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Toxicity of three pesticides on larval instars of *Osmia cornuta*: preliminary results

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Abstract

The effects of three pesticides on the preimaginal development of *Osmia cornuta* (Latreille) were investigated. All three pesticides are considered harmless to adult bees: two of them, Stroby ® (a.i. kresoxim-methyl) and Cuprocaffaro/WG ® (a.i. copper oxychloride) are fungicides, and one, Tecomag® (*Quassia amara* extract), is an insecticide.

Eggs/larvae of *O. cornuta*, provided with their maternal provision, were placed inside gelatine capsules. The following groups, each of them exposed to a different treatment, were established: 30 eggs/larvae, the maternal provision being added with 1 µl Stroby® at field dose (14 g/hl); 29 eggs/larvae, the maternal provision being added with 1 µl Cuprocaffaro/WG ® at field dose (100 g/hl); 29 eggs/larvae, the maternal provision being added with 1 µl Tecomag ® at field dose (400 cc/hl); 30 eggs/larvae, the maternal provision being added with 1 µl of deionised water (untreated control); 20 eggs/larvae were left on their maternal provision and transferred in gelatine capsules, thus acting as non-manipulated control. The toxic impact of the three pesticides has been evaluated by recording and comparing the final mortality in each group, i.e. percent larvae that did not finish cocoon spinning.

Tecomag ® showed the most toxic effect (82.8% final mortality), followed by copper oxychloride (44.8% mortality). The final mortality of eggs/larvae treated with Stroby® (13.3%) did not differ significantly from the one recorded for both the untreated and the non-manipulated controls (10.0% and 9.9%, respectively). Stroby may thus be considered harmless to *O. cornuta* larvae. No manipulation damage was evidenced, being the mortality of the non-manipulated control group identical to the water-treated control.

Key words: *Osmia cornuta,* larvae, kresoxim-methyl, copper oxychloride, *Quassia amara*, pear pollination.

Introduction

Fungicides have relatively low toxicity for bees, thus spraying of crops during bloom is allowed and for most plant protection products the prevention time, i.e. the time elapsed from spraying, is about one hour (Kubik *et al*., 1999). However, even though fungicides may not affect bees, residues can be found in pollen grains and nectar collected by bees from treated plants (Kubik *et al*., 1999). The progeny may thus be exposed to contaminated provisions with severe risks for its development. For example it has been reported that the fungicide Captan has morphogenetic effects on adult honeybees exposed as larvae, e.g. very small adults, wing malformations, in some cases wingless, stunted bodies, crippled legs and wings (Jay, 1964; Atkins and Kellum, 1986). All these effects are likely to severely affect the ability of the adults to perform duties within the colony and forage effectively and were observed at realistic levels of exposure. It is also possible that foragers are ejected from the colony after foraging on some fungicides treated crops as a result of chemicals within the formulation altering the perception of the environmental cues on which bees rely for nestmate recognition (Thompson, in press). However, this kind of effect cannot be detected in laboratory studies.

Susceptibility to a compound can also vary among various bee species because of morphological and physiological differences and large differences in toxicity to one species appear within the same insecticide category (Johansen, 1971; Ahmad and Johansen, 1973; Tasei, 2002).

In Northern Italy several studies evidenced that wild bees are affected by intensive agricultural management, especially by the use of chemicals for crop protection (Radeghieri *et al*., 1998; Porrini *et al*., 1999). Recent studies found that *Osmia cornuta* (Latreille) (Hymenoptera Megachilidae) can perform a good pollination service in pear orchards (Maccagnani *et al*, 2003), but its foraging activity is seriously threatened by the use of fungicides during pear flowering and its reproductive period is abruptly interrupted by the use of insecticides for *Hoplocampa* spp control immediately after pear flowering. For some pesticides their toxicity to immatures of other Megachilid bee species is known (Torchio, 1983; Tasei and Carré, 1985). We thus decided to make a preliminary investigation on the toxicity effects of three pesticides on preimaginal instars of *O. cornuta* The pesticides tested were two fungicides, Stroby® (kresoxim-mehtyl) and Cuprocaffaro/WG ® (copper oxychloride), commonly used on pear in Integrated Pest Management in the Emilia-Romagna Region (Italy), and one insecticide, Tecomag® (*Quassia amara* L.) (Simaroubaceae), used in organic farming.

Materials and methods

In the middle of May 2002, eggs and larvae of *O. cornuta* were retrieved from pedotrophic nests placed in net tunnels of brussels sprouts for pollination service. *O. cornuta* provisions were carefully separated from their egg/larva and transferred into gelatine capsules (type "000"). A 1 µL-drop of test solution was applied in the centre of the upper surface of the provisions. As soon as the droplet had been absorbed by the provision, the egg/larva was repositioned in the centre of the upper surface of the provision.

The following groups, each of them exposed to a different treatment, were established:

- 30 eggs/larvae, the provision being added with 1 µl Stroby® at field dose (14 g/hl);
- 29 eggs/larvae, the provision being added with 1 µl Cuprocaffaro/WG ® at field dose (100 g/hl);
- 29 eggs/larvae, the provision being added with 1 µl Tecomag ® at field dose (400 cc/hl);
- 30 eggs/larvae, the provision being added with 1 µl of deionised water (untreated control);
- 20 eggs/larvae were left on their maternal provisions and placed in gelatine capsules to evaluate the mortality due to manipulation in the treated groups, i. e. the removal and the repositioning of eggs/larvae (non-manipulated control).

The groups' composition on the base of the various pre-imaginal instars is reported in table 1.

Provisions with eggs/larvae were incubated in complete darkness at 23±1°C, R.H.= 70±10%. Development of immatures was checked regularly (up to four times a day during the first larval instars). For each treatment, individuals that died during the various larval instars were counted until the end of cocoon spinning. χ^2-Test in contingency tables 2x2 was used to compare final mortality rates (percent larvae that did not finish cocoon spinning) in the three Pesticide treatments with those in the two Control treatments.

Results

Final mortality was highest for eggs/larvae exposed to Tecomag® (82.8%), followed by Cuprocaffaro /WG ® (44.8%) and finally by Stroby® (13.2%).

Mortality rates recorded for both Tecomag® and Cuprocaffaro /WG ® were significantly higher than those registered for the two Controls (untreated and non-manipulated), whereas no significant differences emerged between eggs/larvae exposed to Stroby® and the two Controls (table 2). Also, mortality in the two Control groups did not differ significantly from each other.

Because of the high heterogeneity of the initial eggs/larvae population (table 1), mortality rates of the different larval instars have not been reported.

Discussion and conclusions

Quassia amara L. (Simaroubaceae) (bitterwood) is a small tropical tree. Chemicals are extracted from bark and leaves and are usually used on pear against larvae of *Hoplocampa* spp. (Hymenoptera Tentredinidae) (Vergnani *et al*., 2001). Quassin (a. i. of Tecomag®) has insecticide and larvicide properties (Grenand *et al.,* 1987) and acts by ingestion and contact. The high final mortality recorded for the Tecomag® treatment indicates that sprays against *Hoplocampa* spp. on pear could compromise the survival of *O. cornuta* larval progeny (table 2). Even though Tecomag® may be used by organic and IPM growers in Italy, its application could compromise the reproductive activity of *O. cornuta*.

Copper oxychloride (Cuprocaffaro /WG ®) is used as a fungicide and also against fire-blight, *Erwinia amylovora* (Burrill) Winslow *et al*., on pear (Aysan *et al*., 1999). It acts on fungi spores through mechanisms of cellular toxicity on the respiratory processes and denatures also the cellular membrane. Also the use of this product should probably be avoided, since high final mortality was recorded (table 2). In a previous cage study on red rape (Ladurner *et al*., 2002), a high larval *O. cornuta* mortality was recorded. All caged plants were treated with Klartan® (tau-fluvalinate), a pirethroid without side effects on bees and usually used against aphids (Muccinelli, 2000), and copper. It's well known that copper salts inhibit pollen grain germinability; recent studies based on comparative hystochemical analysis of the pear pollen from anthers, *O cornuta* provisions and *O.cornuta* digestive tracts showed that osmotic processes and plasmolysis of pollen grains are essential for the pollen to be digested in the intestine (Ladurner *et al*. 1999; Cresti *et al*. 2001; Maccagnani *et al.* 2002). This preliminary study seems to indicate that copper may affect pollen viability and thus the survival of *O. cornuta* larvae.

Also Stroby® (kresoxim-methyl) is used on pear against various fungal diseases (Politi, 1997). It is a wide action spectrum fungicide characterised by high antisporulant activity. The Stroby® treatment seems not to harm *O. cornuta* immatures. The use of this fungicide during bloom could thus be compatible with the establishment of a permanent population of *O. cornuta* in a pear orchard.

No manipulation damage was evidenced, being the mortality of the manipulated control group identical to the untreated control.

Table 1. Number of eggs/larvae exposed to the different treatments.

Treatment	Eggs	1st larval instar	2nd larval instar	3rd larval instar	4th larval instar	Total larval instar
Tecomag®			4	17	8	29
Cuprocaffaro/WG®	3	3	4	3	17	29
Stroby®			4	8	18	30
Manipulated control			4	12	14	30
Untreated control	1	10	9			20

Table 2. Final mortality in each treatment (%) (different letters indicate statistically significant differences; p<0.05).

Treatment	Final mortality (%)
Tecomag®	82.8 a
Cuprocaffaro/WG®	44.8 b
Stroby®	13.2 c
Untreated control	10.0 c
Non-manipulated control	9.9 c

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