LABORATORY ASSESSMENT OF COUMAPHOS AS A POTENTIAL ALTERNATIVE TO FIPRONIL FOR USE IN SMALL HIVE BEETLE, *AETHINA TUMIDA* MURRAY (COLEOPTERA: NITIDULIDAE) REFUGE TRAPS

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Summary

Australian bee keepers need an effective means to control small hive beetle infestations in their hives. A refuge trap comprising a two piece rigid plastic shell encasing a fipronil-treated corrugated cardboard insert has proved effective but coumaphos is a potential alternative to fipronil that requires investigation. In laboratory bioassays using treated corrugated cardboard, comparison of the LC50s of coumaphos and fipronil suggested that fipronil was approximately 400 times more potent in killing beetles than coumaphos. Beetles exposed to filter papers treated with a concentration of coumaphos known to cause approximately 100% mortality within 48 h (10 g L^{-1}) were knocked-down, or dead within 4 h or exposure. This was comparable with the rate at which fipronil (0.3 g L^{-1}) caused similar effects.

Keywords: harbourage, insecticidal control, fipronil, honey bee

INTRODUCTION

Outside its native southern African distribution, the small hive beetle, *Aethina tumida* Murray is a damaging pest of honey bee colonies. Primary damage is through the activity of the larvae that feed on brood, pollen and honey causing it to ferment (Lundie 1940). Secondly, bees may abscond from infested hives and damaged hives may not recover (Ellis *et al.* 2003). Finally, stored supers of honey or extracted comb are also susceptible to damage by larvae and adult beetles (Elzen *et al.*, 1999). Small hive beetle is now well established throughout coastal eastern Australia and has been taken inland in infested hives.

Several control strategies for small hive beetle have been developed or are under development. These include various oil traps and insecticide treatment of soil adjacent to infested hives. An earlier study demonstrated the effectiveness of harbourages containing fipronil-treated corrugated cardboard inserts in both laboratory bioassays (Levot and Haque 2006) and subsequent field trials (Levot 2008). Despite the excellent control achieved with this device in commercial hives, and with no detectable fiprole (fipronil and its toxic metabolites) residues in honey (Levot 2008), industry has not yet sought product registration. There have been reports from Europe suggesting that sub-lethal concentrations of insecticides such as imidacloprid and fipronil have contributed to bee colony collapses (Chauzat *et al.* 2006) and to reduced foraging (Colin *et al.* 2004), poor olfactory learning behaviour in honey bees (Decourtye *et al.* 2005, El Hassani *et al.* 2005) and to slight decreases in sucrose responsiveness (El Hassani *et al.* 2005). Repeated daily exposure to sub-lethal concentrations of fipronil caused high bee mortality (Aliouane *et al.* 2009) probably indicating a cumulative effect. Moreover, the results obtained with the sub-lethal doses of fipronil tested by these researchers did not indicate a minimum no observable effect concentration threshold (Aliouane *et al.* 2009).

It is possible that the concerns emanating from Europe will prevent or further delay, commercialisation of a fipronil-treated small hive beetle harbourage. Although fipronil was shown to be superior to several other insecticides in terms of both efficacy (Levot and Haque 2006) and physico-chemical characteristics such as low vapour pressure (Colliot *et al*. 1992) alternative insecticides should now be considered. In the United States of America coumaphos impregnated (100 g kg^{-1}) plastic strips (Checkmite+™) normally used to control varroa mite, may be stapled under pieces of stripped-back corrugated cardboard and placed on the hive bottom board to control small hive beetle (Elzen *et al.*, 1999, Neumann and Hoffmann 2008). This strategy has proved to be very efficient in reducing numbers of live small hive beetles (Neumann and Hoffmann 2008). However, in an Australian situation they can be considered unnecessarily hazardous to bees and honey as varroa does not occur here. An alternative might be to enclose coumaphos-treated cardboard inserts inside the plastic shell of the small hive beetle harbourage described by Levot (2008).

Here the results of laboratory bioassays that measured the efficacy of coumaphos-treated cardboard against adult small hive beetles are presented for comparison with previously published data for fipronil (Levot and Haque 2006). Data includes speed at which the proposed use rates of fipronil or coumaphos caused knock-down and death of adult beetles exposed to treated surfaces.

MATERIALS AND METHODS

Insecticides

Technical grade (968 g kg^{-1}) coumaphos was diluted in 950 g L^{-1} ethanol to produce a 20 g L^{-1} stock solution that was subsequently serially diluted in ethanol to produce a concentration range for testing (0.890-10 g L^{-1}).

Formulated fipronil (Nufarm Regent® 200SC g L^{-1}) was diluted in water to produce a 0.3 g L^{-1} solution.

Coumaphos-treated harbourage bioassay

Pieces (9 x 9 cm; mean weight 4.209 g) of 4 mm core-fluted cardboard (Australian Corrugated Box Company, Wetherill Park) were immersed in the coumaphos solution such that they became saturated. Excess liquid was poured from the cards which were then air-dried. The volumes of solution before and after card treatment were recorded so that an estimate of the dose applied per card could be calculated. When dry the cards were covered with 50 µm thick adhesive-backed aluminium foil such that only the open ends of the corrugations were exposed. Untreated cards acted as controls.

Five bioassays were conducted on separate days. On each occasion duplicate treated cards comprising eight coumaphos concentrations (16 cards) and controls (ten cards) were placed inside sealed plastic arenas (18 x 12 x 7 cm) whose internal sides had been coated with fluon to keep the beetles on the flat bottom surface. A 2 x 2 cm piece of wet paper towel was placed on top of each foil covered card to increase humidity inside the arenas. Approximately 20 adult beetles (minimum age 7 days) were tipped into each container. The containers were kept in an illuminated incubator run at 27± 0.5°C. Mortality was assessed after 48 h. Data was analysed according to the probit method of Finney (1970).

Speed of intoxication bioassay

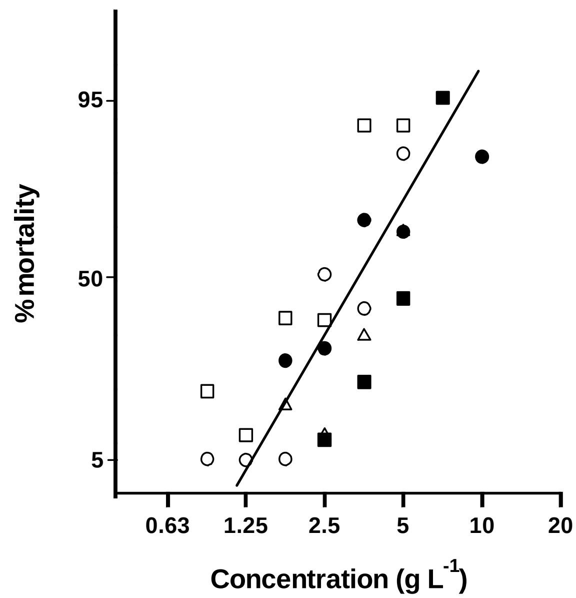
Labelled filter papers (Whatman No. 1; 9 cm diameter) were arranged on a clean glass sheet. One mL of solution (10 g L^{-1} coumaphos in ethanol or 0.3 g L^{-1} fipronil in water) was applied to each of five papers. This coumaphos concentration was chosen as likely to achieve 100% mortality within the bioassay interval. The fipronil concentration was that used to date in all field testing. Untreated papers acted as controls. Papers were air-dried overnight then placed individually into the large shell of a 9 cm diameter polystyrene petri-dish. Approximately ten adult beetles were transferred onto the paper and trapped inside the petri-dish by fitting the lid. The lid was perforated with several ventilation holes. The petri-dishes were held shut with elastic bands and were transferred to an illuminated incubator at 27± 0.5°C. Beginning 30 min later and then again at 1, 2, 3, 4, 6 and 8 h after placement of the beetles on the treated surfaces, the dishes were inspected and at each time-point the numbers of alive (walking), knocked-down (alive but unable to walk) or dead beetles were recorded.

RESULTS

Coumaphos-treated harbourage bioassays

On average each card retained 4.54 mL of solution. This suggests that cards treated with the highest application rate (10 g L^{-1}) retained approximately 45 mg of coumaphos (10.74 mg per g cardboard). Control mortality was negligible in each bioassay. Analysis of the data indicated an LC50 of 3.3 g L^{-1} (95 % fiducial limits; 2.9 - 3.8 g L^{-1}) and an LC95 of 8.2 g L^{-1} (6.5 – 11.7 g L^{-1})(Figure 1).

Figure 1. Adult small hive beetle responses to exposure to coumaphos-treated corrugated cardboard in replicated laboratory bioassays.



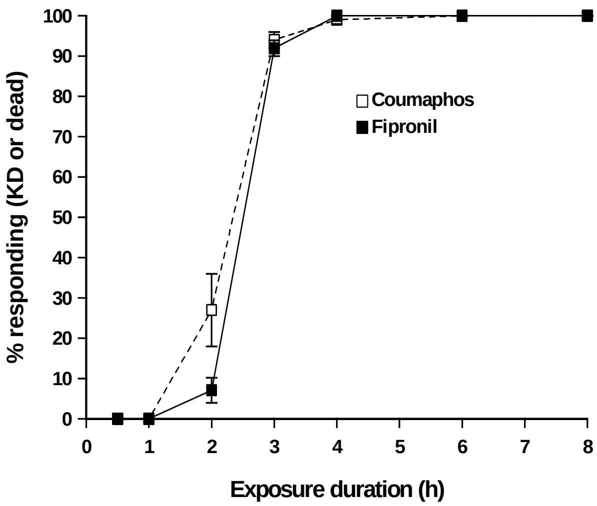
Speed of intoxication bioassays

Neither fipronil nor coumaphos had obvious effects on the adult beetles in the first hour after beetles were exposed to the treated filter papers (Figure 2). However, many beetles were knocked-down in the third hour of exposure. After 4 h exposure response (knock-down or death) was maximal and by 6 to 8 h all but a few beetles exposed to either insecticide were dead.

DISCUSSION

Coumaphos was toxic (LC50 3.3 g L^{-1})(Figure 1) and relatively fast-acting at the concentration tested (Figure 2), to adult small hive beetles via contact. Fast-acting insecticides control infestations quickly and reduce the likelihood of beetles moving around the hive after contact with a treated surface. These results, together with the fact that coumaphos is used in the United States of America to control small hive beetles suggests that it

Figure 2. Rate at which adult small hive beetles were knocked down (KD) or killed when exposed to filter papers containing dried residues of fipronil (0.3 g L^{-1}) or coumaphos (10 g L^{-1}).



could be considered as a possible treatment for the cardboard inserts of the proposed harbourage should an alternative to fipronil become necessary. However, earlier no-choice treated filter paper screening bioassays (Levot and Haque 2006) did indicate that coumaphos was only a tenth as potent as fipronil at LC50. Moreover, in a free-choice treated harbourage bioassay fipronil (LC50 0.0082 g L^{-1}, Levot and Haque 2006) was approximately 400 times more effective in killing beetles than coumaphos (LC50 3.3 g L^{-1}). The high mortalities recorded for the highest coumaphos concentrations tested clearly indicates that beetles freely entered the treated harbourages and stayed long enough to take up a lethal dose with many beetles dying outside the harbourages. It is possible these beetles deliberately evacuated the harbourages after detecting the insecticide. If this is true the absolute amount of coumaphos per g of cardboard (10.74 mg at the highest concentration tested) compared to fipronil (0.004 mg for cards treated with 0.3 g L^{-1} solution), may be responsible. At this concentration coumaphos residues formed a conspicuous white dust on the cardboard.

For varroa control intimate contact between infested bees and coumaphos-impregnated plastic strips (Checkmite+™) dislodges and kills the mites and when used like this for up to seven days is reported to leave no measurable residues in honey and wax (Sanford *et al.* 1999). However, although having relatively low toxicity to bees (Klochko *et al.* 1994, seen in Abstract only) and causing only minor effects on odour learning and bee memory (Weick and Thorn 2002) the use of coumaphos is not without risk, and without the complication of having to consider varroa infestations, Australian bee keepers have the opportunity to use insecticides in a much less potentially hazardous way. The small hive beetle harbourage (Levot 2008) has features that eliminate access to the insecticide-treated cardboard insert by bees or by users of the device. When assembled, the harbourage has a rigid acrylic plastic protective covering for the treated cardboard insert that resists distortion and is very robust. The encapsulation of the insecticide treated cardboard insert in a plastic shell means that compounds other than fipronil could be substituted should this be deemed necessary. Coumaphos is one possible alternative. However, in the context of trying to control small hive beetles inside the bee colony, the only advantage coumaphos has over fipronil, is its low toxicity to bees. Conversely, fipronil has several advantages. In particular fipronil has far superior toxicity to small hive beetles and a vapour pressure of only 3.7 x 10^{-4} mPa compared to 1.3 x 10^{-2} mPa for coumaphos (e-Pesticide Manual 2002). Low vapour pressure is considered important for minimising the chance of insecticide permeating wax or being transferred to honey. The choice of fipronil as the preferred insecticide for treatment of the cardboard inserts for the small hive harbourage is sound but it is clear that the proposal to use fipronil in this way is not without controversy. Data indicates that although less toxic to beetles than fipronil and possessing a higher vapour pressure, coumaphos could be considered as an alternative treatment for beetle control. If used in the small hive beetle harbourage it would pose little risk to bees or users of the device especially compared with the use pattern adopted overseas.

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