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The effects of 20-hydroxyecdysone, metyrapone and dithiodiglucose on survival and reproduction of the aphid, *Myzus persicae* (Rhynchota Aphididae)

Václav NĚMEC[1], Jan HAVELKA[1], Nadia BALDASSARI[2], Piero BARONIO[3]
[1]*Institute of Entomology, Czech Academy of Sciences, Czech Republic,*
[2]*Scienze e Tecnologie Alimentari, Università di Bologna, Cesena, Italy,*
[3]*Dipartimento di Scienze e Tecnologie Agroambientali - Entomologia, Università di Bologna, Italy*

Abstract

The effects of systemically applied metyrapone, 20-hydroxyecdysone and dithiodiglucose on reproduction, development and survival were followed in the aphid *Myzus persicae* (Sulz.). These substances were applied as water solutions, which penetrated through the leaf stem. Most dramatic changes in aphid performance occurred at the second half of the experimental period, i.e. between days 6-12, when a noxious effect was manifested even in the controls. Metyrapone showed the most deleterious effect on survival and reproduction. This substance also caused necrotization of tobacco leaves. Dithiodiglucose and 20-hydroxyecdysone in concentration 1 mg/ml protected somehow the leaves from necrosis compelled by aphid sucking. The reproduction index and number of survivals was lower than in the control test. The higher concentration (2 mg/ml) rapidly decreased reproduction and survival rate, and dramatically increased mortality, particularly in the case of dithiodiglucose, when the trial had to be stopped after 6th days.

Key words: *Myzus persicae*, systemic application, metyrapone, 20-hydroxyecdysone, dithiodiglucose, reproduction, mortality.

Introduction

Interactions between plants and insects have been developing since the appearance of these organisms. The self-defense systems developed by plants are both mechanical (thorns, sharp hairs) and chemical. The latter included various substances with deterrent, repellent or toxic effects (for a review see Rosenthal and Bernbaum, 1991). Some plants contain substances with the activity of insect hormones (Sláma, 1969, 1993, Sláma *et al*., 1974) or hormonal antagonist (Bowers *et al*., 1976). For example, 20-hydroxyecdysone is a common ecdysone analogue of plant origin. This steroid was found in various unrelated plant species like the fern, *Polypodium vulgare* (Jizba *et al*., 1967), *Podocarpus nakai* (Nakanishi *et al*., 1966), *Leuzea (Rhaponticum) carthamoides* (Baltaev and Abubakirov, 1987) and others (for a review see Adler and Grebenok, 1995).

Our study compares effects of 20-hydroxyecdysone as a representative of insect hormone agonist, metyrapone as hormonal antagonist and dithiodiglucose (DTDG) as a metabolical antagonist. The latter two compounds are synthetic, but thiosugars do occur in plants of the *Brassicaceae* family as glucosinolates (thioglycosides) in which sugar moiety is conjugated to an indole derivatives (Gmelin and Virtanen, 1961). Metyrapone is still used in human medicine for testing some endocrine malfunctions. The aim of this study was to find a suitable substance and way for the control of aphids.

Materials and methods

The tests were performed on the aphid, *Myzus persicae* (Sulz.) that sucked on tobacco leaves. Each leaf was cut off from its maternal plant and the petiole was submerged in water (control) or water solution of a tested compound. Three adult virginoparae were transferred by small wet brush on a medium size tobacco leaf and let to reproduce. The tests were performed at 22 °C and 18/6 light-dark regimes. Each trial was repeated two times on five tobacco leaves. Tested substances were taken up by the leaves through their veins and were consumed by the aphids.

Metyrapone (2-methyl-1, 2-di-3-pyridyl-1-propanone, purchased from Aldrich Co.) was applied as water solutions in concentrations 1mg/ml or 10mg/ml. Sonication was used to obtain these solutions that were poured in 20 ml glass bottles. The petioles of tobacco leaves were submerged in the tested solution and the bottle was closed with a cotton plug. The tests with other compounds were prepared in the same way.

20-OH-ecdysone (20 E) was isolated from the roots of plant, *Leuzea carthamoides (Asteraceae)* in the Institute of Entomology, Czech Academy of Science by Dr. P. Šimek and refined to 99% purity. It was applied in concentrations 1 mg/ml and 2 mg/ml in the way as described above.

The sugar antimetabolite dithiodiglucose (DTDG) was kindly provided by Prof. J. Kocourek (Department of Biochemistry, Faculty of Sciences at Charles University in Prague). It was applied in the same concentrations and way as described for 20 E.

The tested aphids were scored daily, recognizing: a) living and dead aphids, b) nymphs and adults. The dead aphids were removed. Each trial was performed separately in a cage covered with dense net. Collected data were statistically processed (using program Statistica, '99 edition, Stat Soft Inc., Microsoft Excel 97) as seen in tables 1 and 2. The "reproduction index" represents ratio of the mean number of F1 nymphs to the mean number of F0 adult females.

Results

Water and 10% ethanol were used in our pilot control trials. Since we found a noxious effect of 10% ethanol on the tobacco leaves (necrotization) and, due to that, also on the reproduction and survival of tested aphids, we abandoned the use of 10% ethanol as a solvent for tested substances. The relatively high mortality among the control aphids sucking on leaves submerged in water was most probably a consequence of deleterious effect of the tobacco leaves, particularly in the second half of the experimental time, i.e. between days 6 and 12 (see tables 1 and 2).

Metyrapone proved to exert a high deleterious effect on aphids. These effects were not only direct (toxic) but also indirect, due to necrotization of the leaves. Metyrapone restricted reproduction and decreased the number of survivals. The effects were dose-dependent. The reproduction index, mortality and the number of survivals in the trials with concentration 1mg/ml were little lower than in the controls, whereas concentration 10 mg/ml caused a significant decrease in reproduction and the mortality was nearly 90%.

The low concentration (1mg/ml) of 20 E had a favorable effect on aphid reproduction and survival and also on the tobacco leaves. The second highest number of survivals, occurrence of winged aphids and no necrotization of the leaves were recorded in this test. The higher 20E concentration (2 mg/ml) decreased the number of survivals by 70% and mortality increased nearly to 50%.

Dithiodiglucose (DTDG) applied in the lower dose (1 mg/ml) it compensated somehow the noxious effect of aphid sucking on tobacco. We have scored in this trial the highest number of survivals and also the highest rate of reproduction; the mortality was 50%. The higher concentration (2 mg/ml) decreased the number of survivals by 73%. Mortality was 83% within the first 6 days of the trial, so we have interrupted the test.

Discussion

Metyrapone exerted the most deleterious effect on both tobacco leaves and the aphids sucking on them. This substance is known as inhibitor of P-450 monooxigenases, which participate in the biosynthesis of juvenile hormone and ecdysone (Feyreisen and Hoffmann, 1977, Darvas *et al*., 1987). Thus, the resulting effect might have reflected the lack of ecdysone and juvenile hormone. Metyrapone also evoked necrosis of tobacco leaves and thereby restricted the nutrition of aphids. The higher doses of metyrapone (10 mg/ml) showed evident toxic effect to the aphids and induced large necrotization of the tobacco leaves, which resulted in 90% mortality. It should be noted that orally applied metyrapone accelerated development in the larvae of *Spodoptera littoralis* (Boisd.) and *Galleria mellonella* (L.) (Gelbič and Němec, 2001).

Thioglucosides are important growth factors in the plant genus *Brassica* (Gmelin and Virtanen, 1961).

Table 1. Effects of 10% ethanol, metyrapone, 20-hydroxyecdysone (20 E) and dithiodiglucose (DTDG) on reproduction, surviving and mortality in the aphid, *Myzus persicae* 6 days after application

Substance	Concentration	Reproduction	Surviving aphids	Mortality %
Water	---	13.2 ± 2.3	213	8.3 ± 2.3
Ethanol	10%	4.5 ± 1.0	82	0.0
Metyrapone	1mg/ml	8.8 ± 3.5	88	31.8 ± 14.8
Metyrapone	10mg/ml	4.1 ± 2.0	46	10.9 ± 4.8
20 E	1mg/ml	12.9 ± 3.7	167	18.0 ± 12.5
20 E	2mg/ml	5.7 ± 2.2	68	0.0
DTDG	1mg/ml	9.7 ± 2.2	160	36.3 ± 8.1
DTDG	2mg/ml	5.2 ± 4.8	45	83.0 ± 17.0

Table 2. Effects of 10% ethanol, metyrapone, 20-hydroxyecdysone (20 E) and dithiodiglucose (DTDG) on reproduction, surviving and mortality in the aphid, *Myzus persicae*, 12 days after application

Substance	Concentration	Reproduction	Surviving aphids		Mortality %
Water	---	11.8 ± 4.5	300	+	60.7 ± 24.3
Ethanol	10%	12.7 ± 4.7	176	+	25.7 ± 13.7
Metyrapone	1mg/ml	15.3 ± 5.9	268	+	38.4 ± 16.0
Metyrapone	2mg/ml	1.5 ± 1.2	14	+	88.0 ± 13.0
20 E	1mg/ml	14.3 ± 5.1	426	x	38.4 ± 16.0
20 E	2mg/ml	11.6 ± 6.4	139	x	45.5 ± 27.0
DTDG	1mg/ml	20.1 ± 6.1	520	x	50.0 ± 0.0

+ leaf necrotization, x occurrence of winged forms.

Systemically applied DTDG in concentrations 10 mg/ml, 1 mg/ml, 0.1 mg/ml and 0.001 mg/ml to the aphid, *Acyrtosiphon pisum* (Harris) by means of hydroponically cultivated pea, *Pisum sativum* restricted reproduction and evoked the appearance of winged aphids, even DTDG did not penetrated in the plant (Němec *et al*., 1984). Orally administered DTDG to the larvae of *S. littoralis* induced also juvenilizing effects as described Gelbič and Němec (1982). But the principal effect of DTDG was similar as in the case of the monomer, 1-thio-D-glucose: interference with the metabolism of D-glucose (Shankland *et al*., 1968; Jiráček and Němec, 1975).

In our trials, DTDG influenced the infested aphids directly, because it could penetrate into the tobacco leaves. The lower concentration (1 mg/ml) revealed beneficial effects on both leaves and aphids. We have scored the highest number of survivals and reproduction index (number of progeny per one female, see table 2). The higher tested concentration (2 mg/ml) was harmful. Since aphid mortality reached 83% during the first 6 days, the trial, was discontinued (see table 1).

The 20E hormone evokes various disturbances in insect development (apolysis without molting, ecdysial death etc. for survey see Sláma *et al*., 1974; Kubo *et al*., 1983) when injected or ingested. Our trials offered an opportunity to study the direct effect of ingested 20E on the aphid *M. persicae.* It should be mentioned that *L. carthamoides,* which contains high amount of phytoecdysteroids (including 20E) (Baltaev, 1994) was introduced in the Czech Republic, it became associated with some consolidated entomofauna (Zelený *et al*., 1997), regardless of the high content of phytoecdysteroids. *M. persicae* occurred there only rarely.

According to the presented data (see table 2) the low concentration of 20E (1 mg/ml) compensated somehow the noxious effects of tobacco and also prevented leaf necrosis. The beneficial effects were indicated by the high reproduction index, number of survivals and low mortality. The concentration (2 mg/ml) decreased significantly the number of survivals. Another effect, which was observed, was appearance of winged form of aphids (as in the trials with DTDG). Study of other hormonal agonists and antagonists is under way.

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Authors' addresses: Václav NĚMEC (corresponding author, e-mail: Nemecv@entu.cas.cz), Jan HAVELKA, Institute of Entomology, Czech Academy of Sciences, Branišovská 31, České Budějovice, 37005 Czech Republic; Nadia BALDASSARI, Scienze e Tecnologie Alimentari, Università di Bologna, via Ravennate 1020, 47023 Cesena, Italy; Piero BARONIO, DiSTA - Entomologia, Università di Bologna, via Fanin 42, 40127 Bologna, Italy.

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