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Assessment of the synergy and repellency of pyrethroid/fungicide mixtures

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Abstract

There has been considerable concern over the last few years about the potential for synergism between pyrethroids and fungicides applied to flowering oilseed rape. Field data from honey bee (*Apis mellifera* L.) poisoning incidents in the UK have shown that mixtures of pyrethroids with fungicides that are not synergistic under laboratory conditions have been involved. This study aimed to determine if the mixing of fungicides with pyrethroids alters the repellent properties of the pyrethroid and thus increases the risk to honey bees when applied to flowering crops. Interpretation and regulation may be difficult if it is unclear whether synergism or a change in repellency is responsible for the incidents. Synergy and repellency of realistic combinations of two pyrethroids and eight fungicides were tested. Synergy was tested using a standard acute toxicity test method and repellency was tested using a novel *in vitro* test method. The results showed that *in vitro,* certain combinations of pyrethroids and fungicides did significantly increase the risk posed by pyrethroids alone, due to a reduction in the repellency of the pyrethroid. Further work is now in progress to see if this reduction in repellency seen in the laboratory occurs under semi-field conditions.

Key words: synergy, pyrethroid insecticides, fungicides, repellency.

Introduction

There has been considerable concern over the last few years about the potential for synergism between pyrethroids and fungicides applied to flowering oilseed rape. Synergy can be defined as greater than additive toxicity i.e. the toxicity of a mixture is greater than the sum of the toxicity of the mixtures components. Colin and Belzunces (1992) and Pilling and Jepson (1993) have shown synergism to occur with mixtures of pyrethroid insecticides and fungicides when applied to honey bees in the laboratory with increases in toxicity in the range of 10 to 100 fold. The use of tank mixes when applying agrochemicals is common agricultural practice and synergism may occur by design or by accident where different classes of pesticide are combined. However, many studies have investigated mixtures of pesticides unlikely to be encountered by wildlife or at unrealistic ratios of components (Thompson, 1996).

Where mixtures are applied to flowering crops or crops containing flowering weeds honey bees may be exposed. In 1997 two incidents, which involved the approved use of the pyrethroid alpha-cypermethrin in tank mixes with fungicides were reported to the UK Wildlife Incident Investigation Scheme (WIIS) (Brobyn, 2001). The repellent nature of pyrethroids is important in limiting the exposure of honeybees to this highly toxic group of insecticides. It is not clear whether the poisoning incidents were due to increased synergy or to a reduction in the repellency of the pyrethroids. Therefore, it is important to determine if increased exposure of honeybees to pyrethroids is occurring by reducing the repellent nature of the insecticide, i.e. the risk assessment based on repellency is valid. In this study synergy and repellency of realistic combinations of two pyrethroids and eight fungicides were tested. The pyrethroids investigated were alpha-cypermethrin and lambda-cyhalothrin. They were chosen because they are the second and third most used insecticides on oilseed rape (cypermethrin is the most used) (Garthwaite and Thomas, 1999) and are also marketed as ‘bee safe’. The selected fungicides account for over 87% of all the fungicides used on oilseed rape in the UK during the period March to July (Garthwaite and Thomas, 1999). Therefore this study was directed at realistic mixtures likely to be encountered by honeybees both in terms of mixtures and ratio of components.

Materials and methods

The test items used in the study were commercial formulations of two pyrethroid insecticides and eight fungicides, details of the pesticides are given in table 1. The pesticide formulations were kindly supplied by the agrochemical companies.

For both the synergy and repellency tests worker honey bees (*Apis mellifera* L.) were obtained from National Bee Unit colonies that had not been treated with antibiotic or varroacides within 4 weeks of the start of the study. Bees were collected by gently shaking into wire holding cages (50-70 per cage) and placed in an incubator at 25± 1°C and 65±5% relative humidity until required.

Synergy

An initial study was undertaken to determine the dose-response relationship of the pyrethroid pesticides. Alpha-cypermethrin (Contest) doses used were 1.0, 0.50, 0.25 and 0.125 μg formulation /bee and lambda-cyhalothrin (Hallmark) doses used were 2.0, 1.0, 0.50 and 0.25 μg formulation /bee. Each fungicide was also tested to determine if there was any mortality at the maximum application rate. Groups of control bees were dosed with 1g l^{-1} Triton X-100. The tests were undertaken in a petri dish cages with 10 adult worker bees and 3 replicates per dose according to OECD guideline 214 (1998). Mortality and sublethal effects were recorded at 24 and 48 hrs and the LD_{50} and 95% confidence limits determined using the CSL Probit 1 program (Finney, 1971).

To determine whether synergy was occurring between the pyrethroids and fungicides the fungicide was applied to the bees as a mixture with the pyrethroid. Thus the pyrethroid toxicity tests were rerun with each of the fungicides added to the test doses at the same ratio as would occur in tank mixes for application in the field. The pesticide dilutions were prepared as a mixture in 1g l^{-1} Triton X-100 in water within 2 hrs of use. Mortality and sublethal effects were recorded at 24 and 48 hrs and the LD50 and 95% confidence limits determined using the CSL Probit 1 program (Finney, 1971).

Repellency

A pilot study was undertaken to determine if a choice or no-choice test design was the most appropriate method for testing repellency in the laboratory, i.e. demonstrating repellency with least variability between replicates, and the number of replicates required. The pilot tests resulted in selection of a test design that comprised in a clear plastic box (approx 12 cm by 8 cm). A pre-weighed glass sucrose feeder was placed in the centre of a piece of filter paper (4 cm by 7 cm). The filter paper on which the feeder was placed was treated with pyrethroid, fungicide, pyrethroid and fungicide or untreated for each run of the test, i.e. all the assessments were comparable with the pesticides alone. There were three replicates per treatment. The filter paper was treated by applying a known volume of the pesticides dissolved in water and allowing the filter paper to dry prior to placing in the test cage. The amount of pesticide applied was determined by reference to the maximum application rate (g ai/ha or g formulation/ha assuming 200 l/ha) on flowering crops. When combinations were applied they were pre-mixed prior to application. Sucrose solution (50% w/v aqueous solution) was placed in each feeder. The bees were placed in the incubator to starve for 1.5-2 hrs prior to the test, knocked down for 2 mins with CO_2 and then ten adult worker bees were placed in the test cages. The cages were held on the bench in the light at 20 ± 1°C during the test. The bees were allowed access to the feeder until the control bees had consumed close to 100% of the test feed or for a maximum of 4 hrs (whichever was shorter). If the control intake was less than 50% then the test was repeated. At the end of the test period the cages were placed in the freezer overnight. The feeder was weighed to determine the amount of sucrose consumed and the amount consumed in the cages treated with pesticide was compared with the concurrent control cages.

Results and discussion

Toxicity

The increase in toxicity (decrease in LD_{50}) for the pyrethroids and their combination with fungicides is shown in figure 1. The maximum increase observed was a 6.7 fold increased in the toxicity of lambda-cyhalothrin in the presence of prochloraz (Sportak). Six of the eight fungicides increased the toxicity of lambda-cyhalothrin and three increased the toxicity of alpha-cypermethrin. The maximum increase observed in combination with alpha-cypermethrin was 2.2 fold with prochloraz (Sportak).

Repellency

The amount of sucrose consumed, as an indirect measure of the repellency of the treated filter paper on which it was placed, is expressed as a percentage of the control intake (figure 2). Both alpha-cypermethrin (Contest) and lambda-cyhalothrin (Hallmark) showed significant repellency although the scale of the repellency differed. Alpha-cypermethrin (Contest) showed 80% repellency whereas lambda-cyhalothrin (Hallmark) showed only 40%. The only fungicide which showed significant repellency was Compass (55%) (iprodione and thiophanate-methyl) and no other fungicide showed repellency when compared to controls.

Table 1. Pesticides and rates used in mixtures

Pesticide	Formulation (type/ nominal a.i. content)	Max application rate (formulation /ha)	Usage of a.i. on oilseed rape (ha) (March-July[1])
Pyrethroids			
Alpha-cypermethrin	Contest (WG, 15% w/w)	0.133 kg	120,667
Lambda-cyhalothrin	Hallmark (CS, 100 g/l)	0.075 l	52,882
Fungicides			
Iprodione and thiophanate-methyl	Compass (SC, 15.5%/15.5% w/w)	3.00 l	222,454
Carbendazim	Derosal (WG, 80% w/w)	0.625 l	282,913
Prochloraz	Sportak (EW, 450 g/l)	1.10 l	12,117
Chlorothalonil	Bravo (SC, 500 g/l)	3.00 l	2,773
Flusilazole	Sanction (EC, 400 g/l)	0.500 l	58,658
Difenconazole	Plover (EC, 250 g/l)	0.500 l	13,155
Propiconazole	Tilt (EC, 250 g/l)	0.500 l	2,977
Tebuconazole	Folicur (EW, 250 g/l)	1.00 l	139,649

[1] Garthwaite and Thomas, 1999

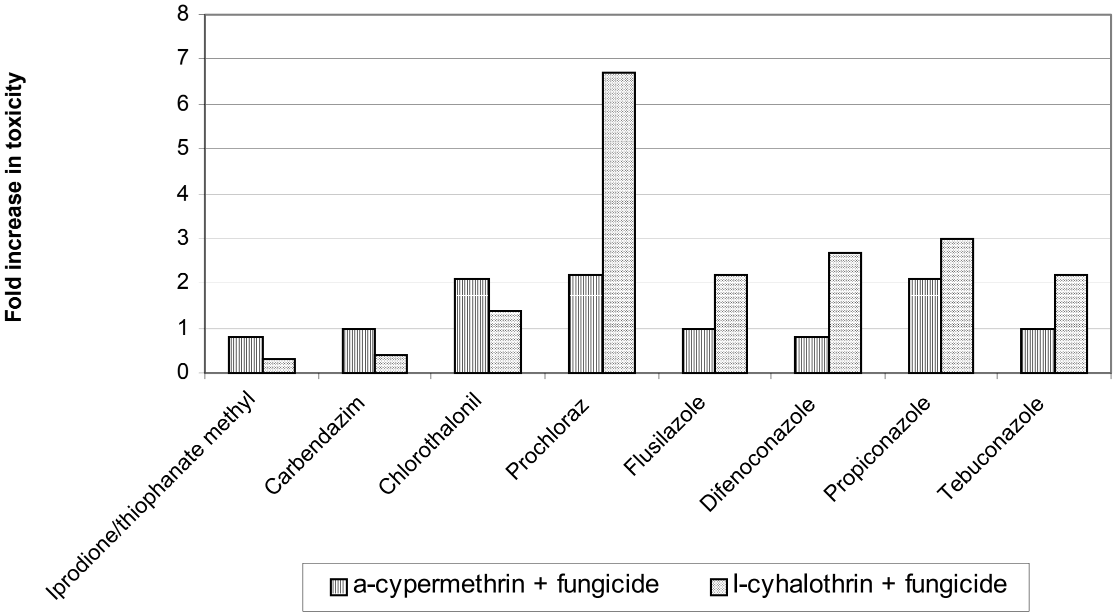

Figure 1. Increase in toxicity to honeybees (decrease in LD_{50}) for mixtures of fungicides and pyrethroids compared with pyrethroid alone.

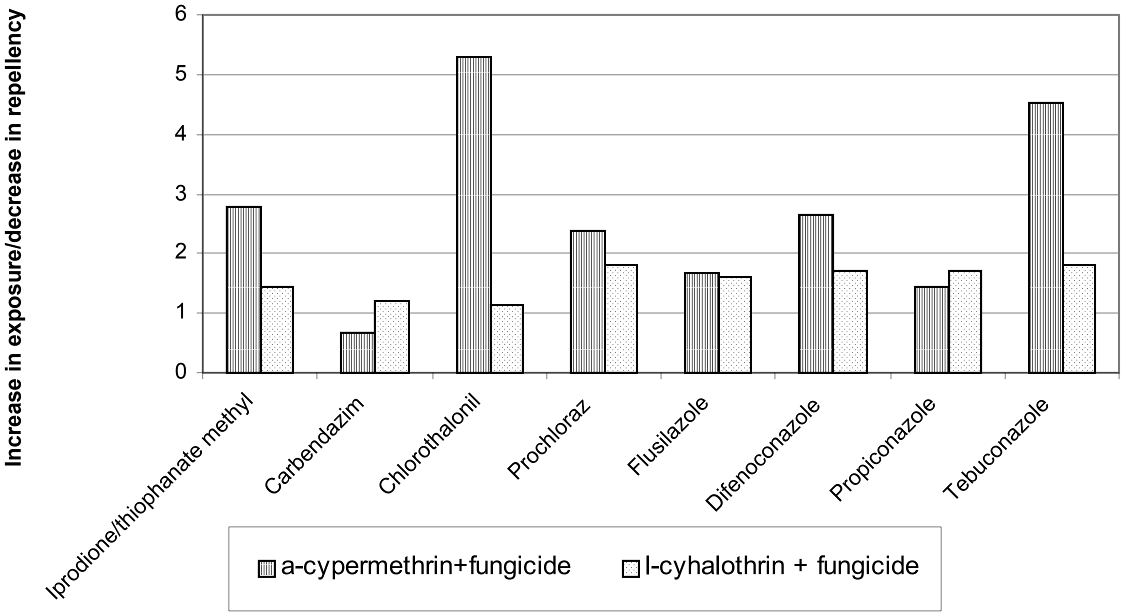

Figure 2. Increase in exposure/decrease in repellency of honeybees offered feed on filter paper treated with mixtures of fungicides and pyrethroids compared with pyrethroid alone.

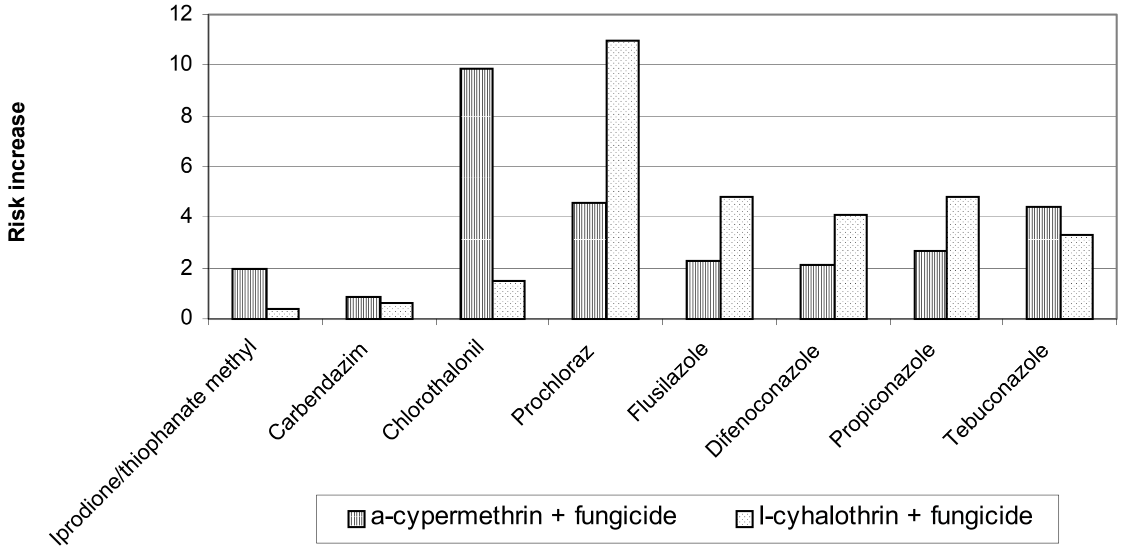

Figure 3. Increase in risk (toxicity x exposure) to honeybees for mixtures of fungicides and pyrethroids compared with pyrethroid alone.

For both pyrethroids three fungicides significantly decreased the repellency of each of the pyrethroids, but they were not the same fungicides. Chlorothalonil (Bravo), difenoconazole (Plover) and tebuconazole (Folicur) significantly decreased the repellency of alpha-cypermethrin (Contest). Prochloraz (Sportak), flusilazole (Sanction) and propiconazole (Tilt) significantly decreased the repellency of lambda-cyhalothrin (Hallmark). The greatest decline in repellency was observed with alpha-cypermethrin and chlorothalonil (Bravo) where repellency was significantly reduced ($p<0.001$) from a mean of 80% to a mean of 6%.

The risk posed to honeybees by a pesticide is a product of the intrinsic toxicity of the pesticide and the exposure of the bee to it. Therefore increased risk can be related to an increase in exposure or an increase in toxicity for the pesticide. The consequent predicted increase in risk associated with combinations over the pyrethroid alone are summarised in figure 3. This predicts that chlorothalonil significantly increases the risk posed by alpha-cypermethrin due to a reduction in the repellency of the pyrethroid. Therefore the repellency and toxicity of the combination of chlorothalonil and alpha-cypermethrin will be tested in a semi-field trial with chlorothalonil alone and alpha-cypermethrin alone as the controls.

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