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Bumblebees can be used in combination with juvenile hormone analogues and ecdysone agonists

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Abstract This study examined the lethal and sublethal effects on the beneficial insect *Bombus terrestris* by two classes of insect growth regulators (IGRs) that are commercially used in agriculture to control pest insects. Three juvenile hormones analogues (JHAs) (pyriproxyfen, fenoxycarb and kinoprene) and two ecdysone agonists or moulting accelerating compounds (MACs) (tebufenozide and methoxyfenozide) were tested. The bumblebee workers were exposed to the insecticides via three different routes of exposure: dermally by topical contact, and orally via the drinking sugar water or the pollen. In the first series of experiments the IGRs were applied at their respective maximum field recommended concentration (MFRC). These risk hazard tests showed that the tested IGRs caused no acute toxicity on the workers, and any compound had an adverse effect on reproduction (production of males). In addition, larval development was followed in the treated nests compared with the controls. After application of the two MACs and the JHA fenoxycarb no adverse effects were observed on larval development. However, in the nests where the workers were exposed to the JHAs pyriproxyfen and kinoprene higher numbers of dead larvae were scored. These larvae were third and fourth instars, implying a lethal blockage of development before metamorphosis. In a second test, a series of dilutions was made for kinoprene, and these results revealed that only the MFRC caused a toxic effect on the larval development. On the other hand, kinoprene at lower concentrations (0.0650 mg ai/l) had a stimulatory effect on brood production. It was remarkable that ovaries of such treated dominant workers were longer and contained more eggs than in the controls. In a last experiment, the cuticular uptake was determined for a JHA and MAC to evaluate to what extent worker bees accumulate these classes of IGRs. Cuticular uptake ranged from 34 to 83% at 24 h after topical application. Overall, the obtained results indicate that the tested IGRs at their recommended concentration are safe to be used in combination with *B. terrestris*.

Keywords Bumblebee · *Bombus terrestris* · IGR · Fenoxycarb · Kinoprene · Pyriproxyfen · Methoxyfenozide · Tebufenozide · Survival · Sublethal effects · Larval growth · Brood production · Ovarial growth · Cuticular uptake

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Introduction

Insect growth regulators (IGRs) are more selective insecticides compared to the conventional pesticides due to their interference with specific insect targets, namely the insect endocrinology. Therefore, these chemistries are used worldwide in environmentally friendly integrated pest management (IPM) programs. One major class of IGRs is the juvenile hormone analogues (JHAs). JHAs are non-neurotoxic insecticides

that work as contact and stomach poisons. They functionally resemble the juvenile hormones (JHs) (Retnakaran et al. 1985). In insects JHs are responsible for the regulation of metamorphosis, a unique process in insects, and for the synthesis of vitellogenin (Hartfelder 2000). Therefore these compounds are responsible for an incomplete metamorphosis, ovicidal effects and blocking of the embryonic development (Retnakaran et al. 1985). In addition they interfere with the moulting of the larval stages. JHAs as fenoxycarb, kinoprene and pyriproxyfen are used at present in the control of public health pests and in greenhouses against whiteflies (Tomlin 2004). A second important class of IGRs is the ecdysone agonists or moulting accelerating compounds (MACs). MACs primarily work by ingestion, but also by contact. They become active by binding on the receptor site of the insect moulting hormone 20-hydroxyecdysone, the ecdysone receptor (EcR) (Dhadialla et al. 2005). Therefore a disturbance of the insect's endocrinology causes a cessation of feeding and premature lethal moulting, and the insect will never develop into an adult. The three major compounds of this class are methoxyfenozide, tebufenozide and halofenozide for the control of Lepidoptera (Tomlin 2004).

Bumblebees such as *Bombus terrestris* are economically important pollinators in greenhouses (like tomatoes, sweet peppers) and in fruit production (Goulson 2003). Up until now there are only a few studies on the effects of JHAs and MACs on bumblebees (Tasei 2001). However for pollinators as honeybees *Apis mellifera* the effects of JHAs have recently been investigated into detail, and these compounds were found very harmful for the brood (Thompson et al. 2005). In contrast the MACs, highly active against lepidopteran pests, are reported safe for beneficial insects such as honeybees.

This study aimed to assess the lethal and sublethal effects of two major classes of IGRs on *B. terrestris* bumblebees. Three JHAs: pyriproxyfen, fenoxycarb and kinoprene, and two MACs: methoxyfenozide and tebufenozide, were tested, and the effects were explored via three different routes of exposure. The tests evaluated if the IGRs cause acute toxicity on the workers, and if any compound has an adverse effect on reproduction (production of males). Subsequently, the survival and development of larvae was followed in the nests. In addition, a dissection of the ovaries was done to give more insight in the mode of action of the JHA kinoprene and its effects on reproduction. The results of these tests allow to draw conclusions about the compatibility of the different IGRs tested with *B. terrestris*. In a last experiment the uptake through the cuticle was investigated. Here we used a ^{14}C-isotope of pyriproxyfen as representative of the class of JHAs and ^{14}C-halofenozide of the class of MACs. This was to investigate the impact of pharmacokinetics on the toxicity of JHAs and MACs.

Materials and methods

Insecticides

Commercial compounds of the three JHAs and the two MACs were used to evaluate the effects on worker bumblebees. Table 1 presents an overview of the five IGRs tested, their commercial name, type of formulation and percentage of active ingredient (ai), and their MFRC in % of formulation and corresponding amounts in mg ai/l.

Insects

In all experiments, artificial nests were used each containing five *B. terrestris* workers. The workers were supplied from colonies held at Biobest N.V. (Westerlo, Belgium). The nests were produced in-house and were made of transparent plastic (15 cm wide, 15 cm deep,

Table 1 List of the three JHAs (fenoxycarb, kinoprene and pyriproxyfen) and two MACs (methoxyfenozide and tebufenozide) tested, their commercial name, type of formulation and percentage of active ingredient (ai), and their maximum field recommended concentration (MFRC) in % of formulation and corresponding amounts in mg ai/l

CSI	Commercial name	Formulation: type and %AI	MFRC (in %)	MFRC (in mg ai/l)
Fenoxycarb	Insegar®	25% WG	0.04%	100
Kinoprene	Enstar®	65% EC	0.1%	650
Pyriproxyfen	Admiral®	10% EC	0.025%	25
Methoxyfenozide	Runner®	24% SC	0.04%	96
Tebufenozide	Mimic®	24% SC	0.1%	240

WG: wettable granules; EC: emulsifiable concentrate; SC: suspension concentrate

10 cm high). At the center of the nest there was a drinking place and brood area. Under the nest a container with 500 ml sugar water was provided. After 1 week, the dominant worker started to produce eggs that develop into males. The nests were kept under standardized laboratory conditions in the dark at 28 ± 2°C and 60 ± 10% RH, and commercial pollen and sugar water were provided as food (Mommaerts et al. 2006).

Bioassay to assess effects on survival, growth and reproduction

The different IGRs were tested via three different routes of exposure. Adult worker bees were exposed via contact by topical application and orally via drinking sugar water and eating pollen (Sterk et al. 1995). For each insecticide four nests were treated each containing five workers. The nests were followed during a period of 11 weeks and once per week the number of workers was scored to evaluate acute toxicity. In addition, the amount of brood and brood care, egg hatching, the number of dead larvae removed from the nest and the number of males were scored weekly in each nest as biological endpoints of effects on reproduction and larval growth.

In the first series of tests, the different insecticides were applied as aqueous solutions at their maximum field recommended concentration (MFRC) (Table 1). For negative controls, workers were treated with water or fed on untreated diet (sugar water), and as positive control with imidacloprid at its MFRC (200 mg ai/l). For a contact application, 50 µl of the aqueous concentration was topically applied on the dorsal thorax of each worker with a micropipette. Alongside the contact assays, worker bumblebees were treated orally via the provision of drinking of sugar water treated with the IGRs at their respective MFRC. Hereto, each nest was exposed ad libitum to 500 ml of this concentration over a period of 11 weeks. Bumblebees can also be exposed orally to insecticides via the pollen that serve as a protein food source. Pollen was sprayed with the prepared concentration of each IGR at its respective MFRC until saturation and then supplied ad libitum to the nests. For each treatment, we started with four nests each containing five workers. Then for the different routes of exposure, means ± SEM were analysed by one-way ANOVA and separated by a Tukey–Kramer post hoc test ($P = 0.05$) using SPSS 10.0 software.

As an effect was observed for kinoprene in the previous tests using the MFRC, a dose–response test was started for this JHA with dilutions of its MFRC at 1/1, 1/10, 1/100, 1/1000 and 1/10,000. The bumblebees were treated as described above with the JHA via the pollen and also by contact to evaluate an effect on larval growth and the production of males. Where possible, the results obtained were analysed using non-linear sigmoid curve fitting, and the activity of each treatment was evaluated based on the medium–response concentration (LC_{50} values and corresponding 95% confidence interval) using GraphPad 4 software (Smagghe et al. 2001).

Effect of kinoprene on ovarial length and number of eggs

For this experiment two nests with each three workers were started up. The workers of one nest were topically treated with 1/10,000 of the MFRC of kinoprene while the other nest served as negative control. After 5 weeks the two dominant workers were killed in the freezer and dissected in phosphate buffer (pH 7.6). The numbers of eggs in the two ovaries of the treated female were counted under the binocular. In addition, the length of the two ovaries of the dominant worker was measured and compared with the controls.

Cuticular uptake of ^{14}C-isotopes in worker bees after contact treatment

Adult worker bumblebees of *B. terrestris* were individually treated by applying 1 µl of an acetone solution containing ^{14}C-pyriproxyfen (spec. act. 15 mCi/g; Sumitomo, Osaka, Japan) and ^{14}C-halofenozide (spec. act. 15 mCi/g; Rohm and Haas, PA, USA) on the dorsal thorax with a Hamilton microapplicator (Bonaduz, Switzerland). The average amount of radioactivity per worker was 28,907 ± 811 dpm for ^{14}C-pyriproxifen and 5466 ± 28 dpm for ^{14}C-halofenozide. The workers were killed by freezing after 24 h of treatment. To assess the amount of ^{14}C-labelled pyriproxyfen and halofenozide the workers were washed in 2 ml acetone for 1 h in a 20 ml plastic scintillation vial as was before optimised by Perez-Farinos et al. (1998). This was repeated 2 times and five replicates per insecticide were done. Then, acetone was concentrated to dryness and the amount of radioactivity determined with 10 ml Ultima Gold (PerkinElmer LifeScience, the Netherlands) in a Wallace 1414 WinSpectral Liquid Scintillation Counter. The amount of radioactivity penetrated was expressed as a mean percentage ± SEM of the total amount of radioactivity applied (Smagghe et al. 2001).

Results

Lethal effects on the workers by the IGRs at their MFRC

After topical and oral application of the worker bees, no lethal effects were scored for the three JHAs and two MACs at their MFRC. At the end of the test period, i.e. 11 weeks, the number of dead workers was not higher than in the controls (0–5%) (data not shown).

Sublethal effect on reproduction of the nest by the IGRs at their MFRC

Figure 1 shows the number of males produced as biological endpoint of reproduction in the treated nest. Topical contact treatment of pyriproxyfen, fenoxycarb and kinoprene at their respective MFRC on the number of worker produced did not cause any effect on the reproduction as after 11 weeks the number of males produced did not differ significantly ($P > 0.05$) from those of the controls. Also oral treatment via drinking sugar water and pollen with the three tested JHAs at their MFRC had no negative effect. In contrast with kinoprene via sugar water only, male production tended to be stimulated, however, this was not significant $P > 0.05$.

In the nests exposed to the MFRC of tebufenozide and methoxyfenozide via the three different routes there was no significant ($P > 0.05$) effect observed on the number of males produced (data not shown).

Lethal effect on the larvae by the IGRs at their MFRC

Via the three routes of exposure, any abnormality in larval growth was measured for fenoxycarb in the treated nests (Fig. 2). For pyriproxyfen, no negative effects were observed after treatment by contact and drinking sugar water; however, with treated pollen a high number of larvae was killed (38.0 ± 1.5) compared to controls (6.0 ± 2.3). Typically, worker bees removed these dead larvae from the nests and in most cases these dead larvae were third and fourth instars. Similarly, kinoprene killed a significantly high amount of larvae after contact and via pollen

For the two MACs, methoxyfenozide and tebufenozide, via the three routes of exposure no adverse effects were observed compared to controls (data not shown).

Dose–response assay for kinoprene

As an effect was observed for kinoprene in the previous tests at its MFRC, a dose–response test was started with dilutions of the MFRC at 1/1, 1/10, 1/100, 1/1000 and 1/10,000 and results are given in Fig. 3. The results showed that only in the nests treated with the highest concentration of kinoprene tested (1/1 of the MFRC) by contact and in pollen a significantly ($P < 0.05$) higher number of dead larvae was scored. GraphPad Prism software estimated a respective LC_{50} for contact and pollen of

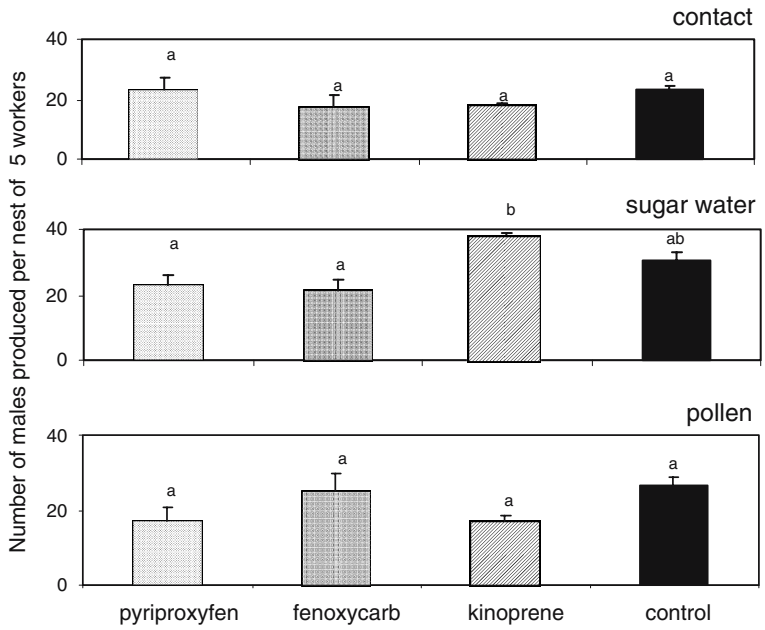

Fig. 1 Effect of the three JHAs on reproduction of *B. terrestris*, when treated at their respective MFRC by topical contact and orally via sugar water and pollen. The numbers of males produced were scored after 11 weeks of treatment and are based on four replicates. ANOVA resulted in one group for contact: $F = 0.916$; df = 14; $P = 0.465$, in two groups for sugar water: $F = 5.189$; df = 14; $P = 0.018$, and in one group for pollen: $F = 2.826$; df = 14; $P = 0.088$. Means ± SEM per route of exposure that are followed by a different letter (a-b) are significantly different (Tukey–Kramer post hoc with $P = 0.05$)

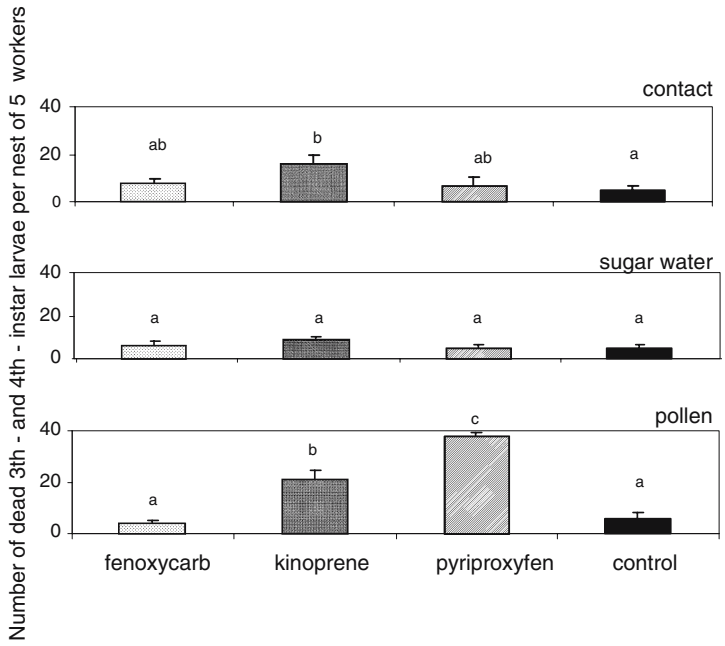

Fig. 2 Effect of the three JHAs on larval growth of *B. terrestris*, when treated at their respective MFRC by topical contact and orally via sugar water and pollen. The cumulative numbers of dead larvae that were removed from the nest after 11 weeks of treatment are based on four replicates. ANOVA resulted in two groups for contact: $F = 3.834$; df = 13; $P = 0.046$, in one group for sugar water: $F = 1.180$; df = 12; $P = 0.371$, and in three groups for pollen: $F = 57.053$; df = 14; $P < 0.001$. Means ± SEM per route of exposure that are followed by a different letter (a-c) are significantly different (Tukey–Kramer post hoc with $P = 0.05$)

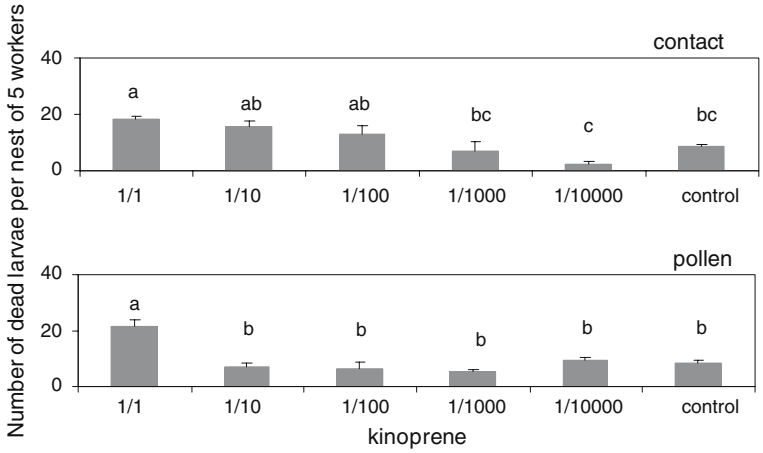

Fig. 3 Effect of different concentrations kinoprene on larval growth 11 weeks after topical contact and oral treatment via pollen. The scored numbers of dead larvae are based on four replicates. ANOVA resulted in three groups for contact: $F = 7.355$; df = 23; $P = 0.001$, and in two groups for pollen: $F = 20.14$; df = 23; $P < 0.001$. Means ± SEM per route of exposure that are followed by a different letter (a-c) are significantly different (Tukey–Kramer post hoc with $P = 0.05$)

524×10^6 mg ai/l (corresponding to 800,000/1 of the MFRC) and 28,300 mg ai/l (corresponding to 44/1 of the MFRC); however, these concentrations are result of extrapolation as the calculated values fall far outside of the concentration range tested.

In addition, in the nest treated topically with the lowest concentration, namely 1/10,000 of the MFRC, a significantly ($P < 0.1$) higher number of males was produced after 11 weeks (Fig. 4). In these nests, the number of dead larvae was equal to controls nests.

Dissection of the ovaries

To explain the stimulatory effect on production of males after a topical application of very low concentrations of kinoprene (1/10,000 of the MFRC), the ovaries of the dominant worker from such treated nests were evaluated. After dissection, treated and control ovaries consisted of four ovarioles, but it was apparent that the dominant worker ovaries were 2 times longer (27 ± 1 mm) as compared to those of dominant workers from control nests (14 ± 1 mm) (Fig. 5). In

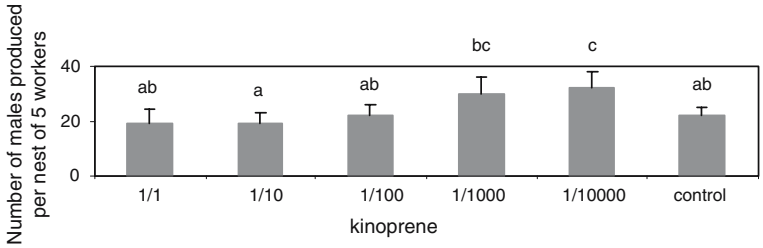

Fig. 4 Effect of different concentrations kinoprene reproduction (numbers of males produced) after 11 weeks topical contact. ANOVA resulted in three groups ($F = 5.3$; df = 18; $P = 0.07$). Means ± SEM that are followed by a different letter (a-c) are significantly different (Tukey–Kramer post hoc with $P = 0.1$)

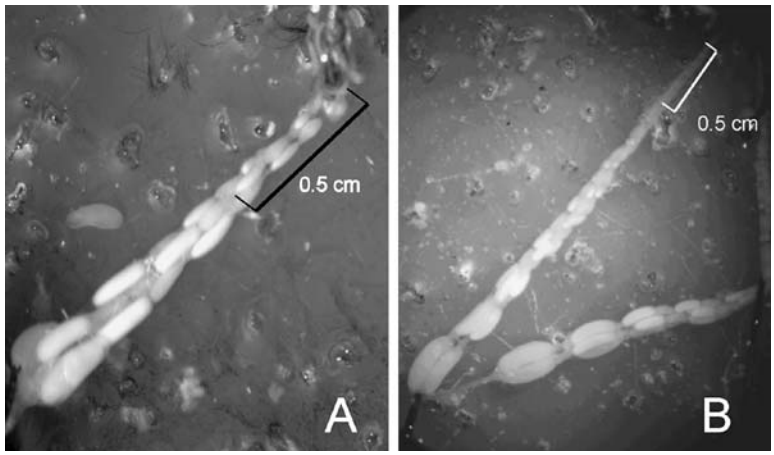

Fig. 5 The ovaries of a dominant worker bumblebee were about 2 times longer and contained 2.6 times more eggs after topical contact with 1/10,000 of the MFRC of kinoprene (**B**) than in the control group (**A**)

addition, the number of eggs was counted under the binocular, and treated dominant females contained 2.6 times more eggs (78 ± 3 eggs) per ovary than those of the control nests (30 ± 3 eggs).

Cuticular uptake of ^{14}C-isotopes in worker bees after contact treatment

In a last experiment the uptake after contact for the JHA pyriproxyfen and the MAC halofenozide was tested. At 24 h after topical administration, only 34 ± 3% of the total amount of pyriproxyfen had penetrated. In contrast, the uptake of halofenozide yielded a much higher percentage of 83 ± 2.

Discussion

For the class of JHAs, our results with fenoxycarb, kinoprene and pyriproxyfen confirm that these IGRs cause no acute toxicity on adult bumblebees agreeing with previous studies of De Wael et al. (1995) and Gretenkord and Drescher (1996). Hence, our experiments demonstrated that exposure of *B. terrestris* to the three JHAs at their MFRC via the three different routes of exposure had no effect on brood production in treated nests. In contrast to these results obtained with bumblebees, fenoxycarb is found to be highly toxic for honeybees. Thompson et al. (2005) reported most severe effects after treatment with this JHA in *A. mellifera*. Fenoxycarb caused brood mortality and was detrimental for the colony viability and the ability to overwinter. It is suggested that the JHA provokes the induction of precocious foraging in the exposed individuals and reduces so the number of nurse bees. These long-term effects are apparent when fenoxycarb is used in the spring and the early summer. From these different studies it is clear that a compound can be toxic for honeybees and not for bumblebees and/or vice versa (Thompson et al. 1999). Therefore it is of interest that both species should be considered in an ecotoxicological risk assessment for pesticides.

As IGRs are known for their strong larvicidal and ovicidal/reproductive activities it is necessary to explore these effects (Mommaerts et al. 2006). In the nests that were treated with the highest concentration tested (MFRC) of kinoprene and pyriproxyfen a high number of dead third- and fourth-instar larvae was measured, suggesting a blockage before metamorphosis due to the JHA. Similar detrimental effects have

been reported in other pest and beneficial insects by several other investigators (Hoddle et al. 2001; Schneider et al. 2004). Therefore, the current observations confirm the physiological mode of action of pyriproxyfen and kinoprene as JHA in *B. terrestris*. In addition, we observed a stimulatory effect on the reproduction in the nest. However this was only the case with kinoprene and only with the lowest concentration of 0.065 mg ai/l. To explain this unique phenomenon on brood production, dissection of dominant females demonstrated that the treatment with kinoprene resulted in 2 times longer ovaries that contained about 2.6 times more eggs as compared to the controls. But until now there is no information for kinoprene on the underlying molecular mechanism to explain this stimulatory observation. But for JHs it is well known that they regulate female fertility by stimulating the vitellogenin synthesis in the fat body and its uptake by growing oocytes (Hartfelder 2000). In honeybees Pinto et al. (2000) suggested that a high JH titer inhibited the vitellogenin synthesis in workers, and a low titer permits the onset and the accumulation of vitellogenin in the hemolymph. Studies on *B. terrestris* reported that the dominant egg-laying bee has a significantly higher titer of JH (Bloch et al. 2000). In previous experiments of van Doorn (1989) treatment of bumblebee workers with JH caused a dose-dependent increase in oocyte length. Therefore we hypothesize that the low concentrations of the JHA kinoprene had the same function in reproduction as JHs. As a consequence it is suggested that low concentrations of endocrine interacting compounds like JHAs have to be documented carefully in an ecotoxicological risk hazards report.

In the current study, the three economically important JHAs were tested for their effects on brood production and larval development via a worst case exposure scenario for the three routes of exposure: i.e. each compound was applied at its MFRC in a single contact administration, and as a continuous treatment via the drinking sugar water and pollen. Under these stringent conditions, the three tested JHAs, fenoxycarb, kinoprene and pyriproxyfen, were found not to cause a detrimental effect on production of males. This conclusion agrees with those of De Wael et al. (1995) who reported that fenoxycarb and pyriproxyfen did not cause any damage to bumblebee larvae in the nest brood. But it should be remarked that in the latter study worker bees were fed only for 24 h on treated sugar water and pollen and then the brood production was followed for 5 weeks. Although we did not see a negative effect on brood production it should be reported that pyriproxyfen and kinoprene at their respective MFRC caused a higher larval mortality (20–22%) compared to 10% in controls. However, the latter effect on larval growth is inferior as the total number of males produced by worker bees exposed to the two JHAs at their MFRC is significantly equal ($P > 0.05$) to the control nests. Besides, these negative effects were seen with the highest concentration tested, i.e. 650 mg ai/l for kinoprene and 25 mg ai/l for pyriproxyfen. As a consequence, it is unlikely that bumblebees will be exposed at such high concentrations in the field. Taken all together, it can be concluded that the three JHAs tested are safe to be used in combination with *B. terrestris*.

For the MACs with a dibenzylhydrazine structure as methoxyfenozide, tebufenozide and halofenozide these IGRs are used specifically for the control of lepidopteran pests. In this study it was clear that this class of IGRs had no adverse effects on the different biological endpoints of adult survival, nest reproduction and larval growth in *B. terrestris*. Recently, Thompson et al. (2005) reported on long-term effects of tebufenozide in honeybee colonies. In agreement with our current results, these authors too found that this IGR had no impact on honeybee colonies and queen development. As reviewed by Dhadialla et al. (2005), an important process in the selectivity of this class of IGRs is the specific binding of the MAC molecules to the target EcR that is governed by a lock-and-key principle. For instance, in targeted Lepidoptera pests the binding affinity is high according to Carlson et al. (2001), whereas binding is low/not detectable in non-targeted insects. Based on the current worst case exposure tests, it can be concluded that the use of the two tested MACs is compatible with bumblebees *B. terrestris*.

In a last experiment of the project we have studied the cuticular penetration rate of the JHA pyriproxyfen and the MAC halofenozide. The pharmacokinetic results showed that the MAC was accumulated to a very high percentage of 83% after cuticular administration. However, this class of MACs has no negative effect on *B. terrestris* when topically applied at the MFRC. Similar results were obtained for methoxyfenozide and tebufenozide by Schneider et al. (2004). They reported that after a topical application of these compounds on *Hyposoter didymator* about 60% was absorbed after 24 h. However, the two MACs were not toxic for this beneficial parasitic wasp. In another beneficial insect the lacewing, *Chrysoperla carnea*, larvae had penetrated through the cuticle only 10% of tebufenozide at one day after topical contact (Medina et al. 2002) and this low penetration helps in explaining its no-toxicity. As is also suggested for these two other beneficial insects, we believe that the MACs are not

able to bind on the insecticidal target site of the EcR of bumblebees and as such cause no adverse effects on *B. terrestris*. Although the so far available EcR sequences (http://www.ncbi.nlm.nih.gov) show a relatively strong conservation of the ligand-binding pocket, there exist divergent residues lining the binding pocket, namely 326, 368 and 379. These respective residues are isoleucine, methione and isoleucine in honeybee *A. mellifera* and also in other insects and non-insects/arthropods that show no/low susceptibility for tebufenozide. In contrast, in Lepidoptera (exemplified by *Heliothis virescens*, *Choristoneura fumiferana* and *Spodoptera frugiperda*, three important pest caterpillars in agriculture, horticulture and forestry) that show a high sensitivity for tebufenozide and methoxyfenozide, these residues of Ile326, Met368 and Ile379 are replaced by a methionine and two valine residues, respectively. As also discussed by Wurtz et al. (2000) especially the presence of a isoleucine at position 326 in non-sensitive species generates steric contacts between the γ-methyl group of the Ile-residue and the C5-methyl group at the B-ring of tebufenozide or the C4-ethyl group of its B-ring, depending on the orientation of tebufenozide. This can most likely account for the no toxicity of the MACs against honeybees and bumblebees. Nevertheless, we also suggest here, in agreement with Wurtz et al. (2000) and Ogura et al. (2005), that in addition to the structure of the EcR ligand-binding pocket other factors like pharmacokinetics and metabolism may help in determining the toxicity spectrum of the MACs. For the JHA pyriproxyfen, our study demonstrated that lower amounts of 34% were accumulated after contact, and that this compound caused no negative effects as was also the case for halofenozide. So for pyriproxyfen, it is reasonable that a low uptake after cuticle contact may explain that this compound is harmless for *B. terrestris*. In contrast, in a previous study of De Clercq et al. (1995) small amounts of pyriproxyfen were ingested in nymphs of the soldier bug, *Podisus maculiventris*, nonetheless it was very toxic. On the other hand, Medina et al. (2002) found that, although there was a high cuticular uptake of pyriproxyfen in nymphs and adults of *C. carnea*, it was not toxic due to a rapid clearing by excretion. Therefore, we hypothesize that as we observed for pyriproxyfen, the no toxicity of the three JHA tested may be due to a low uptake in the body of the worker bumblebees. However, next to penetration the presence of active metabolites and elimination via the insect excretion system are important to understand their activity and selectivity toward beneficial and non-target species.

In general, we are convinced of the importance to explore the short and long-term effects of pesticides, especially those that interact with the growth, development, reproduction and endocrinology of insects. Despite the high amount of work, it is recommended to do this on a more species-by-species basis. In addition, for IGRs it is suggested that these compounds have a substantially larger impact on the population level of bumblebees as the reproductive rate of bumblebees is lower than that of honeybees. The insights obtained here in this study are helpful in preventing pesticide incidents and thus in the loss of biological diversity.

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