Developing guidelines for monitoring non-target effects of Bt crops

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Introduction

Possible consequences from the widespread planting of Bt crops have been reconsidered in light of laboratory studies showing that both non-target herbivores and beneficial insects may be adversely affected without feeding directly on Bt crops (Hilbeck *et al.* 1998, Losey et *al.* 1999, Jesse and Obrycki 2000, Dutton et *al.* 2002, Ponsard et al. 2002). Though not proving any harm to the environment, these laboratory studies suggest assessing the unintended effects of Bt crops is more complex than anticipated. However, because Bt crops are not grown in controlled environments, field trials will likely continue to be the final standard by which their benefits and risks are assessed.

Such field studies generally detect no differences in levels of non-target groups (Pilcher et *al.* 1997, Acciarri et *al.* 2000, Al-Deeb and Wilde 2003, Al-Deeb et *al.* 2003, Jasinski et *al.* 2003) or indicate Bt crops promote greater populations of non-target organisms relative to other pest management approaches (Orr and Landis 1997, Riddick et *al.* 2000, Reed et *al.* 2001). Conversely, studies indicating "no effect" may simply be unable to detect differences among treatments due to one or more aspects of their design (i.e. have low statistical power). Recent U.S. Environmental Protection Agency (EPA) scientific advisory panels note several specific problems with field studies (EPA 2001, 2002) that reduce the likelihood that any real effects of Bt on non-target groups, adverse or beneficial, will be found. Critical issues that influence the ability of a field trial to detect possible effects of Bt crops include the selection of appropriate taxa, replication of treatments, plot size, and data analysis.

Choice of taxa

Selection of representative taxa is the most basic requirement for non-target field research. The possibility of indirect effects demands a broader range of non-target organisms be monitored than only those shown to be susceptible to the Bt toxin(s) by laboratory testing. However, it is impractical to assess effects on all species in a community, so appropriate taxa must be chosen. Representative taxa may comprise different levels of classification. That is, if species within a higher taxon (i.e. genus or family) are ecologically similar, identification to species-level may not be necessary. General

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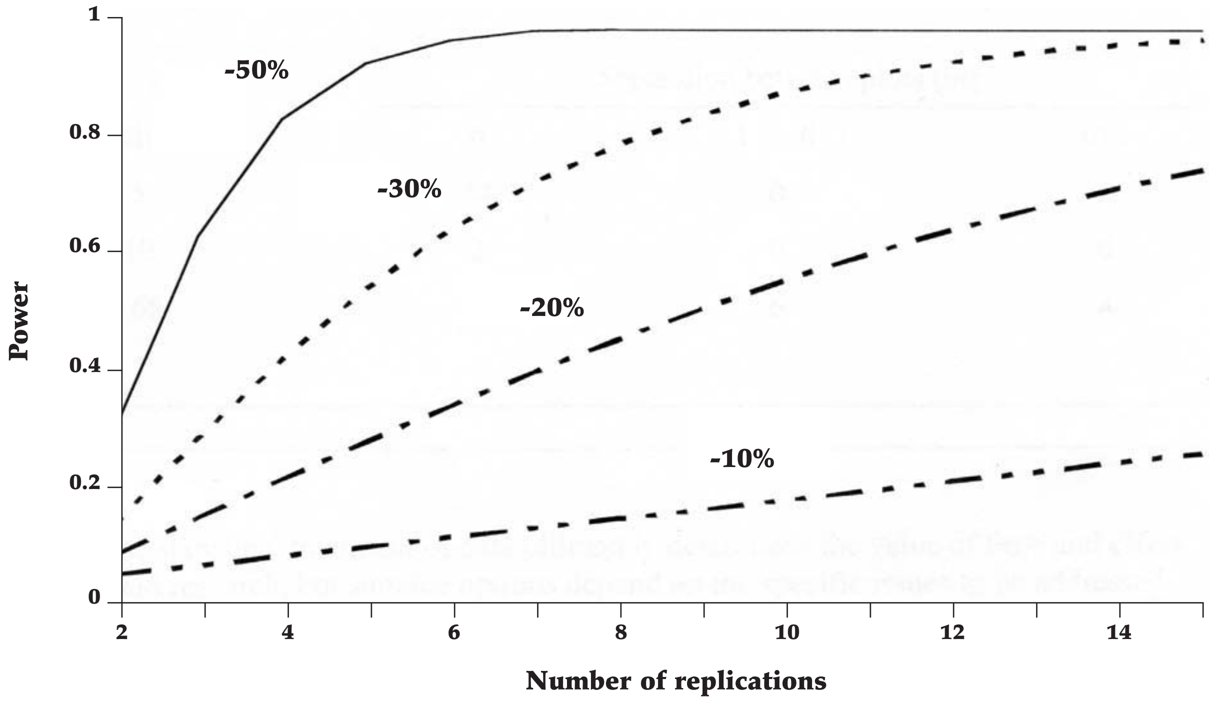
criteria for selection of taxa should include likelihood of exposure or susceptibility to expressed Cry proteins, functional importance, abundance, and ecological diversity. Each of these criteria is essential for assembling a group representative of the non-target community. Taxa susceptible or exposed to Bt toxins should be considered because they are most likely to be impacted by any unintended effects. Functionally important (e.g. predators, decomposers) and abundant groups should be included because they are likely to cause secondary effects within the community if their abundance changes. Abundant taxa also may allow easier detection of effects than would rare species. Finally, though taxonomic diversity should arise from observing the other criteria, breadth of taxa may be important for interpretation of results. For example, if sampling focuses only on predators and parasitoids, it may be difficult to distinguish direct toxicity effects from indirect density-dependent responses to their prey and host species.

Replication of treatments

Adequate replication is another key consideration for field tests of non-target effects because of the relationship between replication and power. Simply put, the power of a test is the probability of detecting a real difference among treatments (i.e. probability of correctly rejecting a false null hypothesis). While power may be affected by choice of taxa, sampling method, or timing of sample collection, power is consistently enhanced by increasing replication. If effects on non-target taxa are expected to be large (e.g. with broad-spectrum insecticides), statistical analysis with high power (> 0.80) is possible with relatively few replicates of each treatment. However, the potential non-target impacts of Bt crops are expected to be relatively subtle (Wold *et al.* 2001), meaning that the low levels of replication generally used in field crop research are likely inadequate to detect differences between Bt crop production and alternative pest management strategies (Bourget *et al.* 2002; Figure 1). For this reason, preliminary or historical data should be used wherever possible to estimate the number of replicates needed to detect differences in non-target taxa with considerable power (see Perry *et al.* 2003). If a *post-hoc* assessment concludes an experiment lacked adequate replication to reveal effects at the desired magnitude (e.g. a 20% effect), retrospective analysis may help to determine the detectable effect size for a test (e.g. to possibly exclude larger effects; Thomas and Juanes 1996).

Table 1. *Plot width and separation in 30 Bt non-target field trials. Data from 14 published, peer-reviewed studies. Experiments analyzed independently are considered separate trials.*



Plot size

The influence of plot size on the evaluation of non-target effects is another challenge to researchers. Small plots may give misleading results (Cantelo 1986, Witmer et al. 2003), in part because various non-target species establish in or re-colonize disturbed areas at different rates (Jepson 1989, Jepson and Thacker 1990). However, limitations of time and labor may dictate that replicated scaled-down plots within a single field be used as a substitute for field-sized replicates. Prior to registration of Bt varieties, small plots also may be required due to restrictions of experimental use permits or seed availability of Bt hybrids. The greatest concern when assessing non-target effects of Bt crops is that any treatment effects will be masked by the movement of insects between plots. This is a serious risk when the width of plots is less than the distance that individuals of indicator taxa might commonly move in its daily activity (i.e. the 'micro' scale, as defined by Jepson 1989). The likelihood of such a problem also is increased when plots are positioned adjacent to each other without any separation between them. Non-target studies that use field plots as an alternative to whole-field replication of treatments appear to commonly use such small, adjacent plots (Table 1). Very small or narrow plots may be acceptable for less mobile microfauna (e.g. mites, nematodes, collembolans), but are a poor substitute for larger crawling or flying insects likely to enter or exit experimental plots daily. For robust evaluation of mobile arthropods, a minimum plot size that is both practical and experimentally sound should be used.

Table 1. *Plot width and separation in 30 Bt non-target field trials. Data from 14 published, peer-reviewed studies. Experiments analyzed independently are considered separate trials.*

	Separation between plots (m)		
Plot width (m)	0	1-10	10+
1-5	14	0	0
5-10	2	0	0
10-65	4	6	4

Data analysis

An appropriate statistical treatment of data ultimately determines the value of time and effort invested in field research, but suitable options depend on the specific issues to be addressed. Researchers might be interested in comparing Bt crops to other management options based on abundance of non-target herbivores, rates of predation or parasitism, composition of functional groups or entire arthropod community; these comparisons also may be made at one or two specific points in time, or over a series of dates (i.e. a time-series). This diversity of hypotheses makes recommending a standard approach to analysis inappropriate, but problems with common methods in non-target analysis should be considered. For example, the practice of conducting separate analyses of several trials sharing common experimental treatments may have a number of consequences. Conducting more separate analyses or comparisons increases the occurrence of type I errors, the likelihood of detecting effects that are not actually present. When results of several such analyses do not agree, the interpretation of results also is made more difficult. Further, when effects are relatively subtle, analyzing trials separately is akin to reducing replication; small effects are then less likely to be detectable. Similar problems may stem from the separate analysis of each species included in a study; though this may be appropriate when there is reason to suspect their responses to treatments will differ (e.g. congeners are related but have very different feeding habits). Unintended consequences also may result when collected time-series data are pooled prior for analysis. This approach may conceal differences at critical periods by averaging. The removal of time as a component of analysis also makes any significant results more difficult to interpret. Alternative approaches that may preclude these common problems and offer increased statistical power include the combined analyses of similar trials when possible, or multivariate procedures such as principal response curves (PRC; Van den Brink and ter Braak 1999), which can permit combined analyses of time-series data on several indicator taxa.

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