Cyromazine Effects on *Opius concolor* SZÉPL. (Hymenoptera: Braconidae) Applied through Laboratory Host *Ceratitis capitata* WIED. (Diptera: Tephritidae)

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(Received 9 February 1996; Accepted 16 July 1996)

The effects of topical treatments with five concentrations (0.00001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 1 mg/ml and 10 mg/ml) of cyromazine on *Ceratitis capitata* WIED. (Dip., Tephritidae) and indirect effects of these treatments on a larval parasitoid, *Opius concolor* SZÉPL. (Hym., Braconidae), were studied. The results obtained showed that topical treatments on the third larval stadium of *C. capitata* did not affect the development. Further, at the concentrations used here, cyromazine did not affect the development or parasitism of *O. concolor*.

Key words: cyromazine, *Opius concolor*, *Ceratitis capitata*, parasitism, parasitoid development

INTRODUCTION

Common pesticides disturb the balance between hosts, predators, and parasitoids. They can sometimes produce secondary outbreaks of pests. Thus, selective pesticides are needed for modern pest management programs. Development and use of insect growth regulators (IGRs) is one of the more promising techniques in dipteran control. More pesticides with adverse effects on insect metamorphosis have become available in recent years and are being introduced into IPM-programs. Whereas most of these insecticides are considered to be more or less harmless against various beneficial organisms, like parasitoids, some of the recently developed compounds showed detrimental effects (CROFT, 1990; GRENIER and GRENIER, 1993; HASSAN, 1992; HASSAN et al., 1991; STOLZ, 1990).

Cyromazine is a specific and effective IGR in control of dipteran populations. Cyromazine was discovered by Ciba Geigy Ltd. in the mid-1970s and was originally developed under the trade name of VETRAZIN® as a blowfly control agent. Further applications of cyromazine are in the control of fly larvae in manure, either by topical application (NEPOREX®) or as feed-through in poultry (LARVADEX®), as well as in crop protection (TRIGARD®) (GRAF, 1993). Its specificity for dipteran species makes it particularly valuable in IPM programs. Moreover, its negative effects on natural enemies are negligible. Cyromazine is an IGR of the triazine class (Ciba-Geigy División Agricultura, 1988) that, when applied to adult diet of the Mediterranean fruit fly, *Ceratitis capitata* WIED., negatively affected egg hatching and subsequent preimaginal development, and as a consequence, caused a reduction of *C. capitata* populations (JIMENEZ-PEYDRO et al., 1995).

The direct effects of cyromazine on the adults and pupae of *Opius concolor* SZÉPL. have been studied by JACAS et al. (1992) and JACAS and VIÑUELA (1994 a, b). However, the

indirect effects on the fruit fly parasitoids are unknown.

In the present work, laboratory tests were conducted to show the indirect effects on the preimaginal development of the parasitoid *O. concolor* when cyromazine is applied to parasitized larvae of the laboratory host *C. capitata*.

MATERIALS AND METHODS

The rearings of *C. capitata* and *O. concolor* were carried out in a climatic chamber at $25 \pm 2^\circ\text{C}$, 65–70% relative humidity and a photoregime of 16 h light, according to the methods described by JIMENEZ-PEYDRO et al. (1995) for *C. capitata* and by AVILLA (1982) for *O. concolor*.

Technical cyromazine (95% a.i.) was diluted in water to obtain the desired concentrations; 1% acetone was added to avoid further precipitation. Five increasing cyromazine concentrations (Table 1) were topically applied to parasitized and non-parasitized 3rd-stadium *C. capitata* larvae. Cyromazine (1 μl) was topically applied to the abdomen of larvae using a 10 μl Hamilton microsyringe.

The treatment on parasitized 3rd-stadium *C. capitata* larvae was made 24 h after parasitization. Each specimen, briefly anesthetized by cold (10 min at 4°C), was placed on a glass plate protected by a piece of filter paper to avoid contamination, and cyromazine solution was carefully applied to the abdomen. The insects were isolated in glass containers under the same conditions previously described for the mass-rearing.

Three replicates with 20 larvae for each concentration, and one untreated control were done. Daily observations were made until adult parasitoid emergence.

For *C. capitata*, the parameters studied were larval mortality, percentage of pupation, adult emergence and morphogenetic anomalies. For *O. concolor*, the percentage of parasitism, defined as the percentage of adults of the parasitoid emerging from the host, preimaginal development duration and morphogenetic anomalies were investigated.

Statistical analyses. The data were all analyzed with a one-way analysis of variance (ANOVA); means were separated using FISHER's Protected Least Significant Differences (FISHER's PLSD) (STATVIEW II™, 1987). All percentage data were transformed to arcsine square root of proportion before analysis of variance. In all cases, untransformed means and standard errors (SE) are reported. Treatment effects were considered significant at $p < 0.05$.

RESULTS

1. Effects on *Ceratitis capitata*

Larval mortality, pupation and adult percentages are shown in Table 1. Topical treatments to 3rd-stadium larvae of *C. capitata* had no effect on development. Larval mortality was between 12.4 and 18.2%, and did not differ significantly from that of the control (14.2%). Percentage of pupation of *C. capitata* did not differ significantly from the control for any of the concentrations tested. The percentage of pupation observed ranged between 81.8 and 87.6% for the concentrations tested. In a high number of pupae, pupation was not complete, and some pupae died, but this also occurred in the control. The percentage of adult emergence did not differ significantly from the control and ranged between 62.1 and 67.1% at the concentrations tested. We found some adultoids of *C. capitata*. The adultoids were also found in the control, and adultoid percentages of the treated trials (between 2.3 and 5.1%) did not differ from that of the control (4.9%).

Table 1. Larval mortality, pupation and adult emergence percentages of *Ceratitis capitata*

Concentration (mg/ml)	% Larval mortality ± SE ^a	% Pupation ± SE ^b	% Adult emergence ± SE ^c
Control	14.2 ± 2.1 a	85.8 ± 2.1 a	65.7 ± 3.3 a
0.00001	13.7 ± 0.9 a	86.3 ± 0.9 a	66.7 ± 2.0 a
0.01	17.9 ± 1.3 a	82.1 ± 1.3 a	64.2 ± 2.2 a
0.1	12.4 ± 1.2 a	87.6 ± 1.2 a	67.1 ± 4.8 a
1	18.2 ± 2.2 a	81.8 ± 2.2 a	62.1 ± 5.1 a
10	16.0 ± 1.0 a	84.0 ± 1.0 a	65.3 ± 3.0 a

Means ± SE followed by the same letter within this column are not significantly different [$p > 0.05$; FISHER's PLSD test (STATVIEW II™, 1987), arcsine transformation].

^a % Larval mortality = (No. of death larvae/No. of larvae) × 100.

^b % Pupation = (No. of pupae/No. of larvae) × 100.

^c % Adult emergence = (No. of adults/No. of pupae) × 100.

Table 2. Percentage of parasitism by *Opius concolor*

Concentration (mg/ml)	% Parasitism ± SE ^a
Control	58.7 ± 2.4 a
0.00001	56.8 ± 3.8 a
0.01	55.5 ± 3.2 a
0.1	56.1 ± 4.2 a
1	57.8 ± 3.1 a
10	55.9 ± 4.8 a

Means ± SE followed by the same letter are not significantly different [$p > 0.05$; FISHER's PLSD test (STATVIEW II™, 1987), arcsine transformation].

^a % Parasitism = (No. of adults of *O. concolor*/No. of larvae of *C. capitata*) × 100.

2. Effects on *Opius concolor*

The percentage of parasitoid adults obtained from the topically treated larvae of *C. capitata* are shown in Table 2. The percentage of parasitism obtained was very similar in all concentrations and did not differ from the control. These results show that the percentage of effective parasitism did not differ between treated and control trials.

The duration of preimaginal development in all cases was between 19–22 days. In consequence, it is possible to conclude that topical treatments to the host had no effects on the duration of preimaginal development at any of the concentrations tested. Further no significant anatomical effects in adults of *O. concolor* were observed. In all of the cases studied 5–10% of the individuals had incomplete emergence or some malformation, but these anomalies were also found in the same proportion in the control.

DISCUSSION

Results of a preliminary study showed that cyromazine added to the diet of the last larval instar of *C. capitata* WIED. has significant effects on the development of this species (JIMENEZ-PEYDRO et al., 1995). When cyromazine is applied to larval food, a significant decrease is found in adult eclosion and the decrease was concentration dependent. When the application is on the pupa it causes a delay of adult eclosion and in many cases the adult

does not normally eclose from the pupa (STARK et al., 1992). In contrast, topical treatments with cyromazine has no effects on the survival of treated larvae and adult emergence. Two types of morphological abnormalities have been found: adults appear with abnormalities in wing formation and with difficulties in mobility, and adults with only wing buds and abnormal legs cannot eclose from the puparium, but these abnormalities have also been observed in the control and in mass-rearing.

Cyromazine topically applied to the host does not show any harmful effect on the development of *O. concolor* in laboratory tests. In the present trials, the average mortality and adult eclosion rates did not differ from those of the untreated control. Pupation and adult eclosion occurs normally.

Contradicting results were found in the literature about the effect of cyromazine on natural enemies. There are non-toxic effects of cyromazine when applied to immature stages of *Chrysocharis parski* (PARRELLA et al., 1983) and *Diglyphus* spp. (PARRELLA et al., 1987). AVILLA et al. (1990) found that cyromazine has a slightly harmful effect on the survival of *Encarsia tricolor* females and concluded that this compound should be considered as inoffensive for this parasitoid and compatible for the IPM programs. VAN DE VEIRE (1994) concluded that cyromazine was compatible with *Encarsia formosa* for the greenhouse whitefly *Trialeurodes vaporariorum*. This compound was classified as harmful in the 6th joint testing program of the IOBC/WPRS Working Group, "Pesticides and Beneficial Organisms" (HASSAN et al., 1991). BLÜMEL and STOLTZ (1993), after a study of cyromazine effects in *Phytoseiulus persimilis*, concluded that cyromazine should be considered as slightly to moderately harmful for this predator.

JACAS et al. (1992) concluded that cyromazine has no effect on parasitism potential of the females treated and that this compound should be considered as slightly harmful (category 2 of IOBC criteria) (HASSAN et al., 1991) for this parasitoid. In a subsequent study, JACAS and VIÑUELA (1994 a, b), after modification of the laboratory method for study of cyromazine effects on *O. concolor*, concluded that cyromazine should be considered as harmless for this parasitoid (category 1 of IOBC criteria). In accordance with these authors, our study shows that topical treatment with cyromazine had no negative impact on the development of *O. concolor* in laboratory tests. According to the IOBC evaluation criteria (HASSAN, 1994; HASSAN et al., 1991), cyromazine should be considered harmless (category 1) for *O. concolor* when topically applied to *C. capitata*.

In conclusion, topical treatment with cyromazine of the host-parasitoid complex *C. capitata*-*O. concolor* had no effect on the development of this parasitoid, but further studies of the effects of cyromazine on the reproduction and progeny development of *O. concolor* are necessary to conclude that this product is compatible with *O. concolor* in Olive Fruit Fly IPM programs.

ACKNOWLEDGEMENTS

Our thanks to Dr. R. SEND (Ciba Geigy Ltd., Basel, Switzerland) and to Mr. J. TICÓ (Ciba Geigy, Barcelona, Spain) for providing the active compound. We thank Dr. J. PIEDADE-GUERREIRO and Mrs. I. GUARDADO (Instituto de Investigação Científica Tropical, Lisboa, Portugal) for providing *C. capitata* stock and Dr. A. JIMÉNEZ (Departamento Protección Vegetal, CIT-INIA, Madrid) for providing the *O. concolor* stock.

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