MINI REVIEW

The impact of transgenic plants on natural enemies: a critical review of laboratory studies

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Accepted: 12 November 2004

Key words: transgenic plants, natural enemies, predators, parasitoids, laboratory tests, life history, stress factors, genetic modification

Abstract

We reviewed laboratory tests which studied the impact of genetically modified plants on arthropod natural enemies. A total of 18 species of predators and 14 species of parasitoids have been tested, most in only a few experiments. Certain groups (braconid wasps) or species (the green lacewing, *Chrysoperla carnea*) have attracted much effort, while representatives of others, including whole orders (e.g., Diptera), have never had a species tested. We conclude that laboratory tests are not the 'worst case' scenarios intended by the experimental designs, and are not often ecologically realistic: they typically provided ad libitum feeding, no prey choice, single prey type, no combination of stress factors and usually uniform temperatures. None of these are representative of field conditions, yet most could be easily mimicked in more complex laboratory tests. In most cases (94.6%), the studies were unable to indicate the level of power required to detect any impact. Small sample size and large variability are factors that mask all but very large differences in potential effects. For predators, 126 parameters were quantified, most commonly including survival/mortality (37 cases), development time (22), and body mass/size (20). For parasitoids, 128 parameters were quantified, the majority involving lectins or proteinase inhibitors. Most frequent measurements were: fecundity (23 experiments), adult longevity, extent of parasitism (17 each), body size, mortality, and larval development time. An aggregative scoring (summarising all quantified parameters) indicated that the laboratory tests quantified a remarkable number of cases (30% for predators, 39.8% for parasitoids), where the impacts of the genetically modified plant were significantly negative. These involve various parameters, organisms, test methods, and significance levels, but collectively they indicate that the use of genetically modified crops may result in negative effects on the natural enemies of crop pests.

Introduction

Agriculture is an important environmental quality driver (Hails, 2002), and its effect is not likely to diminish in the future (Tilman et al., 2001). Furthermore, it has been realised that life on Earth depends on the proper functioning of several large-scale ecological processes, many of which provide humanity with irreplaceable benefits, termed 'ecosystem services' (Daily et al., 1996). We know that loss of biodiversity threatens these benefits, but exactly what type and amount of biodiversity is necessary for unimpaired, sustained ecological functioning (and productivity) is unknown: the diversity–productivity relationship is currently hotly debated in ecology (Loreau et al., 2002). Considering these future scenarios and uncertainties, agricultural innovations are increasingly scrutinised for their potential environmental impacts (Hails, 2002). This also holds for the growing of genetically modified (GM) crop plants, a recent but fast-spreading (Shelton et al., 2002) agricultural innovation.

The first production of transgenic plants resistant to insects offered expectations as a means of pest control that could lead to a reduction in pesticide use in intensive cropping systems. On the other hand, claims of potential negative impacts were made, and the growing of transgenic

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crops was therefore linked to regulations and limitations, both before and after release (see Conner et al., 2003). The type of risk assessment, the formulation of the requirements, and the necessary level and complexity of the risk assessment procedure is variable, 'in flux', and often hotly debated.

The scope of the current review is to systematically and critically survey the published, peer-reviewed literature regarding the impact of GM plants on natural enemies. We restricted our attention to arthropod natural enemies tested in laboratory studies (there are virtually no studies on nonarthropod natural enemies of pests or weeds). This does not include any judgement about the importance of such studies, nor of the significance of these impacts relative to impacts from gene flow, resistance evolution, or socio-economic effects. We contend, however, that natural pest control is an important enough ecosystem service that the effects of any GM plant should be tested before the field release of such a plant. As a consequence, a stock-taking of our knowledge in this area 10 years after the start of the commercial growing of GM plants should be useful. We intend to devote special attention to the ecological realism of these laboratory studies, including the selection of species, study conditions, study duration, and statistical/experimental design.

Selection of studies

Previous reviews of empirical research have been varied, some only considering the ecological impacts of narrower or broader sense; others (Dale et al., 2002; Conner et al., 2003) aiming to be wider, also including economic, legal, and ethical aspects. The reviews sometimes include a significant portion of non-peer reviewed sources (e.g., Fontes et al., 2002), or even documents available on the Internet. In our view, the inclusion of non-peer reviewed results in this highly charged field is to the detriment of all involved. The advantage of accessing such studies before, rather than after the time lag that the peer-reviewed literature normally imposes on publication, does not counteract the peril of lacking or improper quality assurance.

Based on a review of these studies, it is evident that if generalisations were to be made under our current state of knowledge, they would risk outrunning the available data. No published laboratory study exists on major predatory groups such as predatory flies and staphylinids, polyphagous predators such as spiders, or social predators such as wasps and ants. We suggest feasible ways to improve the ecological realism of laboratory tests to check the impact of GM crops, and urge the consideration of elementary behavioural and ecological factors to create ecologically realistic 'worst case scenarios' that more closely approximate the most likely situation.

Evaluation methods

For laboratory tests, we surveyed and tabulated the arthropod species studied, their taxonomic affiliation, the test plant, the genetic modification, experimental conditions, number of true replications, the duration of the study, the inclusion of behavioural aspects, and the characters quantified. We sorted these characters into five classes, showing significant negative ($P<0.05$), non-significant negative ($0.49>P>0.05$), neutral ($P>0.5$), non-significant positive, or significant positive effects. To arrive at a rough overall assessment, a 'bean-counting algorithm' was used. This simply summarised the number of cases (characters quantified) in each of the above 'effect' classes. Consequently, studies that quantified more parameters had a higher impact on the final scores.

Statistical analyses

Only two of the 14 reviews published since 2000 discussed experimental design and data analysis. Marvier (2002), using a few field release applications prepared by the biotechnology industry, illustrated serious shortcomings concerning sample size, statistical power, and the duration of experiments. Statistical power analyses can enhance research planning, as well as clarifying the interpretation of the results. For risk-related research, type II errors may become more important than type I errors (Hill & Sendashonga, 2003), thus a power analysis is especially useful. The magnitudes of type I and type II errors are not independent. The relationship between the two types of errors can be simply expressed as a decreasing function: as $\alpha \rightarrow 0$, β will grow for $0<\alpha<1$, meaning that the tendency to erroneously reject the null hypothesis will increase. It is common statistical practice to minimize type I errors. This is, despite the repeated protests of statisticians (e.g., Sokal & Rohlf, 1995), often automatically set by most statistical packages at values as low as $P = 0.05$. Therefore, if a type II error is not explicitly calculated via a power analysis (e.g., Steidl et al., 1997), the researcher has no control over this type of error. This very important point was rarely considered in the description of the experimental design in the research papers considered in the present review. Steidl et al. (1997) contend that the use of a retrospective power analysis calculated with experimental data is meaningless, as it yields no information other than that provided by the original hypothesis. Some authors (e.g., Lundgren & Wiedenmann, 2002; Romeis et al., 2004) published retrospective power analyses. This, however, is incorrect, as it is affected by the interdependence of type I and II errors (Hoenig & Heisley, 2001) and the chosen type I error rate automatically determines the power of the experiment. For

this reason, the new specialist journal, Environmental Biosafety Research, does not accept the use of retrospective power analyses (Andow, 2003). An alternative use of a retrospective power analysis for experiments where the null hypothesis was not rejected, is in the calculation of effect sizes other than the observed effect size. This was chosen, for example, by Romeis et al. (2004), who tested detectable effects at a level of 20%. However, these results are to be taken cautiously, as they only respond to a question on a single hypothetical value and cannot answer broad questions about the validity of the null hypothesis. Pseudoreplication is another problematic statistical phenomenon that also occurs in the GMO biosafety research. Examples of pseudoreplication in laboratory studies with *Chrysoperla carnea* are discussed in Andow & Hilbeck (2004).

Various test methods were sometimes used by different authors to test effects on the same parameter (e.g., mortality). It might be useful to restate here the main difference between non-parametric and parametric tests. Parametric tests, such as least square differences or Student's t-test, require that the data have a 'normal distribution' defined by the parameters of their means and variance. Therefore, they represent a correct choice if two major prerequisites are satisfied: (1) the data are normally distributed and independent, and (2) their variance is homogeneous (homoschedasticity). In case of clearly different data structures, or even in cases of ambiguity, it is advisable to use non-parametric tests (Sokal & Rohlf, 1995). This was the choice in several papers evaluated in the present review. Nevertheless, parametric tests were sometimes used for measuring insect mortality (e.g., Couty & Poppy, 2001; Duan et al., 2002), and in a few cases it is not clear whether the assumption about normal distributions was properly considered (e.g., Sims, 1995).

The number of specimens tested varied widely among the studies (see Tables 1 and 3), with a greatest total of 240 lacewing larvae used by Hilbeck et al. (1999). The use of few replicates makes it less likely that an effect could be detected. Especially for parasitoids, the number of individuals used was sometimes extremely low (e.g., Schuler et al., 2003; Pruetz & Dettner, 2004). Increasing the sample size is the best means that scientists have to reduce the overall error rate of their experiments. The optimal sample size, however, can only be determined after a specific detection limit has been chosen (Marvier, 2002).

A final point worth considering is the acceptable level of baseline mortality in the control treatments of laboratory experiments. In any experiment, a certain level of mortality, caused by the artificial laboratory conditions, is unavoidable. Such mortality in the experimental studies we reviewed was quite variable. A mortality rate of around 15–20% (e.g., Zwahlen et al., 2000; Burgess et al., 2002) can be considered acceptable, while several experiments reported a much higher mortality in the control groups (e.g., Down et al., 2003). Differences can even be found when the same biological system was used. In laboratory experiments with larvae of the lacewing *Chrysoperla carnea*, first instar mortality when feeding on *Spodoptera littoralis* from control plants varied between 16% (Hilbeck et al., 1998a) and 27% (Dutton et al., 2003). Apart from the biological significance of possible suboptimal experimental conditions, a high level of mortality in the control groups could affect the outcome of the statistical analyses and mask a significant effect. In laboratory feeding tests, restricting the pre-test variability in size or mass in the experimental populations could be considered, in order to improve the powers of the procedure to detect treatment effects.

Laboratory tests on predators

The 26 published papers considered in this section included 35 experiments on 18 species in three insect orders (Table 1). Most of these (17 studies) were on Heteroptera (involving 11 species), seven on Neuroptera (all on *C. carnea*), and 11 on beetles (eight on Coccinellidae, three on Carabidae, involving three species each in the two families). *Chrysoperla carnea* (of which only the larvae are predatory) is the most often studied predator species. Apart from this, more than a single study was done on only a handful of species: two coccinellids (*Adalia bipunctata*, and *Coleomegilla maculata*, three and four studies, respectively) and a few heteropterans (*Perillus bioculatus* – three studies, *Geocoris puncticeps, Orius tristicolor, O. insidiosus* – two each). Typically, a laboratory test on predators was of short duration, performed at a constant temperature, with unlimited access to a single type of prey, under a no-choice feeding regime (Table 1). The majority of studies (18) involved Bt-toxin, either in artificial diet, or in GM plants. Six of the Bt-related studies involved plant- or (mostly) pollen-feeding species.

A total of 126 parameters were quantified in the laboratory tests. Most of these were connected to development, general biology, or fitness, which we classified into nine major groups (Table 2). Most commonly, survival/mortality (37), development time (22), and body mass/size (20) were measured. Surprisingly, prey consumption was measured in only 13 cases. Reproduction-related measurements were taken in 12 cases.

Employing a 'bean-counting algorithm' (see above) for these admittedly incomplete data, 135 assessments could be categorised (Table 2), with the majority (47.4%) showing no significant response (neutral). However, a positive effect was found in 16 cases (12% of total tests, half of them

Table 1 A summary of the laboratory test conditions and parameters measured on predatory natural enemies

Order/family	Species	Plant[a]	GM/ analogue	n/test	Temperature		Feeding type: single/ multiple	Prey quantity: ad libitum/ limited	Choice yes/no	Duration in days/no. generations	Parameter measured	Ref.
					Variable?	°C						
Heteroptera												
Anthocoridae	*Orius tristicolor*	Cotton	Bt	10–30	No	25	Single	ad lib.	No	1 gen.	Longevity, mortality	1
	O. tristicolor	Potato/plant	Bt	20	No	25	Single	ad lib.	No	1–13 days	Mortality	2
	O. insidiosus	Maize/pollen	Bt	39	No	26	Single	ad lib.	No	1 gen.	Dev. time, mortality, adult mass	3
	O. insidiosus	Diet	Bt	67	No	24	Single	ad lib.	No	23 days	Mortality, mass, body size, plant feeding, mortality, dev. time	4
	O. majusculus	Maize	Bt	3	No	25D:20N	Single	ad lib.	No	1 gen.	Mortality, dev. time	5
Lygaeidae	*Geocoris puncticeps*	Potato/plant	Bt	20	No	25	Single	ad lib.	No	1–13 days	Mortality	2
	G. punctipes	Cotton	Bt	10–30	No	25	Single	ad lib.	No	1 gen.	Longevity, mortality	1
	G. pallens	Potato/plant	Bt	20	No	25	Single	ad lib.	No	1–13 days	Mortality	2
	Lygus hesperus	Potato/plant	Bt	20	No	25	Single	ad lib.	No	1–13 days	Mortality	2
	Zelus renardii	Cotton	Bt	10–30	No	25	Single	ad lib.	No	1 gen.	Longevity, mortality	1
Miridae	*Cyrtorhinus lividipennis*	Rice	Bt	80	Yes	28D:23N	Single	ad lib.	No		Mortality, dev. time	6
Nabidae	*Nabis* sp.	Cotton	Bt	10–30	No	25	Single	ad lib.	No	1 gen.	Longevity, mortality	1
	Nabis sp.	Potato/plant	Bt	20	No	25	Single	ad lib.	No	1–13 days	Mortality	2
Pentatomidae	*Perillus bioculatus*	Potato	OCI PI	30–35	Yes	25D:15N	Single	ad lib.	No	10 days	Enzyme activity, mortality, moult/dev. time, mass, mass w/prey, survival w/ prey, moulting w/prey	7
	P. bioculatus	Potato	OCI PI	60	No?	?	Single	ad lib.	No	5 days	Enzyme activity	8
	P. bioculatus	Diet	OCI PI	12	No	22	Single	limited	No	28 days	Mortality, consumption, egg laying, days to maturity, fecundity, egg mass, egg viability, satiation level, enzyme activity	9
	Podisus maculiventris	Spiked prey	GNA	30–40	No	20	Single	ad lib.	No	1 gen.	Mass, larval growth, mortality, dev. time, egg prod., egg viability	10
	P. maculiventris	Potato	GNA/ CpTI	20–27	No	20	Single	ad lib.	No	1 gen.	Adult mass female – male, dev. time female – male, fecundity, CpTI, fecundity, GNA, viability	10

Table 1 Continued

Order/family	Species	Plant[a]	GM/ analogue	n/test	Temperature		Feeding type: single/ multiple	Prey quantity: ad libitum/ limited	Choice yes/no	Duration in days/no. generations	Parameter measured	Ref.
					Variable?	°C						
Neuroptera												
Chrysopidae	*Chrysoperla carnea*	Maize	Bt	20	No	22–25	Single	ad lib.	Yes	N/A	Preference, prey consumed, feeding time	11
	C. carnea	Maize	Bt	60	No	25	Single	ad lib.	No	1 gen.	Mortality, dev. time, mass	12
	C. carnea	Maize	Bt	200	Yes	25D:20N	Single	ad lib.	No	1 gen.	Mortality, dev. time	13
	C. carnea	Diet	Bt	150	Yes	25D:20N	Single	ad lib.	No	1 gen.	Mortality, dev. time	14
	C. carnea	Diet	Bt	240	Yes	25D:20N	Single	ad lib.	No	1 gen.	Mortality, dev. time	15
	C. carnea	Maize	Bt	70	No	25	Single	ad lib.	No	1 gen.	Mortality, dev. time	16
	C. carnea	Maize/pollen	Bt	43	No	24	Multiple	ad lib.	No	1 gen.	Mortality, dev. time, adult mass	3
Coleoptera												
Carabidae	*Nebria brevicollis*	Diet	BPTI	97	No	23	Single	ad lib.	No	1 gen.	Enzyme activity, mortality, mass, consumption	17
	Harpalus affinis	Diet	BPTI	54	No	23	Single	ad lib.	No	1 gen.	Consumption 24–48 h	18
	Lebia grandis	Potato	Bt	8–10	No	25	Single	ad lib.	No	3 days	Prey mass eaten,% prey offered eaten	19
Coccinellidae	*Adalia bipunctata*	Potato	GNA	12–24	No	20 (?)	Single	ad lib.	No	1 gen.	Consumption, egg production, egg viability, adult longevity	20
	A. bipunctata	Diet	GNA	25	No	21	Single	ad lib.	No	1 gen.	Mortality, dev. time, larval mass, consumption	21
	A. bipunctata	Diet/potato	GNA	25	No	21	Single	ad lib.	No	1 gen.	Aphid consumption, L1 duration, L2 mass, L1 survival, larval survival, adult mass, no. egg/ female, egg viability, early adult mortality, adult mortality	22
	Coleomegilla maculata	Maize/pollen	Bt	30	No	27	Single	ad lib.	No	1 gen.	Mortality, dev. time, adult mass, no. eggs laid, adult mortality	23
	C. maculata	Maize/pollen	Bt	36	No	25	Single	ad lib.	No	2 gen.	Mortality, dev. time, mass, adult mobility, adult survivorship, fecundity	24

Table 1 Continued

Order/family	Species	Plant[a]	GM/ analogue	n/test	Temperature		Feeding type: single/ multiple	Prey quantity: ad libitum/ limited	Choice yes/no	Duration in days/no. generations	Parameter measured	Ref.
					Variable?	°C						
	C. maculata	Maize/pollen	Bt	45	No	24	Single	ad lib.	No	1 gen.	Dev. time, mortality, adult mass	3
	C. maculata	Potato	Bt	4–15	No	26	S/M	ad lib.	No	1 gen.	Prey consumption, mass, % prey offered, dev. time, adult mass, % mortality, % pupal mortality, adult fecundity	25
	Harmonia axyridis	Oilseed rape	OCI PI	30	No	20	Single	ad lib.	No	2 gen.	Mortality, mass, prey consumption, enzyme activity, fecundity, viability, dev. time	26

1, Ponsard et al. (2002); 2, Armer et al. (2000); 3, Pilcher et al. (1997); 4, Al-Deeb et al. (2001); 5, Zwalen et al. (2000); 6, Bernal et al. (2002); 7, Bouchard et al. (2003a); 8, Bouchard et al. (2003b); 9, Ashouri et al. (1998); 10, Bell et al. (2003); 11, Meier & Hilbeck (2001); 12, Dutton et al. (2002); 13, Hilbeck et al. (1998a); 14, Hilbeck et al. (1998b); 15, Hilbeck et al. (1999); 16, Lozzia et al. (1998); 17, Burgess et al. (2002); 18, Jørgensen & Lövei (1999); 19, Riddick & Barbosa (2000); 20, Birch et al. (1999); 21, Down et al. (2000); 22, Down et al. (2003); 23, Duan et al. (2002); 24, Lundgren & Wiedenmann (2002); 25, Riddick & Barbosa (1998); 26, Ferry et al. (2003).

[a]In the 'plant' column, the notation 'diet' indicates the use of an artificial diet with the GM product or analogous active ingredient incorporated. Abbreviations: Bt – *B. thuringiensis* toxin; OCI PI: oryzacystatin, protease inhibitor; GNA: snowdrop (*Galanthus nivalis*) agglutinin; CpTI: cowpea trypsin inhibitor; BPTI: bovine pancreatic trypsin inhibitor; D:N: daytime : nighttime (temperature); gen.: generation; N/A: not applicable; ad lib.: ad libitum feeding.

Table 2 The relative distribution of reaction classes, from significantly[a] negative to significantly positive, of the different parameters quantified in laboratory tests of GM plant impacts on predatory insects

	Relative no. of cases (%)[b]					
Parameter	Negative significant	Negative non-significant	Neutral	Positive non-significant	Positive significant	Total no. of tests
Survival/mortality	15.5	18	56	10	–	39
Development time	28	12	56	–	4	25
Body mass/size	22	17	48	9	4	23
Prey consumption	33	–	58	–	8	12
Reproduction	42	8	42	8	–	12
Enzyme activity	44	–	–	–	66	9
Longevity	60	–	40	–	–	5
Egg viability	80	–	–	20	–	5
Behaviour	50	–	50	–	–	4
Total	30	11	47.5	6	6	135

[a]Significance level was set at $P<0.05$.

[b]The lines are percentage of cases of the number of tests that quantified the given parameter. Overall total refers to all the parameters in all tests.

significant), whereas a negative impact was registered for 55 (41%) cases (30% of all tests were significantly, and 11% non-significantly negative). The relative distribution of the five classes per parameter was typically highest in the 'neutral' class, and was skewed towards the negative (Table 2). The characters with the lowest frequency in the 'non-responsive' class that can be considered the most sensitive were longevity, reproduction, and egg/progeny viability. Enzyme activity, while always sensitive, was an equivocal character: the activity of certain enzymes decreased, while that of others increased, both significantly (Table 2).

Laboratory tests on parasitoids

We analysed 18 papers published between 1999 and 2004. All except one of these were studies on Hymenoptera, involving 14 species, mostly Braconidae (13 studies on eight species). The genus *Cotesia* was the most frequently studied, with *C. flavipes* in three studies, and three other species in one study each (Table 3). Four studies were done on *Eulophus pennicornis* (Eulophidae), and three on *Aphelinus abdominalis* (Aphelinidae). The only non-hymenopteran involved was *Lydella thomsoni*, a parasitoid fly of the European corn borer, *Ostrinia nubilalis* (Manachini, 2003). However, this last-mentioned work relied on studying parasitoids from field-collected hosts and thus we did not include it in the detailed analysis. The larger number of studies on a small number of species was probably due to experimental difficulties, and the more specific host requirements of parasitoids vs. predators. Interestingly, the plants used in the tests were rarely commercially available varieties: only four studies (three using maize and one cotton) used commercially available plant lines. The use of plants with proteinase inhibitors and lectins (13 studies), often using artificial diets instead of plants (11 studies, Table 2), differed from the predator tests, in which Bt-plants or the Bt-toxin were mostly used.

A total of 128 parameters were quantified, 31 on Bt-plants and 97 on plants/diets containing lectins or protease inhibitors. More than 15 different reference variables were used, which we classified into nine classes. Most frequently, some measure of fecundity (23 experiments) was quantified, followed by adult longevity, extent of parasitism (17 each), body size, mortality, and larval development time (Table 4). Host acceptance or behavioural characteristics were also studied (Table 4), but choice was not frequently investigated. Tests were mostly done under a constant temperature; only two studies (Ashouri et al., 2001; Bell et al., 2001) used variable temperatures.

The majority of parameters in both plant classes (Bt-plants and other GM plants) showed significant negative impacts (57% and 32% for Bt-plants and other GM plants, respectively). The next largest group was that of the nonaffected (neutral) parameters (27% and 35%, respectively), followed by the non-significant negative impacts (13% and 22%, respectively). Overall, 54.6% of the parameters examined indicated a significant (39.8%) or non-significant (14.8%) negative impact of the examined transgenic plant or trait on the organism studied. Only 12.5% of the parameters showed a positive response (Table 4).

Table 3 A summary of the laboratory test conditions and parameters performed on parasitoid natural enemies

Hymenopteran family	Species	Plant/ artificial diet[a]	GM/ analogue	n/test	Temperature		Feeding type: single/ multiple	Quantity: ad lib./ limited	Choice: yes/no	Duration: days/no. generations	Parameter measured	Ref.
					Variable?	°C						
Aphelinidae	*Aphelinus abdominalis*	Diet	GNA	12	No	23	Single	ad lib.	No	1 gen.	Fecundity, longevity, dev. success	1
	A. abdominalis	Diet	GNA	6–15	No	23	Single	ad lib.	No	?	% parasitism, dev. time, sex ratio, mass, resistance to starvation	2
	A. abdominalis	Diet	GNA	10	No	23	Single	ad lib.	No	1 gen.	Mass, longevity, fecundity, sex ratio	3
Braconidae	*Aphidius ervi*	Diet	GNA	15	No	23	Single	ad lib.	No	1 gen.	Mass, dev. time, mortality, adult resistance to starvation, sex ratio	4
	Aphidius nigripes	Potato	Bt Cry3A	12/?	Yes	12–22	Single	N/A	No	2 gen.	Mortality, host acceptance, dev. time, adult mass, fecundity, life span, sex ratio	5
	A. nigripes	Potato	OCI-PI	12/?	Yes	12–22	Single	N/A	No	2 gen.	Mortality, dev. time, size, fecundity, life span, sex ratio	5
	A. colemanni	Sucrose	GNA	?/28–156	No	23	Single	ad lib.	No	1 gen.	Host acceptance, time feeding, longevity, fecundity, emergence, sex ratio	6
	Cotesia flavipes	Maize	Bt Event 176	5	No	27	Single	?	No	1 gen.	Mortality, no. par./host, par. mass (cocoon/host), food consumption by host	7
	C. flavipes	Sugarcane	GNA	2/8/100	No	22–25	N/A or Single	limited	Yes	1 gen.	Behaviour, % parasitism, no. adult par., sex ratio	8
	C. flavipes	Sugarcane	GNA	?	No	30	Single	limited?	No	2 gen.	Host acceptance, suitability, no. par/cocoon, emerging adult/host, dev. time, female longevity, egg load, sex ratio	9
	C. glomerata	Sucrose	GNA	?–157	No	23	Single	ad lib.	No	1 gen.	Behaviour, longevity, time feeding	6
	C. marginiventris	Cotton	Bt Event 531	40	No	27	Single	?	No	2 gen.	Mortality, dev. time, longevity, size, sex ratio, fecundity	10
	C. plutellae	Oilseed rape	Bt	4	No	26	Single	ad lib.	Yes	1 gen.	Choice, % parasitism, emergence	11

Table 3 Continued

Hymenopteran family	Species	Plant/ artificial diet[a]	GM/ analogue	n/test	Temperature		Feeding type: single/ multiple	Quantity: ad lib./ limited	Choice: yes/no	Duration: days/no. generations	Parameter measured	Ref.
					Variable?	°C						
	Parallorhogas pyralophagus	Maize	Bt	59	No	25	Single	ad lib.	No	2 gen.	Mortality, adult longevity, dev. time, brood size, egg load, size, sex ratio	12
	P. pyralophagus	Sugarcane/ diet	GNA	40–80	No	25?	Single	ad lib.	No/Yes	1 gen.	% parasitism, host selection	13
	P. pyralophagus	Sugarcane/ diet	GNA	20–46	No	25	Single	ad lib.	No	2 gen.	Adult longevity gen. 1, gen. 2, size, fecundity, gen. 1, gen. 2, dev. time, mortality	14
Trichogrammatidae	*Trichogramma brassicae*	Sucrose	GNA	?/30–160	No	25	Single	ad lib.	No	1 gen	Behaviour, time feeding, longevity, fecundity, emergence, sex ratio	6
Encyrtidae	*Copidosoma floridanum*	Cotton	Bt Event 531	?	No	27	Single	?	No	1 gen.	Development, pupal mass, size, % parasitism	10
Eulophidae	*E. pennicornis*	Diet/ potato	CpTI	20	No	20–25	Single	ad lib.	No	1 gen.	% parasitism, no. offspring, no. par. host, mortality, sex ratio, longevity, egg load, size	15
	E. pennicornis	Potato	GNA	2	Yes	15–30	Single	limited	No	17 days/F1	% parasitism, no. pupae/ host, no. adult/host, sex ratio, emergence success, size, longevity, fertility, viability	16
	E. pennicornis	Diet	GNA/ CpTI/ ConA	30	No	25	Single	ad lib.	No	1 gen.	Longevity, fecundity, potential fecundity	17
	E. pennicornis	Diet	GNA	6–20	No	25	Single	ad lib.	No	2 gen.	% hosts parasitised, no. par./ host, dev. time, egg load, female size, longevity, egg viability	18

1, Couty & Poppy (2001); 2, Couty et al. (2001a); 3, Couty et al. (2001b); 4, Couty et al. (2001c); 5, Ashouri et al. (2001); 6, Romeis et al. (2003); 7, Pruetz & Dettner (2004); 8, Setamou et al. (2002a); 9, Setamou et al. (2002b); 10, Baur & Boethel (2003); 11, Schuler et al. (2003); 12, Bernal et al. (2002); 13, Tomov et al. (2003b); 14, Tomov et al. (2003a); 15, Bell et al. (2001a); 16, Bell et al. (2001b); 17, Bell et al. (2004); 18, Bell et al. (1999).

[a]In the 'plant/artificial diet' column, the notation 'diet' indicates the use of an artificial diet with the GM or analogous active ingredient incorporated. Abbreviations: Bt – *B. thuringiensis* toxin (with the toxin type indicated if specified. In other cases, the plant transformation event is identified); OCI PI: oryzacystatin, protease inhibitor; GNA: snowdrop (*Galanthus nivalis*) agglutinin; CpTI: cowpea trypsin inhibitor; ConA: concanavalin A; ad lib.: ad libitum feeding; gen.: generation(s); N/A: not applicable.

Table 4 The relative distribution of reaction classes, from significantly[a] negative to significantly positive, of the different parameters quantified in laboratory tests of GM plant (or GM product in artificial diet) impacts on parasitoids

	Relative no. of cases (%)[b]					
Parameter	Negative significant	Negative non-significant	Neutral	Positive non-significant	Positive significant	Total no. of tests
Fecundity	48	13	30	4	4	23
Longevity	59	6	12	12	12	17
% parasitism/no. parasitoids emerged	35	18	24	18	6	17
Body mass/size	33	13	47	–	7	15
Host acceptance, feeding, diff. behaviours	27	–	60	–	13	15
Mortality/emergence	50	–	43	7	–	14
Development time	50	33	17	–	–	12
Viability	–	66	–	33	–	3
Sex ratio	17	33	42	8	–	12
Overall total	39.8	14.8	33.6	7.0	5.5	128

[a]Significance level was set at $P<0.05$.

[b]The lines are percentage of cases of the number of tests that quantified the given parameter. Overall total refers to all the parameters in all tests.

Discussion

Species selection vs. taxonomic composition of natural enemies

Van Driesche & Bellows (1996) listed the main groups of arthropod natural enemies. Among parasitic/parasitoid groups, there are 12 families of Diptera, the most important being the Tachinidae. Hymenoptera is a hyperdiverse order, with eight superfamilies and >36 families containing parasitoid species, among them economically important families such as Encyrtidae, Aphelinidae, Trichogrammatidae, Ichneumonidae, and Braconidae (Godfray, 1994). There are nine orders of predators, with Hemiptera, Neuroptera, Coleoptera, Diptera, and Hymenoptera being the most numerous and important. Among the arachnids, mites (27 families) and spiders (60 families) contain predators (van Driesche & Bellows, 1996).

Against this background, the list of families and species on which laboratory tests have been performed (Tables 1 and 3) is limited. Several important groups of natural enemies have hardly (Diptera), or never (ants, Formicidae) been tested. The same organism in different countries on different plants or in different situations has rarely been examined. Some groups or even species have been studied frequently, while on others hardly any reports can be found. In the published tests, Neuroptera, represented by a single species – the green lacewing, *Chrysoperla carnea* (note that the species identity is not always ascertained, see Henry et al., 2002) – makes up nearly 20% of all predator tests. Hemiptera (11 species) make up close to 50% of species on which tests have been published. There have been very few studies on beetles (but see Jørgensen & Lövei, 1999; Burgess et al., 2002). There are virtually no published tests on very important groups such as parasitoid flies (but see Manachini, 2003), and several wasp families. Among predators, the lack of work on spiders, social predators, and flies is the most striking omission. Reasons to choose particular species are often not well substantiated, and seem governed by opportunity, ease of access, or ease of culturing rather than the systematic screening of several potential candidates, even though this has repeatedly been suggested (e.g., Cowgill & Atkinson, 2003).

Test conditions

Worst case scenarios have been suggested for laboratory testing (e.g., Dutton et al., 2003), where the organisms are exposed to more extreme conditions than they would encounter under field conditions, i.e., in 'reality'. At the same time, in order to show some relevance to the real world, such tests should also be ecologically realistic (Lövei, 2001). For example, early tests using the green lacewing, *C. carnea* (Hilbeck et al., 1998a,b, 1999), have been criticised for their choice of prey organism, i.e., for lack of ecological realism (Hilbeck et al., 1998a; Dutton et al., 2002). However, published laboratory experiments can be considered 'worst case scenarios' only with respect to the candidate toxin concentrations applied. This is the strict consequence of a restricted, ecotoxicological view that has limited relevance to GM ecological impact assessment studies (Ervin et al., 2003; Andow & Hilbeck, 2004). Numerous studies on *C. carnea* (Hilbeck et al., 1998b; Romeis et al., 2004) and those on *A. bipunctata* (Birch et al., 1999; Down et al., 2003) reinforce that significant impacts cannot be fully explored by following an ecotoxicological conceptual framework. This is a serious shortcoming of a recently

suggested testing approach for non-target impacts of GM crops (Dutton et al., 2003). For example, predators under field conditions experience variable temperatures, prey choice, several different prey types, food shortage, and predation risk. All these factors constitute variable stresses, and the impact of an additional potential stress (toxic or suboptimal prey, as a consequence of feeding on a GM plant) is modulated by them. A 'worst case' should include the important elements of such field conditions as they arguably contribute to a 'less than optimal' environment. Some of these conditions are difficult to simulate under controlled conditions, but several (variable temperature, prey shortage, or mixed feeding) are not, and we recommend that these should be included in the test conditions.

Selection of response parameters and the evaluation of their responses

A wide range of response parameters were employed for measuring the potential impacts of GM crops, but only a few have been used frequently. We can assume that all response parameters were selected because of a direct link to the fitness of the test organisms. Due to the restricted range of the organisms, we would strongly emphasise that generalisations should only be made with caution. What is not clear is how sensitive the selected parameters are to changes in conditions, and whether a lack of response (such as the majority of parameters quantified in predator tests) means a real lack of risk, or a lack of sensitivity. It is also extremely difficult to assess how important a certain parameter is for fitness. If two such parameters indicate opposite impacts, which one should be accepted? If no binary (accept/reject) decision is to be taken, how could they be reconciled? We feel that our current database is simply too sparse to provide us with a valid basis to answer this important question.

We have chosen to 'score' published tests. If several criteria were examined, all parameters were classified as +/– and an 'aggregate score' was calculated (see Evaluation methods). This approach has several tacit assumptions. These include that the selected species are equally important as natural enemies and all studied parameters have the same influence on fitness. This is clearly not the case. The simple 'aggregating' evaluation technique is just a first, very crude approximation. We consider this only a temporary and not a final nor a completely satisfactory technique. Nevertheless, the overall skew towards negative impacts (Tables 2 and 4) is a signal that we ought to consider seriously. The negative impacts are too numerous to just explain them away as non-significant or non-relevant. It should be remembered that all of them were selected because they were assumed to be fitness-related. We cannot retrospectively de-couple them from fitness, arguing that they are not ecologically important.

Physiological and behavioural parameters are probably more sensitive in showing impacts than parameters such as mortality or population growth rate. Another type of sensitive measure is the 'integrative' type, in which an accumulated impact is quantified. Progeny viability over a representative period of time can be considered such a case. Several processes in the mother must operate at (reasonably) normal levels and intensity to produce viable offspring. As a consequence, a number of conditions and their impact are 'cumulatively expressed' in such traits. At least in some situations, the inclusion of such parameters should be considered a standard requirement for the pre-release testing of non-target impacts of GM crops.

Immature natural enemy stages are usually more sensitive and have narrower tolerance limits than adults. For example, ground beetle larvae have weakly chitinised, predatory larvae that typically live in the soil (Lövei & Sunderland, 1996). They are more sensitive to moisture and feeding conditions than adults. Moreover, they often represent the overwhelming majority of individuals of a given species under field conditions. Methodological difficulties can pose practical problems for culturing immature stages and using them for biosafety tests, but in several situations they can and should be considered as test organisms and preferred over the less sensitive adults.

Conclusions

In conclusion, we emphasise the need for a clear, consistent approach for selecting laboratory test species, which considers their ecological role in the agro-ecosystem including the GM crop (Birch et al., 2004). We emphatically stress that ecological communities have a structure, and that not all species are equally important (Lawton, 1992). There are neither theoretical nor practical grounds for demanding all-inclusive tests. Our current situation is analogous to the results presented by Malcolm (1992). This author measured the growth and mortality of the larvae of nine species of aphid predators raised on two host species, viz., an alkaloid-accumulating aphid (*Aphis nerii*) and a non-accumulating (*Acyrtosiphon pisum*) one, kept on the same host plant, *Nerium oleander*. Included were three species each of ladybirds (Coccinellidae), lacewings (Chrysopidae), and hover flies (Syrphidae). Malcolm (1992) obtained only three types of response: no effect, a partial effect, or a fatal, toxic effect. The taxonomic affinity of test organisms, however, did not predict the outcome. Likewise, we can expect a finite number of impact types of GM plants, but with our current level of knowledge we are unable to predict the impact on a selected natural enemy group or

species without testing. Therefore, we infer that we are in great need of a larger body of empirical data. These should be systematically collected, including species in taxa not studied thus far. They should be tested under ecologically more realistic laboratory 'worst case' scenarios, choosing sensitive and reliably measurable response parameters over realistic time scales. We should consider that multiple stresses are the norm under field conditions, not the exception, that organisms often react in non-linear ways to combined stresses (Stamp et al., 1997), and we should at least attempt to mimic these conditions in laboratory tests. These altered practices, several of which are easily achievable, would hopefully improve our powers of prediction regarding the potential ecological impacts of growing GM crops.

Acknowledgements

This work was partially funded by a grant from D.J.F. to G.L.L. We thank Ms J.-Y.Guo for help with collecting material for the review and obtaining Chinese publications, and three anonymous reviewers, whose comments have improved the manuscript.

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