

Can biological control benefit from genetically-modified crops? Tritrophic interactions on insect-resistant transgenic plants

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Abstract. The use of recombinant DNA technology to develop genetically-modified crops is considered as a major breakthrough for agriculture by many scientists. However, some scientists, and an even larger proportion of the general public, are sceptical about the advantages and are even more concerned about the potential risk of this new technology. To evaluate this novel technology, cost-benefit analyses are needed in which the real risks are measured and judged against the benefits. A tiered risk assessment scheme is described herein. This allows comparisons to be made with other insect-control technologies (e.g. insecticides) and risks to be determined, rather than only hazards being identified. Recombinant DNA technology could allow plants to be designed that are well suited for use alongside biological control programmes. Unfortunately, plant breeders have continued to attempt to breed for total resistance, and biocontrol specialists have ignored the role of the plant in ensuring successful foraging behaviour by insect natural enemies. Although some scientists have highlighted the need to consider both the bottom-up (plant defence) and top-down (biocontrol) control of insect pests, there have been few serious attempts to combine these approaches. As more is understood about the proximate and ultimate causes of direct and indirect defences, the potential exists for engineering plants that combine both strategies. This new possibility for controlling insect pests, which will combine both 'nature's' own defences with man's ingenuity, may stack the odds in our favour in the continual struggle against insect pests.

Key words. *Bacillus thuringiensis*, hazard, inducible signals, natural enemies, partial resistance, physically-mediated interactions, risk, tiered risk assessment.

Introduction

The use of recombinant DNA technology to develop transgenic or genetically-modified (GM) crops is regarded, by many scientists, as a significant breakthrough for food production. However, a majority of the general public remain doubtful about the advantages and are concerned about the potential risks of this new technology (Crawley, 1999;

Poppy, 2000; Wolfenbarger & Phifer, 2000). To evaluate this new technology, cost-benefit analyses in which the real risks are measured and judged against the real benefits are needed. In this review, a risk assessment scheme will be described that adopts a tiered approach and thus allows comparisons to be made with existing approaches to pest management (e.g. insecticides). Thus, risks may be assessed in a quantitative fashion rather than qualitative hazard identification. In addition, the use of recombinant DNA technology to design plants that are well suited for use alongside biological control programmes is described.

Crop plants and insect pests are part of a complex agricultural ecosystem that involves interactions between many trophic levels, often referred to as multitrophic interactions

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(Poppy, 1997). Unfortunately, plant breeders have continued to attempt to breed for total resistance, whereas biocontrol specialists have ignored the role of the plant in ensuring successful foraging behaviour by insect natural enemies. Although some scientists, notably Agrawal (2000) and Cortesero *et al.* (2000), have highlighted the need to consider both the bottom-up (plant defence) and top-down (biological control) management of insect pests, there have been too few serious attempts at combining these approaches, despite a seminal study of the importance of considering tritrophic interactions over two decades ago (Price *et al.*, 1980). As more is understood about the proximate and ultimate function of direct defences (e.g. defence chemicals, trichomes, etc.) and indirect defences (e.g. recruitment of natural enemies), the potential exists for engineering plants that synergize both these defence strategies.

Conventional outlook: assessing the ecological threat

In this section, the conventional view of the effects of GM plants on biological control will be considered. This will be from a risk assessment perspective, primarily focusing on the risks of GM plant to nontarget natural enemies (predators and parasitoids).

The ability to engineer plants containing genes encoding insecticidal proteins offers numerous and obvious benefits. However, as with the conventional insecticide treatments, this new technology has the potential to disrupt biological control through both direct and indirect effects. The interactions between transgenic plants and these beneficial insects need to be assessed via a robust and reliable risk assessment to avoid future problems (Schuler *et al.*, 1999a).

It is important to clarify what is meant by 'risk' before discussing an appropriate assessment scheme for GM plants. All too often, the words risk and hazard are confused, and this can lead to considerable hysteria and panic. A hazard is defined as a situation that, in particular circumstances, could lead to harm, whereas risk is a combination of the probability of occurrence of a defined hazard and the magnitude of the consequences of the occurrence (Department of the Environment, 1995). Identification of a hazard is an essential component of risk assessment but, to quantify the risk, the likelihood of the hazard being realized needs to be determined. For example, if a pollen is identified that kills monarch butterfly larvae, *Danaus plexippus* L., the probability that the larvae will be exposed to toxic quantities of pollen also needs to be assessed. Confusion between hazard and risk led to well-publicised studies suggesting that pollen from transgenic maize could create an environmental catastrophe (Losey *et al.*, 1999). However, the view that risks are not perceived in this fashion by the general public should not be overlooked. Thus, because the hazards linked with GM are so outrageous, the perceived risks are too great for acceptance of the technology. These ideas, discussed by Professor Peter Sandman, are possibly the reason why newspapers, pressure groups and politicians

tend to react to the outrage of the hazard rather than more calmly to the potential risk (Sandman, 1988) (Fig. 1). However, in this review, scientific risk assessment and how it can be used for GM crops will be strictly adhered to. Where appropriate, the broader issues will be alluded to, but the more complex debates about risk and the precautionary principles remain beyond the remit of this review.

Tiered risk assessment

The principal advantage of transgenic plants over conventional insecticides is the high specificity of the insecticidal proteins conferred, such that effects on nontarget insects should theoretically be minimal (MacIntosh *et al.*, 1990). Current commercial insect resistant GM plants rely on the production of toxins derived from the bacterium *Bacillus thuringiensis* Berliner (*Bt*) and, as such, are only resistant to a limited number of insects because of the high degree of specificity of *Bt*. These include European corn borer, *Ostrinia nubilalis* (Hübner), corn earworm, *Heliothis virescens* (F) and south-western corn borer, *Diatraea grandiosella* Dyar and, recently, the corn rootworm complex *Diabrotica* spp. in maize; Colorado potato beetle, *Leptinotarsa decemlineata* (Say) in potato crops; and tobacco budworm, *Heliothis virescens* (F) and cotton bollworm, *Helicoverpa armigera* (Hübner) in cotton. Theoretically, this should limit the exposure of natural enemies to the proteins in contrast to broad-spectrum insecticides, which are toxic to both target and nontarget organisms (Schuler *et al.*, 1998).

Another advantage relates to the reduced environmental exposure. Only those insects actually consuming parts of the plant expressing the protein are exposed, reducing accidental exposure as typically occurs with conventional insecticide spraying. Thus, it could be argued that the overall risk should be low due to a reduced hazard and a reduced likelihood of

RISK = Hazard × Probability

In scientific terms risk is not only in the nature of the hazard but also the probability of this hazard occurring. Thus, travel by aeroplanes may be the most hazardous form of travel but offers the lowest risk.

RISK = Hazard × Outrage

According to Sandman (1988), risks are perceived by the public as the nature of the hazard and how outrageous this hazard is.

Note that the probability of the hazard occurring is not represented in this simple equation which is thought to be more in tune with how risks are perceived.

Fig. 1. The different perceptions of risk according to the scientific and the public viewpoint. Adapted with permission (Sandman, 1988).

exposure. However, risks need to be quantified in a rigorous and robust assessment to ensure that both direct and indirect effects are assessed. For the possible effects on nontarget organisms such as parasitoids and predatory insects, a tiered risk assessment similar to that used in insecticide toxicity testing is advocated for evaluating risks associated with GM crops (Poppy, 2000). This is schematically represented in Fig. 2. A principle advantage of a tiered approach is that the use of relatively simple bioassays can determine a 'worst case scenario' and thus detect 'gross' affects at early stage in the development of a GM plant. Such a system thus combines economics with environmental safety and allows a way forward that can combine these two, often opposing, factors. Any effects observed on individuals during first-tier tests need to be related to population effects, which can be studied at the higher tiers. An example of this would be the detection of a slight reduction in fecundity of individual female insects in a first-tier study. The critical question relates to whether this has any effect on the population dynamics of these insects when more realistic spatial-temporal and environmental fac-

tors are introduced at higher tiers. Obviously, if the hazard observed to individuals at the first-tier is so great then the GM crop can either be abandoned at that stage, or larger-scale extended-laboratory trials can be conducted within the laboratory environment, which many consider as second-tier experimentation. The use of laboratory and semifield testing can assist in the design of field trials that are both difficult and expensive. Finally, tiered testing allows direct comparisons to be made against conventional pesticides because the methods are comparable to those used in pesticide ecotoxicology (Denholm *et al.*, 1998). Although not formally part of the risk assessment scheme for GM plants (regulators need to consider only risks and not the benefits accrued by using one technology as opposed to another), such comparison is a useful tool, both for scientists and the public, when considering the risks and benefits of the technology.

First-tier studies

First-tier studies invariably involve 'worst-case scenario' experiments whereby the test organisms are given no choice as to whether or not they feed on the transgenic plant material. Providing that these studies are part of a tiered assessment, such hazard identification does have enormous value because of the importance of initially detecting any direct effects of the foreign protein on any stage of the nontarget insect. For example, if a predator or parasitoid should ingest pollen, nectar and or honeydew produced by or on the GM crop, does mortality increase? Conclusions about risk cannot be based entirely on the results of first-tier investigations. Although not directly related to biocontrol issues, no-choice and unrealistic dosing scenarios led to conclusions that pollen from *Bt* maize may pose risks to the larvae of monarch butterfly (Losey *et al.*, 1999; Niiler, 1999). Important lessons must be learnt from this for all risk assessment of GM crops to nontarget organisms; namely, that it is not possible to draw conclusions about these risks to nontarget populations in the field based entirely on the initial results of laboratory findings. In response to these concerns, a comprehensive risk assessment was carried out on exposure of monarch butterflies to transgenic maize pollen. The data indicate that, although *Bt* maize pollen can be toxic to early larval instars (Hellmich *et al.*, 2001), any exposure adjacent to maize fields is likely to be negligible (Sears *et al.*, 2001). Gatehouse *et al.* (2002) produced a comprehensive review of the findings of these and other studies and demonstrated that *Bt*-expressing maize posed little risk to other nontarget insects, including beneficial insects such as pollinators and natural enemies.

In addition, 'worst-case scenario' experiments demonstrated that, when the generalist predator green lacewing larvae *Chrysoperla carnea* (Stephens) were fed *Bt*-fed *Spodoptera littoralis* (Boisduval) and *O. nubilalis*, this resulted in higher mortality relative to controls (Hilbeck *et al.*, 1998a). Furthermore, direct toxicity to *C. carnea* larvae was reported when *Bt* toxins were incorporated in

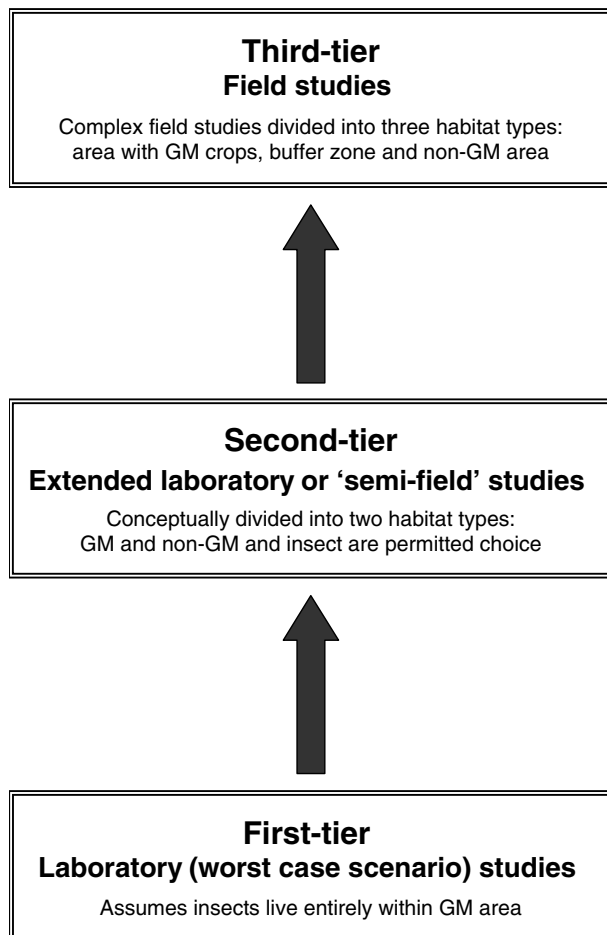


Fig. 2. Structure of a possible three tiered risk assessment for genetically-modified (GM) crops. This scheme is in the style of the Environmental Protection Agency testing of agrochemicals, thus allowing comparative risk assessment.

an artificial diet (Hilbeck *et al.*, 1998b). However, the validity of these studies is questionable because the lacewings were 'forced' to consume nonpreferred prey (Poppy, 2000), thus representing a suboptimal diet that could lead to undesirable effects either being magnified or actually occurring in the first place. Recent work by the same scientists showed that the lacewings preferentially feed on aphids when offered a choice of insects feeding on *Bt* (Meier & Hilbeck, 2001). Because the aphids are not ingesting the *Bt* or being affected by the *Bt*, there would likely be no direct or indirect effect of *Bt* maize on lacewings in higher tier experiments or first-tier experiments that offer a choice of diets.

In a study of the effects of snowdrop lectin, *Galanthus nivalis* agglutinin (GNA), on ladybirds, Down *et al.* (2000, 2003) revealed that small effects on development were observed. Ladybird larvae fed GNA-containing aphids were found to be smaller than controls, but ate 40% more aphids. GNA did not have direct toxic effects on developing ladybird larvae, but the effects observed may be due to the the GNA-fed aphids representing a suboptimal diet. This highlights the need to discriminate between indirect and direct effects when studying tritrophic interactions (Ferry *et al.*, 2003) and the need to consider behavioural factors because they alter the probability of exposure.

The effect of GNA in transgenic potato plants on a parasitoid of the tomato moth, *Lacanobia oleracea* L., was also investigated. The ability of *Eulophus pennicornis* (Nees) to parasitize the moth larvae was not altered by the presence of GNA in the diet of the host (Bell *et al.*, 1999, 2001). Also in first-tier experiments, Couty *et al.* (2001a) found that the aphid parasitoid, *Aphelinus abdominalis* (Dalman), excretes most of the GNA ingested. Sublethal effects of GNA were also studied and no detrimental effect was observed. However, GNA had an indirect host-size-mediated effect on the sex ratio and the size of parasitoids developing in GNA-fed aphids (Couty *et al.*, 2001a). These experiments demonstrate the power of first-tier bioassays in elucidating the importance of direct and indirect effects in determining cause and effect (Couty & Poppy, 2001; Couty *et al.*, 2001a,b,c), which are essential for making informed judgements about a new technology. They also demonstrate that, by including appropriate biological factors into simplistic assays, much more powerful ideas can be developed for assessing in higher tiers.

First-tier testing by Schuler *et al.* (1999b,c) showed that mortality of *Cotesia plutellae* Kurdjumov, a parasitoid of the diamondback moth *Plutella xylostella* (L.), was almost 100% when developing on oilseed rape expressing the *Bt* transgene. If inferences were drawn from this alone, then it would be assumed that the transgenic plant affects the parasitoid directly. However, the parasitoids died owing to insufficient time to complete their development within *P. xylostella* larvae reared on *Bt* oilseed rape because the host died as a result of the toxin. However, when *Bt*-resistant diamondback moths were used in similar trials, there was no difference between parasitoid mortality developing on *P. xylostella* feeding on wild-type oilseed rape and the GM

oilseed rape (Schuler *et al.*, 1999c). This illustrates the need to link any effects with their precise cause. In this example, the host insect was killed by the plant and, in effect, this means that the plant has been successfully engineered for insect-resistance.

Second-tier studies

By increasing the spatial and temporal complexity of the bioassays, second-tier studies invariably allow observations at the population level. They are typically conducted in an extended laboratory-type setting, glasshouse or an 'enclosed cage' within a larger field. They offer an intermediate scale of risk assessment between 'worst-case scenario' testing and full-scale field testing. Their design should allow the investigation of the effects of transgene expression in the crop plant on populations of insects.

In the previously mentioned study by Schuler *et al.* (1999c), second-tier testing in a wind tunnel demonstrated that *C. plutellae* parasitoids selectively oviposited in *Bt*-resistant *P. xylostella*, which has obvious implications for *Bt*-resistance management. The susceptible insects caused less damage to *Bt* oilseed rape plants than the resistant *P. xylostella*. Therefore, the host-plant volatiles that the parasitoids utilize for host location provided a stronger signal from the plants on which the *Bt*-resistant caterpillars were feeding. Experiments using mixtures of GM and wild-type oilseed rape plants in large cages showed no differences in parasitism rates of the aphid *Myzus persicae* (Sulzer) by the parasitoid *Diaeretiella rapae* (McIntosh) on GM and untransformed plants (Schuler *et al.*, 2001). In this study, two transgenic proteins were investigated, the δ -endotoxin Cry1Ac and the proteinase inhibitor oryzacystatin I (OC-I) from rice, neither of which had effects on the aphid host but, more importantly, had no deleterious effects on parasitoid emergence. These experiments are an important component in the risk assessment process because they provide a link between simple bioassays and field-scale experimentation.

Third-tier studies

The final tier in the risk assessment should comprise field trials, preferably conducted at a large scale over more than one season. The most notable example of these is the recently completed Farm Scale Evaluations (FSEs) of GM crops in the U.K. (Firbank *et al.*, 1999; Firbank *et al.*, 2003). This project principally focused on assessing the impacts on farmland biodiversity of changing management practice when using GM herbicide-tolerant (GMHT) beet, maize and oilseed rape. The monitoring and assessment of invertebrates was an essential component of the sampling because they were used as measures of ecosystem functioning, biomass for feeding higher trophic levels, keystone species and sensitive indicators of environmental change. This illustrates the importance of invertebrates, in particular

insects, in assessing the environmental impacts of GM crops, even if the plant has not been engineered to directly affect insects.

From data collected over 3 years in the FSEs, in general, weed biomass was reduced under GMHT management in both beet and oilseed rape, but increased in maize compared to conventional treatments. Not surprisingly, changes in weed resource availability had significant effects on higher trophic levels including natural enemies (Hawes *et al.*, 2003) because of the decreased abundance of host insects (i.e. herbivores). The GMHT treatments also had effects on the adjacent field margins, where there were reductions in both bee and butterfly abundance. A likely explanation for this is the reduced nectar availability in GMHT-tilled margins and crop edges (Roy *et al.*, 2003). This finding could also be highly significant for natural enemies because they too depend on nectar and pollen as alternative food resources (Jervis & Kidd, 1996). Possibly the most important outcome of the FSEs is the demonstration that invertebrate groups in agroecosystems, including natural enemies, are highly sensitive to changes in weed communities arising from altering management regimes, regardless of whether these are novel herbicides or GM crops (Hawes *et al.*, 2003).

Other field trials have also been undertaken to assess the potential impact of transgenic *Bt* maize in the U.S.A. on several beneficial insects, including coccinellids, chrysopids and anthocorids. There were no significant differences in the overall density of beneficial insect populations between *Bt* and non-*Bt* maize (Pilcher *et al.*, 1997). Other work using *Bt* maize has found no effect of the transgene protein on natural enemy populations (Orr & Landis, 1997). However, in one investigation by Wold *et al.* (2001), there was a trend for non-*Bt* treatments to have a higher number of *Coleomegilla maculata* (DeGeer) larvae than *Bt* treatments, although the authors emphasize that this may be due to relatively subtle population effects. Wilson *et al.* (1992) also provided field evidence that *Bt* cotton has no effect on chrysopids. For many of these studies, the sampling of beneficials was incidental to recording effects on pest species and sampling was only performed three or four times in a season. It is important that field-testing of GM crops is robust and does not depend upon incidental recordings of natural enemies or on 'snapshot sampling' (Crawley, 1999).

The ultimate aim of the tiered risk assessment is to provide some quantitative measure of the risk of the GM crop. It is also important to link any effect with a precise cause and to differentiate between direct and indirect effects. Although such points may appear semantic to those investigators who just want to know whether there is any effect or not, they are critical in not only assessing the crop, but also in deciding how to manage the crop and perhaps, most importantly, in allowing the public to judge the costs versus the benefits.

Applications in the new millennium

A principal advantage of using GM plants to manage insect pests is the potential for a significant reduction of insecti-

cide usage. The aim of integrated pest management (IPM) is to become less reliant upon synthetic insecticides, especially as a prophylactic measure. The use of *Bt* varieties in the U.S.A. has dramatically reduced the amount of chemical pesticides applied to cotton (Ferber, 1999). Naturally, this reduction should have immediate benefits for biocontrol by allowing greater numbers of natural enemies to survive. This has been reported in China, where GM cotton has a significantly higher abundance of parasitoids and predators than conventionally sprayed cotton (Cui & Xia, 2000).

Looking forward to the next 5 years, possibly the most important crop to have GM insect resistance incorporated would be rice, with some two billion people depending on it for their staple diet. Stem borers are a serious problem in rice in Asia, causing estimated losses of 2.3% of the total yield (Savary *et al.*, 2000). Yellow stem borer, *Scirpophaga incertulas* (Walker), and striped stem borer, *Chilo suppressalis* (Walker), are the major stem borer pests in Asia. Currently, the Philippines and China are testing *Bt* rice against several lepidopteran pests that are difficult to target with conventional insecticides (Shu *et al.*, 2000; Tu *et al.*, 2000). Effects of a transgenic *Bt* rice crop on higher trophic levels need to be fully realized before release. The effects on parasitoids that target stem borer should be investigated, although the most significant of these are egg parasitoids [e.g. *Tetrastichus schoenobii* Ferriere and *Telenomus rowani* (Gahan)] (Kim *et al.*, 1986), which are unlikely to be affected by a *Bt* toxin expressed in the plant. Care also needs to be taken to prevent gene introgression into red rice and wild rices (e.g. *Oryza nivara* and *O. rufipogon*), which could become 'superweeds' (Cohen *et al.*, 2000) should fitness-enhancing transgenes introgress into these species.

If comparative risk assessments were used in the above cases, then GM should be compared with conventional pest control and biocontrol. However, many may view GM technology as an expensive component in an IPM programme, particularly in developing countries where other components (i.e. farmer and extension training) should be improved. This illustrates the need to consider additional socio-economic factors when developing IPM programmes. Whether GM can be included in such systems remains to be seen because it is both costly to develop and expensive for the end-user. It is likely that the most important advancements using this technology remain poorly developed because of the poor investment in and/or return from the most significant pest problems in developing countries.

Beyond *Bt*: a new way of thinking in the post-*Bt* era

All currently available insect resistant GM plants are resistant to a limited number of herbivorous insects because of the high degree of pest specificity of *Bt*. Rather than depend solely on crop plants that confer insect resistance by the production of *Bt* toxins, there is the potential to use and boost the direct and indirect defences of the plant to enhance biological control. It is feasible to combine engineered crop plants with biological control. This section will

discuss those plant traits that may be advantageous to enhancing biocontrol and outline how recombinant DNA technology may help in this novel way of controlling insect pests. Plant characteristics that enhance the performance of biological control agents are discussed at length by Cortesero *et al.* (2000) and are summarized in Fig. 3. Ecological and agricultural implications are discussed by Agrawal (2000). Typically, conventional breeding has rarely selected for these traits and may have led to them being selected against. Several examples will be cited where conventional plant breeding has had significant direct or indirect effects on the fecundity, mortality and behaviour of natural enemies, thus illustrating that plant breeding and biological control should not be developed in isolation. Whereas, in previous breeding techniques, it has been difficult to select for the traits proposed, the precision of genetic engineering coupled with the knowledge obtained will make such strategies a reality.

Can we exploit herbivore inducible signals to natural enemies?

The use of biocontrol agents in arable crops has had limited success because of the problem of attracting and maintaining a high enough density of predators and parasitoids in the crop before the levels of the pest become

economically damaging. Natural enemies are known to respond to host-plant volatiles, particularly when herbivores are present. Consequently, by manipulation of herbivore-induced plant signals, it may be possible to attract predators and parasitoids to their prey and hosts (Vet & Dicke, 1992; Tumlinson *et al.*, 1993; Karban & Baldwin, 1997; Paré *et al.*, 1999).

Cotton is one of the model systems that has been well researched and both the biosynthetic pathways (Paré & Tumlinson, 1997a, 1997b) and elicitor (Paré *et al.*, 1998) have been elucidated. In the future, this could enable the the crop to be altered to respond more strongly or more quickly to herbivore attack. Recent research has also demonstrated the importance of these signals in cotton growing in the wild (De Moraes *et al.*, 1998). Interestingly, 'wild' naturalized cotton produces seven-fold more signal volatiles than commercial varieties (Loughrin *et al.*, 1995), although this is not the case with the wild relative of maize, teosinte (Gouinguéné *et al.*, 2001). Traditionally breeding wild cotton with modern cultivars is difficult, but it may be possible to genetically engineer the biosynthetic pathway responsible for enhanced signalling into the modern cultivars. Although there is some debate concerning the fitness benefits of plants signalling to higher trophic levels, recent work on wild *Nicotiana* by Kessler & Baldwin (2001) has clearly demonstrated the advantages of plants indirectly

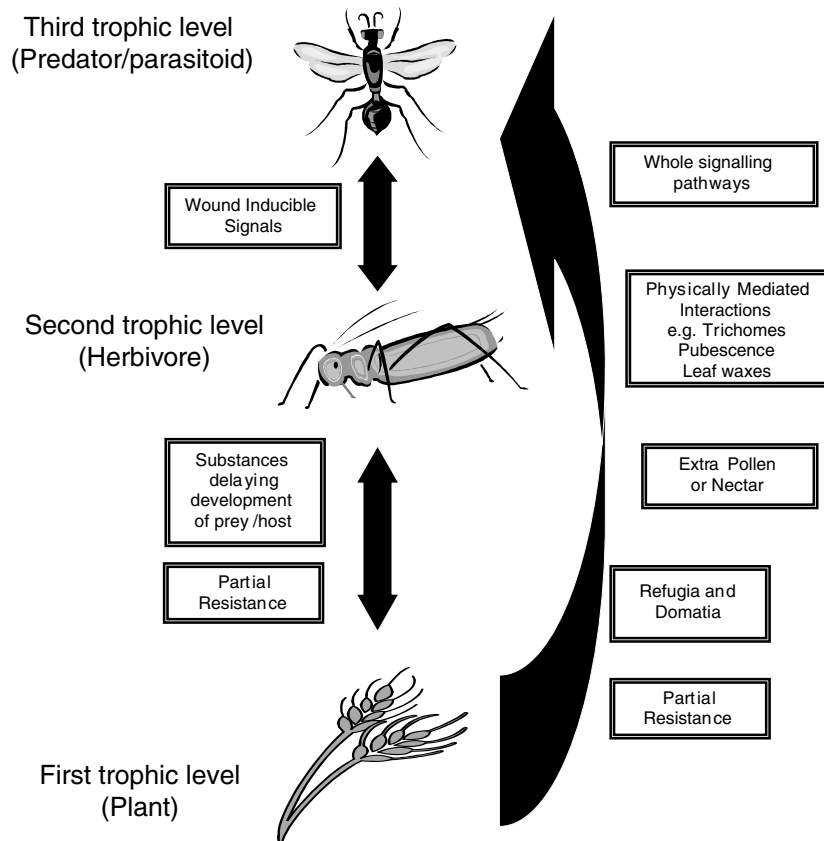


Fig. 3. The role of plant attributes in enhancing biological control and the trophic level at which they act.

defending themselves via signalling to parasitoids. A few studies have found that conventional breeding has been of benefit to pest species and their natural enemies (Benrey *et al.*, 1998), with evidence being provided from work on caterpillars of the small white butterfly, *Pieris rapae* (L.) and its parasitoid, *Cotesia glomerata* (L.). In choice experiments, *C. glomerata* was more responsive to the crop plants than to related wild plants. This suggests that the plant volatiles used by the parasitoids for host location have not diminished as a result of plant domestication. Thus, situations need to be considered on a case-by-case basis just as with the risk assessment of GM crops. Of critical importance, genetic engineering may allow previously intractable traits to be incorporated into elite varieties and allow attempts at selection programmes for partial resistance, which is often quite advantageous but not preferred by classical traditional breeding (see below).

Terpenoid compounds play an important role in attracting natural enemies. Maize seedlings release large amounts of terpenoid volatiles after they have been damaged by beet armyworm, *Spodoptera exigua* (Hübner). Artificially damaged seedlings do not release these volatiles in significant amounts unless oral secretions from the caterpillars are applied to the damaged sites. Females of the parasitic wasp *Cotesia marginiventris* (Cresson) use these plant-derived volatiles to locate hosts (Turlings *et al.*, 1990, 1995). Further work has shown that terpenoid production is not confined to the sites of damage but occurs throughout the plant (Turlings & Tumlinson, 1992). It is widely assumed that these plant defence systems are induced through the octadecanoid pathway (Thaler, 1999) and thus manipulation of this pathway could allow better signalling to parasitoids.

The effects of enhanced allelochemical production on natural enemies has been well studied in the Brassicaceae. Bradburne & Mithen (2000) examined the response of the parasitoid, *D. rapae*, to two lines of *Brassica oleracea* that differed in the glucosinolates emitted following herbivory. By enhancing the production of but-3-enyl isothiocyanate in the plant, the attraction of the parasitoid was increased. Similarly, the effects of glucosinolates from *Brassica napus* and *Sinapsis alba* were investigated on the aphids, *M. persicae* and *Brevicoryne brassicae* (L.) and the ladybird *Adalia bipunctata* (L.). Although both species of aphids were positively influenced by host-plant species, some negative effects on the ladybirds were reported. These effects were attributed to the glucosinolates (Francis *et al.*, 2000). In additional work, *B. napus*, *Brassica nigra* and *S. alba* were used as host plants for the same species of aphid. When reared on plants with higher glucosinolate concentrations, *B. brassicae* was found to be more toxic to *A. bipunctata* than *M. persicae* (Francis *et al.*, 2001). This demonstrates the importance of plant allelochemicals on third trophic levels and should be borne in mind when considering plant manipulation, whether by genetic modification or conventional breeding.

Constitutive expression of plant signals that attract parasitoids or predators in undamaged plants is to be discouraged. Natural enemies, in particular parasitoids, have

remarkable powers of learning (Vet & Groenewold, 1990; Vet *et al.*, 1990; Vet & Papaj, 1991) and are likely to learn quickly that a plant producing a particular allelochemical, but that has no host or prey associated with it, is unprofitable (Powell, 1986). It is probably more appropriate to use external chemical signals to switch on plant genes when necessary (Pickett & Poppy, 2001) or to ensure that they are fully switched on after small amounts of herbivory. Finally, because of genetic diversity in parasitoid populations, the phenotypic plasticity expressed by many individuals and their varying physiological state can result in substantial variations in the response to chemical cues (Lewis & Martin, 1990). Thus, the manipulation of parasitoids by exploiting herbivore-induced plant signals will not be an easy challenge, but the possibilities offered by genetic engineering may encourage us to revisit this promising research field.

Partial resistance

Agricultural ecologists have often suggested that partial resistance could be a more sustainable way of controlling pests (van Emden, 1986; Gould *et al.*, 1991). It is argued that this puts less selection pressure on the insect to develop resistance and that it can allow insect pests to be kept below an economic injury level rather than eliminated. Trying to encourage plant breeders to breed for partial resistance is extremely difficult, in spite of the fact that total resistance is rarely generated. One of the few successful examples of total resistance is single gene resistance [e.g. hessian fly, *Mayetiola destructor* (Say)] resistance in wheat (Cox & Hatchett, 1986). This approach is fraught with difficulties in terms of resistance management that typically relies on a 'refuge' composed of nonresistant plants to conserve susceptible alleles in the pest insect population. The current range of *Bt* crops also utilizes the single gene resistance approach, which is why prophylactic resistance management strategies are necessary (Roush & Shelton, 1997; Shelton *et al.*, 2000; Tabashnik *et al.*, 2000) and has led to some refugia where 50% susceptible plants are grown. Thus, polygenic partial resistance could be a useful way forward, providing the plants combine an element of resistance with limited trade-offs in terms of yield and other desirable characteristics.

By looking at the achievements of conventional breeding, the possibilities and limitations of using transgenes to confer pest resistance can be examined. A review by Verkerk *et al.* (1998) focused on the compatibility of partial plant resistance and biological control, citing a number of incidences that support contrasting tritrophic theories. One notable example was in conventionally bred partially resistant wheat, where the size of the aphid, *Metopolophium dirhodum* (Walker), was reduced by 5%. However, the knock-on effect of this on the third trophic levels was a 34% size reduction in the parasitoid, *Aphidius rhopalosiphii* De Stefani-Perez. Parasitoid emergence was also reduced by 10–30% and fecundity lowered by 26% (Gowling, 1989;

van Emden, 1995). This was due largely to increased restlessness of the aphids on the partially resistant cultivars, particularly in the presence of the parasitoids. A similar situation was found in partially resistant Brussels sprouts, where syrphid larvae were the natural enemy and *B. brassicae* occupied the second trophic level (Gowling, 1989). In an investigation by Fuentes-Contreras *et al.* (1998), the interactions between *A. rhopalosiphii* and the entomopathogenic fungus *Erynia neoaphidis* (Remaudiere & Hennebert) developing on the grain aphid, *Sitobion avenae* (F.) were studied on resistant and susceptible wheat cultivars. Parasitoid survival was again lower on the resistant cultivar than the susceptible, although this depended on the timing of parasitoid oviposition and fungal infection (Fuentes-Contreras *et al.*, 1998). The above examples demonstrate once more the need to investigate the effects of modifying plant genetics on third and possibly even fourth trophic levels if resistance is used as a strategy to combat pests.

Physically-mediated interactions

Morphological traits in plants, such as trichomes, foliar pubescence, waxiness and toughness, can also influence natural-enemy behaviour and this could also be genetically engineered into crops. Many wild ancestors and/or relatives of crop plants have a very different physical appearance. Wild potatoes and wild cabbage plants are much hairier than their commercially produced relatives, and the role of this on insect/plant interactions is just beginning to be investigated in detail. The opportunities offered by GM and mutant *Arabidopsis* will increase our understanding of these traits. This knowledge should allow us to determine whether manipulating the physical traits of plants may be a useful way of increasing resistance via direct defence or enhancing indirect defence by natural enemies. However, in spite of the optimism of molecular biologists (Mitchell-Olds, 2001), it is questionable whether the principal form of defence in *Arabidopsis* is its unapparent and ephemeral nature.

It is interesting to note that most of the examples cited in the literature describe only the negative effects on natural enemies. For example, leaf pubescence has been observed to influence parasitoid behaviour in tobacco (Elsley & Chaplin, 1978), potato (Obrycki *et al.*, 1983) and cotton (Treacy *et al.*, 1986). Furthermore, on cucumber varieties with a high trichome density, there was a strong negative influence on parasitism of the whitefly, *Trialeurodes vaporariorum* (Westwood) by *Encarsia formosa* Gahan (van Lenteren *et al.*, 1995). In other research, Eigenbrode *et al.* (1995) demonstrated that waxiness of cabbage plants affects *C. carnea*, *Hippodamia convergens* Guerin-Meneville and *Orius insidiosus* (Say). It was suggested that waxes accumulated on the tarsi of these predators, thus decreasing their mobility.

The increasing of leaf domatia is also an important physically mediated interaction by which natural enemy impact can be enhanced. Leaf domatia are small structures on the leaf or plant, which often harbour predaceous

arthropods that are potentially beneficial to the plant. Agrawal & Karban (1997) and Agrawal *et al.* (2000) simulated leaf domatia in cotton plants and demonstrated an overall yield increase of 30% in those plants with leaf domatia and hence predatory mites.

A great deal of potentially valuable morphological traits have been conventionally bred out of modern crop cultivars. By looking at wild relatives, it may be possible to select those traits that are beneficial to natural enemies and reinsert those genes into the crop cultivars by transformation.

Pollen/nectar production

Adult parasitoids and some predators typically utilize a different food source to their larvae and this is usually in the form of floral nectar, extrafloral nectar, pollen and honeydew (Jervis & Kidd, 1996). Genetic modification of crop plants could be utilized to enhance resources for natural enemies that might enhance biocontrol.

A classic example in which the resource requirements of adult parasitoids were ignored was when cotton varieties were conventionally bred without extrafloral nectaries. This was to prevent sooty mould formation on the developing bolls. The nectariless cotton had increased bollworm, *H. zea* damage due to a lack of parasitoids in the field, which depended upon the nectar for food (Treacy *et al.*, 1987). Further work by Stapel *et al.* (1997) found that the presence of extrafloral nectaries on cotton increases the retention time of the parasitoid, *Micropplitis croceipes* (Cresson) and, consequently, parasitization of *H. zea*.

In generalist natural enemies, such as ladybirds, lacewings and hoverflies, the adults often require floral resources in addition to prey items and, in the case of the syrphids, floral resources are always necessary (Jervis & Kidd, 1996; Gurr *et al.*, 1998; Wratten *et al.*, 1998; Sutherland *et al.*, 2001). However, there are often problems in ensuring that these generalist natural enemies move from the resource provided as wildflower strips to the crop, where the natural enemies are necessary to manage pest populations. Genetically-modified crops that provide suitable resources for the adults could ensure that both adult and larval food resources are located on the same plant.

Conclusions

Manipulating plants to assist natural enemies has now become technically possible. Many of the scientific solutions suggested above are feasible using conventional means, but have been difficult to adopt due to a reluctance for such pest management programmes. Use of recombinant DNA technology will permit easier genetic modifications and a more rapid transfer of technology to the end user (i.e. the farmer). Previously, conventional breeding for insect resistance in a crop has proved difficult and is usually attained by trial and error, which is not the best way

forward in what is a continual struggle against insect pests. This invariably has meant exposing thousands of breeding lines to the pest and scoring their level of innate resistance. Evidently, this is highly labour intensive, although there have been some notable successes (e.g. the brown plant hopper, *Nilaparvata lugens* (Stål) in Asia) (Sogawa, 1982). As more is understood about the proximate and adaptive causes of plant defence against insects, it will be possible to move away from random screening towards targeted breeding programmes including, where appropriate, the use of transgenes and genetic engineering.

This new technology will not only bring new opportunities, but also will undoubtedly raise new risks and concerns about the role of agriculture in the environment. It is vital that the environmental risks are fully assessed using the tiered risk assessment outlined in this review so as to reduce the potential for disruption to ecological systems. This system provides a rigorous and robust framework that will prove essential in winning the confidence of the general public who are being asked to judge GM plants. By providing a more accurate measure of risk, the benefits of GM crops can be considered against the risks and a comparison can be made with alternative pest management strategies, such as synthetic insecticides or the use of 'organic' control measures.

The final challenge will be to accept that science is only one part of the equation that also includes both moral and ethical issues. However, science should be at the hub of any discussion and provide accurate information essential for informed debate and dialogue. Although it is sometimes easier to adopt a simple definition of the precautionary principle to stop scientific advances, decisions about scientific advances are necessary to assess the wisdom of maintaining the present system of agricultural production. Scientists should enter this new century with excitement about what can be achieved, but should also be responsible about how these opportunities are used and not misused. As entomologists, we certainly live in interesting times!

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