INSECTICIDAL CONTROL OF ADULT SMALL HIVE BEETLE, *AETHINA TUMIDA* MURRAY (COLEOPTERA: NITIDULIDAE) IN LABORATORY TRIALS

G.W. Levot and N.M.M. Haque

Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries,
PMB 8, Camden NSW 2570, Australia
Email: garry.levot@dpi.nsw.gov.au

Summary

Treated filter paper laboratory bioassays were used to identify insecticides with potential for the control of Small Hive Beetle adults. Coumaphos, diazinon, temephos, flumethrin, tau-fluvalinate, methomyl, imidacloprid and fipronil were evaluated. Fipronil was the most potent and was selected for further testing in a novel delivery system. This system, based on aluminium foil covered, insecticide treated cardboard took advantage of the beetle's preference to harbour inside corrugated cardboard. Dose-response lines for beetles exposed to cardboard that had been treated with various concentrations of fipronil demonstrated that residues deposited by application of 25 mg L^{-1} (or greater) solutions were lethal to adult beetles. Results of bioassays with cardboard that had been stored at room temperature for up to 84 days indicated that toxicity slowly dissipated over time. Placement of a single covered piece of treated cardboard on the bottom board of artificially infested boxes of comb caused the death or moribundity of 98.4% of beetles within 7 days.

Keywords: Small Hive Beetle, insecticidal control

INTRODUCTION

Small Hive Beetle (*Aethina tumida* Murray (Coleoptera: Nitidulidae)) has cost millions of dollars in lost export markets and damage to European honey bee hives in a number of American States (Hood 2000). Primary damage is by larvae that feed on brood, pollen and honey causing it to ferment. In addition, stored honey or extracted comb can also be ruined by the feeding and subsequent fouling caused by larvae and adult beetles (Elzen *et al.* 1999a). Hepburn *et al.* (1999) reported bees absconding from Small Hive Beetle infested hives.

Small Hive Beetle was detected at Richmond in western Sydney in October 2002 (Fletcher and Cook 2002). Since then it has spread into Queensland (Anon. 2003) and Victoria as well as eastern New South Wales (Sommerville 2003). Reports from bee keepers and limited industry surveys suggest that hive losses in eastern Australia have been considerable in some locations but minimal in others.

In 2002 a Steering Committee formed by Animal Health Australia developed a national management plan for Small Hive Beetle. This plan (Anon. 2003) aimed to slow the spread of the Small Hive Beetle and minimise the damage caused by Small Hive Beetle by identifying and implementing chemical and non-chemical control strategies. Opportunities for insecticidal intervention occur in the hive where larvae feed and adults harbour and lay eggs, and in soil in front of the hive where wandering larvae pupate. In the United States of America (USA) insecticidal control of the Small Hive Beetle relies on permethrin soil drenches (Hood 2000) and the in-hive use of coumaphos impregnated plastic strips (Check Mite^{+TM} strips, Bayer) (Elzen *et al.* 1999b) that are used primarily for varroa mite control. Apart from a Pesticide Order to allow the use of permethrin for the treatment of larvae in the soil there are no approved insecticide treatments for use in Australia and there is significant concern that without a safe, effective insecticidal product aimed at adult beetles, Small Hive Beetle damage may become excessive and impact significantly on the productivity and profitability of bee keeping. Here we report the results of laboratory bioassays with several candidate insecticides with potential for control of Small Hive Beetle. Our aim was to develop an in-hive insecticide treatment that would control Small Hive Beetles below economically damaging levels without affecting bees and without leaving unacceptable residues in honey.

MATERIALS AND METHODS

Beetles

Sufficient Small Hive Beetles for use in the bioassays were reared in the laboratory on a diet of pollen, Torula yeast and honey (Haque and Levot 2005). Adult beetles at least one week old were used in the tests.

Filter paper bioassay

A treated-surface, self-dosing bioassay was developed. One mL of insecticide in acetone was applied to 9 cm diameter filter papers (Whatman No. 1) arranged on clean glass sheets. After drying, the papers were placed in the larger shell of 9 cm polystyrene petri dishes. Approximately 10 adult beetles were removed from the rearing boxes and placed on the filter paper inside each petri dish along with a 2 cm length of plastic drinking straw filled with damp absorbent cotton wool as a

moisture source. Elastic bands were used to hold the smaller shell of the petri dish in place as a lid. The sides of smaller shell were treated with fluon to prevent the beetles moving off the treated paper and the flat surface had small holes in it for ventilation.

Pilot bioassays were used to identify concentration ranges expected to span 0-100% mortality of beetles. Full dose-response bioassays were then conducted using tau-fluvalinate, coumaphos, flumethrin, diazinon, methomyl, temephos, imidacloprid and fipronil. Larvae were considered to have responded it they were dead or moribund when assessed after 24 h. Each bioassay was replicated at least once. The probit method of Finney (1970) was used to calculate the concentration lethal to 50% of the population (LC50) and the raw dose-response data were used to determine the minimum effective concentrations (the minimum concentration causing 100% response of beetles). Results of these bioassays were used to rank the candidate insecticides in terms of potency against adult Small Hive Beetles. Fipronil was the most potent of the insecticides tested and was selected for further investigation in a novel delivery system.

Treated cardboard bioassay

Replicated laboratory bioassays were conducted with pieces of fipronil-treated 'C'-flute cardboard (Australian Corrugated Box Pty. Ltd., Wetherill Park, NSW; Product Code no. CO3 RL150,) covered with 50 µm thick adhesive-backed aluminium foil (Precision Paper Coating Pty. Ltd. Villawood, NSW) such that only the open ends of the corrugations were exposed.

Formulated fipronil (Regent 200SC®; 200 g L^{-1} fipronil; BASF Australia Ltd.) was diluted in water to produce a serial range of concentrations from 0.8-50 mg fipronil L^{-1}. Each concentration was run in duplicate and cards immersed in water acted as controls. Pieces of cardboard (9 x 9 cm) were immersed in each solution and allowed to air dry standing up. On average each piece of cardboard retained about 6 mL of the aqueous solution. When dry each piece of treated cardboard was covered with the adhesive-backed aluminium foil as before. Cards were placed inside sealed plastic arenas (18 x 12 x 4 cm) with approximately 20 adult beetles. The containers were kept in an illuminated incubator run at 28°C. Mortality was assessed after 48 h and LC50s calculated as before.

An additional trial was undertaken to assess the shelf-life of the fipronil treated cardboard. Cards (9 x 9 cm) were treated as before, allowed to dry, marked with the treatment concentration and then stored in an open rack at room temperature on a bench in the laboratory. Beginning 1 day after treatment and repeated at 7, 14, 28, 56 and 84 days after treatment pairs of cards from each concentration group were wrapped in adhesive backed foil and placed inside the plastic containers with approximately 20 adult beetles. The containers were kept in an illuminated incubator run at 28°C. Mortality was assessed after 48 h and LC50s calculated as before.

Prototype harbourage evaluation

Laboratory trials were conducted in which larger (20 x 20 cm) treated (300 mg fipronil L^{-1} of water), or untreated (controls) prototype harbourages were placed on the bottom board of hive boxes of stored comb. Each piece of cardboard retained approximately 20 mL of insecticide solution. About 100 adult beetles were released into the boxes which were then sealed with adhesive tape and stored at 29°C. Mortality was assessed when the boxes were opened 7 days later.

RESULTS

Filter paper bioassay

Control mortality was less than 5% in all bioassays and beetle mortality generally increased with concentration for each insecticide. The confidence limits around the LD50s and LD95 suggest that the variability between replicate bioassays was not excessive considering that treatment relied on the movement of the beetles over the treated surface (Table 1). Results suggest that fipronil (mean LC50=0.03 mg mL^{-1}), diazinon (mean LC50=0.03 mg mL^{-1}) and methomyl (mean LC50=0.05 mg mL^{-1}) were the most toxic insecticides tested and about an order of magnitude more efficacious than coumaphos (mean LC50=0.32 mg mL^{-1}), temephos (mean LC50=0.37 mg mL^{-1}), imidacloprid (mean LC50=0.31 mg mL^{-1}) and flumethrin (mean LC50=0.24 mg mL^{-1}). Tau-fluvalinate had low toxicity to Small Hive Beetles (mean LC50=11.6 mg mL^{-1}) (Table 1).

Treated cardboard bioassays

Beetles exposed to cardboard treated with 25 (or higher) mg fipronil L^{-1} were either dead or moribund within 24 h while those exposed to 1.5 (or less) mg L^{-1} were unaffected. The mean LC50 was 8.2 mg L^{-1}. There was no control mortality in either bioassay. Results of similar bioassays conducted with cardboard that had been treated up to 84 days earlier indicate that toxicity of the treated cardboard declined over time. According to the markings on the treated cards, the LC50 to the beetles exposed to 84 day old residues was almost double that for 1 day old residues. Whereas one day old cards that had been dipped in a 12.5 mg L^{-1} solution were lethal to all adult beetles, by day 84 these

Table 1. Dose-responses of adult Small Hive Beetles exposed to insecticide treated filter papers.

Insecticide	No. of replicate bioassays	Mean LC50 (95% CL) (mg L^{-1})	Mean LC95 (95% CL) (mg L^{-1})	Lowest concentration tested causing 100% mortality (mg L^{-1})
Tau-fluvalinate	2	11.6 (9.7-15.1)	43.3 (28.2-94.5)	>10
Coumaphos	2	0.32 (0.25-0.39)	0.59 (0.46-1.06)	1.0
Flumethrin	4	0.24 (0.20-0.29)	1.03 (0.72-1.86)	1.0
Diazinon	2	0.03 (0.02-0.04)	0.04 (0.03-0.08)	0.05
Methomyl	2	0.05 (0.03-0.08)	0.10 (0.07-0.23)	0.25
Imidacloprid	2	0.31 (0.23-0.50)	1.77 (0.88-12.4)	>0.5
Temephos	2	0.36 (0.34-0.38)	0.49 (0.47-0.57)	>0.71
Fipronil	4	0.03 (0.02-0.04)	0.10 (0.06-0.45)	0.1

Table 2. Toxicity of fipronil (200 g L^{-1}) 'aged' treated cardboard harbourages to adult Small Hive Beetles in laboratory bioassays.

Age of treated harbourage (days)	Slope (SE)[1]	LC50 (95% FL) (mg L^{-1})	LC95 (95% FL) (mg L^{-1})	Lowest 'marked'[2] concentration tested causing 100% mortality (mg L^{-1})
7	1.6 (1.1)	4.5 (3.1-6.5)	45 (14-146)	12.5
14	2.7 (0.5)	4.5 (3.8-5.5)	18 (12-26)	25
28	7.0 (2.0)	6.5 (5.8-7.3)	11 (9-14)	50
56	4.5 (0.7)	8.1 (7.0-9.3)	19 (15-24)	50
84	2.8 (0.3)	8.4 (7.0-10.1)	33 (22-50)	50

1. Standard Error
2. This is the concentration of the solution used to treat the cards.

Table 3. Toxicity of fipronil (200 g L^{-1}) treated cardboard harbourages to adult Small Hive Beetles in artificially infested hive boxes.

Treatment		No. of beetles exposed to treatment	No. of beetles moribund or dead after 7 days	% moribund or dead after 7 days
Control		87	15	17.2
Treated	Rep. a.	101	99	99
	Rep. b.	106	106	100
	Rep. c.	103	101	98.1
Corrected[1] mean % moribund or dead after 7 days				**98.4**

1. Corrected for control mortality (Abbott 1925)

cards killed only 70% of beetles. The minimum application concentration that provided 100% response of beetles at day 84 had been treated with a 50 mg L^{-1} solution (Table 2).

Prototype harbourage evaluation

The laboratory trials with a single fipronil treated corrugated cardboard harbourage placed on the bottom board of sealed boxes of comb demonstrated that 98.4% of beetles were killed or moribund within 7 days (Table 3).

DISCUSSION

Insecticides for testing were selected either because they were reported to be less toxic to bees, were registered for use in hives overseas, have the desirable characteristics of low vapour pressure and/or low water solubility or were thought to have particular merit for use as a treated surface treatment. With our intention of eventually developing an insecticide treated harbourage rather than a baited trap for use in hives, it was most relevant to test insecticides for their contact activity against adult beetles. The treated surface bioassay developed for this study was suitable for assessing the relative potency of contact insecticides against *A. tumida*. The mean LC50s and minimum effective concentrations (Table 1) suggest that fipronil and diazinon were superior to the other insecticides. Diazinon is under regulatory review and may not be available in the future and so perhaps should be discounted. In contrast to the fipronil and diazinon results, coumaphos which is used in the USA for treatment of both varroa mite and Small Hive Beetle was an order of magnitude less effective but if varroa enters Australia it is likely that coumaphos-impregnated Check-Mite+™ strips will be imported for use in Australian bee hives. Several of the other insecticides, for example temephos, imidacloprid or methomyl also showed effectiveness similar to, or better than coumaphos. The pyrethroid alternative tau-fluvalinate was considered to have potential for use against Small Hive Beetle as dried residues of this insecticide have relatively low-toxicity to bees (Lefebvre and Bassand 1999). Unfortunately Small Hive Beetle was also able to tolerate it, and to a lesser extent flumethrin, another pyrethroid miticide used for varroa control in the USA.

Adult Small Hive Beetles seek out dark crevices and prefer secure contact with solid material rather than remain exposed. For example, given the opportunity, adult beetles will congregate preferentially in the fluted section of corrugated cardboard. Based on these observations we thought it likely that a refuge trap rather than a food-based trap might be worthy of investigation. Fipronil was selected as the primary candidate with potential for in-hive use. Regent 200SC® was suitable for treating corrugated cardboard (Tables 2, 3). It appears the beetles are not irritated by, and perhaps unable to detect fipronil residues on the cardboard as they readily entered treated harbourages and were killed (Table 3). This is a great advantage when control relies on contact between the pest and the treated surface. Although no beetle survived exposure to concentrations higher than 25 mg L^{-1} in the laboratory bioassays there may be advantages in terms of useful life during service and shelf-life prior to placement in hives, by using a considerably higher concentration to treat the cardboard should such a product be developed commercially.

The prototype corrugated cardboard lethal harbourages used in these bioassays were simple to make and required no special equipment, however, the commercial development and use of such devices should not be considered unless they are shown to be safe to bees and bee products. Results of preliminary trials in NSW Department of Primary Industries research hives suggest that, at this time, this is not the case (Levot unpubl. data). Further work is needed to refine the delivery device, determine a suitable insecticide concentration and to enlist commercial interest in registering a product. Until this support is gained and the technical aspects are resolved apiarists must rely on non-chemical in-hive traps, physical removal of beetles, cold-room storage of frames of comb and permethrin soil drenches, to control Small Hive Beetles.

ACKNOWLEDGMENTS

The co-operation and advice provided by Mr. Bruce White, retired Apiary Officer NSW Department of Primary Industries is gratefully acknowledged. This research was jointly funded by the Rural Industries Research and Development Corporation (RIRDC) and the NSW Department of Primary Industries.

REFERENCES

Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. *Journal of economic entomology* **18**: 265-267.

Anon. (2003). *Small Hive Beetle National Management Plan*. Animal Health Australia.

Elzen, P., Baxter, J., Eischen, F. and Wilson, W.T. (1999a). Biology of the Small Hive Beetle. *American Bee Journal* **139**: 310.

Elzen, P.J., Baxter, J.R., Westervelt, D., Randall, C., Delaplane, K.S., Cutts, L. and Wilson, W.T. (1999b). Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honey bees in the western hemisphere. *Apidologie* **30**: 361-366.

Finney D.J. (1970). *Probit Analysis.* Cambridge University Press, Cambridge, 318 pp.

Fletcher, M.J. and Cook, L.G. (2002). Small Hive Beetle. Agnote DAI-288, NSW Agriculture. 3pp.

Gillespie, P., Staples, J., King, C., Fletcher, M.J. and Dominiak, B.C. (2003). Small Hive Beetle, *Aethina tumida* (Murray) (Coleoptera: Nitidulidae) in New South Wales. *General and Applied Entomology* **32**: 5-7.

Haque, N.M.M. and Levot, G.W. (2005). An improved method of laboratory rearing the Small Hive Beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae). *General and Applied Entomology* **34**: 29-31.

Hepburn, H.R., Reece, S., Neumann, P., Moritz, R.F.A. and Radloff, S.E. (1999). Absconding in honeybees (*Apis mellifera*) in relation to queenstate and mode of worker reproduction. *Insectes Society* **46**: 323-326.

Hood, W.M. (2000). Overview of the Small Hive Beetle, *Aethina tumida*, in North America. *Bee World* **8**: 129-137.

Lefebvre, B. and Bassand, D. (1999). Bee selectivity of tau-fluvalinate in tank mix with difenoconazole. Short, medium and long term effects under semi-field conditions. *Proceedings of the Fifth International Conference on Pests in Agriculture*, Part 3, Montpellier, France. pp 713-719.

Sommerville, D. (2003). *Study of the Small Hive Beetle in the U.S.A.* Rural Industries Research & Development Corporation, Canberra.

This page left blank intentionally