## EFFECT OF SURFACTANTS ON HONEY BEE SURVIVAL

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#### ABSTRACT

The toxicity of surfactants applied topically and orally to honey bees (*Apis mellifera* L.) was determined by laboratory bioassays. Eleven surfactants (Citowett<sup>®</sup>, Pulse<sup>®</sup>, Boost<sup>®</sup>, Codacide oil<sup>®</sup>, Contact<sup>®</sup>, Raingard<sup>®</sup>, Peptoil<sup>®</sup>, Sunspray<sup>®</sup>, Ethokem<sup>®</sup>, Multifilm<sup>®</sup> and Uptake<sup>®</sup>) were applied topically to anoxiated bees. Anoxiating bees and spraying them with water had no significant effect on their survival. Four surfactants (Citowett<sup>®</sup>, Pulse<sup>®</sup>, Boost<sup>®</sup> and Ethokem<sup>®</sup>) were toxic when applied topically. Ethokem<sup>®</sup> and Boost<sup>®</sup> also showed oral toxicity. Field trials are necessary to assess the actual impact of surfactants. As some surfactants were demonstrated to be toxic to bees in laboratory trials, which suggests they may be toxic when used in the field, they should go through the agrichemical registration process and honey bee warning labels should be included where appropriate. **Keywords:** honey bee, *Apis mellifera*, surfactants, toxicity.

#### **INTRODUCTION**

Surfactants are used with fungicide, herbicide and insecticide sprays to aid the penetration of the active compound through the waxy layer on plant surfaces and insect exoskeletons by reducing the surface tension. As surfactants are not classed as pesticides they are usually not required to be registered and carry honey bee (*Apis mellifera* L.) warning labels. However, concern has been expressed by beekeepers over bee deaths in the Bay of Plenty and Canterbury regions and surfactants have been implicated. The symptoms reported were dead bees outside hives and wet chilled bees clinging to flowers.

Surfactants have been reported to be toxic to insects (Tattersfield and Gimingham 1927; Corey and Langford 1935; Wolfenbarger *et al.* 1967; Imai *et al.* 1994) but have not been tested on honey bees. As soapy water can kill bees (Sames *et al.* 1990), it would not be suprising that surfactants may also be toxic. The product information available on the more than 25 surfactants available in New Zealand does not provide information on their toxicity to honey bees. The aim of this investigation was to determine whether surfactants are likely to be toxic to bees in the laboratory and to determine if there is a potential problem with their use.

#### **METHODS**

### **Topical Application**

Eleven products with surfactant activity were tested: Citowett<sup>®</sup>, Pulse<sup>®</sup>, Boost<sup>®</sup>, Codacide oil<sup>®</sup>, Contact<sup>®</sup>, Raingard<sup>®</sup>, Peptoil<sup>®</sup>, Sunspray<sup>®</sup>, Ethokem<sup>®</sup>, Multifilm<sup>®</sup> and Uptake<sup>®</sup>. They were chosen to represent those products with high usage, a range of product types or had been implicated in bee deaths.

All compounds including water controls were applied using a Burkard Potter spray tower at a high volume equivalent to approximately 2000 litres/ha unless otherwise specified. The rate applied was calculated by measuring the change in weight of a Petri-dish containing the bees before and after treatment. Each of the compounds was tested over a range of concentrations above and below the maximum recommended rate (Table 1).

New Zealand Plant Protection 53:230-234 (2000)

Surfactant	Recommend concentration	Highest concentration tested	Lowest active concentration	Lowest concentration with 100% mortality
Citowett®	0.02 - 0.025%	0.1%	0.04%	_
Pulse®	0.1 - 0.2%	0.2%	0.02%	0.04%
Boost®	0.1 - 0.5%	0.5%	0.01%	0.05%
Codacide oil®	0.04 - 0.3%	0.5%	_	_
Contact®	0.025 - 0.1%	0.1%	_	_
Raingard®	0.02 - 0.045%	0.18%	-	_
Peptoil®	0.5 - 1.0%	4.0%	_	_
Sunspray®	0.5 - 1.0%	4%	_	_
Multifilm®	0.025%	2%	_	_
Uptake®	0.5%	0.1%	_	_
Ethokem®	0.2 - 0.5%	2.0%	0.5%	-

TABLE 1:Summary of recommended concentration for each surfactant tested,<br/>the highest concentration tested, the lowest concentration showing<br/>activity (killed bees) against honey bees, and the lowest concentration<br/>resulting in 100% mortality.

Adult bees were removed from a hive and lightly anoxiated with CO<sub>2</sub>. Twenty were then placed in the base of a Petri-dish, sprayed with the required treatment while anoxiated, and then transferred to a cage. The cages (40 mm × 100 mm × 100 mm) were constructed of wood with two sides covered in 2 mm nylon mesh, with a gravity feeder allowing bees access to a 2 M sucrose solution. The cages were immediately transferred to a controlled environment room at 20°C (constant darkness) and the number of dead bees counted after 24 h unless otherwise specified. Mortality was defined as bees found not moving when observed over several minutes. All the bioassays were conducted between 11 am-3 pm.

To determine the effect of  $CO_2$  anoxia on bee mortality, bees were anoxiated and 20 were placed in each of 20 cages and permitted to recover at ambient temperatures (21°C). The bees in ten of the cages were anoxiated again while in their cages, immediately prior to being sprayed. The cages were laid on their sides with one of the two mesh sides uppermost and sprayed with Ethokem® (1300 litres/ha rate equivalent). The cages were immediately transferred to a controlled environment room.

To evaluate the effect of water volume, 12 cages of 20 bees were sprayed with varying rates of tap water (52 - 5,305 litres/ha rate equivalent). In addition 12 cages of 20 bees were sprayed with varying amounts of Boost<sup>®</sup> at two concentrations (0.1% (recommended rate) and 0.01%).

#### Oral toxicity of surfactants

Bees were given 10 ml of 2 M sugar syrup alone or with either 0.5% Ethokem<sup>®</sup>, 0.1% Pulse<sup>®</sup>, 0.5% Boost<sup>®</sup> or 0.025% Citowett<sup>®</sup> (10 cages of 20 bees for each treatment). Mortality was determined after 16, 24, 40, 65 and 84 h. To establish whether the results of the previous trial could be due to starvation bees were given either 10 ml of 2 M sugar syrup with 0.5% Ethokem<sup>®</sup>, 10 ml of 2 M sugar syrup alone or were not fed (10 cages of 20 bees for each treatment). Mortality was determined after 2, 5, 14, 16, 18, 21, 25 and 41 h.

Students t-tests were used for all analyses. Because this research is a preliminary evaluation of the potential effects of surfactants on honey bees, probit analysis was considered to be unnecessary and would not have affected the interpretation of the results.



Log percent concentration

FIGURE 1: Effect of Pulse®, Boost®, Ethokem®, Citowett® concentration applied topically on bee mortality. The arrows represent the recommended rate.

#### **RESULTS AND DISCUSSION**

There was no significant difference (P>0.05) in the percent mortality between those bees which had (mean = 39.5%, S.E. = 6.75) and had not (mean = 29.0%, S.E. = 3.75) been anoxiated when sprayed. The amount of water used also had no effect on bee survival. Only five bees (1.25%) died within 24 h of being sprayed with water. These deaths did not appear to be related to the volume of water used as 2 bees died at the lowest rate and 3 at an intermediate rate. This was unexpected because it is often recommended that growers do not spray during the middle of the day as bees may become chilled and die.

Four of the 11 surfactants tested (Table 1) (Citowett®, Pulse®, Boost® and Ethokem®) were toxic to bees (Fig. 1). Citowett® did not cause honey bee deaths at the recommended concentration. Pulse® and Boost® resulted in 100% mortality at the recommended concentration. The bees that died when sprayed with Citowett®, Boost® or Pulse usually did so within an hour of being sprayed. Unlike the other surfactants that exhibited activity against bees, those that were sprayed with Ethokem® did not die until more than 2 h after being sprayed.



PLATE 1: Bees sprayed with water at an equivalent rate of 2,000 litres/ha.



PLATE 2: Bees sprayed with 0.1% Boost® at an equivalent rate of 2,000 litres/ha.

#### Spray Drift and Non-Target Impacts

Bees sprayed with water did not appear to be uniformly wet, as the water remained as discrete droplets on their bodies (Plate 1). Those sprayed with surfactants at high enough concentration to cause mortality, appeared wet (Plate 2), except for Ethokem® which caused water to remain as discrete droplets. These symptoms are very similar to bees described as chilled and suggest that they may be caused by the use of surfactants.

Mortality was observed at 951 litres/ha rate equivalent at 0.1% Boost<sup>®</sup> concentration and at 2,590 litres/ha at 0.01% concentration (Fig. 2). 100% mortality was observed at 2,132 litres/ha (0.1% concentration). In the absence of field trials it is difficult to assess the actual amount of surfactant with which a bee would come in contact. There will be the initial amount sprayed, plus further surfactant that will be picked up after visiting wet flowers or by falling onto wet grass. Because of the quick knockdown of some of the surfactants, the bees are likely to die in the field so the only symptom noticeable to beekeepers will be reduced hive strengths.



# FIGURE 2: Honey bee mortality (%) when sprayed with different amounts of two concentrations of Boost® (0.1% (recommended rate) and 0.01%).

Bees fed Ethokem® and Boost® had significantly higher (P<0.05) mortality from 16 h onwards, than those fed Pulse®, Citowett® or only sugar syrup (Fig. 3). The bees fed Ethokem® in sugar syrup had significantly higher mortality (P<0.05) than bees fed sugar syrup only and starved bees indicating that the increased mortality was due to oral toxicity and not due to the bees being repelled from their food and starving. Further tests are required to determine if some of the seven surfactants that did not show contact activity may show oral toxicity.

From these trials it is not possible to predict the full implications of the oral toxicity observed. The surfactant was the only food the bees had access to in these trials. If sprayed on flowers they would be exposed to lower concentrations. However, the use of a surfactant would increase the probability of the nectar being contaminated. Field trials are necessary to assess the full impact of the oral and contact activity of surfactants.

Some insecticidal compounds are reported to be 'bee safe' and are recommended to be used during flowering, e.g. *Bacillus thuringiensis* (Trade names Agree® 50EP



FIGURE 3: Oral toxicity of Ethokem®, Boost®, Citowett® and Pulse® to honey bees. The vertical lines are standard error bars.

and Dipel®). However, it is recommended (New Zealand Agrichemical manual) that a surfactant is added when spraying kiwifruit or brassicas. If the surfactant chosen is one of those showing activity against bees then the *B. thuringiensis* spray will no longer be 'bee safe'. The same applies to the many fungicides and herbicides that carry no bee safety warnings and are recommended to be used with surfactants.

The results of this laboratory study suggest that surfactants may be causing bee mortality in the field and it would therefore be beneficial if they went through the registration process and if necessary carried appropriate warning labels. Where the pesticide label recommends the use of a surfactant, unless the surfactant is 'bee safe', a warning that the surfactant may be hazardous to bees should be included and labels should be read carefully.

#### ACKNOWLEDGEMENTS

This research was funded by the Beekeeping Industry Trust Fund.

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