

## THE USE OF HONEY BEES TO DISSEMINATE AN INSECT PATHOGENIC FUNGUS FOR CONTROL OF INSECT PESTS ON OILSEED RAPE

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### Introduction

Honeybees obtain both nectar and pollen from the flowers of oilseed rape, and many beekeepers in the UK move their colonies to rape crops for pollination and honey production (WILLIAMS et al., 1993; CARRECK et al., 1997). Honey bees have hairy bodies adapted for the transport of pollen grains, but have also been shown to carry fungal and bacterial spores (BATRA et al., 1973; HARRISON et al., 1980; SANDU & WARAICH, 1985; THOMPSON et al., 1990). In recent years, various trials have demonstrated that honeybees can be used to disseminate some biological control agents. These have included the fungus *Cliocladium roseum* applied to strawberry flowers to suppress the mould *Botrytis cinerea* (PENG et al., 1992), and the bacteria *Pseudomonas fluorescens* and *Erwinia herbicola* to apple flowers to control fire blight caused by the bacterium *Erwinia amylovora* (THOMPSON et al., 1990, 1992). Honeybees have also been used to transmit insect diseases such as the bacterium *Bacillus thuringiensis* for control of the banded sunflower moth (*Cochylis hospes*), and *Heliothis* nuclear polyhedrosis virus to control corn earworm larvae (*Helicoverpa zea*) on crimson clover (GROSS et al., 1994). There appear, however, to be no reports of bees being used to transmit insect-pathogenic fungi which, unlike bacteria and viruses, have the advantage that they do not need to be ingested to cause infection, being able to penetrate the cuticle of the host insect directly.

The pollen beetle (*Meligethes aeneus*), and the cabbage seed weevil (*Ceutorhynchus assimilis*) are important pests of oilseed rape throughout Europe, and visit the flowers to feed on pollen. Work at IACR-Rothamsted had shown that some isolates of the insect pathogenic fungus *Metarhizium anisopliae* were highly pathogenic to insect pests of oilseed rape (BUTT et al., 1992; 1994), demonstrating their biocontrol potential, if an efficient method of dissemination of fungal spores to the target insect could be developed.

Experiments at IACR-Rothamsted during 1993-1995 used honeybees to disseminate spores of the bacterium *Bacillus subtilis* onto sunflower heads to control grey mould (*B. cinerea*). Although the honey bees carried the spores to the flowers, the bacterium did not provide effective control of the fungus in the field (H.A. McCARTNEY, V.J. CHURCH, J. BUTTERWORTH, I.H. WILLIAMS, N.L. CARRECK & J.R. SIMPKINS, unpublished).

The method appeared, however, to be suitable for disseminating spores of *M. anisopliae*, providing the fungus was not harmful to the bees. A protocol for testing the effects of fungal agents on honeybees had been developed at IACR-Rothamsted (BALL et al., 1994), and tests showed that *M. anisopliae* strain V245, although demonstrated to be harmful to bees in the laboratory, was less harmful than other strains tested (BUTT et al., 1994). This paper describes field trials in winter and spring-sown crops of oilseed rape to investigate the use of foraging honeybees to disseminate *M. anisopliae* spores to the flowers of oilseed rape for pest control, and to determine its effects on the bees under field conditions.

### Materials and methods

Four field trials were carried out at IACR-Rothamsted during 1997 and 1998 in both winter and spring oilseed rape. In each trial, nine insect-proof cages (2.7 x 2.7 x 1.8 m high) were erected over the flowering crop naturally infested with pollen beetles. A small (c. 5000 workers) colony of honeybees equipped with inoculum disseminators (described by BUTT et al., 1988) was placed in each of six of the cages. Inoculum of *M. anisopliae* V245 was prepared (BUTT et al., 1998), placed in three of the hives and replenished at 48-h intervals unless poor weather prevented bee foraging.

In order to assess mortality of pollen beetles, ninety beetles were collected from each cage on four dates. They were incubated at 23°C, and mortality was recorded daily for 14 days. Dead beetles were removed and placed in Petri dishes lined with moist filter paper to encourage the growth of *M. anisopliae* and external spore formation.

In 1998, to assess the effects of the fungus on the bees, the number of adult bees and the number of sealed brood cells in each colony were estimated using photographic standards (JEFFREE, 1951; M.A.F.F., 1998) before being placed into the cages, and after their removal from the cages at the end of the trial. This gave an indication of the impact of the fungus on the health of the colony. Samples of dead bees were also collected weekly from dead bee traps placed below the hive entrances (BAILEY, 1967), or from the floor of the cages. These dead bees were placed in Petri dishes to encourage the growth of the fungus, as before. External spore formation would indicate that the bees had died due to fungal infection.

## Results and discussion

Bees were effective agents for the dissemination of *M. anisopliae* to pollen beetles (Table I). Mortality was significantly greater in beetles from cages supplied with bees and fungal inoculum than in those from control cages without fungal inoculum, in both the winter and the spring rape trial (BUTT et al., 1998). Results in 1988 showed a similar effect on seed weevil adults (WILLIAMS, in preparation).

Table I

Mortality (%) of pollen beetles on oilseed rape when exposed to honeybees with and without *M. anisopliae* (standard errors in brackets) in 1997

Sample date	Pollen beetles only	Pollen beetles + bees	Pollen beetles + bees + <i>M. anisopliae</i>
<i>Winter rape</i>			
1/5	7(4.5)	10(5.4)	61(8.7)
7/5	7(4.4)	0	60(8.2)
12/5	0	3(3.3)	23(7.7)
15/5	3(3.7)	5(4.7)	45(9.8)
<i>Spring rape</i>			
24/6	23(4.9)	23(4.4)	99(1.1)
1/7	8(2.8)	8(2.8)	69(4.8)
8/7	3(2.8)	3(1.9)	27(4.6)
15/7	8(2.6)	8(2.8)	60(5.1)

When bee populations were measured, a reduction in the population of bees and brood occurred in nearly all of the colonies over the course of the trial. The largest colonies declined the most, but there were no significant differences amongst the treatments. PINZAUTI (1994) has reviewed the performance of honeybee colonies in confined areas such as cages and plastic tunnels, and found that such declines are normal and that it is common for large colonies to suffer more severe population reduction than small ones.

Between 10% and 30% of the dead bees from cages with fungal inoculum did show external spore formation after incubation at 23°C, compared with 0% in cages without fungal inoculum. It is not clear, however, whether this represented *M. anisopliae* living on the dead bee, or whether the fungus had actually caused mortality. Even if the fungus had actually caused mortality, whether this represents a significant effect on the foraging ability of the colony would be dependent on the reduction in the life of individual foraging bees that it caused. Honeybee colonies have a complex age structure (RIBBANDS, 1953), and for the first few weeks of life, young bees are occupied in tasks within the brood nest, and are, therefore, unlikely to come into contact with the *M. anisopliae* spores. Furthermore, the brood nest itself has temperatures normally maintained at 35°C ± 0.5°C (SIMPSON, 1961), unfavourable for the growth of the fungus. It is only during the final, and most hazardous phase of the adult bees life, that of guard duties followed by foraging that the bees will come into contact with the spores, and also experience lower temperatures more suited to the development of the fungus. BUTT et al. (1994) found that when bees were inoculated with *M. anisopliae* V245 and maintained at 30°C the mean LT<sub>50</sub> was 8.5 ± 0.1 days. WINSTON (1987) stated that worker bees in the field forage for an average of only 4-5 days before death although when confined in cages they undoubtedly can live for much longer (BALL, CARRECK & MARTIN, in preparation). Probably any foragers succumbing the fungus would already be nearly at the end of their natural lives, and thus the adverse effect on the foraging capability of the colony is likely to be small.

It is likely that the most valuable application of this system will be by combining its use with an early flowering variety of oilseed rape as a border strip around the main commercial crop. This would concentrate both pest and bee activity to the trap crop and reduce pest numbers before the main crop came into flower.

## Acknowledgements

We thank Liz Isger, Amber Measures, Graham Stenning and Emma Clowes for assistance with fieldwork, and Susanne Clark for statistical analyses. This work was funded by the UK Ministry of Agriculture, Fisheries and Food IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

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