Journal of Insect Physiology 58 (2012) 335–341

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Effects of a methanolic extract of the plant *Haplophyllum tuberculatum* and of teflubenzuron on female reproduction in the migratory locust, *Locusta migratoria* (Orthoptera: Oedipodinae)

Fatma Acheuk [a,*], Michel Cusson [b], Bahia Doumandji-Mitiche [c]

[a] Département de Biologie, Faculté des Sciences, Université M'hamed Bougara, Avenue de l'indépendance, 35000 Boumerdes, Algeria
[b] Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du PEPS, Quebec City, Quebec G1V 4C7, Canada
[c] Département de Zoologie Agricole et Forestière, Ecole Nationale Supérieure d'Agronomie, 16200 El-Harrach, Alger, Algeria

ARTICLE INFO

Article history:
Received 6 October 2011
Received in revised form 7 December 2011
Accepted 9 December 2011
Available online 21 December 2011

Keywords:
Haplophyllum tuberculatum
Teflubenzuron
Benzoylphenyl ureas
Locusta migratoria
Methanolic extract
Reproduction
Vitellogenesis
Ecdysteroid titers
Radioimmunoassay

ABSTRACT

The effects of a methanolic extract of the plant *Haplophyllum tuberculatum* (ME-Ht) and of teflubenzuron (TFB) were compared on several reproductive variables and ecdysteroid titers in the females of *Locusta migratoria*. The test products were administered orally to newly emerged females at doses of 1500 (ME-Ht) and 10 μg/female (TFB). The methanolic extract and TFB had comparable effects on several of the variables examined. Both significantly delayed the first oviposition and reduced fecundity and fertility. ME-Ht and TFB also displayed similar effects on ovarian growth, vitellogenesis and ecdysteroid titers. Both treatments induced a drop in hemolymph protein levels as well as a reduction in vitellogenin uptake by oocytes. This delay in oogenesis was accompanied by a resorption of terminal oocytes. However, whereas TFB completely blocked egg hatch, ME-Ht only had a modest inhibitory effect on this variable. Hemolymph and ovarian ecdysteroid titers, as measured by radioimmunoassay, were similar and low in both control and treated females, except for a peak observed only in control females at the end of vitellogenesis. We discuss the functional significance of the observed effects in the context of the putative modes of action of the methanolic plant extract and TFB.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Insect reproduction and its hormonal regulation constitute potential targets for the development of bio-rational insecticides. In this perspective, the identification of molecules that interfere with insect reproduction and development, and target the associated endocrine regulatory processes has been the focus of fruitful investigations in the past few decades (Dhadialla et al., 2005). This research has led to the development of "insect growth regulators" (IGRs), which disrupt growth, development and reproduction by either mimicking key hormones such as juvenile hormone (JH) and 20-hydroxyecdysone (20E), or by inhibiting chitin synthesis (Dhadialla et al., 1998, 2005).

Effects similar to those of synthetic IGRs have been reported for secondary plant metabolites, which constitute a potential source of naturally occurring endocrine disruptors for insect control. Céspedes et al. (2005) and Rharrabe et al. (2009) have shown that plant metabolites can have various molecular targets when interfering with metamorphosis and reproduction. Research focusing on the modes of action of many of these metabolites has shown that their insecticidal and IGR activities are largely due to their anti-feeding (Feng et al., 1995) and inhibitory effects on enzymes and metabolism as a whole (Kubo and Kloche, 1983; Céspedes et al., 2000, 2001), with the exception of precocenes, which have a direct inhibitory effect on JH biosynthesis (Pratt and Bowers, 1977).

In an effort to identify novel pest-control products that are both environmentally acceptable and effective for the management of the migratory locust, *Locusta migratoria*, we assessed the effects of a methanolic extract of the above-ground portion of the perennial herb *Haplophyllum tuberculatum* (Rutaceae) on several reproductive variables in locust females. This plant is common in the Adrar region of Algeria, where it escapes feeding damage by *L. migratoria* (unpublished observations). Assayed against various organisms, *H. tuberculatum* extracts have been observed to display insecticidal (Mohsen et al., 1989), nematicidal (Onifade et al., 2008), antifungal and antibacterial (Sheriha et al., 1985; Al-Burtamani et al., 2005) properties. The plant's chemical composition has been shown to vary as a function of geographic location and time of collection, and has so far been found to include alkaloids, lignans, flavonoids and essential oils (Khalid and

* Corresponding author.
E-mail address: fatma.acheuk@yahoo.fr (F. Acheuk).

0022-1910/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2011.12.004

Waterman, 1981; Sheriha and Abouamer, 1984; Sheriha et al., 1985; Al-Yahya et al., 1992; Al-Rehaily et al., 2001; Al-Yousuf et al., 2005; Al-Burtamani et al., 2005; Javidnia et al., 2006).

Reproduction and its endocrine regulation in *L. migratoria* have received considerable attention. Depending on rearing temperature, vitellogenin (Vg) first appears in the hemolymph of adult females between the 5th and 9th days after emergence, and its titers reach a peak of 25–30 mg/mL just before the onset of the second vitellogenic cycle (Chinzei and Wyatt, 1985). Although vitellogenesis in *L. migratoria* is primarily under the control of JH (Dhadialla and Wyatt, 1983; Wyatt et al., 1987; Wyatt, 1988), a neurohormone, the "ovary maturing parsin" (Lom-OMP), has been shown to promote Vg synthesis and vitellogenesis in adult females through a mechanism independent of JH, possibly through an ecdysteroidogenic effect (Girardie and Girardie, 1996; Girardie et al., 1998). However, although ecdysteroids play a role in the control of reproduction of many insects (Lafont et al., 2005), 20E is not essential for Vg production in adult locusts but appears to potentiate the JH-induced transcription of the Vg gene (Girardie et al., 1998). Follicular cells are the site of ecdysteroid production in *L. migratoria* and a high proportion (95%) is incorporated in eggs as polar or apolar conjugates (Lagueux et al., 1977, 1981; Gande and Morgan, 1979; Hagedorn, 1985), and can be bound to proteins such as vitellin (Hoffmann, 1980; Hagedorn, 1985; Tawfik et al., 1999). These conjugates are a source of ecdysteroids during embryogenesis (Lagueux et al., 1977), and their hydrolysis, in the eggs, is responsible for the peak of free ecdysone observed during embryonic development (Lagueux et al., 1981), where they are involved in the initiation of morphogenetic movements and the induction of cuticulogenesis (Lagueux et al., 1979).

For the present study, we assessed oocyte growth, vitellogenesis, oviposition, fecundity, fertility and ecdysteroid titers in *L. migratoria* females treated with a *H. tuberculatum* extract at emergence. Given that preliminary work showed similarities between the effect of this extract, on the variables considered here, and those documented for desert locusts, *Schistocerca gregaria*, treated with the benzoyl phenyl urea (BPU) diflubenzuron (Tail et al., 2008, 2010), we compared the effects of the extract to those of teflubenzuron, another IGR of the BPU family. BPUs are a group of insecticides that disrupt insect molt through their inhibitory effect on chitin synthesis (Cohen, 1987; Graf, 1993). Studies focusing on diflubenzuron, the first BPU to be commercialized, have shown that this molecule can also affect reproduction in adult females, with reports pointing to reductions in both fecundity and fertility (Lim and Lee, 1882; Soltani, 1987; Soltani and Soltani-Mazouni, 1992). Here we show that the two treatments have similar, yet distinct effects on the variables examined.

2. Materials and methods

2.1. Insect rearing

Mass rearing of *L. migratoria cinerascens* was carried out in the laboratory according to the method of Pener et al. (1989), using adults collected in the field in the Adrar region, Algeria. Larvae and adults were held in $45 \times 50 \times 50$ cm cages at 30–32 °C, 50–70% RH, and under a 12 h:12 h, L:D photoperiod. Locusts were fed an *Avena sterilis*-based diet, complemented with wheat germ.

2.2. Treatments

Teflubenzuron (TFB; Nomolt®) was provided by the *Service de Lutte Antiacridienne* of the *Institut National de la Protection des Végétaux (INPV)*, Algeria. The rutacea *H. tuberculatum* was collected in the biotope of the migratory locust, in the Adrar region. The methanolic extract of the above-ground portion of the plant (ME-Ht) was prepared from leaves and stems dried in the shade and ground into a fine powder. The extraction was carried out by macerating the powder for 3 days in methanol, followed by filtration and evaporation at 40 °C. The dried extract was kept at 4 °C and resuspended in ethanol (30 mg/mL) just before being used for treating insects.

Newly emerged females (0–12 h post emergence) were treated with TFB (10 μg/female) or with the crude methanolic extract of *H. tuberculatum* (1500 μg/female), by forced feeding of an ethanol solution (50 μL) using a micropipette, without anesthesia. Controls were given absolute ethanol only. Doses were chosen in preliminary assays on the basis of their sub-lethal effects on reproduction.

2.3. Measurement of reproductive variables

Mating pairs were established for each newly emerged female (treated or control individual) using males of the same age, by introducing a pair into a $25 \times 25 \times 30$ cm cage at the beginning of the experiment. The rearing of these pairs was done under the same conditions described above for mass rearing. The time of first oviposition, the number of ootheca, the number of eggs per ootheca and hatching success (eggs incubated at 30 °C) were recorded for each pair.

To assess the effects of TFB and ME-Ht on oocyte growth, five females were dissected each day (from the seventh day after treatment up to the first oviposition) to measure the length of the terminal oocyte using an ocular micrometer fitted to a dissecting microscope. For each dissected female, we measured the length of 10 terminal oocytes in each ovary, for a total of 20 oocytes/female. Ovarioles exhibiting resorption bodies were also counted to calculate the rate of oocyte resorption.

2.4. Gel electrophoresis of hemolymph and ovarian proteins

Hemolymph and ovaries were obtained from three females in each of the control and treated groups at 8 and 12 days post-treatment. Phenyl methyl sulfonyl fluoride (PMSF; final concentration: 0.1%) was added to hemolymph (20 μL) collected from metathoracic legs using a graduated capillary pipette. Ovaries were homogenized and centrifuged at 9600*g* in a Fresco microcentrifuge (Heraeus) for 10 min, and the supernatant was then recovered. Proteins were fractionated by SDS–PAGE (Laemmli, 1970) on a 10% gel, followed by staining using Coomassie brilliant blue.

2.5. Sample collection and extraction of ecdysteroids

Hemolymph (10 μL) was collected from the metathoracic legs of control and treated females, using a graduated capillary pipette. Each sample was transferred to 300 μL methanol and stored at −20 °C until processed. For extraction, each sample was first centrifuged at 2400*g* for 10 min, followed by collection of the supernatant and resuspension of the pellet in 50 μL methanol, for a second extraction. The two supernatants thus generated were pooled and submitted to evaporation of methanol in a vacuum concentrator (Centrivap™, Labconco). With respect to ovaries, they were collected from control and treated females, quickly weighed, and then transferred to 500 μL methanol and stored at −20 °C until processed. Just before titer determination, ovaries were homogenized (in methanol), after which the temperature of the homogenate was raised to 60 °C for 10 min, followed by centrifugation at 9600*g* for 10 min. The supernatant was collected and the pellet reextracted twice in 200 μL methanol. The supernatants were pooled and dried in a vacuum concentrator as described above. The extract was then resuspended in methanol and partitioned twice against hexane to remove lipids (Dinan and Rees, 1981). The methanolic phase was

Download English Version:

https://daneshyari.com/en/article/2840664

Download Persian Version:

https://daneshyari.com/article/2840664

Daneshyari.com