Possible Effects of Genetically Modified Plants on Insects in the Plant Food Web

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Abstract: During the last years, there has been increasing focus on the environmental effects of genetically modified plants, not only hybridization and gene flow, but also effects on insects. A general overview of possible effects of genetically modified plants on insects is presented. Insects from different levels of the plant food web are included: herbivores (pests and non-pests), pollinators, predators/parasitoids and decomposers. Each relationship is illustrated by current research and discussions.

Key words: genetically modified plants, insects, food web, aphids, parasitoids.

Introduction

About 10 years ago, the first genetically modified plant (gm plant) was commercially available, the tomato Flavr Savr in U.S.A. According to a new ISAAA (International Service for the Acquisition of Agro-biotech Applications) report, the situation in 2002 has been radically changed:

- 587 000 km^2 is planted with gm plants, engaging about 6 million farmers. This is an increase from 2000 of about 34% in km^2 and 71% in number of farmers.
- About 62% of the gm area had herbicide tolerant soya and about 13% had Bt-maize. In addition gm cotton and gm oil seed rape (canola) are commercially important.
- 99% of the commercially produced gm plants are grown in 4 countries: USA (66%), Argentina, Canada and China. Europe has until now refused to release gm plants commercially, with a moratorium since 1998. Small-scale field trials (research) are nevertheless allowed in most countries, but not in Norway.

The gm plants produced for commercial use so far is called first-generation gm crops. They have been design for agronomic benefit, i.e. the farmer and the agrobiotech multinational companies have been the main beneficiaries. Two main type of gm plants, or a combination of the two, have until now been available commercially:

- Herbicide resistant plants. The plants tolerate herbicides. Examples are glyphosate resistant plants of soya, oilseed rape, cotton and maize.
- Insect resistant plants. They are constructed to reduce insect pests.

The commercially available ones produce a toxic Cry protein, due to an inserted gene from the bacterium *Bacillus thuringiensis* (Bt plants). The toxin kills insect species within some taxonomic groups, e.g. Lepidoptera and Coleoptera. Examples are Bt cotton against tobacco and cotton budworms, Bt maize (=corn) against European corn borer and Bt potato against the Colorado beetle.

Included in insect resistant gm plants are also plants that produce non-toxic substances that nevertheless make them less digestible for phytophagous insects, mainly Lepidoptera, Diptera, Coleoptera and Homoptera. Examples are plants containing lectins, protease-inhibitors, alfaamylase inhibitors or plant enzymes. Such plants are not commercially available yet. Examples are lectin plants of potato and tobacco.

Some, but not all, gm-crops have an increase in yield and a reduction in herbicide or insecticide use. However, many of the figures are disputed

(Slater et al. 2003).

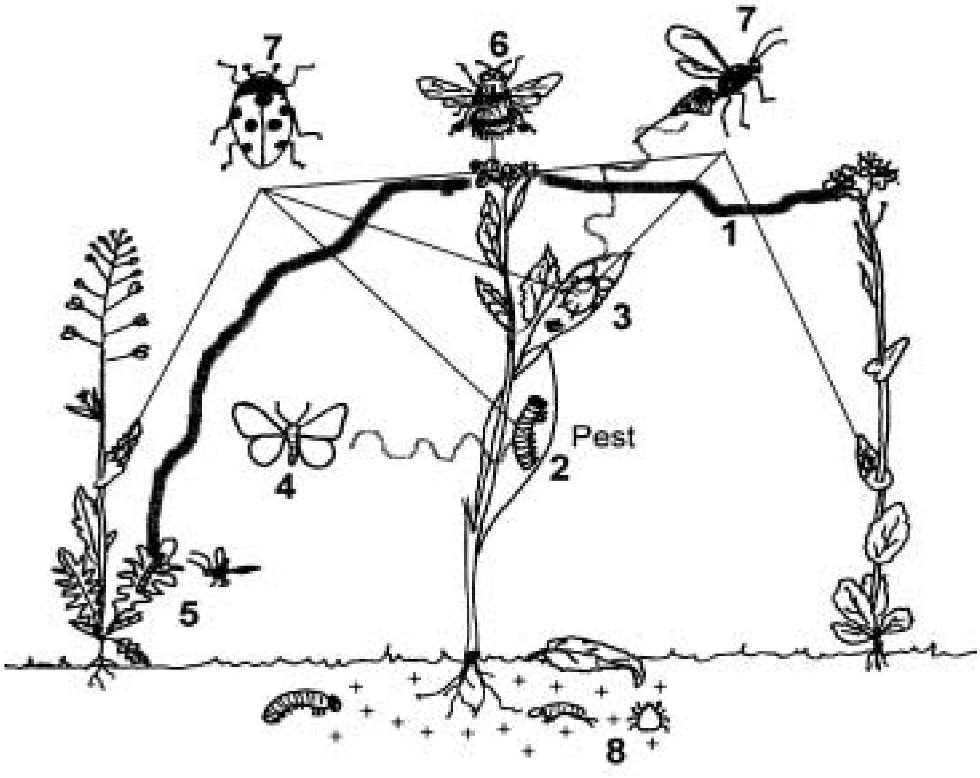
Potential effects of gm plants on the environment have been seriously discussed for some years, particularly the possibility of hybridization between the gm plants and their wild relatives, and also horizontal (non-sexual) gene transfer. Only the last years has concern about ecological effects in the plant food web been raised. Insects are often key organisms in such food webs, with herbivores (including pollen eaters), carnivores and decomposers. Wolfenbarger and Phifer (2000) give a review paper on ecological risks and benefits of gm plants.

Commercial companies, like Monsanto and Syngenta, develop the gm plants and have the patent rights on them. When they apply for commercial release of such plants into the fields in Europe, they must enclose documentation, or risk analysis, showing that the plants do not harm the environment. Such "documentation" has until now been very poor, or even absent with respect to insects (Ulltveit-Moe, 2001). An international working group, initiated by dr. Angelica Hilbeck in Switzerland, are now working to construct guidelines for such obligatory pre-testing (GMO Guideline Project, IOBC Global Working Group "Transgenic organisms in IPM and Biocontrol"). One of the 5 main topics is non-target and biodiversity effect.

The present paper gives a rough overview of some relationships between gm plants and insects in the food web. Such a simplified web may also be a relevant basis for a pre-testing design. For each relationship, examples from current research are indicated. Some preliminary results from our own laboratory experiments are also presented, to exemplify pre-testing studies.

General environmental effects

Figure 1 gives a presentation of some main links in the food web of a gm plant. For simplicity, one could imagine a Bt-plant, constructed to kill an insect pest (number 2 in fig.1). A wild relative of the gm plant and a not related wild plant also grow within the area. The numbers below refer to the numbers in figure1.



Not relative GM-plant Wild relative

Figure 1. Potential effects of genetically modified plants (in the middle, e.g. Bt plants) on insects and mites at different trophic levels: 1 – hybridization/invasion; 2 – resistance; 3 – secondary pests; 4 – new pests; 5 – reduced biodiversity; 6 - pollinators affected; 7 - natural enemies affected; 8 – soil organisms affected; + - Bt poison.

1. The gm plant: Hybridization with wild relative or spreading.

Closely related species may hybridize. The related species may be a wild herb. Swedish and Danish researchers are studying the natural gene flow between the cultivated B*rassica napus* (oilseed rape) and its wild herb relatives, like the weedy *Brassica rapa*, wild radish and wild mustard (Ekbom and Jørgensen, pers. com). If genes for herbicide tolerance thus are transferred to wild weed relatives, a "superweeed" may result. However, the wild relative could, rather than be a weed, be the wild origin of important human food plants. Bio-contamination of such wild, ecologically suited varieties with stray genes from their gm-relatives is a major concern in the tropics. Recently, wild maize in Mexico seems contaminated by genes from Bt-maize, although these findings are heavily debated in Nature and other (Mellen, 2003).
The gm plants themselves may also spread and become «superweeds»in a new crop environment. This has occurred in Canada, where various gm-strains with resistance for different herbicides have hybridized to multiresistant canola and thereafter spread into wheat fields, becoming an herbicide tolerant weed here. Infestation of gm plants into other fields represents also a great problem for farmers that grow ecologically and do not allow gm plants. Swedish researchers are looking at the invasive potential of gm oilseed rape, expressing lectin in the anthers to reduce the attack of the pollen beetle (*Meligethes aeneus*) (Ekbom, pers. com.).

2. Insect pests develop resistance to the gm-trait.

It was to be expected that the insect pests will develop resistance to Bt poison even faster than to insecticides, because the insects are exposed to the poison throughout the plant's life, or even longer.
There is agreement that insect resistance will be a major problem, as with lepidopteran pests (e.g. *Ostrinia nubialis*) on Bt maize and Bt cotton.
The "solution" until now has been to delay resistance development by planting a certain, and increasing, part of the cultivated area with the not- modified variety. For some cultures up to 50% has been proposed. In such cases, one may question the benefit of the method. Also the genetic assumptions have been questioned (is the resistance gene recessive or dominant?).
Another recent problem is that some resistant insects (i.e. *Plutella xylostella*) may have increased fitness on gm plants by using the toxin as a supplementary food protein (Sayyed et al., 2003).

3. The technology may result in secondary pests.

Not all herbivores are susceptible to e.g. Bt-plants. Secondary pests may result from decreased competition from the target pest. This is not a new problem, but should not be forgotten also in the gm debate.
The Potato leafhopper (*E. fabae*) takes over for the Colorado beetles in Bt potato fields in USA (Riddick et al., 1998), either because the leafhoppers are favoured by less spraying against the Colorado beetles, and / or less competition from the beetles.

4. Altered plant chemistry in gm-plants may result in new pests.

Gene modifications change the plant chemistry and the "new" plant may thus send out new signals (often volatiles) to potential pests that previously did not attack the plant seriously (or deterrent signals may be reduced).
Scientists in Germany investigate whether terpene signals will change as a result of the gene modification in Bt maize, with possible effects on potential pests among Lepidoptera.
Recently, gm lectin potato plants have shown reduced levels of bitter-tasting chemicals, making the plants more vulnerable to a range of insect pests, including the potato leafhopper. The reduced level may actually stimulate the potato aphid to feed (Birch et al., 2002).

5. Reduced biodiversity.

Until now, the gm trait has been expressed in all or most parts of the plant, including in the pollen.

Vulnerable or threatened phytophagous non-target insect species may happen to eat poisonous gm-pollen, for instance blown onto their host plants. Both such herbivores and thereby their specialist natural enemies, like parasitoids, may be victims, and in worst case will become extinct if populations are reduced below a certain threshold.

An illustration is a famous and much discussed paper in Nature in 1999, where larvae of the monarch butterfly died by eating pollen from Bt-maize. The concern is that such pollen may infest the leaves of their host plant, the milkweed, and that the leaf eating larvae may by chance also ingest poisonous pollen (Losey et al., 1999). The heavy discussion of Losey's paper stimulated to further studies on the real exposure such pollen may have in the larval habitat of the butterfly. The plant strain is also important, since different strains express different degree of poison, which also can be differently allocated among plant parts.

The large Farm-Scale Evaluation project (FSE) currently conducted in United Kingdom will compare biodiversity in herbicide tolerant gm-crops and conventional crops of beet, oilseed rape and maize (Firbank et al., 2003).

6. Pollinators are affected.

Insects pollinate 80% of the plants that humans grow for their own use.

GM- pollen may harm pollinators like honeybees, bumblebees and lepidopterans. Also many predators and parasitoids eat pollen (syrphids, coccinellids, lacewings, parasitic wasps etc).

Surprisingly few reliable studies have been published on the effect of gm-pollen on pollen eating insects. Some studies on adult honeybees found no effects of protease inhibitors in the pollen (Girand et al., 1998) or Bt toxin (Malone et al., 2001). Others found some concentration-dependent negative effects of protease inhibitors on adult honeybees (Burgess et al. 1996, Picard-Nizou et al., 1997; Pham-Delegue et al., 2000; Malone et al., 2001). Danish and Swedish researchers are now looking at the effect of lectin pollen from transgenicoilseed rape on honeybee larvae (Ekbom, pers. com.).

Honeybees may be affected in other ways: It is claimed that German scientists in 2000 showed that genetic material from gm canola had been transferred to bacteria in the gut of the honeybee, i.e. horizontal gene transfer had occurred (ref.: http://www.beekeeping.com/articles/afb_gm.htm)

For the future, one should generally avoid that the gm-trait is expressed in the pollen or nectar, although such plants may be efficient against pollen eating pests. A new technique, using modified chloroplast-DNA, is believed to eliminate or reduce the gene-expression in the pollen, since chloroplasts are normally inherited maternally (Slater et al., 2003). However, although very infrequent, transfer of DNA between chloroplasts and the nucleus, and thereby to the pollen, have recently been demonstrated in tobacco (Huang et al., 2003).

7. Natural enemies are affected.

Both polyphagous insects (many predators) and specialists (many parasitoids) may be affected. There can be effects on vulnerable or threatened species, and on important natural enemies, with reduced effect on pests as a result. Two types of effects are possible:

Direct effect: Changed plant signals/chemistry disturb the host location process of the natural enemy. Such topics are involved in the international EU-project: "Effects and mechanisms of Bt transgenes on biodiversity of non-target insects: pollinators, herbivores and their natural enemies" (Bt-BioNo Ta). One study looks for instance on the effects of volatiles from infested Bt maize and oilseed rape on parasitoid behaviour by use of wind tunnels and electroantennograms.

Indirect effect: Eating prey containing substances derived from the gm plant affects natural enemies. Not only susceptible, dying prey may be eaten, which can harm the predators, but probably more seriously also non-susceptible prey that nevertheless contain «new» material from the gm plants. Some studies show no harmful effect on predators /parasitoids, others show reduced survival, developmental rates and fecundity when natural enemies eat healthy herbivores from gm-plants. The latter case has for instance been shown on lacewings (*Chrysoperla carnea*) eating lepidopterous larvae (*Spodoptera littoralis*) on Bt-maize (Hilbeck et al., 1998, Dutton et al., 2002) and on the 2- spot ladybirds eating healthy aphids on lectin potatoes (Birch et al., 1999). However, Down et al. (2003) recently found less effect on this ladybird, the discrepancies being partly due to methodological differences and different GNA levels in the two studies.

Also host products like honeydew may contain the transgene product, and can thus have effects on the large number of insects eating honeydew. Such effects seem species specific (Romeis et al., 2003).

Looking for effects on non-targets, case-by-case studies are obviously necessary.

Preliminary results from our tritrophic studies on lectin potatoes with *Myzus persicae* and *Aphidius colemani* show no distinct effects of the gene manipulation, neither on aphid fecundity, mortality and population development, nor on parasitoid colonization behaviour on infested and un-infested plants. However, more replicates are needed to verify this, supplied with other types of experiments as well.

Many studies, like ours, are done in the laboratory, feeding the natural enemies with herbivores from the gm plants and from control (not gm) plants. However, searching behaviour of the natural enemy in the field is also important. The study of Schuler et al. (1999) is an example, showing that parasitoids in Bt oilseed rape fields prefer to oviposit in healthy larvae of the diamond moth (*Plutella xylostella*), i.e. in larvae on non- modified plants or in Bt-resistant larvae, rather than in susceptible larvae on Bt plants.

8. Soil-living organisms, including decomposers, are affected.

PSRAST (2001) give a broad discussion on the current knowledge of non-target effects of gm-plants on soil microbes. Exudates from leaves and roots on Bt plants continue long after the plant has wilted, more than 200 days is noted (Zwahlen et al., 2003). There is a possibility of horizontal transfer of transgenic vector genes from transgenic crops to soil microorganisms, but increase knowledge is necessary to demonstrate nontarget effects in the soil ecosystem.

Cowgill et al. (2002) found that cystatins against nematode pests had some effects on non-targets like bacteria and fungi in the potato rhizosphere, but during the two years the soil functioning was not affected.

Studies should be performed on other soil-living organisms as well, e.g. effect of Bt plants on Lepidoptera larvae or pupae.

Concluding remarks

To avoid at least the most obvious risks on insects by releasing gm plants, there is international agreement that we need:

- Case-by-case studies.
- Study the sublethal effects over several generations, not only spontaneous or acute lethal effects
- Three-tiered risk assessment (Poppy, 2000):

1. Laboratory studies: "worst case scenario", small scale
2. Semi-field studies: field cages, glasshouses, population scale
3. Field studies: large scale.

- A certain minimum number of tests on each step.
- Use the gm plant itself in tests, not only the chemical products (e.g. toxin).
- Indicator species should at least be chosen among rare species, pollinators. natural enemies (both predators and parasitoids of both target and non-target pests), host related non-target species.
- Compare risk with other available agricultural practices (conventional, IPM, ecological etc)

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