Wissenschaftlerkreis
Grüne
Gentechnik e.V.

Gentechnisch veränderte Pflanzen und Bienen

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Gentechnisch veränderte, "transgene" Pflanzen (GV-Pflanzen) tragen Erbinformationen anderer Lebewesen (Mikroorganismen, Pflanzen oder Tiere) oder sind mit gentechnischen Methoden hergestellt, die sie mit neuen Eigenschaften ausstatten. Seit 1996 werden GV-Nutzpflanzen nicht nur in den USA in grossem Massstab angebaut, mittlerweile sind es weltweit mehr als 100 Millionen Hektar. Der Schwerpunkt des Anbaus ist bisher Nord- und Südamerika sowie Asien. In Europa wird erst ein kleiner Flächenanteil mit GV-Pflanzen bepflanzt, vor allem mit insektenresistentem Bt-Mais. In Deutschland wurden 2006 knapp 1000 Hektar Bt-Mais angebaut.

Ein entscheidender Aspekt bei der Zulassung von GV-Pflanzen, neben der gesundheitlichen Unbedenklichkeit für Tier und Mensch, ist ihre Umweltverträglichkeit. Dabei muss sichergestellt sein, dass z. B. eine Maissorte die gegen den Maiszünsler geschützt ist, möglichst wenig negative Auswirkungen auf Nicht-Ziel-Organismen hat, also auf andere Insekten und Lebewesen, die sich in Maisfeldern oder deren Umgebung aufhalten. Ein besonderes Augenmerk gilt dabei aufgrund ihrer wichtigen ökologischen und wirtschaftlichen Rolle den Bienen. Sowohl US-amerikanische als auch europäische Zulassungsbehörden verlangen Unterlagen, welche die Abschätzung eines potentiellen Risikos für Bienen ermöglichen. Für insektenresistente Pflanzen bedeutet dies, dass Bienen oder ihre Larven im Labor einer hohen Konzentration des in den Pflanzen produzierten Wirkstoffes ausgesetzt werden, die weit über dem auf dem Feld zu erwartenden Mengen liegen – nur wenn sich hier kein Gefährdungspotential abzeichnet, erhalten die GV-Pflanzen eine Anbaubewilligung.

Unabhängig von den Zulassungsverfahren haben sich auch zahlreiche Forscher immer wieder mit möglichen Auswirkungen transgener Nutzpflanzen auf Bienen beschäftigt – dieses Dokument soll einen Überblick über den derzeitigen Wissensstand liefern.

Bienen und gereinigter Bt-Wirkstoff

Viele insektenresistente Nutzpflanzen tragen eine Erbanlage, welche ursprünglich aus dem Bodenbakterium *Bacillus thuringiensis* stammt. Dieses Gen befähigt das Bakterium oder auch die GV-Pflanze, ein Eiweiss mit insektizider Wirkung zu produzieren. So kann sich die GV-Pflanze selbst gegen Frassinsekten schützen. Es existieren verschiedene Varianten des Gens. Die unterschiedlichen Eiweisssubstanzen wirken sehr spezifisch auf ganz bestimmte Insektengruppen.

Cry3B, ist eines der Eiweisse, das eine Wirkung gegen Käfer hat. Um herauszufinden ob Cry3B darüber hinaus ungünstige Auswirkungen auf Honigbienen haben könnte, wurden Bienenkolonien mit Zuckersirup gefüttert, welchem eine hohe Konzentration von Cry3B beigefügt war – mehr als 1000- mal mehr als in den Pollen von Bt-transgenen Pflanzen zu erwarten ist. Über zwei Monate wurde die Entwicklung der Larven, das Gewicht der Puppen sowie die Gesamtzahl der Bienen in den so gefütterten Stöcken beobachtet. Hierbei zeigten sich keine toxischen Effekte auf die Bienenlarven, und auch das Gewicht der Puppen war nicht beeinträchtigt (Arpaia 1996). Hieraus wurde geschlossen, dass für transgene Nutzpflanzen mit dem Cry3B-Wirkstoff keine negativen Auswirkungen auf Bienen zu erwarten sind. Sehr ähnliche Versuche wurden auch für zahlreiche andere Bt-Wirkstoffe durchgeführt, so auch für das in den MON810-Maispflanzen produzierte Cry1Ab (Babendreier et al. 2005). In keinem Fall wurden negative Auswirkungen der Bt-Eiweisse auf die Bienengesundheit veröffentlicht (Malone et al. 1999, Malone & Pham-Delègue 2001, 2002, Keil et al. 2002, Malone 2004, O'Callaghan et al. 2005, Sanvido 2006).

Auch andere Aspekte der Bienenentwicklung wurden betrachtet. So untersuchten verschiedene Forscher mögliche Auswirkungen von Bt-Eiweissen auf die Futterdrüsen der Arbeiterinnen, in denen die eiweissreiche Nahrung für die Bienenlarven produziert wird. Weder für Cry1Ba (Malone et al. 2004) noch für Cry1Ab (Babendreier et al. 2005, 2006) konnten hier schädliche Auswirkungen des gereinigten insektiziden Wirkstoffes gefunden werden. Das Flugverhalten wurde ebenfalls durch Cry1Ba nicht beeinflusst (Malone et al. 2001). In einem Fall wurde ein möglicher Einfluss von Cry1Ab auf die Futtersuche der Bienen berichtet, allerdings wurde dieser Einzelversuch im Winter und ohne direkten Vergleich mit unbehandelten Bienen durchgeführt und seither nicht wiederholt, so dass hieraus keine klaren Aussagen abgeleitet werden konnten (Ramirez-Romero 2005). Sogar die Darmflora der Bienen, die für Ihre Gesundheit wichtig ist, wurde auf ihre Empfindlichkeit gegen Cry1Ba geprüft: auch hier war kein Effekt zu sehen (Babendreier et al. 2007). Zusammengenommen kann festgestellt werden, dass bisher keine Nachweise von möglichen Gesundheitsgefahren für Bienen durch Bt-Eiweisse, wie sie in gentechnisch veränderten Nutzpflanzen eingesetzt werden, in der wissenschaftlichen Fachliteratur beschreiben wurden.

Hummeln und gereinigter Bt-Wirkstoff

Da Hummeln ebenfalls eine wichtige ökologische Rolle spielen, wurden neben Bienen (und vielen anderen Nicht-Ziel-Organismen) auch Hummeln auf ihre Empfindlichkeit gegen Bt-Eiweiss untersucht. Wenn die Hummeln über ihr Futter gereinigtes Cry1Ac-Eiweiss aufnahmen, konnte keine Auswirkung auf ihr Fressverhalten, Gewicht, Koloniegrösse und Zahl und Geschlechterverteilung der Nachkommen beobachtet werden (Morandin & Winston 2003).

Bienen und Bt-Mais

In ihrer natürlichen Umgebung sammeln Bienen Nektar und Blütenpollen, die zur eigenen Ernährung und der Ernährung der Brut dienen. Maisfelder sind dabei, solange Alternativen vorhanden sind, wenig attraktive Futterquellen (Trachtpflanzen). In Europa wird gegenwärtig als gentechnisch veränderte Nutzpflanze nur insektenresistenter Mais mit dem Konstrukt MON810 (Protein Cry1Ab) in nennenswertem Umfang angebaut. Verschiedene Untersuchungen haben gezeigt, dass der Nektar selbst nur wenig Protein enthält, und daher praktisch frei auch von Bt-Eiweiss ist (Malone & Pham-Delègue 2001). Der Hauptweg, auf dem Bienen in Europa in Kontakt mit Bt-Eiweiss gelangen können, ist daher der Verzehr von Bt-Mais-Pollen. Wie viel davon nehmen Bienen nun tatsächlich auf? Für eine Abschätzung ist es zunächst einmal wichtig zu wissen, wie viel Maispollen Bienen allgemein fressen oder an ihre Larven verfüttern. Um dies zu untersuchen, wurden Bienenvölker in grossen Flugzelten mit Maispflanzen ohne Zugang zu anderen Futterpflanzen gehalten, und der Darminhalt der Larven anschliessend mikroskopisch untersucht. Es stellte sich heraus, dass Bienenlarven nur sehr wenig Maispollen direkt verzehren - weniger als 5% des Proteinbedarfs der Larven wurde so gedeckt (Babendreier et al. 2004, 2006). Ihre Exposition gegenüber dem in den Pollen von insektenresitentem Mais enthaltenen Bt-Wirkstoff ist daher sehr gering.

Eine direkte Verfütterung von Pollen der (in Europa noch nicht zum Anbau zugelassenen) transgenen Maissorte Bt11 (Cry1Ab) oder TC1507 (Cry1F) an Bienenlarven zeigte, dass diese Behandlung keine Auswirkungen auf die Sterblichkeit der Larven oder Puppen, oder das Puppengewicht hatte (Hanley et al. 2003). Auch der Verzehr von MON810-Maispollen (Cry1Ab) durch Bienen über 10 Tage zeigte keine Auswirkung auf ihr Überleben, oder die Entwicklung ihrer Futtersaftdrüse (Babendreier et al. 2005, 2006), oder ihre Darmflora (Babendreier et al. 2007). Ein direkter Kontakt mit Bt-Maisblüten zeigte keine ungünstigen Auswirkungen auf die Sterblichkeit der Bienen (Bailey et al. 2005).

Insgesamt scheint die direkte Aufnahme von Bt-Maispollen durch Bienen oder ihre Brut keine ungünstigen Auswirkungen zu haben, wie auch viele weitere Studien und Übersichtsartikel belegen(Keil et al. 2002, Malone 2004, Malone et al. 2004, O'Callaghan et al. 2005, Sanvido et al. 2006).

Bienen und herbizidtoleranter Raps

Gegenwärtig wird in Europa kein gentechnisch veränderter Raps kommerziell angebaut. In den USA und in Kanada dagegen ist der Einsatz verbreitet, dort wachsen etwa 4,8 Millionen Hektar herbizidtoleranter (HT)-Raps. Diese Pflanzen sind unempfindlich gegen bestimmte Breitband-Herbizide, und ermöglichen so den Landwirten eine effizientere Unkrautkontrolle mit geringerem Arbeitsaufwand. Da Nektar und Pollen von Raps ein wichtiges Bienenfutter sind, wurden auch mögliche Auswirkungen dieser Pflanzen auf Bienen untersucht.

In Nektar und Pollen verschiedener gentechnisch veränderter Rapspflanzen konnte das kodierte Eiweiss nicht nachgewiesen werden (Pham-Delègue et al. 2002), im Allgemeinen ist die Wahrscheinlichkeit dass Bienen diese Substanzen aufnehmen wohl eher gering. In Feldversuchen in Kanada wurden Bienenstöcke in der Nähe grosser Felder mit transgenem oder konventionellem Raps aufgestellt. Es zeigte sich, dass die Bienen gleichviel Pollen beider Sorten sammelten. Es konnte kein Einfluss auf das Überleben der Larven oder das Puppengewicht festgestellt werden. Auch die Anzahl der Bienen, die nach einem Sammelflug in den Stock zurückkehrten, unterschied sich nicht (Huang et al. 2004). Verhaltensstudien zeigten, dass Bienen in Feldversuchen nicht zwischen transgenem und konventionellem Raps unterscheiden, und beide Sorten gleich gerne anfliegen (Pierre et al. 2003).
Da herbizidtolerante Rapspflanzen eine spätere aber wirksamere Unkrautbekämpfung ermöglichen, finden sich in Feldern mit transgenem HT-Raps und üppigem Hybridwuchs zu Ende der Saison in der Regel weniger blühende Unkräuter. Diese Felder sind daher für auf derartige Pflanzen spezialisierte Bienen weniger attraktiv als konventionelle oder Bio-Raps-Felder, auf denen auch bei den Unkräutern eine höhere Artenvielfalt herrscht (Haughton et al. 2003, Bohan et al. 2005, Morandin & Winston 2005). Allerdings ist die Attraktivität eines Rapsfeldes für Bienen im Sommer nach Ende der Blütezeit generell gering. Es ist unwahrscheinlich, dass die beobachteten Unterschiede zwischen Feldern mit herbizidtoleranten GV-Raps und herkömmlichen Pflanzen eine Auswirkung auf die Gesamtpopulation der Bienen haben, da diese sich ihre Futterquelle innerhalb eines grösseren Bereiches auswählen können. So zeigten sich bereits am Feldrand nur noch geringe Unterschiede in der Bienen- und Hummelzahl (Roy et al. 2003).

Bienen und andere transgene Pflanzen

Auch verschiedene andere, in Europa im Moment nicht kommerziell angebaute gentechnisch veränderte Pflanzen wurden auf mögliche Auswirkungen auf Bienen untersucht. Bei herbizidtoleranten Zuckerrüben wurden bei grossen Feldversuchen in England ähnliche Beobachtungen wie mit HT-Raps gemacht: aufgrund geringerer Zahl von Unkräutern waren tendenziell weniger Bienen und Hummeln auf diesen Feldern zu finden. Gerade andersherum verhielt es sich bei HT-Mais, hier fanden sich etwas mehr Bienen und Hummeln, da in diesen Feldern eine gezielte Unkrautkontrolle zu Beginn der Wachstumsperiode eine grössere Artenvielfalt im Lauf des Sommers ermöglichte (Haughton et al. 2003). Die beobachteten Effekte auf die Anzahl der Bienen waren bereits an den Feldrändern dann kaum noch zu beobachten (Roy et al. 2003).

In der Literatur finden sich Untersuchungen auch zu anderen Pflanzen, wie z. B. insektenresitenter Bt-Baumwolle. Negative Einflüsse zugelassener transgener Pflanzen auf Bienen wurden auch hier nicht beschrieben (Liu et al. 2005, Velkov et al. 2005).

Transgene Pflanzen und Bienensterben

Im Frühjahr 2007 wurde aus den USA über ein neuartiges Bienensterben berichtet – zahlreiche Völker verliessen ihre Stöcke und verschwanden spurlos. Auch aus verschiedenen europäischen Ländern wie Deutschland und der Schweiz wurden ähnliche Beobachtungen gemeldet. Dieses in den USA als "Colony Collapse Disorder" CCD bekannte Phänomen wurde in populären Medien auch als "Bienen-AIDS" bezeichnet. Die Ursachen sind noch weitgehend unklar, obwohl intensiv an einer Aufklärung gearbeitet wird.

Da in den USA mittlerweile 40% der Maisfelder mit insektenresistenten, gentechnisch veränderten Bt-Sorten bestellt werden, wurde vereinzelt auch vermutet dass ein Zusammenhang zwischen dem Anbau von Gentech-Pflanzen und dem Bienensterben bestehen könnte. Als Hinweis hierfür wurde auch eine Beobachtung an der Universität Jena im Rahmen eines Sicherheits-Forschungsprogrammes mit transgenem Bt-Mais herangezogen. Bei gesunden Bienen wurden hier weder Hinweise auf akute noch auf chronische toxische Wirkungen des Bt-Mais gefunden. Bei einem Versuch wurden die Bienen jedoch von Parasiten (Mikrosporidien) befallen und so stark gesundheitlich beeinträchtigt, dass der Versuch schliesslich abgebrochen werden musste. Bei diesen bereits erkrankten Bienen schien der Verzehr von Bt-Mais-Pollen den Gesundheitszustand weiter zu verschlechtern. Allerdings konnte diese Einzelbeobachtung in den weiteren Versuchen nie wiederholt oder belegt werden. Aufgrund der fehlenden Reproduzierbarkeit wurde dieses Resultat auch nie in einer Fachzeitschrift veröffentlicht, es steht allerdings auf dem Internet zur Verfügung ("Auswirkungen von Bt-Maispollen auf die Honigbiene", http://www.biosicherheit.de/de/sicherheitsforschung/68.doku.html).

Gegen einen unmittelbaren Zusammenhang zwischen dem Anbau gentechnisch veränderter Pflanzen und dem Bienensterben sprechen jedoch zahlreiche Punkte. Bereits in der

Vergangenheit, bevor GV-Pflanzen überhaupt landwirtschaftlich genutzt wurden, wurden immer wieder Bienensterben beobachtet. Heutzutage fehlt eine geographische Übereinstimmung zwischen den Anbaugebieten von GV- Pflanzen und den von dem Bienensterben betroffenen Regionen. So wird CCD in den USA aus vielen Bundesstaaten gemeldet, in denen kaum Gentech-Pflanzen wachsen, aus manchen Hochburgen der Biotech-Landwirtschaft dagegen liegen keine Berichte hierüber vor. In Europa wird das Bienensterben zudem in Gebieten beobachtet, in denen gar keine GVO-Pflanzen angebaut werden, so z. B. in der Schweiz. Als auslösender Faktor für das Bienensterben CCD kommen gentechnisch veränderte Nutzpflanzen daher nicht in Frage, in Expertenkreisen werden vielmehr Belastung durch Chemikalien, Krankheitserreger oder andere Stressfaktoren oder eine mangelnde genetische Vielfalt der Bienenvölker diskutiert.

Gentransfer im Darm der Honigbiene

Im Jahr 2000 berichteten verschiedene Medien über Resultate einer Arbeitsgruppe aus Jena, die einen Hinweis auf einen möglichen Gentransfer von gentechnisch verändertem, herbizidtolerantem Raps auf Bakterien im Darm der Honigbiene gaben. Es wurde vermutet, dass die Mikroorganismen der Darmflora ein Gen der Rapspflanze aufgenommen hatten, und so selber resistent gegen das Herbizid geworden waren. Bei der Überprüfung dieser Resultate durch externe wissenschaftliche Gutachter konnten jedoch nicht alle offenen Fragen zu den Experimenten ausgeräumt werden, so dass diese Resultate bisher noch nicht in einer Fachzeitschrift veröffentlicht wurden.

Um diesem Hinweis nachzugehen, untersuchten Wissenschaftler, in welchem Umfang transgene Rapspollen von Honigbienen, Mauerbienen (einer Wildbienenart) und Hummeln gesammelt wird. Es zeigte sich, dass alle drei Arten durchaus Pollen von transgenen Rapspflanzen sammeln und verzehren; ein direkter Kontakt mit der Darmflora der Bienen ist also gegeben (Sick et al. 2004). 40 % dieser Mikroorganismen stellten sich.in der Tat als unempfindlich gegen das Herbizid heraus. Eine genauere molekularbiologische Untersuchung zeigte jedoch, dass keines dieser untersuchten Mikroorganismen das Transgen aus dem Raps aufgenommen hatte, sondern dass es sich um bereits natürlich vorhandene Resistenzen handelte (Mohr et al. 2007).

Zusammenfassung

Bisher existieren in der wissenschaftlichen Fachliteratur keine Hinweise auf mögliche direkte oder indirekte Schädigungen von Bienen durch gegenwärtig zugelassene gentechnisch veränderte Pflanzen. Dies ist das Resultat zahlreicher Labor- und Feldversuche, bei denen eine unter natürlichen Bedingungen mögliche Exposition gegenüber den GVO-Pflanzen bzw. ihren Produkten zum Teil stark übertrieben wurde. Veränderte Anbaumethoden, wie sie z. B. mit herbizidtoleranten Pflanzen möglich sind, können saisonal die Zusammensetzung der Unkrautarten innerhalb der Felder beeinflussen – dies ist allerdings auch bei konventionellen Anbaumethoden der Fall und keine direkte Folge der gentechnischen Veränderung. Bei mobilen Insekten wie den Bienen, die einen grossen Lebensraum haben, sind hiervon keine negativen Auswirkungen zu erwarten.

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Anhang: detaillierte Literaturangaben zu gentechnisch veränderten Pflanzen und Bienen

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> To assess the possible hazard for honey bees of CryIIIB protein from a Bacillus thuringiensis Berl. derived gene, the toxin was supplied, mixed in supplemental syrup, to Apis mellifera L. colonies. Two different toxin concentrations were used at levels of about 400 and 2000 times higher than the expected protein content in pollen from Bt-transgenic plants. Hives were sampled every week to record larval survival and pupal dry weight. Frames of bees were counted at the beginning and the end of the experiment as an index of colony strength. No toxic effects on larvae were observed. Pupal weight was not significantly affected by diet regime. These results indicate that transgenic crops producing CryIIIB toxin may represent a suitable environment for pollinators.

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> In order to assess the potential impacts of transgenic plants on larvae of the honey bee, Apis mellifera, information on pollen consumption is needed. We here report on experiments that were conducted with small bee colonies kept in field cages (8 Å~ 14 m) containing only flowering maize plants as protein source. Fully grown worker bee larvae were found to contain between 1720 and 2310 maize pollen grains in their gut before defecation, corresponding to 1.52-2.04 mg of pollen consumed per larva. On average, 74.5% of pollen grains were completely digested while 23.3% were partially digested and 2.2% remained undigested. Our data indicate that the contribution of the protein by directly feeding larvae with pollen is less than 5% in relation to the total amount of protein necessary for complete larval development. We suggest that our measurement for pollen consumption should be taken into account when establishing dose regimes to assess the risk that transgenic plants pose for honey bee larvae.
> http://dx.doi.org/10.1051/apido:2004016 (full text PDF available)

Babendreier D, Kalberer NM, Romeis J, Fluri P, Mulligan E, Bigler, F 2005. Influence of Bt-transgenic pollen, Bt-toxin and protease inhibitor (SBTI) ingestion on development of the hypopharyngeal glands in honeybees. *Apidologie* 36:585-594.

> In order to assess the risks of transgenic crops for honey bee colonies, we studied the development of hypopharyngeal glands of adult workers. We introduced 50 newly emerged adult bees into small, queenright colonies of c. 250 bees. Bees were fed either Bt-transgenic maize pollen (MON 810) or a sugar solution containing either purified Bt-toxin (Cry1Ab, 0.0014% w/v) or Kunitz soybean trypsin inhibitor (SBTI) at two concentrations (0.1% and 1% w/v). Neither the Bt maize pollen nor the Bt toxin showed any effect on bee survival or on the development of hypopharyngeal glands after a period of 10 days' feeding. In contrast, treatment of newly emerged bees with SBTI (0.1 and 1%) for 10 days significantly reduced the mean weights of the hypopharyngeal glands and the mean diameter of the glands' acini. While small amounts of Bt toxin were detected by ELISA in the hypopharyngeal glands of bees fed the Bt-sugar solution, SBTI could not be detected in gland samples by Western blotting.
> http://dx.doi.org/10.1051/apido:2005049 (full text PDF available)

Babendreier D, Romeis J, Bigler F, Fluri P 2006. Neue Erkenntnisse zu möglichen Auswirkungen von transgenem Bt-Mais auf Bienen. *Forschungsanstalt Agroscope Liebefeld-Posieux ALP, Schweiz.*

> Insektenresistente, transgene Nutzpflanzen sind gezielt mit insektentoxischen Eigenschaften ausgestattet, welche sich gegen Schadinsekten richten. Das Insektentoxin kann auch im Polen gebildet werden, der den Bestäuberinsekten als Nahrung dient. Honigbienen sind als Bestäuber von vielen Kultur- und Wildpflanzen sowohl ökologisch als auchökonomisch von grosser Bedeutung. Desshalb müssen transgene Nutzpflanzen bienenverträglich sein. In der vorliegenden Studie wurde in einem ersten Experiment die Exposition von Honigbienenlarven gegenüber transgenen Pflanzen untersucht. In einem

zweiten Experiment untersuchten wir die Wirkung von Bt-Mais und dem Bt-Toxin in hohen Konzentrationen auf die Entwicklung der Futtersaftdrüse. http://www.alp.admin.ch/themen/00502/00538/00861/index.html?lang=de (full text PDF available

Babendreier D, Joller D, Romeis J, Bigler F, Widmer F. 2007. Bacterial community structures in honeybee intestines and their response to two insecticidal proteins. *FEMS Microbiology Ecology* 59:600-610.

In this study, the effects of the Bt-toxin Cry1Ab and a soybean trypsin inhibitor (SBTI) on intestinal bacterial communities of adult honeybees (Apis mellifera) were investigated. It was hypothesized that changes in intestinal bacterial communities of honeybees may represent a sensitive indicator for altered intestinal physiology. Honeybees were fed in a laboratory set-up with maize pollen from the Bt-transgenic cultivar MON810 or from the non-transgenic near isoline. Purified Cry1Ab (0.0014% w/v) and SBTI (0.1% or 1% w/v) represented supplementary treatments. For comparison, free-flying honeybees from two locations in Switzerland were analysed. PCR-amplification of bacterial 16S rRNA gene fragments and terminal restriction fragment length polymorphism analyses revealed a total of 17 distinct terminal restriction fragments (T-RFs), which were highly consistent between laboratory-reared and free-flying honeybees. The T-RFs were affiliated to Alpha-, Beta-, and Gammaproteobacteria, to Firmicutes, and to Bacteriodetes. Neither Bt-maize pollen nor high concentrations of Cry1Ab significantly affected bacterial communities in honeybee intestines. Only the high concentration of SBTI significantly reduced the number of T-RFs detected in honeybee midguts, a concentration that also increases bee mortality. Therefore, total bacterial community structures may not be a sensitive indicator for providing evidence for the impact of insecticidal proteins on honeybees at sublethal levels. http://dx.doi.org/10.1111/j.1574-6941.2006.00249.x

Bailey J, Scott-Dupree C, Harris R, Tolman J, Harris B 2005. Contact and oral toxicity to honey bees (Apis mellifera) of agents registered for use for sweet corn insect control in Ontario, Canada. *Apidologie* 36:623-633.

Assays were conducted to compare direct and residual contact and oral toxicities to honey bees of sweet corn insecticides and of Bt-sweet corn. Direct contact assays focusing on LC50 determined that technical grade clothianidin was most toxic, >carbofuran, >imidacloprid=spinosad, >lambda-cyhalothrin, >Bacillus thuringiensis. In residual contact assays, forager age bees were exposed to treated non-transgenic sweet corn tassels. Carbofuran treated tassels caused significant mortality up to 2 and 3 days after treatment (DAT) in 2002 and 2003, respectively. Lambda-cyhalothrin treated tassels had no impact on honey bees in 2002; in 2003, their toxicity was significantly higher than the untreated control tassels for 1 DAT. In both years, spinosad, imidacloprid and clothianidin or Bt-sweet corn tassels had no impact on honey bee mortality. Pollen collected from insecticide field treated corn and fed to honey bees had no impact on mortality. http://dx.doi.org/10.1051/apido:2005048

Bohan DA, Boffey CWH, Brooks DR, Clark SJ, Dewar AM, Firbank LG, Haughton AJ, Hawes C, Heard MS, May MJ, Osborne JL, Perry JN, Rothery P, Roy DB, Scott RJ, Squire GR, Woiwod IP, Champion GT. 2005. Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. *Proceedings of the Royal Society B-Biological Sciences* 272:463-474.

We evaluated the effects of the herbicide management associated with genetically modified herbicide-tolerant (GMHT) winter oilseed rape (WOSR) on weed and invertebrate abundance and diversity by testing the null hypothesis that there is no difference between the effects of herbicide management of GMHT WOSR and that of comparable conventional varieties. For total weeds there were few treatment differences between GMHT and conventional cropping, but large and opposite treatment effects were observed for dicots and monocots. In the GMHT treatment, there were fewer dicots and more monocots than in conventional crops. At harvest, dicot biomass and seed rain in the GMHT treatment were one-third of that in the conventional, while monocot biomass was threefold greater and monocot seed rain almost fivefold greater in the GMHT treatment than in the conventional. These differential effects persisted into the following two years of the rotation. Bees and butterflies that forage and select for dicot weeds were less abundant in

GMHT WOSR management in July. Year totals for Collembola were greater under GMHT management. There were few other treatment effects on invertebrates, despite the marked effects of herbicide management on the weeds.
http://dx.doi.org/10.1098/rspb.2004.3049 (full text PDF available)

Hanley AV, Huang ZY, Pett WL 2003. Effects of dietary transgenic Bt corn pollen on larvae of Apis mellifera and Galleria mellonella. *Journal 0f Apicultural Research* 42: 77-81.

The effects of dietary transgenic Bt corn pollen on 4-5-day-old honey bee worker larvae were examined. We measured larval and pupal mortalities, pupal weight, and haemolymph protein concentration of newly emerged adults after they were fed (as larvae) various pollens (mixed bee pollen, non-transgenic corn pollen, Cry1A(b) or Cry1F corn pollen). There were no significant differences in all the parameters tested between larvae fed transgenic Bt corn pollen Cry1A(b) or Cry1F and non-transgenic corn pollen. We also evaluated Bt corn pollen as a potential control for wax moth larvae in a laboratory study. We fed first instar wax moth larvae three types of pollen: non-transgenic corn pollen, Cry1A(b) corn pollen, and Cry1F corn pollen. We found that the mortality of larvae fed Cry1F corn pollen was significantly greater than the mortality of larvae fed Cry1A(b) corn pollen or non-transgenic corn pollen ($P<0.05$). In each trial Cry1F fed larvae showed 100% mortality. Our studies suggest that transgenic Bt corn pollen does not pose a threat to honey bee larval development and has the potential to serve as an alternative control for wax moth.
http://www.cyberbee.net/huangpub/2003JAR.pdf (full text PDF available)

Haughton AJ, Champion GT, Hawes C, Heard MS, Brooks DR, Bohan DA, Clark SJ, Dewar AM, Firbank LG, Osborne JL, Perry JN, Rothery P, Roy DB, Scot, RJ, Woiwod IP, Birchall C, Skellern MP, Walker JH, Baker P, Browne EL, Dewar AJG, Garner BH, Haylock LA, Horne SL, Mason, NS, Sands RJN, Walker MJ 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. *Philosophical Transactions of The Royal Society of London Series B-Biological Sciences* 358: 1863-1877.

The effects of the management of genetically modified herbicide-tolerant (GMHT) crops on the abundances of aerial and epigeal arthropods were assessed in 66 beet, 68 maize and 67 spring oilseed rape sites as part of the Farm Scale Evaluations of GMHT crops. Most higher taxa were insensitive to differences between GMHT and conventional weed management, but significant effects were found on the abundance of at least one group within each taxon studied. Numbers of butterflies in beet and spring oilseed rape and of Heteroptera and bees in beet were smaller under the relevant GMHT crop management, whereas the abundance of Collembola was consistently greater in all GMHT crops. Generally, these effects were specific to each crop type, reflected the phenology and ecology of the arthropod taxa, were indirect and related to herbicide management. These results apply generally to agriculture across Britain, and could be used in mathematical models to predict the possible long-term effects of the widespread adoption of GMHT technology. The results for bees and butterflies relate to foraging preferences and might or might not translate into effects on population densities, depending on whether adoption leads to forage reductions over large areas. These species, and the detritivore Collembola, may be useful indicator species for future studies of GMHT management.
http://dx.doi.org/10.1098/rstb.2003.1408 (full text PDF available)

Huang ZY, Hanley AV, Pett WL, Langenberger M, Duan JJ 2004. Field and semifield evaluation of impacts of transgenic canola pollen on survival and development of worker honey bees. *Journal of Economic Entomology* 97:1517-1523.

A 2-yr field trial (2001 and 2002) and 1-yr semifield trial (2002) were conducted to evaluate the effect of transgenic herbicide (glyphosate)-tolerant canola Brassica napus L. pollen oil larval and adult honey bee, Apis mellifera L., workers. In the field trial, colonies of honey bees were moved to transgenic or nontransgenic canola fields (each at least 40 hectares) during bloom and then sampled for larval survival and adult recovery, pupal weight, and hemolymph protein concentrations. No differences in larval survival, adult recovery, and pupal weight were detected between colonies placed in nontransgenic canola fields and those in transgenic canola fields. Colonies placed in the transgenic canola fields in the

2002 field experiment showed significantly higher hemolymph protein in newly emerged bees compared with those placed in nontransgenic canola field; however, this difference was not detected in the 2001 field experiment. In the semifield trial, bee larvae were artificially fed with bee-collected transgenic and nontransgenic canola pollen and returned to their original colonies. Larval survival, pupal survival, pupal weight, and hemolymph protein concentration of newly emerged adults were measured. There were no significant differences in any of the parameters measured between larvae that were fed transgenic canola pollen and those fed nontransgenic corn pollen. Results from this study suggest that transgenic canola pollen does not have adverse effects on honey bee development and that the use of transgenic canola does not pose any threat to honey bees.
http://cyberbee.msu.edu/huangpub/2004JEE.pdf (full text PDF available)

Keil S, Romeis J, Fluri P, Bigler F (2002). Sind Honigbienen durch den Einsatz von insektenresistenten transgenen Pflanzen einem Risiko ausgesetzt? *Forschungsanstalt Agroscope Liebefeld Posieux ALP, Schweizerisches Zentrum für Bienenforschung*.

Transgene Pflanzen mit schädlings- und krnakheitsresistenten Eigenschaften erhalten fremde Gene, die ursprünglich aus anderen Pflanzen, Tieren oder Mikroorganismen stammen und in das Erbgut eingebaut werden. Diese Gene können beispielsweise Proteine bilden, die genetisch veränderte Pflanzen vor Insektenfrass oder Pilzbefall schützen. Ziel ist es, den Einsatz von Pflanzenschutzmitteln zu verringern und dadurch ein niedrigeres Gefährdungspotential für die Umwelt, den Anwender und den Konsumenten zu erreichen.
http://www.alp.admin.ch/themen/00502/00538/00861/index.html?lang=de (full text PDF available)

Liu YH, Chen SL, Liu YH, Chen SL. 2001. Influences of transgenic insect-resistant plants on honeybees. *Entomological Knowledge*. 38:258-262.

Transgenic cotton containing a synthetic version of the insecticidal toxin gene from Bacillus thuringiensis subsp. kurstaki has been planted in China in a large scale and may have adverse impacts on honeybees. The pollens of the transgenic cotton and the non-transgenic parental cotton were collected and their impacts on worker bees were tested. Experimental results showed that the transgenic cotton pollen had no oral acute toxic effects on worker bees. No significant difference was observed in the superoxide dismutases activity and the longevity of worker bees fed with diets containing the transgenic cotton pollen or non-transgenic parental cotton pollen. The results of our experiment indicate that the transgenic cotton has no direct adverse impacts on honeybees. Further work needs to be carried out to evaluate the long-term and indirect impacts of the transgenic cotton on honeybees.
http://dx.doi.org/10.1007/s10531-004-0824-7

Liu B, Xu CG, Yan FM, Gong RZ 2005. The impacts of the pollen of insect-resistant transgenic cotton on honeybees. *Biodiversity and Conservation* 14: 3487-3496.

Transgenic cotton containing a synthetic version of the insecticidal toxin gene from Bacillus thuringiensis subsp. kurstaki has been planted in China in a large scale and may have adverse impacts on honeybees. The pollens of the transgenic cotton and the non-transgenic parental cotton were collected and their impacts on worker bees were tested. Experimental results showed that the transgenic cotton pollen had no oral acute toxic effects on worker bees. No significant difference was observed in the superoxide dismutases activity and the longevity of worker bees fed with diets containing the transgenic cotton pollen or non-transgenic parental cotton pollen. The results of our experiment indicate that the transgenic cotton has no direct adverse impacts on honeybees. Further work needs to be carried out to evaluate the long-term and indirect impacts of the transgenic cotton on honeybees.
http://dx.doi.org/10.1007/s10531-004-0824-7

Malone LA, Burgess EPJ, Stefanovic D 1999. Effects of a Bacillus thuringiensis toxin, two Bacillus thuringiensis biopesticide formulations, and a soybean trypsin inhibitor on honey bee (Apis mellifera L.) survival and food consumption. *Apidologie* 30 (6): 465-473

Newly emerged adult honey bees, Apis mellifera L., were fed with a pollen-based food containing various additives: purified and activated Cry1Ba delta-endotoxin, from Bacillus thuringiensis Bt4412 (Bt) (1, 0.25 and 0.025 % w/w), Bt biopesticide preparations, Dipel 2X (1 and 0.25 %) and Foray 48B (0.25 %), and Kunitz soybean trypsin inhibitor (SBTI) (1, 0.5 or 0.05 %). The bees received these foods for 7 days and were then given control food without additives for the rest of their lives, Bee survival time was unaffected, and the food was consumed at the same rate as control food For all treatments, except 1 % Dipel, where both survival and food consumption were significantly reduced. A second experiment showed that bees completely deprived of the pollen-based food also had poorer survival than those fed with the control food. Adult bees are unlikely to be harmed by trans genic plants expressing Cry1Ba or SBTI, or by Bt biopesticides that are used as recommended. (C) Inra/DIB/AGIB/Elsevier, Paris.

Malone LA, Burgess EPJ, Gatehouse HS; Voisey CR, Tregidga EL, Philip BA 2001. Effects of ingestion of a Bacillus thuringiensis toxin and a trypsin inhibitor on honey bee flight activity and longevity. *Apidologie* 32:57-68.

To assess potential impacts of transgenic pest-resistant plants, newly-emerged adult honey bees from ten colonies were tagged, placed in cages at 33 degreesC, and fed with 625 mug/g Cry1Ba Bacillus thuringiensis (Bt) toxin or 2.5 mg/g aprotinin proteinase inhibitor in pollen-food (equivalent to 0.25% or 1% of total soluble protein). Control bees were given similar food without additive. All foods were consumed at similar rates. After seven days, all bees were returned to their hives. Subsequent observations showed that Cry1Ba-fed bees did not differ significantly from control bees in the timing of their first flight, the period during which flights took place or in estimated longevity. However, aprotinin-fed bees began to fly and also died about three days sooner than Cry1Ba-fed or control bees. Their flight periods were similar to those of the other bees. The effects of transgenic aprotinin-plants on honey bees will thus depend on gene expression levels in pollen. http://dx.doi.org/DOI: 10.1051/apido:2001111 (full text PDF available)

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

As more transgenic crop plants become commercialized, there is an increasing need for information on their impacts on honey bees and bumble bees. Direct effects on bees may arise upon ingestion of proteins encoded by transgenes, if they are expressed in pollen, nectar or resin. Indirect effects may occur if plant transformation inadvertently changes flower phenotype. This review summarizes current findings on effects of purified transgene product ingestion on adult bee gut physiology, food consumption, olfactory learning behaviour and longevity. Bt, protease inhibitor, chitinase, glucanase and biotin-binding protein genes are discussed. Results from tests conducted in the laboratory with individual adult bees and with colonies in the field are presented. Observations of bee foraging on transgenic plants kept under containment are also summarized. Results so far suggest that transgenic plant impacts on pollinators will depend on a case-by-case analysis of the gene concerned and its expression in the parts of the plant ingested by bees. http://dx.doi.org/DOI:10.1051/apido:2001130 (full text PDF available)

Malone LA, Pham-Delègue MH 2002. Using proteins to assess the potential impacts of genetically modified plants on honey bees. *Honey bees: estimating the environmental impact of chemicals p.290-311, 2002. Editors: Devillers, J.; Pham-Delègue, M. H. Publisher: Taylor & Francis, London, UK*

Genetically modified plants manifest new traits via the expression of foreign proteins encoded by inserted transgenes. For example, cotton modified to contain a Bacillus thuringiensis (Bt) gene and expressing Bt toxin in its leaves and buds will be protected from bollworm attack. Since the protein products of many transgenes can be purified, these "active ingredients" of genetically modified plants can be used in experiments to assess the likely impacts of such plants on bees. Such tests have a number of advantages: they can be conducted prior to the lengthy process of plant modification, the effects of the proteins can be quantified and some tests may be conducted with bees outside strict quarantine conditions. The shortcomings of this approach are that indirect impacts of genetically modified plants on bees, such as pleiotropic effects resulting from changes in plant phenotype, cannot be assessed and that the test conditions may be somewhat artificial, for

example keeping the bees in cages in an incubator. This chapter summarizes current results from bioassays with bees and purified transgene products. Effects of a range of proteins, Bt toxins, protease inhibitors, chitinases, glucanases, and biotin-binding proteins, on adult bee gut physiology, food consumption, olfactory learning behaviour, and longevity are presented.

Malone LA 2004. Potential effects of GM crops on honey bee health. *Bee World* 85: 29-36.

Recent increases in the global area planted in GM crops have been accompanied by rising public awareness of agricultural practices and concern about the environment. Honey bees are widely recognized as important beneficial insects. In most countries, regulators assessing potential risks and benefits from GM plants list the honey bee among the 'non-target' species that need to be considered before these plants are released. Research on honey bees and GM crops has focused on the presence of GM material in honey, the roles that bees may play in the flow of genes from GM crops, and the potential impacts of GM plants on bee health. This article summarizes research on bee health and GM. There is now a considerable body of knowledge on this topic and some well-established techniques for assessing risks to bees before new plants are released.

Malone LA, Todd JH, Burgess EPJ, Christeller JT 2004. Development of hypopharyngeal glands in adult honey bees fed with a Bt toxin, a biotin-binding protein and a protease inhibitor. *Apidologie* 35 (6): 655-664.

To investigate potential impacts of insecticidal transgene products on hypopharyngeal gland development, newly-emerged adult honey bees were kept in cages and fed for ten days with a pollen-food to which one of the following proteins had been added at concentrations equivalent to pollen expressing 1.25% (of total protein) aprotinin, 11.2 muM avidin or 0.3% Cry1Ba protein. Control bees were fed pollen-food without additive, with casein added, or pollen-candy. None of the treatments significantly affected bee survival, or the mean diameters of gland acini, gland mass or protein content on Days 1, 4, 8 or 10. Bees consumed significantly more of the pollen-candy than the other foods. Assays showed no evidence of aprotinin, avidin or Cry1Ba in the glands of bees fed with these proteins. Thus effects of these transgene products on gland development are unlikely, but field trials using transgenic plants are required for a full assessment.
http://dx.doi.org/10.1051/apido:2004063 (full text PDF available)

Mohr KI, Tebbe CC 2007. Field study results on the probability and risk of a horizontal gene transfer from transgenic herbicide-resistant oilseed rape pollen to gut bacteria of bees. *Appl Microbiol Biotechnol.* [Epub ahead of print, 2. 2. 2007;]

Bees are specifically subjected to intimate contacts with transgenic plants due to their feeding activities on pollen. In this study, the probability and ecological risk of a gene transfer from pollen to gut bacteria of bees was investigated with larvae of Apis mellifera (honeybee), Bombus terrestris (bumblebee), and Osmia bicornis (red mason bee), all collected at a flowering transgenic oilseed rape field. The plants were genetically engineered with the pat-gene, conferring resistance against glufosinate (syn. phosphinothricin), a glutamine-synthetase inhibitor in plants and microorganisms. Ninety-six bacterial strains were isolated and characterized by 16S rRNA gene sequencing, revealing that Firmicutes represented 58% of the isolates, Actinobacteria 31%, and Proteobacteria 11%, respectively. Of all isolates, 40% were resistant to 1 mM glufosinate, and 11% even to 10 mM. Resistant phenotypes were found in all phylogenetic groups. None of the resistant phenotypes carried the recombinant pat-gene in its genome. The threshold of detecting gene transfer in this field study was relatively insensitive due to the high background of natural glufosinate resistance. However, the broad occurrence of glufosinate-resistant bacteria from different phylogenetic groups suggests that rare events of horizontal gene transfer will not add significantly to natural bacterial glufosinate resistance.
http://dx.doi.org/10.1007/s00253-007-0846-7

Morandin LA, Winston ML 2003. Effects of novel pesticides on bumble bee (Hymenoptera : Apidae) colony health and foraging ability. *Environmental Entomology* 32 (3): 555-563.

Two experiments were conducted testing for lethal and sublethal effects of the transgenic proteins Cry1Ac and chitinase, and the chemical seed and soil treatment imidacloprid on humble bees (Bombus occidentalis Greene and B. impatiens Cresson, Hymenoptera: Apidae). In the first experiment, B. occidentalis colonies were exposed to realistic residue levels of Cry1Ac, chitinase, and imidacloprid found in pollen. There were no effects on pollen consumption, bumble bee worker weights, colony size, amount of brood, or the number of queens and males produced. In the second experiment, using B. impatiens, we tested the effects of Cry1Ac and two levels of imidacloprid. Similar colony health measures were collected as in the first experiment, but in addition foraging ability of individual bees was tested on complex artificial flowers. There were no differences in colony characteristics among treatments. However, bees in the high-imidacloprid treatment had longer handling times on the complex flowers than bees in the other treatments. No lethal, sublethal colony, or individual foraging effects of these novel pesticides were found at residue levels found in the field, suggesting that humble bee colonies will not be harmed by proper use of these pesticides. Use of an artificial flower foraging array proved to be a sensitive method for detecting sublethal response of bees to pesticides.
http://www.ingentaconnect.com/content/esa/envent/2003/00000032/00000003/art00019

Morandin LA, Winston ML 2005. Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecological Applications* 15 (3): 871-881.

The ecological impacts of agriculture are of concern, especially with genetically modified and other intensive, modern cropping systems, yet little is known about effects on wild bee populations and subsequent implications for pollination. Pollination deficit (the difference between potential and actual pollination) and bee abundance were measured in organic, conventional, and herbicide-resistant, genetically modified (GM) canola fields (Brassica napus and B. rapa) in northern Alberta, Canada, in the summer of 2002. Bee abundance data were collected using pan traps and standardized sweep netting, and pollination deficit was assessed by comparing the number of seeds per fruit from open-pollinated and supplementally pollinated flowers. There was no pollination deficit in organic fields, a moderate pollination deficit in conventional fields, and the greatest pollination deficit in GM fields. Bee abundance was greatest in organic fields, followed by conventional fields, and lowest in GM fields. Overall, there was a strong, positive relationship between bee abundance at sampling locations and reduced pollination deficits. Seed set in B. napus increased with greater bee abundance. Because B. rapa is an obligate outcrossing species, the lack of pollination deficit in the organic (B. rapa) fields likely was due to the high bee abundance rather than a lower dependence of B. rapa on pollinators than B. napus canola. Our study illustrates the importance of wild bees to agricultural production and suggests that some agroecosystems may better sustain wild bee abundance, resulting in greater seed production. Further research on why some cropping systems, such as genetically modified, herbicide-resistant canola, have low wild bee abundance would be useful for management of agroecosystems to promote sustainability of food production.
http://www.esajournals.org/esaonline/?request=get-abstract&issn=1051-0761&volume=015&issue=03&page=0871

O'Callaghan M, Glare TR, Burgess EPJ, Malone LA 2005. Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology* 50: 271-292.

Insect resistance, based on Bacillus thuringiensis (Bt) endotoxins. is the second most widely used trait (after herbicide resistance) in commercial-genetically modified (GM) crops. Other modifications for insect resistance. such as proteinase inhibitors and lectins, are also being used in many experimental crops. The extensive testing on nontarget plant-feeding insects and beneficial species that has accompanied the long-term and wide-scale use of Bt plants has not detected significant adverse effects. GM plants expressing other insect-resistant proteins that have a broader spectrum of activity have been tested on only a limited number of nontarget species. Little is known about the persistence of transgene-derived proteins in soil. with the exception of Bt endotoxins, which can persist in soil for several months. Bt plants appear to have little impact on soil biota such as earthworms, collembolans. and general soil microflora. Further research is required on the effects of GM plants on soil processes such as decomposition. Assessment of nontarget impacts is an essential part of the riskassessment process for insect-resistant GM plants.
http://dx.doi.org/10.1146/annurev.ento.50.071803.130352

Pham-Delègue MH, Jouanin L, Sandoz JC 2002. Direct and indirect effects of genetically modified plants on the honey bee. *Honey bees: estimating the environmental impact of chemicals p.312-326, 2002. Editors: Devillers, J.; Pham-Delègue, M. H. Publisher: Taylor & Francis, London, UK*

In this chapter we consider genetically modified (GM) oilseed rape-honey bee interactions, and some factors that could affect plant attractiveness to bees. We report observations on the foraging behaviour of honey bees in situations of choice between GM oilseed rape expressing different genes and untransformed ones. Studies were conducted under controlled, semi-field, and field conditions, and no differential behaviour was found between GM and control genotypes. To evaluate the risk of direct exposure, we investigated the amounts of gene products expressed in nectar and pollen. In the plant material under test, no transgene proteins were detected, which indicates that the risk of exposure to the proteins is reduced. Differences were found between GM and control genotypes in nectar and floral odour composition. However, it was shown that foragers did not discriminate among the genotypes, and that they could learn the olfactory signals from GM plants as well as from control plants. From these studies, it appears that even though the bees can be exposed to the gene products or subjected to secondary changes in the plant chemistry, these changes do not lead to noticeable modifications in the behaviour of the honey bee for the genotypes tested.

Pierre J, Marsault D, Genecque E, Renard M, Champolivier J, Pham-Delègue MH 2003. Effects of herbicide-tolerant transgenic oilseed rape genotypes on honey bees and other pollinating insects under field conditions. *Entomologia Experimentalis et Applicata* 108 (3): 159-168.

Field experiments were carried out to compare the diversity and density of pollinators foraging on two winter oilseed rape varieties, 'Falcon' and 'Artus', and their respective transgenic counterparts 'Falcon pat' and 'Artus LL', which are tolerant to glufosinate, a non-specific herbicide. The number of insects per 1000 available flowers was counted for the four main pollinators: honeybees, bumblebees, solitary bees, and Diptera. Additionally, on 'Falcon'/'Falcon pat' the foraging behaviour of the main pollinating insects, i.e., honeybees and bumblebees, was observed (number of flowers visited per min, foraging postures, intervariety flights). A pleiotropic effect could affect nectar and pollen production, therefore we measured nectar volume, nectar sugar concentration, and composition for the four plant genotypes, and on 'Falcon' and 'Falcon pat' pollen production was also estimated. The diversity and density of the foraging insect population observed on the transgenic genotypes were similar to that on the non-transgenic controls. Moreover, the foraging behaviour strategy was similar on 'Falcon' and 'Falcon pat', and honeybees flew indifferently across these two genotypes. No difference was found in nectar and pollen between the genotypes. Thus, we may assume that the insects do not discriminate between conventional and transgenic oilseed rape specifically resistant to glufosinate. A case-by-case approach is recommended for other genetic modifications to plants potentially visited by bees.
http://dx.doi.org/10.1146/10.1046/j.1570-7458.2003.00081.x

Ramirez-Romero R, Josette Chaufaux J , Pham-Delègue MH 2005. Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee Apis mellifera, a comparative approach. *Apidologie* 36:601–611.

In a comparative approach, we evaluated the effects of Cry1Ab protoxin, deltamethrin and imidacloprid insecticides on mortality, syrup consumption, foraging activity and olfactory learning capacities of free-flying honeybees. In an indoor flight cage we exposed bee colonies to different syrups containing Cry1Ab protoxin, deltamethrin or imidacloprid at 1000 µg/kg, 500 µg/kg and 48 µg/kg, respectively. Cry1Ab did not affect mortality, syrup consumption or learning capacities. However, foraging activity was reduced during and after the treatment. Deltamethrin and imidacloprid both affected syrup consumption and foraging activity. Deltamethrin also induced a reduction in learning capacities. With the tested concentrations, our study suggests that for honeybees, synthetic insecticides such as deltamethrin may induce a greater hazard than Cry1Ab protein, potentially expressed in Bt corn pollen at concentrations lower than 1000 µg/kg.
http://dx.doi.org/10.1051/apido:2005039

Roy DB, Bohan DA, Haughton AJ, Hill MO, Osborne JL, Clark SJ, Perry JN, Rothery P, Scott RJ, Brooks DR, Champion GT, Hawes C, Heard, MS, Firbank LG. 2003. Invertebrates and vegetation of field margins adjacent to crops subject to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 358 (1439): 1879-1898.

The effects of management of genetically modified herbicide-tolerant (GMHT) crops on adjacent field margins were assessed for 59 maize, 66 beet and 67 spring oilseed rape sites. Fields were split into halves, one being sown with a GMHT crop and the other with the equivalent conventional non-GMHT crop. Margin vegetation was recorded in three components of the field margins. Most differences were in the tilled area, with fewer smaller effects mirroring them in the verge and boundary. In spring oilseed rape fields, the cover, flowering and seeding of plants were 25%, 44% and 39% lower, respectively, in the GMHT uncropped tilled margins. Similarly, for beet, flowering and seeding were 34% and 39% lower, respectively, in the GMHT margins. For maize, the effect was reversed, with plant cover and flowering 28% and 67% greater, respectively, in the GMHT half. Effects on butterflies mirrored these vegetation effects, with 24% fewer butterflies in margins of GMHT spring oilseed rape. The likely cause is the lower nectar supply in GMHT tilled margins and crop edges. Few large treatment differences were found for bees, gastropods or other invertebrates. Scorching of vegetation by herbicide-spray drift was on average 1.6% on verges beside conventional crops and 3.7% beside GMHT crops, the difference being significant for all three crops.
http://dx.doi.org/10.1098/rstb.2003.1404 (full text PDF available)

Sanvido O, Stark M, Romeis J , Bigler F 2006. Ecological impacts of genetically modified crops. Experiences from ten years of experimental field research and commercial cultivation. *ART-Schriftenreihe Nr. 01, Agroscope Reckenholz-Tänikon Research Station ART,Switzerland*.

Excerpt from summary: "There are concerns that insect-resistant GM crops expressing Cry-proteins from Bacillus thuringiensis (Bt) could harm organisms other than the pest(s) targeted by the toxin. The published large-scale studies in Bt-crops assessing possible non-target effects on arthropods have only revealed subtle shifts in the arthropod community, which can be explained by a lack of the target pest resulting from the effective control by the Bt-crops. No adverse effects on non-target natural enemies resulting from direct toxicity of the expressed Bt-toxins have so far been observed in laboratory studies and in the field. There is evidence that the Bt-crops grown today are more target-specific and have fewer side effects on non-target organisms than most current insecticides used."
http://www.services.art.admin.ch/pdf/ART_SR_01_E.pdf (full text PDF available)

Sick M, Kuhne S, Hommel B 2004. Transgenic rape pollen in larval food of bees -- component of a model study on the probability of horizontal plant-to-bacteria gene transfer. [Original Title: Transgener Rapspollen in der Bienennahrung -- Teil einer Modelluntersuchung zur Wahrscheinlichkeit des horizontalen Gentransfers von Pflanzen auf Bakterien.] *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie* vol. 14:423-426

The potential of horizontal transfer of genes from pollen of herbicide-resistant rape (pat gene) to gut bacteria of bees was studied in field trials conducted at the Federal Biological Research Centre for Agriculture and Forestry (BBA Kleinmachnow, Germany) since 2001. Transgenic rape was planted on an area of 1200 m2 (4 plots; each 300 m2). As a part of a joint study with the Federal Research Centre for Agriculture (FAL Braunschweig), BBA Kleinmachnow determined the portion of transgenic rape pollen in bee food. To that end, 3 bee species were established in the field trials: honeybee (Apis mellifera), bumblebee (Bombus terrestris) and red mason bee (Osmia rufa syn. O. bicornis). Samples were taken at 3 stages of flowering, i.e. during early, full and late flower. Depending on the stage of flowering, between 60 and 95% of pollen loads of A. mellifera originated almost totally from rape, but transgenic rape was found only in approximately 10% of the samples. In B. terrestris, between 10 and 30% of samples consisted solely of rape, and another 10-20% contained rape seed pollen at different proportions. Transgenic rape pollen was detected in between 1 and 30% of these samples. The mason bee was the only species where brood cells were studied, and here rape pollen was found in 60 to 80% of the cells. Between 50 and 60% of brood cells contained transgenic rape pollen. QC-PCR is being used with pat-positive samples to determine the proportion of transgenic rape pollen in corbicular pollen of individual bees and in individual brood cells.

Velkov VV, Medvinsky AB, Sokolov MS, Marchenko AI 2005. Will transgenic plants adversely affect the environment?. *Journal of Biosciences* 30:515-548.

Transgenic insecticidal plants based on Bacillus thuringiensis (Bt) endotoxins, on proteinase inhibitors and on lectins, and transgenic herbicide tolerant plants are widely used in modern agriculture. The results of the studies on likelihood and non-likelihood of adverse effects of transgenic plants on the environment including: (i) effects on nontarget species; (ii) invasiveness; (iii) potential for transgenes to 'escape' into the environment by horizontal gene transfer; and (iv) adverse effects on soil biota are reviewed. In general, it seems that large-scale implementation of transgenic insecticidal and herbicide tolerant plants do not display considerable negative effects on the environments and, moreover, at least some transgenic plants can improve the corresponding environments and human health because their production considerably reduces the load of chemical insecticides and herbicides.
http://www.ias.ac.in/jbiosci/sep2005/515.pdf (full text PDF available)