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Effect of low doses of precocene on reproduction and gene expression in green peach aphid

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HIGHLIGHTS

- We tested whether low-doses of precocene, an antagonist of JH, would stimulate *Myzus persicae* reproduction.
- Nymphs treated with certain concentrations of precocene had up to 2-fold increased reproductive outputs when adults.
- Treatments had no measurable effects on JH levels in adults.
- Up to 300-fold increased expression of some genes was observed.
- There was no clear relationship between gene expression and reproductive responses.

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ABSTRACT

Insect reproduction can be stimulated by exposure to sublethal doses of insecticide that kill the same insects at high doses. This bi-phasic dose response to a stressor is known as hormesis and has been demonstrated with many different insect–insecticide models. The specific mechanisms of the increased reproduction in insects following sublethal pesticide exposure are unknown, but may be related to juvenile hormone (JH), which has a major role in regulation of metamorphosis and reproductive development in insects. We tested the hypothesis that exposure to sublethal concentrations of precocene, an antagonist of JH, would not result in stimulated reproductive outputs in the green peach aphid, *Myzus persicae*, as can be demonstrated with many neurotoxic insecticides. We also measured JH titers and the expression of various developmental (*FPPS I*), stress response (*Hsp60*), and dispersal (*OSD*, *TOL* and *ANT*) genes in aphids following exposure to the same precocene treatments. We found that when aphid nymphs were treated with certain sublethal concentrations of precocene, 1.5- to 2-fold increased reproductive stimulation occurred when they became adults, but this effect subsided in the following generation. Precocene treatments to nymphs resulted in no measurable effects on JH levels in subsequent reproducing adults. Although we detected major effects on gene expression following some precocene treatments (e.g. 100- to 300-fold increased expression of some genes), there were no clear relationships between gene expression and reproductive responses for a given treatment.

1. Introduction

Hormesis is a biphasic dose–response characterized by inhibition of biological functions with exposure to high amounts of a stressor, coupled with stimulation of the same biological processes when the organism is exposed to low amounts of the stressor (Calabrese, 2008; Mattson, 2008). The phenomenon has been observed across many taxa of microorganisms, invertebrates, vertebrates, and plants in response to various types of chemical, physical, and biological stressors. Insecticide-induced hormesis is now well documented in insects (Cohen, 2006; Cutler, 2013; Guedes and Cutler, 2013) and usually manifests most obviously as increased reproductive outputs in response to low doses of insecticide that are toxic at higher doses. Hormesis is of relevance in agricultural systems where pest and beneficial insects are frequently exposed to a myriad of chemical stressors and pollutants (Cutler, 2013). For example, insecticide-induced hormesis may be significant in development or management of insecticide resistance, pest resurgences, and secondary pest outbreaks (Morse, 1998; Cutler, 2013; Guedes and Cutler, 2013). Despite many reports of reproductive hormesis in insects, the basis of reproductive

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stimulation following exposure to low doses of insecticide is largely unknown.

Juvenile hormones (JH) have major roles in regulation of metamorphosis and reproductive development in insects, including aphids (Hamnett and Pratt, 1983; Staal, 1986; Mittler, 1991; Peric-Mataruga et al., 2006), and it has been hypothesized that changes in JH could cause or accompany increased reproductive outputs in insects during exposure to low doses of insecticide. Yu et al. (2010) showed that during increases in aphid fecundity following exposure to low doses of insecticide, JH titers increased in some instances. Although increased reproduction has been observed in insects following exposure to low doses of many types of stressors (Cutler, 2013), we are not aware of any such experiments with compounds that interfere with JH. This poses the question: Does a compound that inhibits JH, induce hormetic responses in insects in the form of stimulated reproduction?

The precocenes (precocenes I and II) are plant-derived JH antagonists that in most insects suppress metamorphosis in immature stages, and activation of ovarian growth and other reproductive processes in adult females (Bowers et al., 1976; Pratt and Bowers, 1977). Precocenes affect either the mevalonate pathway in JH biosynthesis, or directly affect the corpora allata (CA), the organ that produces JH (Staal, 1986). Their anti-allatal properties induce precocious metamorphosis in insects, including green peach aphid, *Myzus persicae* (Sulzer) (Bowers et al., 1976; Hales, 1976). We hypothesized that, unlike responses observed with neurotoxic insecticides, low concentrations of precocene would not induce a hormetic response in *M. persicae*, in the form of stimulated reproduction. Probably due to the occurrence of completely developed CA in later instars, this anti-JH agent typically is most effective against insects in mid- to late-instars (Staal, 1986). We therefore used third instar *M. persicae* to initiate the experiments.

To gain insight into potential molecular changes during exposure to sublethal concentrations of precocene, expression of certain dispersal-related (*OSD*, *TOL* and *ANT*) (Ghanim et al., 2006) and stress-related (*Hsp60*) (Parsell and Lindquist, 1993; Karouna-Renier and Zehr, 1999) genes, and a JH precursor (*FPPS I*) gene were analyzed at these concentrations (Keeling et al., 2004; Taban et al., 2009). We hypothesized that *OSD*, *TOL*, and *ANT* gene expression would correspond to fecundity responses, if any and that there would be increased *Hsp60* gene expression in the initial generation of exposure. We also predicted that *FPPS I* gene expression and JH III titers would correspond with reproductive stimulation.

2. Methods and materials

2.1. Plant and insect maintenance

Potato foliage was used in insect rearing and to conduct bioassays (Ayyanath et al., 2013). A continuous source of potato plants, *Solanum tuberosum* L. (cv. Kennebec), was maintained in a greenhouse facility at Faculty of Agriculture, Dalhousie University. Plants in pots (diameter = 12.5 cm) containing Pro-Mix® (Halifax Seed, Halifax, NS) were watered as needed. Cohorts of *M. persicae* were established from a wild population infesting broccoli plants (*Brassica oleracea* L.) in the greenhouse. Clear plastic boxes (37 L × 24 W × 14 H cm) that were lined with moistened paper towels were used to rear aphids in a growth chamber (22 ± 2 °C, 16:8 L:D, 65 ± 5% RH). Every second day, fresh foliage was replaced and the paper towels were replaced every tenth day.

2.2. Chemicals

Precocene (7-methoxy-2,2-dimethyl-3-chromene; Sigma–Aldrich, Oakville, ON) was suspended in acetone (Fisher Chemical, Toronto, ON) to obtain a 1000 mg L^{-1} stock solution. Dilutions were prepared in acetone + olive oil (19:1). Preliminary experiments established a no observable adverse effect concentration (NOAEC) of 3 mg precocene L^{-1}, and precocene concentrations of 0.01, 0.03, 0.1, 0.30, 1.0, 3.0, and 10 mg L^{-1} were used in experiments. Controls consisted of the acetone + olive oil solvent. Fresh stock solutions of test concentrations were prepared for each bioassay replicate (block in time).

2.3. Exposure

Five third instar nymphs each were placed in clean glass Petri plates (9 cm diameter) and sprayed in a Potter tower (Burkard Scientific, Uxbridge, UK) at 78 kPa with 5 ml of control or insecticide solution. After each treatment, aphids were transferred to plastic Petri plates (5.5 cm diameter) lined with a Whatman No. 1 filter paper containing two untreated potato leaf discs (1.8 cm diameter). Leaf discs were replaced every second day with freshly excised leaf discs. Fecundity was recorded every second day for two generations (*G*0, *G*1). The experiment was a randomized complete block design, with precocene concentration being the main factor of interest. Each bioassay had seven precocene concentrations (+ control), and for each there were five Petri dishes, each containing five aphids.

Each bioassay was considered an experimental block, and each bioassay was conducted three times. Repeated measures analyses were conducted using Proc Mixed in SAS (SAS, 2008), with the error terms assumed to be normal with constant variance, but not to be independent. Autoregression (AR (1)) represented the appropriate type of dependence for covariance structure. Residuals were used to verify the assumptions of normal error distribution and constant variance. Data were log-transformed as needed to meet the assumptions. If means were significantly different, they were separated using a LSD test ($\alpha = 0.05$). Backtransformed means are reported as required.

2.4. Gene expression analyses

Cohorts of ten third-instar *M. persicae* were treated in a Potter tower and placed on foliage in Petri dishes as described above. The experiment was a completely randomized design with precocene as the main factor of interest, five Petri plates with ten aphids per concentration, and 3 blocks in time (Ayyanath et al., 2014). From each Petri plate, five aphids were randomly collected at days 4, 13 and 17, representing *G*0 adults, *G*1 second instars and *G*1 adults, respectively. Total RNA was isolated using a RNeasy® mini kit (Qiagen, Toronto, ON) from aphids that were flash frozen in liquid nitrogen. A microgram of the total RNA was used for cDNA syntheses (QuantiTect® Reverse Transcription kit, Qiagen, Toronto, ON) that was stored at −20 °C until further analyses. Quality ($A_{260/280} > 2.0$) and quantity of total RNA was recorded using a Nanodrop ND-1000 (NanoDrop Technologies, Wilmington, DE) and gel electrophoresis (rRNA band intensity: 28 s = 2× 18 s) prior to cDNA syntheses. The primers (Sigma–Aldrich, Oakville, ON) used for quantitative Real-Time (qRT) PCR are listed in Table S1. Internal controls with cycle of threshold (ct) values closer to ct of selected genes were chosen to calculate expression fold-change (Pfaffl, 2001). For quantification of *OSD* and *TOL* genes, the *β-actin* gene was used as an internal control. *Ace* was used as an internal control for *ANT*, *Hsp60*, and *FPPS I* genes. In a volume of 10 μL, Quantitative RT-PCR was performed on a StepOne™ RT PCR System (Applied Biosystems, Burlington, ON, Canada) following the manufacturer's instructions using SYBR green reagent (Applied Biosystems, Burlington, ON). The reaction mixture contained 2X SYBR green reagent master mix, 2 μL cDNA, 2.5 μL ultrapure water and 0.25 μL each of forward and reverse primers (final concentration of 2.5 μM).