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Male hypofertility induced by Paraquat consumption in the non-target parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae)

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ABSTRACT

The effects of a herbicide – Paraquat – on male development and reproduction were tested on the parasitoid wasp *Anisopteromalus calandrae* (Hymenoptera, Pteromalidae) by injecting host larvae with different concentrations of this substance. Data measured were: (1) developmental success, (2) sperm stock in seminal vesicles, (3) ability to copulate and transfer sperm, and (4) offspring production. Both developmental success and sperm in seminal vesicles were reduced in the "Paraquat" groups. However, neither sperm stored in the females' spermatheca, nor offspring production (number of female offspring and sex ratio) differed from controls, whatever the host treatment. The decreased of male sperm reserve in "Paraquat" group is likely to reduce their reproductive success because they can succefully inseminated less female than control males. These males are thus hyporfertiles. Because Paraquat has non-negligible consequences on non-target parasitoid males, it is likely to affect natural and controlled insect populations.

1. Introduction

Hymenopteran parasitoids are well known for their capacity to limit pest population growth (Hawkins et al., 1997), and thus are extensively used as biological control agents (van Driesche and Bellows, 1996). However, crop protection is still largely based on broad-spectrum chemical pesticides which are noxious to beneficial arthropod populations via lethal and sublethal effects (Croft, 1990; Desneux et al., 2007). Thus assessments of potential impacts of pesticides on beneficial arthropods are of primary importance for Integrated Pest Management. Also, information on how parasitoids are exposed to pesticides need to be added (Desneux et al., 2007). Parasitoids can be exposed to pesticides through spray droplets, through residues when foraging for hosts on treated substrate/foliage, or when feeding on contaminated food sources. Exposure during development inside (endoparasitoids) or outside (ectoparasitoids) the host could also occur. Indirect exposure to a toxicant via the host can append during the development of parasitoids, altering their future reproductive success by affecting their size, longevity, oviposition, etc. (Desneux et al., 2007). Sperm production was not studied although it is of main importance in hymenopteran parasitoids.

Paraquat (1,1′–dimethyl-4,4′–bipyridinium chloride or methyl viologen) is a herbicide used throughout the world (Eisler, 1990; Ranjbar et al., 2002). It is a broad-spectrum contact weed-killer and herbage desiccant used for weed control in plantation crops and pre-harvest desiccation (Eisler, 1990). It is known to generate oxidative stress by superoxide formation in cells, which induces membrane lipid peroxidation (Bus et al., 1974). Following exposure to Paraquat, organisms can die or manifest sublethal effects on their physiology, behaviour or lifespan (Eisler, 1990). These sublethal effects could result in alterations to reproduction (Wang et al., 2001). This has mainly been studied in females of Collembola (Subagja and Snider, 1981; Choi et al., 2008), birds (Bauer, 1985), mice (Bauer Dial and Dial, 1987; Eisler, 1990), and Gastropoda (Bacchetta et al., 2002). However, Paraquat also affects male physiological functions in various species, such as mice (Rios et al., 1995) or birds (Bauer, 1985), by reducing various reproduction-related features (sperm reserve, testis size, spermatogenesis, and fertility). To our knowledge, the consequences of Paraquat consumption on the male reproductive functions of non-target insects have never been studied.

Anisopteromalus calandrae Howard (Hymenoptera: Pteromalidae) is a common and cosmopolitan parasitoid (solitary ectoparasitoid, idiobiont) of grain weevils in many tropical and sub-tropical countries (Islam and Nargis, 1994) where Paraquat is used. *A. calandrae* is used as a biological control agent for pests of rice (Ahmed and Kabir, 1995; Lucas and Riudavets, 2002), wheat (Ahmed and Kabir, 1995), maize (Williams and Floyd, 1970; Smith,

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1993), beans and peas (Schmale et al., 2001; Islam and Nargis, 1994). Considering a possibility of environmental risk for parasitoid populations, massive pre- or post-harvest sprayings may induce a contamination of the seed. Paraquat has a strong penetration power in both vegetal and animal (Eisler, 1990). Moreover, the bruchid egg is laid on the seed surface and the larva growth inside the seed afterwards (Amevoin et al., 2005). Thus, it is mainly probable that host larvae contain non-negligible amounts of Paraquat after treatments. However, data on the possible transformation of Paraquat in the insect body are missing.

Anisopteromalus calandrae is a good model for studying how Paraquat ingested during development affects the male reproduction. Male reproductive value (mating capacity, male sperm reserve, sperm stored in spermatheca, outcome of sperm competition, offspring obtained after one mating) is known under standard laboratory conditions (Do Thi Khanh et al., 2005). These authors have shown that the initial sperm stock determines male fitness, because the number of female offspring (only females are issued from fertilized eggs in arrhenotokous parthenogenesis) resulting from mating is influenced by the number of sperm transferred by males.

Using *A. calandrae* males for such experiments one needs to consider the following points: (1) initial sperm stock is mainly constituted during the larval development (Do Thi Khanh et al., 2005). (2) The only way to feed the parasitoid larva with Paraquat is to introduce it into the host larva. (3) To control the amount of Paraquat offered to the parasitoid larva, it has to be injected into the host. For many substances, host injection is a classical technique in parasitoid physiological studies (Mondy et al., 2006).

The present study investigated the effects of Paraquat consumption during male development by assessing male developmental success, sperm output, mating ability in single mating, and the number of female offspring obtained. The results provide a clearer understanding of the consequences of this toxicant on males of non-target insects.

2. Materials and methods

2.1. Insect breeding

The solitary ectoparasitoid *Anisopteromalus calandrae* Howard (Hymenoptera, Pteromalidae) strain was collected from cowpea stocks in Ivory Coast (Africa) and has been bred in the laboratory from about 50 founders since 2000 on larvae of *Callosobruchus maculatus* (Coleoptera, Bruchidae) which develop in cowpea seeds (*Vigna unguiculata*). Experiments and parasitoid rearing occurred in a climatic chamber (light:dark periods 12 h:12 h, temperature 28 ± 5 °C, relative humidity 65 ± 10%).

With respect to natural protandry, experimental virgin males were 24-h-old and virgin females 2-h-old. Females of *A. calandrae* reproduce by arrhenotokous parthenogenesis whereby only female offspring are produced from fertilized eggs (Quicke, 1997; Heimpel and de Boer, 2008). The number of female offspring is thus an indication of male fertility.

2.2. Host preparation

The aim of the study was to assess the effect of ingesting Paraquat (Methyl viologen dichloride hydrate 98%; Aldrich) contained in their host, on the development and reproductive potential of male parasitoids. Host larvae were divided into five treatment groups: no injection (control), injected with 1 µl of distilled water (water), and injected with 1 µl of three different concentrations of Paraquat (PQ): 0.1 g L^{-1} (PQ0.1), 0.25 g L^{-1} (PQ0.25), and 0.5 g L^{-1} (PQ0.5). The "water" group was to show if there were deleterious effect of injection. On average, host larvae contained about 2 ± 0.17 µl of haemolymph (Mondy et al., 2006) and their weight was 9.12 ± 0.20 mg (unpublished data). The injection was performed under a microscope with a micro-capillary connected to a Hamilton microliter syringe. *A. calandrae* is an idiobiont parasitoid which stops the development of its host by killing or immobilizing it. In lab conditions, a pre-test showed that parasitoid larva killed their host before detrimental effects due to Paraquat injection (unpublished data).

2.3. Male development

To obtain male eggs only, virgin *A. calandrae* females were offered gelatine capsules each containing one *C. maculatus* last instar larva (Damiens et al., 2001). Freshly laid eggs were collected and each egg was gently deposited on a previously treated *C. maculatus* larva (*cf.* diet preparation). Parasitoid development took place in individual holes of a Plexiglas plate (light:dark periods 12 h:12 h, temperature 28 ± 5 °C, relative humidity 65 ± 10%). This artificial system allows male larval development to be observed without interference.

For each individual parasitoid, mortality ($n_{control}$ = 121, n_{water} = 105, $n_{PQ0.1}$ = 137, $n_{PQ0.25}$ = 129, $n_{PQ0.5}$ = 76) and development time ($n_{control}$ = 35, n_{water} = 25, $n_{PQ0.1}$ = 57, $n_{PQ0.25}$ = 52, $n_{PQ0.5}$ = 17) were recorded. Lethal and sublethal concentrations of Paraquat were determined.

2.4. Male reproductive potential

Sperm are stored in the seminal vesicles in males and in the spermatheca of females (Quicke, 1997). Stored sperm were counted one day after emergence in males in each treatment group: control (n = 15), water (n = 17), a sublethal concentration PQ0.1 (n = 18), and a lethal concentration PQ0.25 (n = 15) (*cf.* Section 3.1). Because a similar quantity of sperm was contained in each seminal vesicle (unpublished data), only one of the two seminal vesicles was dissected and the sperm dispersed in a drop of saline solution (128.3 mM NaCl, 4.7 mM KCl, 2.3 mM $CaCl_2$). Spermatozoa were exhaustively counted under a fluorescence microscope after ethanol fixation and DAPI staining (Bressac and Chevrier, 1998). The number of sperm was multiplied by two to obtain the total male sperm reserve.

In parasitoids, male size influences sperm reserves (Lacoume et al., 2006). To control for this factor, male size was evaluated by measuring male head width ($n_{control}$ = 23, n_{water} = 15, $n_{P0.1}$ = 23, $n_{P0.25}$ = 23) using a stereomicroscope connected to a computer with picture analysis software (NIH-Image J).

To determine the male mating capacity in single mating, 2-h-old virgin females were individually placed for 24 h with one 1-day-old virgin male (control, water, P0.1 or P0.25). A portion of these females ($n_{control}$ = 16, n_{water} = 17, $n_{P0.1}$ = 17, $n_{P0.25}$ = 15) were then isolated and dissected in order to count the spermatozoa stored in their spermatheca, as described previously.

The other females ($n_{control}$ = 26, n_{water} = 20, $n_{P0.1}$ = 26, $n_{P0.25}$ = 20) were individually placed in an oviposition box with 10 bruchid-infested cowpea seeds, renewed daily, and a source of sucrose solution (10% in water). Each seed contained one to three 18-day-old hosts (last instar larvae) (Chevrier and Bressac, 2002). Egg-laying was carried out over a 7-day period (Bressac et al., 2009). After complete development of parasitoids, the number of daughter offspring was recorded as a measure of male fertility.

2.5. Statistical analysis

The male mortalities were compared by a Chi-square (χ^2, $P < 0.05$) and a Chi-square test with Bonferroni correction (χ^2,