Literature Review on Genetically Modified Plants and Bee Products

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EXECUTIVE SUMMARY

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1. Bees collect pollen, nectar, resins and honeydew from plants and incorporate these into bee products such as honey, pollen and propolis.

2. GM material, i.e. transgene DNA or novel proteins encoded by transgenes, may be present in bee products if it occurs in the plant tissues and secretions collected by bees and if they incorporate it into those products.

3. GM plants have the potential to produce:
*   pollen containing both transgene DNA and novel proteins
*   nectar containing novel proteins, although probably only rarely
*   resins containing novel proteins, although this is not at all certain
*   sap containing both transgene DNA and novel proteins, but whether they would remain after passing through the gut of a sucking insect (to make honeydew) is not known.

4. Pollen, which commonly occurs in honey at concentrations ranging from 20,000 to 100,000 grains per 10 g (and rarely to a maximum of 5 million grains per 10 g), is thought to represent the most likely source of GM material in bee products. If we assume that an "average" pollen grain weighs 0.03 μg, these values are equivalent to honey containing 0.0006% to 0.03% w:w pollen, with a maximum value of 1.5% w:w.

5. GM material has been recorded in honey samples:
*   transgene DNA was detected by PCR in shop-bought honey from regions where field trials of herbicide-resistant GM oilseed rape were grown (Friends of the Earth study)
*   a novel protein (conferring kanamycin resistance) was detected by ELISA in a sample of honey taken from a hive near flowering herbicide-resistant GM oilseed rape in the United Kingdom (MAFF study)

6. GM food labelling legislation allows for a food to contain up to a certain percentage of GM material where its presence is unintentional. At present this percentage is:
*   1% w:w in New Zealand, Australia, the European Union and Saudi Arabia
*   3% w:w in South Korea
*   5% w:w in Japan
There is presently no requirement to label foods containing GM material in Canada or the United States.

7. Honey containing GM material cannot be certified as organic. Organic beekeeping rules in New Zealand do not specifically mention GM crops, but hives must be kept at least 3 km from conventionally-grown crops.

8. There are no GM plants being grown outdoors in New Zealand at present. Of the New Zealand crops for which GM varieties are available commercially overseas or are being developed in laboratories, clover, oilseed rape (canola) and eucalypts are the most likely to be important for honey production. Apple and kiwifruit pollen may be a component of bee-collected pollen and may occur in honey. Pollen from other crops, such as maize, grass, pine, tamarillo, potato, onion and leek, may occur in some honeys, but only as very minor components.

9. Bees have been reported to fly up to 13.7 km, although this was to a single food source in a desert where there was no other forage available. Most authors report 10 km as a maximum distance for a bee foraging flight, that most bees will be found within 6 km of their hive, and that most will have mean foraging distances of about 0.5 to 1.5 km.

10. Strategies to minimise the presence of GM material in bee products may include:
- separating GM and non-GM crops (effectiveness will depend on bee flight distances)
- screening GM crops with bee-proof mesh (not practical except on a very small scale)
- using bee attractants such as a sugar syrup spray on non-GM crops
- using bee repellents such as some pesticides on GM crops
- using GM plants where the transgene is not expressed in pollen, or the transgene occurs only in chloroplasts, or where pollen or flower formation is blocked
- removing pollen grains from honey by filtering after harvest.

11. Although the low levels of pollen in most honeys should ensure that GM labelling would not usually be required for honey sold to New Zealand's main honey markets (even if the bees foraged solely on GM plants), there may be a market reaction to honey from a country where GM crops are grown.

12. Some shipments of honey from Canada, where bees can forage on GM canola and GM food labelling is not required, were rejected by Germany in 1999. This event has received considerable publicity, but the Canadian Honey Council reports that this market has now recovered. Reports of difficulties with honey exports from Argentina, the United States or Australia could not be found.

13. Potential impacts of GM plants on bee health are being actively researched in several countries, including New Zealand. The effects on honey bees of a number of novel proteins which might be expressed in pollen have been determined. No negative effects have been observed thus far for the types of GM plants currently commercially available. Slight negative effects for a small number of novel proteins from GM plants under experimental development have been observed.

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1. INTRODUCTION

Genetically modified (GM) plants manifest new traits via the production (expression) of novel proteins encoded by inserted transgenes (DNA). For example, cotton modified to contain a Bt (*Bacillus thuringiensis*) gene and expressing Bt insecticidal protein in its leaves and buds will be protected from caterpillar attack. Both the transgene DNA and the novel protein in such plants could be considered "GM material".

Bees collect pollen and nectar from plants as food for their colonies. They may also collect honeydew (plant sap that has been ingested and then excreted by sucking insects such as aphids) for food and plant resin to make propolis with which to seal up cracks in the hive. With GM plants, there is a possibility that transgene DNA and/or novel proteins may be present in the plant parts and secretions collected by bees. Hive products intended for human consumption and derived from the plant parts collected by bees include honey (from nectar or honeydew, and containing traces of pollen), pollen and propolis. Other products, such as pure royal jelly, bee venom and beeswax, are secretions from the bees themselves and would not be expected to contain any plant matter. Section 2 examines the evidence for "GM material" from GM plants occurring in hive products.

The relevance of this to the production and marketing of bee products will depend on the tolerance limits for GM material in food. GM labelling requirements in countries to which New Zealand exports honey, and organic food standards, are described in Section 3.

GM plants are now being grown commercially in thirteen different countries. In 2000, the estimated global area planted with these crops was 44.2 million hectares (James, 2000). Ninety nine percent of these crops were grown in the USA, Canada, Argentina and China. Herbicide-tolerant soybean was the most common GM crop, occupying 59% of the global area, followed (in descending order) by Bt corn (maize), herbicide-tolerant canola (oilseed rape), herbicide-tolerant corn, herbicide-tolerant cotton, Bt/herbicide-tolerant cotton, Bt cotton and Bt/herbicide-tolerant corn. In addition to these commercially available varieties, many other plants can now be genetically modified. Field trials are under way overseas for field crops (e.g. wheat, rice, barley, tobacco), flowers (e.g. roses, carnations), trees (e.g. poplar, spruce, sweetgum), oil crops (e.g. sunflower, peanut), grasses, sugar crops (beet and cane), fruits (e.g. apple, cranberry, grape, melon, strawberry) and vegetables (e.g. tomato, potato, broccoli, carrot, eggplant, lettuce, pea) (Hagedorn, 1997). In New Zealand, there is sufficient scientific expertise to modify a number of locally important crop plants, experimentally at least (Christey and Woodfield, 2001).

New Zealand beekeepers rely on a wide range of plants to provide nectar and pollen for their bees (Matheson, 1997). Many of these plants are not commercially cultivated, but are native plants (e.g. manuka, rewarewa, pohutukawa, rata), garden plants (e.g. rosemary, flowering currant), or weeds (e.g. gorse, heather, thistles). Of the commercial crops, white clover is the most important for honey production. Trees grown for shelter (e.g. willow, hawthorn) and for fruit (e.g. apple, pear) and field crops (e.g. brassicas, sunflowers) can also be useful sources of forage for bees. Bees are also used to pollinate crops for fruit, vegetable and seed production (Appendix 1).

There have also been reports of bees visiting plants such as corn, grasses and potatoes in the absence of better sources of forage and of the occurrence of traces of pine pollen in honey. Thus, if modified, some of these plants might represent sources of GM materials that could be collected by bees. Section 4 describes GM plants which could be visited by bees and might be grown in New Zealand. It also examines methods for separating GM and non-GM crops for the purposes of keeping separate the bee products derived from them.

Market reaction to the presence of GM materials in bee products will play an important role in determining the economic significance of any impact that GM plants may have on bee products from New Zealand. Section 5 describes the experiences of some beekeepers in countries where GM crops are grown.

Finally, current scientific knowledge of the potential for GM plants to have impacts on honey bee health is reviewed in Section 6.

2. GM MATERIAL IN PLANT PARTS COLLECTED BY BEES

For the purposes of this discussion, "GM material" is defined as either transgene DNA or novel proteins encoded by transgenes. (This is also the definition used in the Australian and New Zealand Food Authority (ANZFA) regulations concerning food labelling in relation to GM.) Plant parts collected by bees are pollen, nectar, resin or honeydew (sap that has passed through the digestive system of a sap-sucking insect).

In GM plants, transgene DNA may occur in any plant tissue which normally carries DNA, but the novel protein which it encodes will not necessarily be expressed in every tissue, since this process is directed by the promoter (switch) on the gene in question. There are many different promoters available to genetic engineers. Some switch on transgene expression in all parts of the plant, while others switch it off in all but one tissue (e.g. a root-specific promoter).

2.1 Potential for GM Material to be Collected by Bees.

2.1.1 Pollen.

Since pollen is a plant tissue composed of cells capable of protein synthesis, it is reasonable to expect to find transgene DNA in pollen grains and also novel protein, if the transgene's promoter permits it. Table 1 presents currently available data on novel protein expression levels in pollen from GM plants.

Compared to leaves, which are about 2% protein (J. Christeller, pers. comm.), pollen has a high protein content, estimated to be between 8 and 40% (Herbert, 1992), and for this reason transgenes may be expected to express novel proteins at reasonable levels in pollen. However, one of the most commonly-used promoters in GM plants, cauliflower mosaic virus (CaMV) 35S promoter, does not drive pollen expression particularly well. For example, in leaves, this promoter can drive expression of novel proteins such as protease inhibitors up to about 0.4% of total protein, but the same protein will be undetectable in the pollen of the those plants (Bonadé Bottino et al., 1998) (see also Table 1). The highest recorded level of a novel protein expressed in pollen via the CaMV 35S promoter is 0.6 μg per g pollen (fresh weight) for a Bt toxin

in cotton (Greenplate, 1997). This is equivalent to 0.00024% w:w of total soluble protein, assuming the pollen is 25% protein (Table 1).

Higher levels of novel proteins may occur in pollen if the transgene includes a pollen-specific promoter such as that derived from maize (Kozeil et al., 1993). Expression levels as high as 0.0418% w:w of total soluble protein have been recorded for a Bt toxin with this promoter in maize pollen (Kozeil et al., 1993; Table 1). This type of GM Bt-maize (referred to as an event 176 hybrid) was used as the source of insecticidal pollen in the much-publicised early monarch butterfly studies of Losey et al., (1999) and Jesse and Obrycki (1999). Subsequent studies compared the effects of event 176 pollen with that of other Bt-maize hybrids in which the gene was controlled by the CaMV 35S promoter (referred to as MON810 or Bt11 hybrids). Pollen from the latter hybrids had negligible effects on monarch larvae (Stanley-Horn et al., 2001; Hellmich et al., 2001). The registration of maize hybrids derived from event 176 will terminate in the United States in 2001 (Stanley-Horn et al., 2001).

New biotechnological developments aimed at eliminating the problems associated with transgene DNA and expression of novel proteins in pollen are discussed in Section 4. The motivation for these developments centres mainly on concerns about gene flow to other plants and allergens in pollen, rather than for bees and bee products.

In summary, it is possible that GM material could occur in pollen harvested for human use from hives placed where bees may forage from GM crops. The actual concentration will depend on how much GM pollen is taken relative to pollen from other plants and how much transgene DNA or novel protein the GM plant produces in its pollen. Similarly, pollen containing GM material could also be present in honey harvested from bees foraging on GM crops.

2.1.2 Nectar

Nectar is a plant secretion, rather than a tissue, and has no cellular content. As such, transgene DNA is not likely to occur in nectar and there are no records of any RNA or DNA in nectar. Most nectars are also free of protein, being composed principally of sugars and sometimes free amino acids (Baker and Baker, 1973; R. Bieleski. pers. comm.). There are exceptions to this however. Recently, tobacco plants have been found to secrete a limited array of proteins to a concentration of about 0.024% protein of total nectar (Carter et al., 1999). Leek nectar has also been shown to contain two proteins, a lectin and an alliinase, comprising about 0.022% of the nectar (Peumans et al., 1997). Both of these proteins were inactivated or degraded when leek nectar was made into honey by bees, presumably by the action of enzymes in the bee's honey stomach. Incidentally, these authors also found two new proteins in the honey that had no equivalents in leek nectar and concluded that these must have come from the bees themselves (e.g. enzymes secreted by the honey stomach).

Consequently, it is theoretically possible that some GM plants could secrete novel proteins with their nectar, although their concentrations are likely to be very low. Consequently, bees could gather such nectar, but the honey they make from it may not necessarily contain the nectar proteins in active form.

Presumably because of nectar's low protein content, there has been only one record of an examination of nectar from a GM plant. Jouanin et al. (1998) noted that Bowman-Birk soybean trypsin inhibitor (BBI) could not be detected in the nectar (or pollen) of transgenic oilseed rape plants containing the BBI gene under the control of CaMV 35S promoter.

2.1.3 Plant resins and gums

There are no records of DNA or RNA being detected in plant gums or resins. Proteins, however, have been recorded from some of these plant secretions. For example, gum arabic (from *Acacia* species) is 2-4% protein (Menzies et al., 1996) and proteins have been recorded from pea root mucilage and rye root exudate (e.g. Knee et al., 2001; Siciliano et al., 1998). There are no references to the presence of either DNA or RNA in propolis. The composition of propolis varies from sample to sample due to the variety of plant resins and gums utilised by the bees and the collection techniques used by beekeepers to obtain propolis from the hive. One report describes a propolis with a protein content of about 2.5 % (Tuha and Simuth, 1991). Novel proteins from GM plants may conceivably find their way into the gums, exudates and resins that bees collect to make propolis, but there is, as yet, no published evidence to support this idea.

2.1.4 Plant sap and honeydew

Honeydew is excreted by sap-sucking insects such as aphids. As well as the sugars that predominate, phloem sap also contains free amino acids, small peptides and sometimes proteins (Salvucci et al., 1998). For example, phloem exudates from squash, cucumber and castor oil plant have been shown to contain proteins, some of which appear to be important in the transport of plant viruses (Christeller et al., 1998; Kruger et al., 2000; Schobert et al., 2000; Owens et al., 2001). Similarly plant mRNA (messenger RNA) is known to circulate in plants (translocate) via the phloem (Oparka and Santa Cruz, 2000). Thus, phloem sap from GM plants could conceivably contain both transgene mRNA and novel proteins. There are no published studies describing the fate of plant RNA after digestion by plant-sucking insects and no records of RNA or DNA in the honeydew they excrete. However, a DNA-tracking study has shown that squash leaf curl virus can pass intact through the guts and into the honeydew of whiteflies (Rosell et al., 1999). It is commonly thought that sucking insects lack digestive proteases and utilise only the free amino acids in sap for their nitrogen needs (e.g. Rahbé et al., 1995; Sandstrom and Moran, 2001). From this it might be concluded that sap proteins would pass into honeydew intact. However, when whiteflies were fed with labelled cotton leaf proteins, these were digested and excreted only as amino acids in the resultant honeydew (Salvucci et al., 1998). Thus it remains debatable whether or not novel proteins from transgenic plants could find their way into honeydew and if they would persist in honey made from it.

2.2 Records of GM Material in Bee Products

Currently two methods may be used to determine the GM status of foods. With the polymerase chain reaction (PCR) test for transgene DNA, a "primer" (consisting of a piece of DNA with a sequence that could only occur in the transgene) is added to a sample of the food to be tested. If the primer matches any DNA in the sample, then

the PCR will cause this DNA to be "amplified". The amplified DNA can then be stained and visualised to give an indication that the sample contains some transgene DNA. With quantitative PCR, the concentration of transgene DNA in the sample may also be estimated. Theoretically this method is very sensitive and can detect even one or two pieces of transgene DNA in a sample. In practice, its sensitivity will depend on the nature of the food being tested and the transgene DNA sequence that is being sought. For example, a study of GM soyabeans and maize (Lin et al., 2000) showed that PCR using a CaMV 35S primer had a detection limit of 0.1% w:w of GM soyabeans, but with a nos (nopaline synthase) primer the test had a limit of 1%. With a CDPK-cry (maize calcium-dependent protein kinase promoter with Bt toxin) primer, PCR had a detection limit of 0.1% w:w for GM maize and with a cry1Ab (Bt toxin) primer the limit was 2%.

The second detection method uses enzyme-linked immunosorbent assay (ELISA) to detect novel proteins in food. With this method, an antibody to the novel protein in question is prepared and linked to an enzyme which catalyses a reaction resulting in a coloured end-product. The enzyme-linked antibody is added to the food sample and, if the novel protein is present, it links to the protein and cannot be washed away. The colour reagents are then added and the intensity of the coloured end-product gives a measure of the concentration of the novel protein in the food.

Pollen represents the most likely source of transgene DNA and novel proteins in bee products. It is also commonly present in the most widely-consumed bee product, honey. Because of this, the only attempts to measure GM material in bee products have focussed on honey and its pollen "contaminant". There are no records of attempts to detect GM material in honey from honeydew, pollen intended as human food or propolis.

Most New Zealand honeys (including clover honey) contain between 20,000 and 100,000 pollen grains per 10 g (Moar, 1985). This is considered the "normal range", but some honeys have pollen concentrations above or below this range. Molan (1998) states that concentrations as low as 500 grains and as high as 5 million per 10 g are possible. In the United Kingdom, shop-bought honeys were found to contain between 20,000 and 80,000 grains per 10 g (Anon A, 1998). Eady et al. (1995) found 100,000 pollen grains per ml in a commercial honey derived from garden flowers (in the UK).

If we assume that an "average" pollen grain weighs 0.03µg (Stanley and Linskens, 1974), then these figures translate as follows:
- 500 grains per 10 g is equivalent to 0.00015% w:w pollen in honey
- 20,000 grains per 10 g is equivalent to 0.006% w:w pollen in honey
- 80,000 grains per 10 g is equivalent to 0.024% w:w pollen in honey
- 100,000 grains per 10 g is equivalent to 0.03% w:w pollen in honey
- 100,000 grains per ml is equivalent to 0.3% w:v pollen in honey
- 5 million grains per 10 g is equivalent to 1.5% w:w pollen in honey.

The stability of transgene DNA and novel proteins in GM pollen stored in honey has been assessed using pollen from modified tobacco and *Arabidopsis* plants with marker genes on pollen specific promoters (Eady et al., 1995). A PCR test showed that transgene DNA remained "relatively intact" even after seven weeks in a commercial honey sample. Similarly, novel protein was detected unchanged after six weeks in

honey. The authors pointed out that the experimental system they used represented a "worst-case scenario" for the presence of GM material in honey and that "the concentration of a given, potentially toxic pollen-borne protein is expected to be very low in natural honey made from nearby transgenic plants". However, they also pointed out that even vanishingly small quantities of some proteins may cause allergic reactions.

There are only two published studies of attempts to measure GM material in natural honey made by bees foraging near GM plants. The first was carried out by the UK Ministry of Agriculture, Fisheries and Food (now the Department for Environment, Food and Rural Affairs) (Anon A, 1998). In this study a sample of honey was taken from a "hive close to a transgenic oilseed rape field", pollen extracted from it and ELISA used to quantify the amount of npt II protein (which confers kanamycin resistance) present. Two readings from the single sample gave a mean of 1.61 ng per mg (0.00016% w:w) of total protein in the pollen sample. A control sample of non-transgenic oilseed rape honey was not included. Pollen samples taken from two GM tobacco plants containing the same transgene construct (nptII and nos promoter) gave mean readings of 35.1 pg and 1.39 ng of nptII protein per mg of total protein (3.51 x 10^{-6}% and 0.000139%, respectively). This report also stated that DNA could be extracted from a number of commercially available honey samples and from honey derived from transgenic oilseed rape (presumably the same sample used in the protein analysis), but the details of this part of the study are not given in the report.

The second study was commissioned by Friends of the Earth in the UK (Anon B, 2000; Anon C, 2000), who were concerned that pollen from unidentified GM-oilseed rape field research sites could occur in honey without beekeepers' knowledge. They purchased 11 jars of locally produced honey and honey comb from retail outlets in an area of England where GM herbicide-tolerant oilseed rape crops had been trialed. Each sample was checked for oilseed rape pollen content and nine of the samples found to contain significant quantities (actual amounts and detection limits not stated) of this pollen. Sub-samples were taken from these honeys and sent to the Austrian Federal Environmental Agency Laboratory. PCR was used to determine whether any of the samples contained DNA sequences corresponding to the herbicide-tolerance gene (bar or pat gene) and the nos gene promoter or terminator used in such GM oilseed rape plants. Two of the nine samples gave positive results for the pat gene and the nos promoter, suggesting that pollen from GM oilseed rape had found its way into honey. Unfortunately, Friends of the Earth could not afford to have a quantitative analysis conducted which would have shown how much GM pollen might be in the honey (Anon B, 2000). The apparent lack of appropriate controls in this study (honey from a region where only non-GM oilseed rape was grown) is also unfortunate. The bar, pat and nos genes are all derived originally from common bacteria (*Streptomyces* and *Agrobacterium*) (A. Gleave, pers. comm.; Wehrmann et al., 1996), suggesting that they may commonly contaminate natural products such as honey. Bar, pat or nos DNA from these bacteria would also give positive results with the PCR tests conducted. While it may be argued that this is unlikely, inclusion of suitable controls would have removed all doubt from this study.

The results of this honey study have subsequently been used by Friends of the Earth and UK beekeepers to call for a halt to field research on GM plants, particularly a

series of "farm-scale" trials of GM herbicide-tolerant crops which commenced in the UK in 1999. Diamand (1999) gives further details of this campaign.

3. LEGAL STANDARDS AND LABELLING REQUIREMENTS FOR GM MATERIAL IN BEE PRODUCTS.

3.1 GM Food Labelling

Honey has been specifically included in Australian and New Zealand legislation concerning the labelling of GM food (Anon D, 2001), which states that:
- "Honey is not, by definition, a food produced using gene technology under the standard",
- "the standard allows a food or ingredient to have up to 10g/kg (1%) of a GM food where its presence is unintentional. This applies to the presence of pollen from an approved GM food commodity",
- "Outcome: No GM labelling required".

Presumably this 1% rule could apply equally to other bee products such as bee-collected pollen and propolis. As worded, the regulations seem to be stating that honey or other bee products containing up to 1% of pollen containing GM material need not be labelled. This is a more stringent requirement than if honey containing up to 1% of the GM material itself was permitted, since transgene DNA or novel protein will comprise only a fraction of the weight of a "GM" pollen grain (see Section 2.1.1 and Table 1).

A similar situation pertains in the European Union, where small amounts (up to 1%) of GM material that are accidentally present in non-GM ingredients do not have to be labelled (Anon E, 2001). This threshold for accidental inclusion of GM material may change in the future however, since the European Parliament has recently called for a reduction from 1% to 0.5% (Anon F, 2002).

Saudi Arabia also allows up to 1% unintentional inclusion of GM product (Gray, 2002). Since United Arab Emirates and Yemen are also members of the Gulf Cooperation Council, which sets food standards for these countries, it may be assumed that the Saudi Arabian limit also applies.

Japan's Agriculture Ministry requires labelling of GM foods in which GM material is one of the top three ingredients and where it accounts for 5% or more of the food weight (Takada, 1999).

The South Korean government requires labelling where GM content exceeds 3%, but apparently the United States government is applying pressure for this to be raised to 5% (Anon G, 2002).

China has recently (Anon H, 2002) implemented new regulations for GM crop and food imports, requiring labelling of all foods with GM ingredients and certificates of harmlessness to human and non-human animals, and to the environment. United States soybean imports have been granted temporary safety certificates until December 2002.

In Canada and the United States, there are, as yet, no GM labelling requirements for any food, since their food legislation focuses only upon potential impacts on human health, rather than consumer preferences (Anon I, 2001; Anon J, 2001). This situation may change in the future. In May 2002, new draft legislation concerning GM food labelling (Genetically Engineered Food Right to Know Act 2002) was placed before the US Congress (Anon K, 2002).

Between 1996 and 1998 New Zealand honey was exported to European Union countries, the United States, Korea, Malaysia, Singapore, Japan, Hong Kong, Taiwan, Saudi Arabia, Yemen and the United Arab Emirates (Bourn et al., 1999).

Only honey samples with the very highest pollen counts (5 million grains per 10 g) may exceed a 1% pollen content (see Section 2.2 above). Thus the vast majority of honeys produced by bees foraging even solely on GM crops would be exempt from a legal requirement to label for GM content in New Zealand, Australia or the European Union. All such honeys would be exempt from labelling if destined for Japan, South Korea, the United States or Canada. The situation with regard to Chinese markets is not certain.

3.2 Organic Honey Standards

Organic farming prohibits the use of GM crops and honey containing GM pollen cannot be certified as organic (Bourn et al., 1999; Moyes and Dale, 1999). Presumably honey tests are required for certification unless absolute evidence that the bees cannot have visited GM crops can be produced, but this is not explicitly stated in organic food standards such as the United Kingdom Register of Organic Food Standards, which does not quote a figure for accidental inclusion of material from GM crops (Moyes and Dale, 1999; Anon L, 2000).

The only organic honey production standards to specifically mention GM crops are those of the Organic Crop Improvement Association (OCIA) in Canada which recommend (but apparently do not require) that "apiaries shall not be located near flowering crops which have undergone genetic manipulation" (Anon M, undated).

These and other standards specify proximity of hives to conventional crops, with the aim of minimising contamination with conventional pesticides. It is not certain whether these separation distances would also satisfy organic regulators with respect to GM crops and field trial sites.

The 2001 BIO-GRO New Zealand Organic Standards for honey do not specifically mention GM crops (Anon N, 2001). However, they do require that beehives are located more than 3 km away from land used for intensive conventional horticulture or cropping. Interestingly, these standards appear to have been relaxed since 1998 when a 5 km distance from conventional cropping was required (Bourn et al., 1999).

Organic regulations overseas are similar, with restrictions on the location of hives near conventionally-grown crops or other areas which may be sprayed with non-organic pesticides or polluted in some way (e.g. golf courses, urban areas, dumps etc.). A 3 km limit is used for some producers in the European Union (Anon O, 1999), Canada and the United States (OCIA regulations; Anon M, undated). Other

European and US standards (IFOAM, KRAV Sweden, Bioland Germany, Oregon Tilth USA) stipulate only that the hives are not close to areas where conventional pesticides have been used (Bourn et al., 1999). The NASAA Australian standard specifies a 5 km separation distance and the UK Soil Association a 7 km separation distance (Bourn et al., 1999).

It is interesting to compare the recommended separation distances for organic bee hives from conventional crops with information and statements about bee foraging distances (see Section 4.3.1.1 below).

4. MINIMISING INCLUSION OF GM MATERIAL IN BEE PRODUCTS

4.1 GM Crop Plants That Could be Sources of Bee Forage in New Zealand

Table 2 lists crop species grown over sizeable areas in New Zealand and for which GM varieties are either available commercially from other countries or are being developed for research purposes in New Zealand. The relevance of each crop to honey bees, in terms of providing nutrients for the bees or pollination services for the plants, is noted. The plant's role in providing raw materials (nectar, pollen etc.) for a bee product for human consumption is also recorded.

Of the plants listed, clover is the most important for honey production, although canola and eucalypts have potential as honey sources as well. Even though forage and vegetable brassicas have the potential to produce useful nectar for honey, only if grown for seed are they permitted to flower before harvest (Christey and Woodfield, 2001) and so they probably do not contribute greatly to New Zealand bee products. Kiwifruit and apple pollen may make up a significant component of bee-collected pollen products and may also be present in some honeys. Maize (or sweetcorn) pollen may be an unintentional ingredient in honey or harvested pollen, since bees have been observed to collect this pollen (Emberlin et al., 1999). Potato, tamarillo, onion and leek pollen may also represent sources of unintentional pollen in honey, but probably only on a very small scale. Grass pollen is regularly found at low levels in New Zealand honey and some grasses are apparently worked by bees for their pollen (Moar, 1985). Pine pollen is produced in vast quantities and is well-dispersed by wind throughout the environment. As such it may end up in bee hives, but there are no records of bees actively collecting pollen from pine trees. Pine pollen has been detected at an extremely low level (less than one percent of total pollen grains) in a clover honey sample (Moar, 1985).

The following discussion of means for separating GM and non-GM varieties will focus on major New Zealand crops which may be genetically modified and may affect bee products such as honey, pollen or propolis.

4.2 Relevance of Cross-pollination Studies with GM Plants

Cross-pollination between GM and non-GM crops and between GM crops and their wild or weedy relatives is a topic for which there is a large and growing body of scientific literature. These data are used to define separation or isolation distances, buffer zones, border crops and other strategies to minimise unwanted cross-pollination. Such strategies can also use knowledge gained over many years in the development of seed certification systems for conventionally bred crop plants (Christey and Woodfield, 2001). The dispersal of pollen is a critical element in cross-pollination studies and often the role of bees is discussed. Obviously such studies can sometimes provide useful information for determining bee foraging distances and bee behaviour in relation to a crop. However, they are not always entirely relevant in determining if bees will gather GM material and if it will be incorporated into a bee product. For example, the ability of the plants concerned to produce viable hybrid offspring is irrelevant, as is the viability of the pollen (since it will still represent GM

material if it occurs in honey). Important questions not generally considered in such studies include:

- Will bees visit and gather pollen and/or nectar from the crop plant?
- How far will they fly to do this?
- How long will the gathered material remain in the hive?
- How much of the material gathered from the crop plant will be present in the products of the hive?

There are some data available to answer some of these questions and these will be covered in the following sections.

Since there are no relevant available data on bees foraging for plant resins or honeydew, this discussion will focus on nectar and pollen collection.

4.3. Strategies to Minimise Inclusion of GM Material in Bee Products

Some possible strategies to minimise accidental inclusion of GM material in bee products include:

- separating GM and non-GM crops via planting distances or flowering times
- screening the crop to exclude bees
- using bee management techniques that maximise foraging on a particular crop (including attractants and repellents)
- using biotechnological solutions
- using post-harvest honey treatments to remove pollen.

4.3.1 Separation of flowering GM crops and hives

One method for ensuring that bee products derived from GM and non-GM plants are kept separate would be to plant the different crops far enough apart to ensure that bees from a single hive could not visit both. This physical separation of the hive from the undesired flower source is the rationale used in the production of organic honey. Hives must be placed at least 3, 5 or 7 km (depending on the standard used) from non-organically grown crops (see Section 3.2 above for more detail). However, these organic rules recognise that, with this method, some contamination is inevitable and so maximum pesticide tolerance limits are set. It has been suggested that similar limits will need to be set for GM material in organic products (Moyes and Dale, 1999; Christey and Woodfield, 2001), but it appears that a "zero-tolerance" policy is in place thus far.

The required distance for GM/non-GM crop separation would depend on the maximum distance that a bee will travel to forage on that crop and it may vary depending on the relative attractiveness of the crop compared to other flowering plants in the same area. Information on bee foraging distances is summarised below.

Temporal separation of GM and non-GM crops (taking advantage of different flowering times) may also provide a possible means of separation.

Beekeepers have extensive experience in ensuring that their bees preferentially visit a particular plant in flower, since they must do this to produce unifloral honeys. This usually involves siting hives where the desired plant predominates at the appropriate time to capture its "nectar flow". While these methods are adequate for producing

honeys of sufficient floral purity to satisfy consumer demand, they do not allow for the exclusion of pollen from a range of plants from the honey. To be called unifloral, a honey must have at least 45% of its total pollen content from the nominal plant species (Molan, 1998). Thus pollen from other plants commonly occurs in "unifloral" honeys. Concurrent foraging visits to other flowering plants account for only some of this pollen. Contamination may also occur when honey is extracted by crushing combs or using a loosening device (as with thixotropic honeys), since this can release stored pollen from nearby cells in the comb. Re-using comb from which honey was extracted during the previous season can also lead to contamination with "old" pollen.

4.3.1.1 Bee foraging distances

According to Winston (1987), most honey bees in agricultural areas forage within a few hundred metres of their hives, although significant populations have been found at 3.7 km. In forested regions, they forage at a median radius of 1.7 km from the hive and most can be found within 6 km. He points out that bees can be recruited to feeding stations up to 10 km from a hive if there are no competing food sources. Williams (2001) confirms this 10 km maximum flight distance.

According to Gary (1992), bees have a strong tendency to forage at the nearest source for each floral species in an area. He also mentions "distant flight" behaviour in agricultural areas where attractive crops are planted in widely dispersed fields, such that significant bee populations may be found at least 6.5 km from an apiary. Gary notes that bees in a desert will fly up to 13.7 km to a food source if there are no other food sources closer to the hive. This is the maximum bee foraging distance mentioned in the literature.

Moyes and Dale (1999) note mean foraging distances of 1.66 km and 557 m for bees foraging on flowering carrots and onions, respectively, and maximum distances for these crops on 6.17 and 4.25 km. Ramsey et al., (1999) notes bees flying 5 km to reach an oilseed rape field.

In New Zealand, a 5 km distance is generally recognised as being the minimum when shifting hives to ensure that they will not return to their old hive site (Matheson, 1997).

Friends of the Earth recently commissioned a study of pollen dispersal by honey bees from a herbicide-tolerant GM oilseed rape farm-scale field trial site (Emberlin and Brooks, 2001). Pollen traps were placed on six hives, two at each of three apiary sites, up to 4.5 km from the flowering GM oilseed rape crop. There were apparently no other flowering crops in the vicinity. Forty samples of pollen were taken from the traps and examined for oilseed rape pollen pellets (identified by colour and shape). Of these, six samples (presumably one from each hive) with numerous oilseed rape pollen grains were selected and sent for DNA analysis to the Austrian Federal Environment Agency Laboratory. PCR tests for the nos terminator and bar gene (see Section 2.2 above) gave positive results for each of the six pollen samples, suggesting that even bees from the furthest hive had gathered pollen from the trial site. Once again, there was no analysis of control samples of oilseed rape pollen collected from non-GM crops, so that the possibility of microbial contamination giving positive readings for nos or bar DNA cannot be discounted.

The literature thus suggests that a distance of more than 13.7 km from hive to GM crop would give a 100% guarantee that bees would not forage on the crop. However, this figure is derived from an experiment with bees in a desert with no other sources of forage, which is not a realistic agricultural situation. A better approach perhaps would be to define realistic foraging distances for different cropping situations and to assign probabilities that a least one copy of transgene DNA will occur in a pollen or honey sample as a function of distance. This approach supposes a zero tolerance limit for such DNA in these bee products. A less stringent limit would produce a different set of probability values.

4.3.1.2 Accidental inclusion of wind-borne pollen in bee products

A second possible source of GM pollen in hives and bee products could be that from GM crops which produce significant quantities of wind-borne pollen, e.g. ryegrass or pine. The discovery of a tiny amount of pine pollen in a sample of New Zealand clover honey (Moar, 1985) suggests that such an occurrence may not be completely improbable. However, there are no published data on how close a honey-producing hive would have to be to a pine plantation or ryegrass pasture for such pollen to occur in the honey or pollen harvested from that hive.

4.3.1.3 Feasibility of the crop/bee separation approach

Making sure that bees are sufficiently distant from any GM crop site to prevent visits to the crop or accidental occurrence of pollen from that crop in hives during any honey-making season could ensure that no or minimal GM pollen is accidentally introduced into the honey made that season. This would require careful, planned deployment of GM crops, especially clover or oilseed rape. Excellent communication between land-users and beekeepers would also be required. Since such communication is already an important factor in the success of beekeeping businesses, this should be achievable. However, some beekeepers have reported that they have been deterred from shifting to organic honey production by increased complications in dealing with landowners (Bourn et al., 1999), even without a consideration of the possibility of GM crops.

The carry-over from year to year of pollen in frames of empty comb and perhaps other hive equipment may mean that an equipment labelling and "quarantine" system, similar to that already employed in New Zealand for American foulbrood control (Goodwin and Van Eaton, 1999), would need to be implemented to segregate "GM" and "non-GM" hives.

The stringency of the techniques that will need to be used will, of course, be dictated by the tolerance limits for unintentional presence of GM material in bee products set by the countries where the honey will be sold.

4.3.2 Screening the crop to exclude bees

Screening a crop with bee-proof mesh would be practical only for small-scale field trial plots. It may be feasible for commercial crops of extremely valuable GM plants,

for example those grown to produce very valuable proteins for extraction and purification ("biopharming"), where the areas planted may be relatively small.

4.3.3 Bee management techniques to direct bees to visit particular crops

A number of bee management techniques have been developed to enhance bee visits to particular crops, usually in order to increase pollination and/or fruit set. The most obvious method is to simply place hives near the crop and away from other flowering plants. This method's usefulness in relation to the presence of GM material in bee products has been covered in Section 4.3.1. Most of the other methods are based on the application of a bee attractant to the crop. They are summarised in Section 4.3.3.1 below.

A number of chemicals have also been identified as bee repellents. Some are insecticides and their bee-repellent qualities have been noted as a side-effect, whereas others have been used deliberately to keep bees away from potentially harmful insecticides or poisons intended for control of pests. These are summarised in Section 4.3.3.2 below.

Both strategies could be of use where there some GM material can be tolerated, e.g. for honey which at present does not require a GM label in New Zealand, Australian, EU, North American and Asian markets. They would not be suitable for situations where there is "zero tolerance" for GM material in bee products.

4.3.3.1 Bee attractants and other methods to maximise foraging on a crop

Spraying crops with sugar syrup in order to increase bee visits, pollination and fruit set has produced mixed results. Goodwin (1997) reviewed this use of sugar syrup and concluded that it was an unreliable method but potentially useful if further research could improve reliability.

A number of commercial products based on sugar syrup (e.g. Beeline, Bee-Q and BeeLure) are sold for spraying on crops to improve pollination. These also have mixed success and there are many reports of their failure to increase bee visits or seed set (e.g. Burgett and Fisher, 1979; Belletti and Zani, 1981; Rajotte and Fell, 1982; Margalith et al., 1984; Singh and Sinha, 1996; Ambrose et al., 1995).

Other commercial products for this purpose are based bee pheromones (e.g. BeeHere, Bee Scent and QMP). Success has been reported with their use on raspberries (Neira et al., 1997), strawberries (Butts, 1991), cranberry (MacKenzie and Averill, 1992), apple, cherry, pear and plum (Mayer et al., 1989). However, failure has been reported with some crops, such as apricots (McLaren et al., 1992), kiwifruit (Tsirakoglou et al., 1997), watermelon and cucumber (Ambrose et al., 1995).

A second use of sugar syrup is to feed syrup scented with the flowers of the target crops to bees, with a view to recruiting more bees to forage on the crop. Goodwin (1997) also reviewed this technique. He reported that it had mixed success and had received little attention over the last 30 years.

A third approach to improving crop pollination is to increase the number of pollen gatherers in a colony by feeding unscented sugar syrup within the hive (Goodwin, 1997). Increases in pollen collection using this method have been reported for a number of crop plants, although actual improvements in their pollination have not been assessed. Syrup feeding may also increase pollen collection from plants other than the target crop, so this method may have limited use in directing bees to forage only on a particular crop within their flight range.

Finally, breeding crops with increased levels of honey bee attractive floral volatiles (e.g. linalool) has been suggested as a method for increasing bee visits to crops such as alfalfa (Henning et al., 1992).

4.3.3.2 Bee repellents and other methods to prevent bee visits to a crop

Trap crops (non-GM borders grown around a GM crop) are used to minimise pollen flow via insects and wind from a GM crop. These take advantage of the fact that pollen dispersal has a highly leptokurtic distribution (i.e. pollen levels decrease dramatically within metres of the crop and then remain at very low levels over a far greater distance) (Williams, 2001). While this may help to reduce concerns about cross-pollination, its impact on GM pollen presence in bee products is not known. Williams (2001) also suggests that planting a surrounding trap crop of preferred bee forage may have potential as a means of reducing bee visits to a GM crop.

Pollen traps fitted to hives have been tried, but found to be unreliable, as a means of reducing the amounts of insecticide-treated pollen entering a hive from sprayed crops nearby (Erickson et al., 1997). Such traps may be of some use as a means of excluding GM pollen, but this has not yet been investigated.

A number of chemicals have been identified as honey bee repellents. Atkins et al. (1975) reported 42% and 69% bee repellence from flowering crops sprayed with ethyl hexanediol and decylamine, respectively.

Some pesticides appear to have bee repellent properties. For example, some pyrethroid insecticides have been shown to repel bees and this is thought to explain why this chemical causes less mortality in the field than would be expected from laboratory-based toxicity tests (Rieth and Levin, 1987; 1988; de Wael and van Laere, 1987). Fries (1985) noted that cypermethrin reduced oilseed rape pollen collection by honey bees. Orthene sprayed on pre-flowering raspberries resulted in a failure of pollination by honey bees (M. Goodwin, pers. comm.). The fungicide captan, although not toxic to honey bees, repels them if applied to flowering plants (van Praagh and von der Ohe, 1982).

Other compounds have been tested for their ability to repel bees, but not other insects, from insecticides or other poisons sprayed on crops or used in baits. The honeybee pheromone 2-heptanone was tested for this purpose but was found to be impractical and not sufficiently reliable (Reith et al., 1986). Goodwin and Ten Houten (1991) had better success with blackstrap molasses added to 1080/jam baits used to kill possums. They identified oxalic acid as the bee-repellent component of the molasses. However, the use of oxalic acid on flowering crops has not been tested and the possibility of phytotoxicity has not been discounted (M. Goodwin, pers. comm.).

4.3.4 Biotechnological solutions

There are a number of biotechnological approaches which may help to reduce GM material in bee products. Most are being developed in order to minimise cross-pollination (and thus gene flow from GM crops) and some are aimed at improving crop yield or reducing pollen allergenicity problems by eliminating flowering.

Promoters that direct transgene expression to tissues other than pollen or the nectaries could be used to minimise the presence of novel proteins in pollen and nectar. For example, leaf- or root-specific promoters are being developed (e.g. Santamaria et al., 2001; Imura et al., 2001), especially for transgenes encoding insecticidal proteins, so that the proteins occur where pest insects feed and not where beneficial insects such as bees do. However, with this method the transgene will still be present in pollen and will thus continue to represent a potential source of GM material for bee products. The following discussion will focus on methods which may eliminate transgene DNA from pollen.

4.3.4.1 Modification of chloroplast DNA

Commercially available GM plants have been modified via the insertion of a transgene into the plant's nuclear genome, so that every plant cell with a nucleus will contain the new DNA. However, chloroplasts, like some other organelles, contain their own DNA, separate from that contained within the nucleus. This is known as the chloroplast genome. It is possible to insert a transgene into the chloroplast genome so that only plant tissues composed of chloroplast-containing cells will carry the transgene and have the ability to express the novel protein it encodes (e.g. Daniell et al., 1998). Since the leaves, shoots and stems of plants are often the desired sites for expression of new traits (e.g. pest or disease resistance or altered nutritional properties), this method has potential for conferring such traits while avoiding the difficulties that the transgene's presence in pollen may pose (e.g. Lutz et al., 2001; DeGray et al., 2001).

In most flowering plants the chloroplast genome is absent from pollen. Because of this, chloroplast DNA sequences are used to study maternal inheritance in many plants (e.g. Balfourier et al., 2000). However, the conifers are a well-known exception to this and chloroplast genome sequences have been used for paternity analysis in *Pinus radiata* in New Zealand (Kent and Richardson, 1997). Paternal transmission of chloroplasts is also known in carrots (Moyes and Dale, 1999), and on some occasions in some other angiosperms, e.g. runner beans, peas, potatoes, meadow grass (Moyes and Dale, 1999), tobacco, lucerne (Stewart and Prakash, 1998; Daniell et al., 1998) and pelargonium (James et al., 2001). Thus the effectiveness of this method for eliminating GM material from the pollen of GM plants will depend on the plant concerned.

Flowering GM crop species visited by honey bees may be suitable candidates for this method. Scott and Wilkinson (1999) studied rates of maternal inheritance of chloroplast DNA in oilseed rape and concluded that there will be no or negligible pollen-mediated chloroplast-transgene dispersal from this crop. McKinnon et al. (2001) drew a similar conclusion from a study of eucalyptus.

4.3.4.2 GM plants without pollen

Male sterility is used in conventional hybrid plant breeding to control pollination and many crops have natural male sterility systems that can be exploited (Christey and Woodfield, 2001). It can also be introduced into crop plants via genetic modification.

Several different strategies are being investigated, but one of the best known is the barnase/barstar system. With this, a bacterial gene encoding a cytotoxic enzyme, barnase (*Bacillus amyloliquefaciens* RNase) is placed on a tapetum- or pollen-specific promoter so that it is expressed only in the anthers during pollen grain formation. Because it is cytotoxic, barnase disrupts this process so that the plants produce either no pollen, deformed or inviable pollen. Plants can also be modified to carry another gene from the same bacterium called barstar. This encodes a protein which inactivates barnase and blocks its cytotoxic effect. If the barstar gene is driven by an inducible promoter (i.e. one that works only when triggered by the application of a particular chemical), then it becomes possible to switch pollen production back on when desired by spraying with the inducing chemical. A number of GM male-sterile crop plants have now been successfully developed (but apparently not yet commercialised) with the barnase system, e.g. oilseed mustard (Arun et al., 2001), cabbage (Zhu et al., 2001), alfalfa (Rosellini et al., 2001), tobacco (Li et al., 1997), wheat (de Block et al., 1997), soyabean (Guo et al., 1997), poplar (Li et al., 2000) and rice (Zhang et al., 1998).

Obviously male-sterile GM plants completely lacking pollen would not be a source of GM material for honey bees. However, it is not certain whether deformed or inviable pollen would be rejected by foraging honey bees. Bees are known to exhibit preferences among pollen types when presented with a choice, apparently choosing on the basis of odour and physical configuration of the pollen grains (Winston, 1987).

4.3.4.3 GM plants without flowers

GM techniques may also be used to retard or prevent flowering, thus preventing undesirable gene flow from pollen dispersal. There may also be benefits in eliminating flowering from some crops in order to encourage vegetative growth (e.g. forage plant production) and to reduce the production of allergenic pollen (e.g. ryegrass). There are very obvious detrimental implications for honey bees in having non-flowering plants, especially with crops that are important for honey production, such as clover. The loss of flowers even from species that are chiefly wind-pollinated, such as maize, could impact negatively on honey bees that may rely on these plants as a supplementary pollen source. However, if the demand for GM-free bee products is sufficiently high, then the option of non-flowering GM plants may become attractive.

One strategy to prevent flower formation uses the barnase (cytotoxic) gene attached to genes that are expressed only in inflorescences, such as a MADS gene (Lemmetyinen et al., 2001).

There are no reports of the impacts of such plants on honey bees or bee products.

4.3.5 Post-harvest honey treatments

If GM pollen could be removed from honey after harvest, the likelihood of GM material (DNA or protein) occurrence would be greatly reduced or eliminated.

Honey is generally filtered after harvest to remove wax and debris before packaging (comb honey is an obvious exception to this). It is sometimes stated that the filtering of commercial honey reduces the level of pollen to 0.1% or less (Anon P, 2001; Anon Q, undated). This figure accords with most of the reports quantifying pollen content of honey (see Section 2.2), but not all. For example, Eady et al. (1995) reported 100,000 grains per ml in a UK commercial honey (equivalent to about 0.3%) and Molan (1985) gave a maximum pollen concentration of 5 million grains per 10 g of honey (about 1.5%).

In New Zealand, a relatively coarse nylon fabric filter is usually used to filter honey (Matheson, 1997) and this is unlikely to remove all pollen grains, although more sophisticated filtration units that use mesh filters may remove significant quantities of pollen (Bryant, 1987). High pressure filters using a series of paper filters, sometimes with diatomaceous earth added, are available and used in the United States (Tew, 1992). Molan (1998) reported that honey that has been filtered with diatomaceous earth has no pollen left in it.

5. MARKET REACTION TO HONEY FROM COUNTRIES WHERE GM CROPS ARE GROWN

New Zealand beekeepers have expressed concern about the loss of markets for bee products should GM crops be grown in New Zealand and at the Royal Commission on Genetic Modification they called for a moratorium on field releases of GM plants (Anon R, 2001).

Food labelling laws and food standards define acceptable levels of GM material in honey and other bee products intended as food. These levels vary between 1 and 5% from country to country and the organic market apparently requires a complete absence of GM material (see Section 3 for details). Bee products able to comply with these standards should have full access to these markets.

However, consumers will be influenced not only by the legal label but also by other factors that affect their perceptions of a product, such as the country of origin. For example, customers may not be entirely satisfied by the GM food labels in their country and may choose to buy produce from countries known not to grow GM crops. It is not certain how important consumer perceptions of the "GM status" of the country of origin will be in the marketing of bee products, but the experiences of honey producers from countries where GM crops are grown may be instructive.

5.1 Market Reaction to Honey from Canada

GM herbicide-tolerant canola (oilseed rape) has been grown extensively for many years in Canada and at present at least 70% of Canada's canola is GM (H. Clay, pers. comm.). Canola represents a very significant nectar source for Canadian honey producers.

In 1999 some Canadian honey shipments to Germany were banned because traces of GM material were found in a honey sample. Because GM canola is so widely grown and Canada does not require GM food labelling (see Section 3.1 above), Canadian producers could not guarantee that their export product was free of GM canola pollen, even though the Canadian Honey Council pointed out that filtering removed all but 0.1% (w:w) pollen from honey. In their 1998/99 report on the Canadian Honey Situation and Trends, Agriculture and Agri-Food Canada reported that "issues over GMO were a concern for many honey producers who were exporting to certain markets" (Parent and Pearen, 1999). This event was extensively reported and is still quoted in popular articles on GM foods (e.g. Anon P, 2001; Munro, 2002). Agriculture and Agri-Food Canada's most recent advice to Canadian farmers wishing to export to the European Union notes that many Canadian GM varieties of canola are not registered in the EU and so Canadian canola seed cannot be accepted there unless it can be guaranteed GM-free (Anon S, undated). These guidelines mention Germany as an export destination for honey but do not mention any requirement for GM labelling. Heather Clay, of the Canadian Honey Council, states that there was "a temporary drop in sales of Canadian honey to Germany, but the market has since recovered" and that "the large exporters report that it is business as usual in Europe" (H. Clay, pers. comm., 3 June 2002).

5.2 Market Reaction to Honey from Argentina, the United States and Australia.

Argentina is one of the world's biggest exporters of honey (70,363 tonnes in 1997) (Parent and Pearen, 1999; Anon T, 1998). It is also one of the top growers of GM crops, although these are principally soyabeans and maize and not honey-yielding plants (James, 2000; B. Achaval, pers. comm.). Perhaps for this reason there have been no reports of difficulties with exports of honey from Argentina, even to Germany which is its largest export market (Anon T, 1998; B. Achaval, pers. comm.).

In the United States honey is produced primarily for the domestic market; in 1997 it exported only 3,296 tonnes of honey (in comparison, Canada exported 7,407 tonnes in the same year, mostly to the United States and Germany) (Anon T, 1998). Germany, Japan and Yemen are its major markets and there have been no reports of difficulties with these in connection with the GM crops grown extensively in the United States, which include the honey-producing plants, canola and cotton.

GM cotton grown in Australia (150,000 hectares planted in 2000; James, 2000) has apparently not yet posed problems for Australian honey producers (S. Ware; H. Lamb, pers. comm.). GM canola has not yet been released commercially in Australia. Interestingly, the state government of New South Wales, a key canola-producing region, has recently announced plans to introduce legislation which will rule out the establishment of GM-free planting zones (New Zealand Herald, 21 June 2002).

6. POTENTIAL IMPACTS OF GM PLANTS ON BEE HEALTH

Although not directly related to the presence of GM material in bee products, there may be impacts of GM plants on bee health that could affect on the bees' ability to produce honey, pollen and propolis.

There is a growing body of published research on the impacts of GM plants and novel proteins on bee health. This research has recently been reviewed (Malone and Pham-Delègue, 2001; 2002; Pham-Delègue et al., 2002). These reviews are summarised below.

GM plants may have direct or indirect effects on bees. Direct effects are those that arise when a bee ingests a novel protein expressed by a GM plant. Indirect effects may arise if the process of introducing the transgene into the plant results in inadvertent changes to plant phenotype affecting its attractiveness or nutritive value to bees.

6.1 Direct Effects of Novel Proteins on Bees

Potentially, the ingestion of a novel protein expressed in pollen or perhaps occurring in nectar, resin or honeydew from a GM plant may affect bee behaviour, development or survival, or it may have no effect. As pointed out above (Section 2), pollen represents the most likely vehicle for the expression of novel proteins. Adult bees consume significant quantities of pollen during their first week after emergence and they have received the most research attention to date. Bee larvae also ingest pollen, especially during the later instars, but their food is composed in large part of glandular secretions from nurse adult bees.

Many experiments have been conducted in which bees are fed with purified novel proteins at concentrations estimated to approximate or to exceed likely pollen expression levels. Novel proteins with insecticidal properties aimed at making GM plants pest resistant have been the most thoroughly tested. There have also been trials conducted with small colonies of bees and potted flowering GM plants in glasshouses or under mesh in the field. Tests have assessed food consumption by adult bees, adult bee survival, olfactory learning and foraging behaviour in adult bees, larval bee development and survival. Results to date are briefly summarised in Table 3. Supporting references may be found in reviews by Malone and Pham-Delègue.

6.2 Indirect Effects of GM Plants on Bees

Indirect effects of transgenic plants on bees may occur when genetic modification results in an unexpected change in the plant's phenotype. Insertional mutagenesis is one such change. In this case, the random positioning of the transgene in the plant's genome interferes with a gene or suite of genes needed for a "normal" phenotype. For example, an insertional mutagenesis event that resulted in plants without flowers would have a definite negative impact on bees. Less obvious changes, such as alterations in nectar quality or volume would be harder, but not impossible, to detect. Effects due to insertional mutagenesis will vary among different lines of plants derived from separate transformation events and can easily be eliminated by line

selection. Pleiotropic effects represent a second type of inadvertent phenotypic change. In this case, it is not the position of the transgene, but its product, which interferes unexpectedly with a biochemical pathway in the plant to create a phenotypic change. Such changes would occur in all lines of the transgenic plant and could not be remedied by line selection.

Nectar analyses of GM oilseed rape plants have suggested that some modification events may lead to phenotypic changes that could influence bee behaviour (Pham-Delègue et al., 2002). One line of GM oilseed rape expressing a chitinase produced more nectar of higher sugar concentration than the corresponding control line, as did one herbicide-resistant line compared with its control.

6.3 Current and Future Research

There are a number of research teams continuing to investigate the effects of GM plants on honey bees. In New Zealand, HortResearch in collaboration with AgResearch has a programme of work looking at non-target effects of GMOs, including effects on honey bees. Current research aims to determine whether feeding young adult bees with a Bt toxin, a protease inhibitor or a biotin-binding protein affects their ability to develop hypopharyngeal glands. (These glands are important because they secrete food for larval bees.) Similar work is being conducted with Bt-corn pollen and bees in Switzerland (J. Romeis, pers. comm.). In France, the effects of Bt-corn on bee larvae are being determined and an assay of bee defensive behaviour (stinging) is being developed (M. Pham-Delègue, pers. comm.). In Denmark, the effects of protease inhibitors on bee larvae are being assessed (H. Brødsgaard, pers. comm.). In Canada, the effects of Bt-sweetcorn pollen on bees are being determined and work on impacts of GM plants on bumblebees and wild bees is planned (C. Scott-Dupree and M. Winston, pers. comm.).

In New Zealand and overseas, many molecular biologists developing GM plants are moving their emphasis away from "input traits", such as herbicide tolerance, insect resistance, disease resistance or drought tolerance, and towards "output traits", such as altered nutritional qualities, improved processing traits, altered flowering and plant form and the production of valuable proteins in GM plants ("biopharming") (Christey and Woodfield, 2001). The potential effects of these new traits on bees will need to be tested. For example, altering the nutritional qualities of plants could well affect the attractiveness of pollen to honey bees, since lipid profiles have been shown to be important in determining the phagostimulatory and antibacterial properties of some pollens (Singh et al., 1999; Manning, 2001).

REFERENCES

Ambrose, J.T.; Schultheis, H.R.; Bambara, S.B.; Mangum, W. 1995. An evaluation of selected commercial bee attractants in the pollination of cucumbers and watermelons. *American Bee Journal, 135(4)*: 267-272.

Anon, A. 1998. MAFF Project No. 2B 067. Honey from genetically modified plants: integrity of DNA, & entry of GM-derived proteins into the food chain via honey. *Laboratory of the Government Chemist.*

Anon, B. 2000. GM Contamination of Honey. Available at http://www.greenpeace.se/np/s/NPS_pdf/Contaminationofhoney.pdf .

Anon, C. 2000. Press Release: GM Trials Threaten UK Honey. Available at http://www.foe.co.uk/pubsinfo/infoteam/pressrel/2000/20000516185312.html .

Anon, D. 2001. Australia New Zealand Food Authority. User Guide – Labelling Genetically Modified Food. Available at http://www.anzfa.gov.au/foodstandards/userguides/labellinggeneticallymodified fooduserguide/index.cfm .

Anon, E. 2001. Food Standards Agency – GM Labelling. Available at http://www.food.gov.uk .

Anon, F. 2002. The Press Democrat. 10 June 2002. Future of genetically modified corn in question given recent decision. Available at http://www.prnewswire.com .

Anon, G. 2002. Activists oppose promotion of genetically modified products from U.S. *Korea Times*. January 25, 2002. Available at http://www.connectotel.com/gmfood/ .

Anon, H. 2002. China implements its new regulations on genetically modified foods. March 21, 2002. *CropChoice news*. Available at http://www.connectotel.com/gmfood/ .

Anon, I. 2001. Guidance for Industry. Voluntary labeling indicating whether foods have or have not been developed using Bioengineering. Available at http://www.cfsan.fda.gov/~dms/biolabgu.html .

Anon, J. 2001. Health Canada – The safety of genetically modified food crops. Available at http://www.hc-sc.gc.ca/english/media/releases/2001/2001_13ebk5.htm .

Anon, K. 2002. The campaign to label genetically engineered foods. Labelling legislation introduced in Congress! Available at http://www.thecampaign.org/ .

Anon, L. 2000. UKROFS standards for organic food production. Standards for organic livestock and organic livestock products. Available at http://www.defra.gov.uk .

Anon, M. Undated. Organic Crop Improvement Association. Specialty crop certification standards. Available at http://www.honeycouncil.ca/organic.html .

Anon, N. 2001. BIO-GRO New Zealand Organic Standards. Module 4.6 honey and bee products production standard. Available at http://www.bio-gro.co.nz .

Anon, O. 1999. Organic honey standard – European Union. Available at http://www.beekeeping.org/databases/eu_organic_honey_standard.htm .

Anon, P. 2001. Canadian honey banned in Europe. *Ontario Farmer*. 3 April 2001. Available at http://207.229.10.88/ontariofarmer/pages/Info_active/farm_general/farm_gen.html#article2 .

Anon, Q. Undated. Genetically modified organisms (GMO'S). Available on request from ahbic@honeybee.org.au .

Anon, R. 2001. Report of the Royal Commission on Genetic Modification. Available at http://www.gmcommission.govt.nz/RCGM/index.html .

Anon, S. Undated. Guide to the European Union's Regulatory Requirements Facing Canadian Agri-Food Exports. Available at http://ats-sea.agr.ca/info/europe/e2878000.htm .

Anon, T. 1998. Honey Exports. Available at www.fas.usda.gov .

Anon, U. 2000. Bt Plant-Pesticides Biopesticides Registration Action Document, United States Environmental Protection Agency. Available at http://www.epa.gov./scipoly/sap/2000/october/brad_2_scienceassessment.pdf .

Anon, V. 2001. Bt Plant-Incorporated Protectants September 29, 2001 Biopesticides Registration Action Document, United States Environmental Protection Agency. Available at http://www.epa.gov/oppbppd1/biopesticides/otherdocs/bt_brad/Bt_BRAD_II.pdf .

Arun, J.; Panchali, B.; Arumugam, N.; Vibha, G.; Burma, P.K.; Deepak, P.; Jagannath, A.; Bandyopadhyay, P.; Gupta, V.; Pental, D. 2001. The use of a spacre DNA fragment insulates the tissue-specific expression of a cytotoxic gene (barnase) and allows high-frequency generation of transgenic male sterile lines in Brassica juncea L. *Molecular Breeding 8(1)*: 11-23.

Atkins, E.L.; Macdonald, R.L.; Greywood-Hale, E.A. 1975. Repellent additives to reduce pesticide hazards to honey bees: field tests. *Environmental Entomology, 4(2)*: 207-210.

Baker H.G.; Baker I. 1977. Intraspecific constancy of floral nectar amino acid complements. *Botanical Gazette 138*: 183-191.

Balfourier, F.; Imbert, C.; Charmet, G. 2000. Evidence for phylogeographic structure in *Lolium* species related to the spread of agriculture in Europe. A cpDNA study. *Theoretical and Applied Genetics 101(1-2)*: 131-138.

Belletti, A.; Zani, A. 1981. A bee attractant for carrots grown for seed. *Sementi Elette, 27(5)*: 23-27.

Bonadé Bottino, M.; Girard, C.; Le Métayer, M.; Picard-Nizou, A.L.; Sandoz, G.; Lerin, J.; Pham-Delègue, M.H.; Jouanin, L. 1998. Effects of transgenic oilseed rape expressing proteinase inhibitors on pest and beneficial insects. *Proceedings of the International Symposium on Brassicas, Acta Horticulturae 459*: 235-239.

Bourn, D.; Newton, B.; Campbell, H. 1999. Strategies for 'Greening' the New Zealand Honey Industry. An evaluation of the Development of Organic and Other Standards. *Studies of Rural Sustainability Research Report No. 8.* Department of Anthropology, University of Otago.

Brødsgaard et al. 2001. Environmental risk assessment of transgenic plants using honey bee larvae. *Abstracts of the XXXVII International Apicultural Congress 28 October – 1 November 2001. Durban, South Africa. Apimondia 2001.* Produced by Document Transformation Technologies.

Burgett, M.; Fisher, G.C. 1979. An evaluation of Beeline as a pollinator attractant on red clover. *American Bee Journal, 119(5)*: 356-357.

Bryant, T. 1987. Honey filtration unit. *New Zealand Beekeeper 196*: 25-26.

Butts, K.M. 1991. Bee attractants: improving strawberry quality? *Citrus and Vegetable Magazine, 55(3,12)*: 16-17.

Carter, C.; Graham, R.A.; Thornburg, R.W. 1999. Nectarin I is a novel, soluble germin-like protein expressed in the nectar of *Nicotiana* sp. *Plant Molecular Biology, 41*: 207-216.

Christeller, J.T.; Farley, P.C.; Ramsay, R.J.; Sullivan, P.A.; Laing, W.A. 1998. Purification, characterization and cloning of an aspartic proteinase inhibitor from squash phloem exudate. *European Journal of Biochemistry 254(1)*: 160-167.

Christey, M.; Woodfield, D. 2001. Coexistence of genetically modified and non-genetically modified crops. Report prepared for the Ministry for the Environment. *Crop & Food Research Confidential Report No. 427.*

Crane, E.; Walker, P. 1984. Pollination directory for world crops. *International Bee Research Association, London.*

Daniell, H.; Datta, R.; Varma, S.; Gray, S.; Lee, S.B. 1998. Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nature Biotechnology 16(4)*: 345-348.

Daniell, H.; Varma, S.; Stewart, C.N.Jr.; Prakash, C.S. 1998. Chloroplast-transgenic plants: panacea – no! Gene containment – yes! *Nature Biotechnology, 16(7)*: 602.

De Block, M.; Debrouwer, D.; Moens, T. 1997. The development of a nuclear male sterility system in wheat. Expression of the barnase gene under the control of tapetum specific promoters. *Theoretical and Applied Genetics 95(1-2)*: 125-131.

De Gray, G.; Rajasekaran, K.; Smith, F.; Sanford, J.; Daniell, H. 2001. Expression of an antimicrobial peptide via the chloroplast genome to control phytopathogenic bacteria and fungi. *Plant Physiology 127*: 852-862.

De Wael, L.; van Laere, O. 1989. Toxicity and the repellent activity of synthetic pyrethroids towards the honeybee (*Apis mellifera* L.). *Proceedings of the XXXIst International Congress of Apiculture, Warsaw, Poland, August 19-25, 1987.* 209-216. Published by Bucharest, Apimondia Publishing House, Romania.

Diamand, E. 1999. Briefing. Bees, honey and genetically modified crops. Available at http://www.foe.co.uk/resource/briefings/bees_honey_gm_crops.html .

Eady, C.; Twell, D.; Lindsey, K. 1995. Pollen viability and transgene expression following storage in honey. *Transgenic Research 4*: 226-231.

Emberlin, J.; Adams-Groom, B.; Tidmarsh, J. 1999. A report on the dispersal of maize pollen. Available at http://www.soilassociation.org/sa/saweb.nsf/a71fa2b6e2b6d3e980256a6c004542b4/8025 .

Emberlin, J.; Brooks, S. 2001. Pollen movement from a genetically modified oilseed rape crop. Available on request from info@foe.co.uk .

Erickson, E.H.; Erickson, B.H.; Flottum, P.K.; Wyman, J.A.; Wedberg, J.L.; Page, R.E. 1997. Effects of selected insecticide formulations, phased application and colony management strategies on honey bee mortality in processing sweetcorn. *Journal of Apicultural Research 36(1)*: 3-13.

Fearing, P.L.; Brown, D.; Vlachos, D.; Meghji, M.; Privalle, L. 1997. Quantitative analysis of CryIA(b) expression in Bt maize plants, tissues, and silage and stability of expression over successive generations. *Molecular Breeding, 3(3)*: 169-176.

Fries, I. 1985. Honeybees and cypermethrin/deltamethrin in fields of flowering seed rape. *Vaxtskyddsrapporter 32*: 169-178.

Gary, N.E. 1992. Activities and behavior of honey bees. Edited by Graham, J.M. *in:* The Hive and the Honey Bee. Published by Dadant & Sons. 8, 269-372.

Goodwin, M.; Van Eaton, C. 1999. Elimination of American Foulbrood without the use of Drugs. A practical manual for Beekeepers. Published by the National Beekeepers Association of New Zealand, Inc.

Goodwin, R.M. 1997. Feeding sugar syrup to honey bee colonies to improve pollination: a review. *Bee World 78(2)*: 56-62.

Goodwin, R.M.; Ten, H.A. 1991. Poisoning of honey bees (*Apis mellifera*) by sodium flouroacetate (1080) in baits. *New Zealand Journal of Zoology, 18(1)*: 45-51.

Gray, Q. 2002. Saudi Arabia Biotechnology Detailed Information on Saudi Arabian GMF Testing. Available at http://www.fas.usda.gov/gainfiles/200203/135683674.pdf .

Greenplate, J. 1997. Response to reports of early damage in 1996 commercial Bt transgenic cotton (Bollgard™) plantings. *Society for Invertebrate Pathology Newsletter 29* 15-18.

Guo, Z.B.; Lin, J.X. 1997. Genetic engineering of male sterile and fertility restorer in soybean. *Soybean Genetics Newsletter 24*: 50.

Hagedorn, C. 1997. Commercial status of transgenic crops and microorganisms. Available at www.ext.vt.edu/news/0021/cals/cses/chagedor/crplist.html

Hellmich, R.L.; Siegried, B.D.; Sears, M.K.; Stanley-Horn, D.E.; Daniels, M.J.; Mattila, H.R.; Spencer, T.; Bidne, K.G.; Lewis, L.C. 2001. Monarch larvae sensitivity to *Bacillus thuringiensis* purified proteins and pollen. *Proceedings of the National Academy of Sciences USA 98 (21)*: 11925-11930.

Henning, J.A.; Peng, Y.S.; Montague, M.A.; Teuber, L.R. 1992. Honey bee (Hymenoptera: Apidae) behavioral response to primary alfalfa (Rosales: Fabaceae) floral volatiles. *Journal of Economic Entomology, Entomological Society of America 85(1)*: 233-239.

Herbert, E.W.Jr. 1992. Honey bee nutrition. Edited by Graham, J.M. *in*: The Hive and the Honey Bee. Published by Dadant & Sons.

Imura, Y.; Seki, H.; Toyoda, K.; Ichinose, Y.; Shiraishi, T.; Yamada, T. 2001. Contrary operations of Box-I element of pea phenylalanine ammonia-lyase gene 1 promoter for organ-specific expression. *Plant Physiology and Biochemistry 39(5)*: 355-362.

James, C. 2000. ISAAA Briefs No. 21 – 2000 preview. Global status of commercialised transgenic crops: 2000. Available at http://www.isaaa.org/publications/briefs/Brief_21.htm .

James, C.M.; Barrett, J.A.; Russell, S.J.; Gibby, M. 2001. A rapid PCR based method to establish the potential for paternal inheritance of chloroplasts in *Pelargonium*. *Plant Molecular Biology Reporter 19(2)*: 163-167.

Jesse, L.C.H.; Obrycki, J.J. 2000. Field deposition of Bt transgenic corn pollen: Lethal effects on the monarch butterfly. *Oecologia*. Available at athene.em.springer.de.

Jouanin, L.; Girard, C.; Bonadé-Bottino, M.; Le Metayer, M.; Picard Nizou, A.; Lerin, J.; Pham-Delègue, M. 1998. Impact of oilseed rape expressing proteinase inhibitors on coleopteran pests and honeybees. *Cahiers Agriculture 7*: 531-536.

Kent, J.; Richardson, T.E. 1997. Fluorescently labelled, multiplexed chloroplast microsatellites for high throughput paternity analysis in *Pinus radiata*. *New Zealand Journal of Forestry Science 27(3)*: 305-312.

Knee, E.M.; Gong, F.C.; Gao, M.S.; Teplitski, M.; Jones, A.R.; Foxworthy, A.; Mort, A.J.; Bauer, W.D. 2001. Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. *Molecular Plant Microbe Interactions 14(6)*: 775-784.

Kozeil, M.G.; Beland, G.L.; Bowman, C.; Carozzi, N.B.; Crenshaw, R.; Crossland, L.; Dawson, J.; Desai, N.; Hill, M.; Kadwell, S.; Launis, K.; Lewis, K.; Maddox, D.; McPherson, K.; Meghji, M.R.; Merlin, E.; Rhodes, R.; Warren, G.; Wright, M.; Evola, S.V. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*, *Bio/Technology 11*: 194-200.

Kruger, C.; Hell, R.; Stephan, U.W.; Horst, W.J. (ed.); Schenk, M.K. (ed.); Burkert, A. (ed.); Claassen, N. (ed.); Flessa, H. (ed.); Frommer, W.B. (ed.); Goldbach, H. (ed.); Olfs, H.W. (ed.); Romheld, V. et al. 2001. A metal-binding LEA protein trafficks micronutrients in the phloem of *Ricinus communis* L. Plant nutrition: food security and sustainability of agro ecosystems through basic and applied research. *Fourteenth International Plant Nutrition Colloquium, Hannover, Germany.* Published by Kluwer Academic Publishers; Dordrecht, Netherlands. 194-195.

Lemmetyinen, J.; Pennanen, T.; Lannenpaa, M.; Sopanen, T. 2001. Prevention of flower formation in dicotyledons. *Molecular Breeding 7(4)*: 341-350.

Li, S.G.; Liu, Y.L.; Zhu, F.; Luo, Y.Y. 1997. Genetically-engineered male sterile tobacco plants and their sensitivity to temperature. *Acta Botanica Sinica 39(3)*: 231-235.

Li, L.; Qi, L.W.; Han, Y.F.; Wang, Y.C.; Li, W.B. 2000. A study on the introduction of male sterility of antiinsect transgenic *Populus nigra* by the TA29-Barnase gene. *Scientia Silvae Sinicae 36(1)*: 28-32.

Lin, H.Y.; Chiueh, L.C.; Shih, Y.C. 2000. Detection of genetically modified soybeans and maize by the polymerase chain reaction method. *Journal of Food and Drug Analysis 8(3)*: 200-207.

Losey, J.E.; Rayor, L.S.; Carter, M.E. 1999. Transgenic pollen harms monarch larvae. *Nature, 399*: 214.

Lutz, K.A.; Knapp, J.E.; Maliga, P. 2001. Expression of bar in the plastid genome confers herbicide resistance. *Plant Physiology 125*: 1585-1590.

MacKenzie, K.E.; Averill, A.L. 1992. A new honey bee attractant, the queen mandibular pheromone. *Cranberries, 56*: 13-14.

Malone, L.A.; Pham-Delègue, M.H. 2001. Effects of transgene products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.). *Apidologie 32*: 287-304.

Malone, L.A.; Pham-Delègue, M.H. 2002. Using proteins to assess the potential impacts of genetically modified plants on honey bees. Edited by Devillers, J.; Pham-Delègue, M.H. *in:* Honey Bees: estimating the environmental impact of chemicals. Published by Taylor & Francis. 14, 290-311.

Malone, L.A.; Tregidga, E.L.; Todd, J.H.; Burgess, E.P.J.; Philip, B.A.; Markwick, N.P.; Poulton, J.; Christeller, J.T.; Lester, M.T.; Gatehouse, H.S. Effects of ingestion of a biotin-binding protein on adult and larval honey bees. *Apidologie*: in press.

Manning, R. 2001. Fatty acids in pollen: a review of their importance for honey bees. *Bee World 82(2)*: 60-75.

Margalith, R.; Lensky, Y.; Rabinowitch, H.D.; Rapp, R. 1984. An evaluation of Beeline as a honeybee attractant to cucumbers and its effect on hybrid seed production. *Journal of Apicultural Research, 23(1)*: 50-54.

Matheson, A. 1997. Practical Beekeeping in New Zealand. Published by GP Publications.

Mayer, D.F.; Britt, R.L.; Lunden, J.D. 1989. Evaluation of BeeScent as a honey bee attractant. *American Bee Journal, 129(1)*: 41-42.

McKinnon, G.E.; Vaillancourt, R.E.; Tilyard, P.A.; Potts, B.M. 2001. Maternal inheritance of the chloroplast genome in *Eucalyptus globulus* and interspecific hybrids. *Genome 44(5)*: 831-835.

McLaren, G.F.; Fraser, J.A.; Grant, J.E. 1992. Pollination of apricots. *Orchardist of New Zealand, 65(8,20)*: 22-23.

Menzies, A.R.; Osman, M.E.; Malik, A.A.; Baldwin, T.C. 1996. A comparison of the physicochemical and immunological properties of the plant gum exudates of *Acacia senegal* (gum arabic) and *Acacia seyal* (gum tahla). *Food Additives and Contaminants 13(8)*: 991-999.

Moar, N.T. 1985. Pollen analysis of New Zealand honey. *New Zealand Journal of Agricultural Research, 28*: 39-70.

Molan, P. 1998. The limitations of the methods of identifying the floral source of honeys. *Bee World 79(2)*: 59-68.

Moyes, C.L.; Dale, P.J. 1999. MAFF Research Project OF0157. Organic farming and gene transfer from genetically modified crops. Available at http://www.gmissues.org/orgreport.htm .

Munro, M. 2002. Genetic threats blowin' in the wind: scientists warm modified crops are 'escaping and going rogues' (June 7 *National Post*). Available on request from WansbroughD@maf.govt.nz .

Neira, M.C.; Lobos, N.S.; Riveros, M.G.; Carrillo, R.L.; Pessot, R.Z.; Mundaca, N.B. 1997. Insect fauna associated with flowers of raspberries (*Rubus idaeus* L., cv Meeker) and evaluation of the pollination activity of *Apis mellifera* L. under the influence of a pheromonal attractant. *Revista Chilena de Entomologia, 24*: 37-44.

Oparka, K.J.; Santa-Cruz, S. 2000. The great escape: phloem transport and unloading of macromolecules. *Annual Review of Plant Physiology and Plant Molecular Biology 51:* 323-347.

Owens, R.A.; Blackburn, M.; Ding, B. 2001. Possible involvement of the phloem lectin in long-distance viroid movement. *Molecular Plant Microbe Interactions 14(7)*: 905-909.

Parent, G.; Pearen, D. 1999. 1998/99 Canadian honey situation and trends. Available at http://www.agr.gc.ca/misb/hort/honey.html .

Peumans, W.J.; Smeets, K.; van Nerum, K.; van Leuven, F.; van Damme, E.J.M. 1997. Lectin and alliinase are the predominant proteins in nectar from leek (*Allium porrum* L.) flowers. *Planta 201*: 298-302.

Pham-Delègue, M.H.; Jouanin, L.; Sandoz, J.C. 2002. Direct and indirect effects of genetically modified plants on the honey bee. Edited by Devillers, J.; Pham-Delègue, M.H. *in:* Honey Bees: estimating the environmental impact of chemicals. Published by Taylor & Francis. 15, 312-326.

Rahbé, Y.; Sauvion, N.; Febvay, G.; Peumans, W.J.; Gatehouse, A.M.R. 1995. Toxicity of lectins and processing of ingested proteins in the pea aphid *Acrythosiphon pisum. Entomologia Experimentalis et Applicata 76*: 143-155.

Rajotte, E.G.; Fell, R.D. 1982. A commercial bee attractant ineffective in enhancing apple pollination. *Hortscience 17(2)*: 230-231.

Rieth, J.P. 1986. The repellent effect of pyrethroid insecticides on honey bees. PhD dissertation, University of Arizona, USA. X + 100 pp.

Rieth, J.P.; Levin, M.D. 1987. The pyrethroid insecticide hazard to honey bees. *American Bee Journal, 127(11)*: 789-790.

Rieth, J.P.; Levin, M.D. 1988. The repellent effect of two pyrethroid insecticides on the honey bee. *Physiological Entomology, 13(2)*: 213-218.

Rooke, L.; Byrne, D.; Salgueiro, S. 2000. Marker gene expression driven by the maize ubiquitin promoter in transgenic wheat. *Annals of Applied Biology 136(2):* 167-172.

Rosell, R.C.; Torres, J.I.; Brown, J.K. 1999. Tracing the geminivirus-whitefly transmission pathway by polymerase chain reaction in whitefly extracts, saliva, haemolymph, and honeydew. *Phytopathology 89(3)*: 239-246.

Rosellini, D.; Pezzotti, M.; Veronesi, F. 2001. Characterization of transgenic male sterility in alfalfa. *Euphytica 118(3)*: 313-319.

Salvucci, M.E.; Rosell, R.C.; Brown, J.K. 1998. Uptake and metabolism of leaf proteins by the silverleaf whitefly. *Archives of Insect Biochemistry and Physiology, 39*: 155-165.

Sandström, J.P.; Moran, N.A. 2001. Amino acid budgets in three aphid species using the same host plant. *Physiological Entomology 26*: 202-211.

Santamaria, M.; Thomson, C.J.; Read, N.D.; Loake, G.J. 2001. The promoter of a basic PR1-like gene, AtPRB1, from *Arabidopsis* establishes an organ-specific expression pattern and responsiveness to ethylene and methyl jasmonate. *Plant Molecular Biology 47(5)*: 641-652.

Schobert, C.; Gottschalk, M.; Kovar, D.R.; Staiger, C.J.; Yoo, B.C.; Lucas, W.J. 2000. Characterization of *Ricinus communis* phloem profilin, RcPRO1. *Plant Molecular Biology 42(5)*: 719-730.

Scott, S.E.; Wilkinson, M.J. 1999. Low probability of chloroplast movement from oilseed rape (*Brassica napus*) into wild *Brassica rapa*. *Nature Biotechnology 17(4)*: 390-393.

Siciliano, S.D.; Goldie, H.; Germida, J.J. 1998. Enzymatic activity in root exudates of Dahurian wild rye (*Elymus dauricus*) that degrades 2-cholorbenzoic acid. *Journal of Agricultural and Food Chemistry 46(1)*: 5-7.

Singh, P.B.; Sinha, S.N. 1997. Effect of Bee-Q on honeybee visit and seed yield of hybrid sunflower. *Seed Research, 24(2)*: 151-153.

Singh, S.; Saini, K.; Jain, K.L. 1999. Quantitative comparison of lipids in some pollens and their phagostimulatory effects in honey bees. *Journal of Apicultural Research 38(1-2)*: 87-92.

Stanley, R.G.; Linskens, H.F. 1974. Pollen, Biology Biochemistry Management, Springer-Verlag, Berlin, Heidelberg, New York.

Stanley-Horn, D.E.; Dively, G.P.; Hellmich, R.L.; Mattila, H.R.; Sears, M.K.; Rose, R.; Jesse, L.C.H.; Losey, J.E.; Obrycki, J.J.; Lewis, L. Assessing the impact of

Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proceedings of the National Academy of Sciences USA 98 (21)*: 11931-11936.

Stewart, C.N. Jr.; Prakash, C.S. 1998. Chloroplast-transgenic plants are not a gene flow panacea. *Nature Biotechnology, 16(5)*: 401.

Takada, A. 1999. Japan to tighten GMO approval procedures (*Reuters*, 14 December 1999). Available at http://www.connectotel.com/gmfood/ .

Tew, J.E. 1992. Honey and Wax – a consideration of production, processing and packaging techniques. Edited by Graham, J.M. *in*: The Hive and the Honey Bee. Published by Dadant & Sons. 15, 657-704.

Treu, R.; Emberlin, J. 2000. Pollen dispersal in the crops Maize (*Zea mays*), Oil seed rape (*Brassica napus* ssp *oleifera*), Potatoes (*Solanum tuberosum*), Sugar beet (*Beta vulgaris* ssp. *vulgaris*) and Wheat (*Triticum aestivum*). Available at www.soilassociation.org .

Tsirakoglou, V.; Thrasyvoulou, A.; Hatjina, F.; Sfakiotakis, E. (ed); Porlingis, J. 1997. Techniques to increase the attractiveness of kiwi flowers to honey bees. Proceedings of the third international symposium on kiwifruit, Thessaloniki, Greece, 19-22 September 1995. *Acta Horticulturae, 444*: 439-443.

Tuha, M.; Simuth, J. 1991. Some properties of water-soluble proteins of propolis. *Farmaceuticky-Obzor 60(12)*: 539-542.

Van Praagh, J.P.; von der Ohe, W. 1982. The possibility of masking the repellent effect of plant protection compounds not dangerous to bees. *Gartenbauwissenschaft 47(3)*: 114-115.

Wehrmann, A.; van Vliet, A.; Opsomer, C.; Botterman, J.; Schulz, A. 1996. The similarities of bar and pat gene products make them equally applicable for plant engineers. *Nature Biotechnology 14(10)*: 1274-1278.

Wilkinson, J.E.; Twell, D.; Lindsay, K. !997. Activities of CaMV 35S and *nos* promoters in pollen: implications for field release of transgenic plants. *Journal of Experimental Botany 48 (307)*: 265-275.

Williams, I.H. 2001. Bee-mediated pollen and gene flow from GM plants *in:* Benedek, P.; Richards, K.W. (eds). Proceedings 8th Pollination Symposium. *Acta Hort. 561:* 25-33.

Winston, M.L. 1987. The biology of the honey bee. Published by Harvard University Press.

Zhang, X.G.; Liu, R.L.; Kang, L.Y.; Zhu, Y.G.; Tian, P. 1998. Construction of expression vectors of male sterility and its fertility restoration in rice (*Oryza sativa* L.). *Acta Agronomica Sinica 24 (5)*: 629-634.

Zhu, Y.Y.; Gong, J.; Wu, X.G., Yang, H.J.; Shen, G.Z.; Wang, X.Q.; Yin, L.Q.; Lu, G.H.; Wang, J.A.; Wan, X.S. 2001. Preliminary study on flower characteristics and segregation of male sterility of transgenic plants with TA29-Barnase in cabbage (*Brassica oleracea* var. capitata). *Acta Agriculturae Shanghai 17(1)*: 79-82.

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Table 1.
Expression of novel proteins in pollen of GM plants.

Plant	Novel protein encoded by transgene	Promoter	Expression level of novel protein in pollen	Expression level as % of total soluble protein (estimated) [1]	Reference
Maize	Bt toxin	maize pollen-specific and PEP[2] (leaf-specific) promoters	260 - 418 ng/mg (of total soluble protein)	0.026 – 0.0418	Kozeil et al., 1993
Maize	Bt toxin	CaMV 35S[3]	Nil	0	Kozeil et al., 1993
Cotton	Bt toxin Cry 1Ac	CaMV 35S	0.6µg per g fresh weight	0.00024	Greenplate, 1997
Maize	Bt toxin Cry 1Ab	maize pollen-specific and PEP promoters	1100-2400 ng/g fresh weight	0.00044 – 0.00096	Fearing et al., 1997
Arabidopsis (experimental brassica)	GUS (marker protein producing blue colour)	CaMV 35S	Nil	0	Wilkinson et al., 1997
Arabidopsis	GUS	nopaline synthase (NOS)	Nil	0	Wilkinson et al., 1997
Tobacco	GUS	CaMV 35S	<64.6 pmol 4-MU /min/mg of total protein	N/A	Wilkinson et al., 1997
Tobacco	GUS	NOS	<2561 pmol 4-MU /min/mg of total protein	N/A	Wilkinson et al., 1997
Tobacco	nptII (kanamycin resistance)	NOS	1.39 ng/mg of total protein	0.000139	Anon A, 1998
Oilseed rape	Oryzacystatin I	CaMV 35S	Nil	0	Bonadé Bottino et al., 1998
Oilseed rape	Bowman-Birk trypsin inhibitor	CaMV 35S	Nil	0	Jouanin et al., 1998
Oilseed rape	nptII (kanamycin resistance)	NOS	1.61 ng/mg of total protein	0.000161	Anon A, 1998

Maize (Starlink)	Bt toxin Cry 9C	CaMV 35S	0.24 µg/g fresh weight	0.000096	Anon U, 2000
Maize (Bt 11)	Bt toxin Cry 1Ab	CaMV 35S	< 90 ng/g dry weight	N/A	Anon V, 2001
Maize (MON 810)	Bt toxin Cry 1Ab	CaMV 35S	< 90 ng/g dry weight	N/A	Anon V, 2001
Maize (Event 176)	Bt toxin Cry 1Ab	maize pollen-specific and PEP promoters	< 7.1 µg/g of pollen	< 0.00284	Stanley-Horn et al., 2001)
Cotton	Bt toxin Cry1Ac	CaMV 35S	11 ng /g fresh weight	0.0000044	Anon V, 2001

[1] Values expressed as a proportion of fresh pollen weight in the original reference have been converted using the assumption that fresh pollen is 25% protein.
[2] Phosphoenolpyruvate.
[3] Cauliflower mosaic virus 35S promoter.

Table 2.
NZ crops which could be genetically modified and their relevance to honey bees.

NZ crops for which GM varieties might become available[1]	Role of honey bees in relation to crop[2]	Potential hive products from crop
Forage crops		
Perennial ryegrass	Bees known to collect some grass pollen, but only small amounts	
White clover	Very important nectar source and pollen source to a lesser extent; bees required for seed production	Honey; pollen
Forage brassicas (turnip, swede)	Good nectar source and pollen source to a lesser extent; bees required for seed production	Honey; pollen
Grain and arable crops		
Maize	Bees may collect pollen if no other forage available[3]	
Canola	Good source of nectar and pollen (to a lesser extent); bees improve seed production, but not essential	Honey; pollen
Vegetables		
Potato	Bees may visit, but flowers have no nectar, little pollen	
Onion	Bees required for pollination, although the flowers not attractive to them	
Vegetable brassicas	Good source of nectar and pollen (to a lesser extent); bees required for seed production when crop not self-fertile	Honey; pollen
Pea	None	
Leek	Bees required for pollination	
Garlic	None	
Forestry		
Radiata pine	None	
Spruce	None	
Eucalyptus	Ornamental species are good source of nectar and pollen (to a lesser extent)	Honey; pollen; propolis?
Fruit		
Apples	Source of pollen and nectar (to a lesser extent); bees required for adequate pollination	Pollen; propolis?
Kiwifruit	Pollen source; bees required for pollination	Pollen
Tamarillo	Bees may visit and could collect some pollen; flowers self-fertile and have no nectar	
Flowers		
Cyclamen, Lisianthus,	None	

Orchids, Pelargonium, Petunia, Sandersonia, Rose, Carnation		

[1] Based on current NZ GM research (Christey and Woodfield, 2001) and availability of commercial GM cultivars from overseas.

[2] Crane and Walker, 1984; Matheson, 1997

[3] Treu and Emblin, 2000

Table 3.
Effects of novel proteins and GM plants on bees (see Malone and Pham-Delègue, 2001, for details).

Novel protein or GM plant	Type of experiment	Results
Bt toxins (lepidopteran-active)	Larval survival	Not toxic
	Adult survival (in lab and in colony)	Not toxic
	Adult food consumption	No effect
	Adult flight activity (protein fed to colony)	No effect
Bt toxins (coleopteran-active)	Larval survival	Not toxic
	Larval survival, pupal weight (protein fed to colony)	No effects
Bt-corn (lepidopteran-active)	Larval development, adult survival, foraging frequency (in field)	No effects
Serine protease inhibitors	Adult survival (in lab and in colony)	High concentrations reduce survival by a few days; low concentrations have no effect
	Adult digestive proteases	Inhibition of some proteases
	Adult flight activity (protein fed to colony)	Flight activity begins a few days earlier (when fed a high concentration)
	Olfactory learning response	One inhibitor offered in sugar reward reduced ability to learn; others did not
	Larval survival	High concentrations reduce survival[1]
Cysteine protease inhibitors	Adult survival	No effect
Cysteine protease inhibitor-expressing oilseed rape	Foraging behaviour	No effect
Chitinase	Adult survival	No effect
	Olfactory learning response	No effect
	Foraging behaviour (sugar feeder with chitinase added)	No effect
Chitinase-expressing oilseed rape	Foraging behaviour	No effect

β-1,3 glucanase	Adult survival	No effect
	Olfactory learning response	No effect
	Foraging behaviour (sugar feeder with β-1,3 glucanase added)	No effect
Biotin-binding protein (avidin)[2]	Adult survival	No effect
	Adult food consumption	No effect
	Larval development and survival	No effect
Herbicide (glufosinate)-resistant oilseed rape (pat gene)	Larval and adult survival, foraging behaviour (in colony)	No effect

[1] Brødsgaard et al., 2001.

[2] Malone et al., in press.

APPENDIX 1

New Zealand Horticultural Crops: Modes of pollination and value of honey bees.

Crop	Modes of Pollination	Honeybees shown to increase crop yields?	Recommended hives/ha
Apples	Flowers bisexual; most cultivars partly self-sterile; must be cross-pollinated by insects	Yes	At least 2
Kiwifruit	Male and female plants;.cross-pollination essential; fruit size related to pollination success	Yes	8
Grapes	Flowers bisexual; some cultivars partially or wholly self-sterile; complex pollination requirements	Yes	1
Avocado	Flowers bisexual; different timing for pollen release and stigma receptivity; insect and bird pollination reported	Yes	5
Grapefruit	Flowers bisexual; different timing for pollen release and stigma receptivity; insect pollination increases fruit set, but cross-pollination often considered unnecessary; seeds not desirable	No	0
Lemons	Flowers bisexual; self-pollination occurs without insects	No	0
Mandarins	Most cultivars self-sterile; some can produce fruit without fertilisation; fruit production increased with insect pollination; perhaps seeds not desirable?	Yes	4?
Oranges	Flowers bisexual; self-pollination occurs without insects	No	0
Tangelos	?	?	?
Persimmon	Male and female plants; some flowers bisexual; bees visit; some cultivars produce seedless fruit without fertilisation	No	0
Melons	Male and female flowers on same plant or bisexual flowers; insect pollination helpful	Yes (but most in NZ grown in glasshouses?)	0.5-7.5

Feijoa	Flowers bisexual; some self-fertile but need pollinator to transfer, others need cross-pollination	Yes	?
Tamarillo	?	?	?
Passionfruit	Most flowers bisexual but self-sterile; amount of pollen determines fruit size; insects essential	Yes	?
Pears	Flowers bisexual; unattractive nectar; many cultivars self-sterile; many need pollenizer of a different cultivar; insects important	Yes	1-5
Nashi	? same as pear?	?	?
Blackcurrants	Most flowers bisexual; self-fertile, but insects usually required to transfer pollen	Yes	6
Boysenberries and Raspberries	Bisexual flowers; automatic self-pollination can occur; insects can increase fruit set	Yes	0.5-2
Blueberries	Cross-pollination important for fruit set and fruit size	Yes	10
Strawberries	Flowers bisexual; fruit size depends on number of stigmas pollinated; cross-pollination occurs; insects important, but only bees transfer pollen effectively without injuring flower parts	Yes	25+
Apricots	Flowers bisexual; some cultivars have self-pollination, but cross-pollination beneficial; in others cross-pollination is essential; honeybees are main pollinators	Yes	2.5
Cherries	Flowers bisexual; cross-pollination improves yield	Yes	2.5-3
Nectarines	See peach	Yes	1-2.5?
Peaches	Flowers bisexual; self-pollination often occurs; some cultivars require pollenizers and honeybees; bees beneficial even for self-fertile cultivars	Yes	1-2.5
Plums	Flowers bisexual; need for cross-pollination varies among cultivars; honeybees primary pollinators	Yes	2.5
Flower seeds	Depends on species, but most benefit from bees for cross-	Depends on species	

	pollination		
Vegetable seeds	As for flower seeds - see below for some details	Depends on species	
Asparagus	Cross-pollination needed for seed production	Yes (for seeds)	5
Beans	Flowers bisexual; automatic self-pollination in French beans, but honeybees useful for runner beans	Yes (runner beans only)	?
Cabbage, cauliflower	Most flowers self-sterile; bees useful for seed production	Yes (for seeds)	?
Capsicums	Flowers bisexual; may be self-fertile; conflicting evidence about importance of insects	No	0
Carrots	Insect pollination essential for commercial seed production	Yes (for seeds)	?
Curcubits	Cucumber - some flowers bisexual, male and female flowers on same plant; pollinators necessary for seed and fruit production	Yes	<10
Garlic	Flowering rare; flowers sterile	No	0
Kumara	Most cultivars self-sterile; cross-pollination of compatible cutlivars needed for seed production; bees useful for this	Yes (for seeds)	?
Lettuce	Flowers bisexual; mostly self-pollinating; male-sterile cultivars used in hybrid seed production require insect pollinators; info on bees lacking	?	?
Onions	Flowers bisexual; cross-pollination common; flowers not very attractive to bees; bees used in US for seed production	Yes (for seeds)	12-36
Peas	Self-fertile bisexual flowers automatically self-pollinated	No	0
Potatoes	Flowers bisexual; many cultivars produce non-functional pollen; no nectar, little pollen, but bees observed to visit and may cross-pollinate rarely	No	0