[SHORT COMMUNICATION]

Effects of a Pyrethroid on Ovarian Development in Diapause Females of the Two Spotted Spider Mite

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(Received 25 September 2012; Accepted 12 November 2012)

Key words: cypermethrin, neurosecretion, *Tetranychus urticae*, vitellogenesis

The endocrine mechanisms regulating adult diapause and vitellogenesis in mites have not been clarified. Adult females of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) enter diapause without vitellogenesis in response to short daylength and low temperature (Veerman, 1985; Kawakami et al., 2009). In the present study, we topically applied a pyrethroid, cypermethrin (CyM) to diapause females of *T. urticae* as a preliminary approach to the endocrine mechanisms of adult diapause and vitellogenesis. Pyrethroids are synthetic analogues of the pyrethrins, naturally occurring insecticides found in pyrethrum, and have been shown to act primarily on the nervous system (Soderlund and Bloomquist, 1989). In some insects, pyrethroids produce an increase in frequency and an alteration in the pattern of electrical activity of neurosecretory cells in the brain and the corpora cardiaca to release neurosecretory substances (Orchard and Osborne, 1979; Orchard, 1980; Singh and Orchard, 1983). In the soft tick, *Ornithodoros moubata*, pyrethroids induce vitellogenesis probably through stimulating the release of neurosecretory hormones (Chinzei et al., 1989; Taylor et al., 1991). Pyrethroids show a stimulating effect on ovarian development in adult females of the twenty-eight-spotted lady beetle, *Henosepilachna vigintioctopunctata* in photoperiodically induced diapause also (Kono and Ozeki, 1987).

We used a laboratory colony of *T. urticae* originating from females collected in Sapporo, Japan. Newly emerged female adults kept from eggs under 12 h light and 12 h darkness (LD 12: 12) at 20 ± 1°C were collected within 24 h (day 0). Females with orange body color on day 12 had immature ovaries and showed no expression of a vitellogenin gene (Kawakami et al., 2009). These adults were regarded as diapause females, and used for the experiment.

A synthetic pyrethroid, cypermethrin (Wako Pure Chemical, Osaka) was dissolved in DMSO/

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DOI: 10.2300/acari.22.45

acetone (1: 1) at 10.0, 5.0, 2.5, 1.0, or 0.1 $\mu\text{g}/\mu\text{l}$. Diapause females on day 12 were soaked in approximately 5 μl of the CyM solution for 1 min. After volatilization of the solvent, the females were kept under LD 12: 12 h at 20°C. After 18 days, we observed whether the females had laid eggs, and dissected the females without oviposition to observe their ovarian stages under a stereoscopic microscope (SMZ1500, Nikon, Tokyo). Ovarian stages were judged by the presence of yolk deposition and classified into three, i.e., –, +, and ++ (stage 0, stages I and II, and oviposition, respectively, in Kawakami et al., 2009).

Approximately 50% females treated with 2.5–10.0 $\mu\text{g}/\mu\text{l}$ CyM solutions showed vitellogenesis, and more than 50% of them laid eggs within 18 days (Fig. 1). The hatching rate of these eggs was similar to that in eggs laid by nondiapause mites, and larvae hatching from these eggs normally developed to the adult stage. Although normal mites never fell from leaves into water, mites often fell after application of CyM. The mortality by CyM toxicity was dose dependent, and was higher than 50% at dosages of 5.0 $\mu\text{g}/\mu\text{l}$ or more.

CyM induces Vg synthesis in the fat body and vitellin accumulation in the oocytes, but not oviposition in unengorged adult females of *O. moubata* (Chinzei et al., 1989; Taylor et al., 1991). Although the mode of action of the pyrethroid for induction of vitellogenesis is unknown, Chinzei et al. (1989) suggested that the normal endocrinological steps for vitellogenesis could be induced by neurosecretory factors secreted artificially due to the change in electrical activity of neurosecretory cells by the pyrethroid. In diapause adults of *H. vigintioctopunctata*,

Fig. 1. Effect of cypermethrin on ovarian development in diapause females of *Tetranychus urticae*. Diapause females on day 12 were soaked in various doses of cypermethrin or only solvent (DMSO/ acetone). Oviposition and ovarian development were observed in living mites 18 days after the treatment: (++) , oviposition; (+), vitellogenic ovary; (–), nonvitellogenic ovary. ns, $P > 0.05$; ***, $P < 0.001$ by the Fisher's exact probability test for the proportion of vitellogenic females (++ and +) compared with the control group.

pyrethroids stimulate ovarian development and partially induce oocyte maturation and oviposition (Kono and Ozeki, 1987). In the present study in *T. urticae*, CyM induced vitellogenesis in approximately 50% of diapause females and oviposition in some of them. In *H. vigintioctopunctata* and *T. urticae*, it is likely that pyrethroids stimulate the neuroendocrine mechanism for diapause termination or vitellogenesis. Further studies are necessary to clarify which mechanism is affected by pyrethroids in *T. urticae*.

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