethrin was both highly toxic to female and male wasps in terms of longevity. However, longevity of females exposed to cypermethrin tended to decrease more drastically relative to males with increasing dose. The difference is thought to be related partly to differences in size and physiology between the sexes.

General stress responses in arthropods are known to be energetically demanding events (KORSLOOT et al., 2004). The organisms may consume more energy to repair mechanisms and pathological effects may deplete energy reserves. Therefore, the decrease in energy storages of in the host, and subsequently parasitoid larvae resulting from cypermethrin-induced stress may prolong the growth and development of parasitoid progeny. The emergence rate of parasitoids may be reduced due to organ malformations in the larvae or other perturbations. SCHNEIDER et al., (2004) reported a decrease in emergence from parasitized hosts after exposure to spinosad in the endoparasitoid, *Hyposoter didymator* (Thunberg) (Hymenoptera : Ichneumonidae), due to the incapacity for the larvae to produce silk for spinning his cocoon. The neurotoxic effects of cypermethrin may suppress juvenile hormone levels in the host (OPPENOORTH, 1985). Parasitoid larvae synchronizing development with the host by making use of host hormones may have been affected by the changes in the hormonal milieu of the host and display a delay in larval developmental time. The delay in immature development of this parasitoid may also be attributed to the cypermethrin-induced decline in diet quality and to the potency of cypermethrin as an antifeedant (TOMLIN, 2000), resulting in an interference of sufficient food supply from the host.

Because insect behaviour is affected by both the nervous system and hormones, insecticides that attack the nervous system and disrupt the hormonal balance and/or metabolic process in insects can affect behaviour and physiology at levels that do not lead to direct mortality (HAYNES, 1988). Therefore, insecticides decrease the production of offspring because of behavioural modifications in mate location, courtship, and oviposition, or due to physiological effects on egg fertilization, oogenesis, ovulation, spermatogenesis, and sperm motility (HAYNES, 1988). Studies with parasitoids have also shown deleterious effects on reproduction with sublethal doses of insecticides (SUH et al., 2000; TAKADA et al., 2001; XU et al., 2001). It has been reported that malathion applied orally to *Pimpla turionellae* L. (Hymenoptera : Ichneumonidae) females have decreased the hatching rate of wasp eggs (ÖZKAN & EMRE, 1997). As a result, sublethal doses of insecticides can affect the population density and may further inhibit continuity of the generation of wasps in nature by preventing eggs from hatching. This, in turn, may disrupt the effectiveness of parasitoid species in integrated pest management programs.

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