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# ROLE OF BIOTECHNOLOGICAL APPROACHES IN RELATION TO HONEY BEES

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## Introduction:

Transgenic crop plants become commercialized; there is an increasing need for information on their impacts on honey bees and bumblebees. Direct effects on bees may arise upon ingestion of proteins encoded by transgenic, if they are expressed in pollen, nectar or resin. Indirect effects may occur if plant transformation inadvertently changes flower phenotype. Effects of purified transgenic product ingestion on adult bee gut physiology, food consumption, olfactory learning behavior and longevity. Bt, protease inhibitor, chitinase, glucanase and biotin-binding protein genes are discussed. Observations of bee foraging on transgenic plants kept under containment are also summarized.

Honey bees (*Apis mellifera* L.) are the most important pollinators of many agricultural crops worldwide and are a key test species used in the tiered safety assessment of genetically engineered insect-resistant crops. There is concern that widespread planting of these transgenic crops could harm honey bee populations. The bees have decreased in numbers by more than 50 percent over the past 70 years, a trend researchers attribute to pesticides, mite infestations, and loss of genetic diversity. This phenomenon is known as Colony Collapse Disorder (CCD).

The Organic Consumers Association(OCA) blames the modified organisms (GMOs) in crop production, arguing that the pesticides used by Monsanto have contributed to the CCD. However, the OCA's claims are untrue. In fact, GMOs hold the potential to save the honeybee species. Consequently there is a need for information about the impacts of transgenic plants on bees as pollinators and as honey producers.

## Effects of Transgene Products on honey bees

### *Bt* genes

Bt genes are isolated from *Bacillus thuringiensis*, a soil-dwelling bacterium which produces a range of insect-specific toxic proteins. Different strains of *B. thuringiensis* produce different suites of toxins. Usually each toxin is specific to a particular order of insects and Bt genes encoding toxins with lepidopteran, dipteran or coleopteran activity have been isolated. Cultured *B. thuringiensis* spores and vegetative stages have been used for many years in biopesticide preparations where their lack of hymenopterans activity has

ensured a good safety record with bees. Transgenic cotton and corn plants containing lepidopteran-active Bt genes are commercially available, as are coleopteran-active Bt-transgenic potatoes (Anon, 1997, 2000). These plants present single toxins to the insect in a pure and "activated" form, whereas the biopesticide preparations, containing whole bacteria and spores, usually present the insects with mixtures of toxins that need to be activated by conditions in the insect's gut. Because of this, additional testing needs to be undertaken to ensure the safety of transgenic Bt-plants to beneficial insects such as bees. Fortunately, Bt toxins can be purified and activated to resemble the state in which they are expressed in transgenic plants (e.g. Simpson *et al.,* 1997) and these can be used in trials with bees. Purified Cry 1Ac (= CryIA(c), lepidopteran- active toxin fed at a concentration of 20 mg/ml to 1–3 day-old larvae and adults of *Apis mellifera* had no significant effect on the survival of these insects (Sims, 1995). This toxin concentration was more than "100 times the concentration of CryIA(c) protein found in the field as present in pollen and nectar of transgenic cotton" (Sims, 1995), but the author did not give details of these gene expression measurements.

Similar toxicity test results were submitted to the United States' Environmental Protection Agency (EPA) for registration of Bt-cotton. No toxicity was noted in honey bee larvae or adults fed purified Cry1Ac at levels "1700 or 10000 times the levels found in pollen and nectar, respectively, of transgenic insect resistant cotton plants" (Anon, 2000). Honey bee larval tests for the EPA have also revealed no bee toxicity for Cry1Ab and Cry9C (both lepidopteran- active toxins for expression in corn) or for Cry3A (coleopteran-active toxin for potatoes) (Anon, 2000).

**Protease inhibitor genes:**

**a. Tests with purified Protease inhibitors**

Protease inhibitors (PIs) can be isolated from a great number of natural sources, representing plants, animals and microbes. As their name suggests, they are proteins which inhibit protease activity. Honey bees and bumblebees use proteolytic enzymes to digest dietary protein (Winston, 1987; Malone *et al.,* 1998, 2000) and so it is not surprising that some PIs at some concentrations have been demonstrated to have effects on these insects. Serine proteases predominate in these insects and serine PIs, such as soybean trypsin inhibitor, may affect bees more than cysteine PIs, such as oryzacystatin. Purified Bowman-Birk soybean trypsin inhibitor (BBI) fed to foraging (older) honey bees at dose levels of 1, 0.1, 0.01 or 0.001 mg/g of sugar syrup had no effect on bee survival over four days (Belzunces *et al.,* 1994). However trypsin activity levels in foraging bees fed three different doses of BBI in syrup for 3.5 days were significantly different from those in control bees.

**b. Test with transgenic plants**

PIs and bees so far suggest that adult bee gut protease activities may be reduced, with a resultant impact on bee longevity, when bees ingest these proteins. However, the effects will depend on the specificity of the particular inhibitor and the concentration to which the bee is exposed.

**Chitinase genes**

Genes encoding chitin-degrading enzymes have been isolated from a number of sources, including plants, insects and entomopathogenic micro-organisms. Acute toxicity tests with 10-day-old adult honey bees fed sugar solution containing a chitinase purified from

tomato (11 mg per bee) showed that this transgene product had no significant impact on bee survival after 24 or 48 hours (Picard-Nizou *et al.,* 1997). Bees injected with 1.69 mg of chitinase were similarly unaffected.

**β-1,3 glucanase genes**

Glucanase genes have been isolated from a number of different plants, where they form an important part of the plant's response to attack from fungal pathogens (e.g. Neuhaus *et al.,* 1992; Chang *et al.,* 1992; Gottschalk *et al.,* 1998). They have also been isolated from micro-organisms (e.g. Haapalainen *et al.,* 1998; Okada *et al.,* 1998). Transgenic plants expressing β-1,3

glucanase have demonstrated enhanced resistance to fungal pathogens (Jongedijk *et al.,* 1995). This protein is highly unlikely to be harmful to bees, since its substrate, β-1,3 glucan, has not been found in insects. At the colony level, the level of visits to feeders of an artificial flower device set in a flight room, filled with sucrose solution added with 110 μg/ml β-1, 3 glucanase diluted between 100 and 10000 times was weaker as the concentration increased. However, there were no differences in the amounts of solution collected that could be attributed to the type of feeder solution presented (Picard *et al.,* 1991).

**Biotin-binding proteins**

The role of biotin in honey bee or bumblebee nutrition is unknown. Preliminary toxicity tests with newly-emerged adult honey bees fed with pollen-based food containing either 6.7 or 20 μM avidin showed that this protein had no significant impacts on the rate at which bees consumed their food or on their longevity (Christeller *et al.,* 1999).

**Glufosinate resistance genes**

Herbicide resistance is one of the most commonly-used traits in commercial cultivars of transgenic crop plants (Anon, 1997). Since this resistance operates via the production of an enzyme to break down the herbicide and bees lack such substrates, they are extremely unlikely to be harmed by these plants. The impacts on honey bees of transgenic herbicide (glufosinate) resistant oilseed rape have been assessed under semi-field conditions (Chaline *et al.,* 1999).

Results from tests with bees and transgene products so far suggest that direct effects of transgenic plants on honey bees and bumblebees will depend largely upon the type of transgene and the biological activity of the protein it encodes. Thus proteins such as lepidopteran-specific Bt toxins and glucan-degrading enzymes are extremely unlikely to affect bees. Proteins that target more general aspects of insect biology, such as protease inhibitors or chitinases, are more likely to have effects on bees. In these cases, the dosage of transgenic product ingested by the bee is very likely to determine the extent of such effects, if any. Obviously, the concentration of expressed protein in the pollen, nectar or resin of the transgenic plant will influence the extent of its impact on bees. The insecticidal proteins produced by these plants (e.g. Bt toxins, PIs) tend to have lower toxicity to bees, fish and mammals than many registered chemical insecticides, particularly those that act as neurotoxins (e.g. some synthetic pyrethroids and organophosphates) (Walton, 2000). The EPA required only larval toxicity tests for honey bees before registering Bt-cotton plants (Anon, 2000).

The toxin was already known to be specific for Lepidoptera, there were no significant negative effects on bee larvae. While this methodology may be more than adequate for assessing the safety of a Bt toxin, the appropriateness of such a high-dose method for other testing other gene products, which may not be so specific but may still present only an extremely low ecological risk, must be questioned.

**Bee-safety testing schedule should include the following:**

1. Determination of gene expression levels in pollen, nectar and resin.
2. Estimation of the highest potential exposure levels for bee adults (workers and reproductives) and larvae, given the levels of expression determined above and the bees' potential for gathering and ingesting the pollen, nectar and resin of the transgenic plants in question.
3. Toxicity and sub-lethal effects tests conducted with purified proteins and caged bees in the laboratory.
4. Determination of flower attractiveness (e.g. nectar volumes, nectar sugar concentrations, flower structure) as part of the selection of transgenic plant lines for release.
5. Confirmation of results obtained in laboratory tests via field tests, preferably with transgenic plants rather than purified proteins.

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