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Derivation and Interpretation of Hazard Quotients To Assess Ecological Risks from the Cultivation of Insect-Resistant Transgenic Crops

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ABSTRACT: Cost-effective and rigorous risk assessments for chemicals may be based on hazard quotients (HQs): the ratio of a measure of exposure to a substance and a measure of the effect of that substance. HQs have been used for many years in ecological risk assessments for the use of synthetic pesticides in agriculture, and methods for calculating pesticide HQs have been adapted for use with transgenic crops. This paper describes how laboratory methods for assessing the ecotoxicological effects of synthetic pesticides have been modified for the measurement of effects of insecticidal proteins, and how these effect measures are combined with exposure estimates to derive HQs for assessing the ecological risks from the cultivation of insect-resistant transgenic crops. The potential for ecological modeling to inform the design of laboratory effects tests for insecticidal proteins is also discussed.

KEYWORDS: Ecological risk assessment, hazard quotient, transgenic crop, functional group modeling

INTRODUCTION

Transgenic crops producing insecticidal proteins have been in commercial cultivation for over 15 years and are providing economic, human health, and environmental benefits in developed and developing countries.[1,2] Before transgenic crops may be sold commercially, they must undergo regulatory scrutiny, which, among other things, considers whether the ecological risks posed by their large-scale cultivation are acceptable. In the case of insect-resistant transgenic crops, the ecological risk assessments (ERAs) concentrate on the likelihood of harm to nontarget organisms and, in particular, those which provide biological control of agricultural pests or are threatened or endangered species.[3,4] Conservation of biological control organisms is important to maintain the benefits of reduced applications of synthetic insecticides previously used to control the target pest of the transgenic crop; owing to the narrow spectrum of activity of the insecticidal proteins, secondary pests may flourish under reduced pesticide applications unless they are controlled biologically.[2,5]

Risks to nontarget organisms from the cultivation of insect-resistant transgenic crops arise from two sources: first, transformation may introduce harmful unintended changes into the crop, such as increases in the concentrations of endogenous toxins; second, the insecticidal protein may be toxic to nontarget organisms at concentrations that result from cultivation of the crop. The risks posed by unintended changes are assessed by a series of plant characterization studies that compare the chemical composition and gross phenotype of the transgenic crop with suitable nontransgenic crop comparators; if the composition and phenotype of the transgenic crop are not different from those of comparators that pose acceptable ecological risk, then the ecological risk from unintended changes in the transgenic crop may also be considered acceptable.[6]

The ecological risks posed by toxicity of insecticidal proteins produced in transgenic crops are assessed by methods very similar to those used to assess the ecological risks of chemical pesticides: the effects of the proteins on nontarget organisms measured in laboratory studies are compared with concentrations of the proteins to which those organisms are likely to be exposed in the field during or immediately following cultivation of the transgenic crop.[7] If the ratio of the exposure and effect estimates (the hazard quotient, HQ) is below an agreed trigger value, the ecological risks posed by the protein in the crop may be deemed acceptable, and no further studies may be required to complete the risk assessment.

This paper reviews the use of HQs in the ecological risk assessment of synthetic pesticides and how methods used for pesticides have been adapted for ecological risk assessments of insect-resistant transgenic crops. The paper concentrates on adaptations of the laboratory methods used to measure the ecotoxicological effects, because this is an area that has received much attention recently, but also briefly reviews methods to estimate exposure. Finally, potential problems of interpreting HQs in the light of ecological complexity are discussed, and the use of mathematical modeling as a possible solution to these problems is illustrated.

HAZARD QUOTIENTS IN ECOLOGICAL RISK ASSESSMENTS

In Europe and North America, ecological risks from the use of synthetic pesticides are evaluated using tiered risk assessments.

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Central to such ecological risk assessments are laboratory studies, which determine the effects of the active ingredient or the formulated product on a range of organisms. The tested organisms represent the functional or taxonomic groups that may be exposed to the pesticide during normal use and act as surrogates for species that may be exposed, but which are not tested. The groups tested vary among jurisdictions, but in general, the effects of the pesticide are measured on one or more species of mammal, bird, fish, soil invertebrate, foliar nontarget arthropod, and nontarget plant.[8,9]

In the laboratory tests of the effects of the pesticide a common measure is the LC_{50}: the concentration of a compound that kills 50% of a test population.[10] Depending on the physical chemistry of the compound, longer term studies that measure the "no observed adverse effect concentration" (NOAEC), the highest concentration of the compound that has no observed adverse effect, may also be required.[11] The effects are measured under extremely simple conditions; for example, terrestrial arthropods may be exposed to compounds sprayed onto glass plates[12] and aquatic invertebrates to compounds in beakers with no movement of the test solution.[13] These conditions are often referred to as worst-case because exposure to the compound is unavoidable and there is no environmental heterogeneity that may mitigate the effect of the compound.[14]

The worst-case measure of the effect for a species is compared with a worst-case (i.e., very conservative) estimate of exposure for the group represented by that species to the pesticide under the proposed use. Exposure estimates are called the predicted or estimated environmental concentrations (PECs or EECs) of the pesticide. The worst-case EECs for various groups use the highest recommended application rates and the recommended maximum number of applications under the proposed uses, along with conservative assumptions about the movement and degradation of the pesticide in the habitat of the relevant taxonomic or functional group.[13,15] The potential effects of metabolites and bioaccumulation of the pesticide are also assessed if relevant.[15]

In tiered risk assessments, the worst-case exposure estimates and effects measures are referred to as tier 1 data and are combined to give a tier 1 measure of risk: exposure ÷ effect = HQ.[16] The next stage of a tiered risk assessment depends on whether any HQ exceeds the maximum value of the HQ (the trigger value) that decision-makers have decided indicates acceptable risk based on tier 1 data. If an HQ for an organism exceeds its particular trigger value, further "higher tier" studies to evaluate effect and exposure under more realistic conditions, and thereby to further characterize the risk to the taxonomic or functional group represented by that organism, would be required if acceptable risk were to be established. If every tier 1 HQ is below its respective trigger value, the risks are deemed acceptable at tier 1 and no further studies are required.

Tiered risk assessment is a means to identify confidently substances that pose negligible ecological risk without extensive testing, and thereby concentrates experimental and regulatory effort on substances that pose most concern.[8] To gain similar advantages for ERA for the cultivation of insect-resistant transgenic crops, an analogous system of tiered testing and assessment has been developed.[7,17,18] Briefly, the transgenic crop is characterized by molecular, compositional, and morphological analyses that test for potentially harmful unintended changes that may have occurred during transformation; such potentially harmful changes are indications that the transgenic crop may be a problematic weed, invade nonagricultural habitats, or have adverse effects on nontarget organisms.[6] If no potentially harmful changes are detected, the risk assessment can concentrate on assessing the likelihood of harmful effects to nontarget organisms from production of the insecticidal active ingredient; this is done by laboratory effects tests that expose surrogate nontarget organisms to concentrations of the insecticidal active ingredient in excess of environmental concentrations likely to result from cultivation of the crop. If there are no observed adverse effects of the active ingredient under these conditions, that is, EEC/NOAEC (= HQ) < 1, risks from cultivating the crop may be deemed acceptable without further testing.

ADAPTATION OF EFFECTS TESTS FOR INSECT-RESISTANT TRANSGENIC CROPS

Tests for ecotoxicological effects of insecticidal proteins produced by transgenic crops are often based on protocols that have been used routinely in the evaluation of synthetic pesticides.[19] The protocols are modified so that the protein is applied orally, not topically, but in many other aspects, particularly replication, validity criteria, experimental apparatus, and environmental conditions, such as temperature, lighting, and humidity, the protocols are identical. The similar general design of the studies gives confidence that they will supply information of equal quality to that used by regulators of synthetic pesticides; for example, the power of the studies to detect effects of a certain size are well-known and deemed suitable for regulatory decision-making.

During the development and application of tiered ERA for insect-resistant transgenic crops, four aspects of the design of effects studies have received critical attention:[20−23] the suitability of the test substance; confirmation of exposure to bioactive test substance during the study; the test conditions, particularly the length of exposure and whether suboptimal conditions should be used; and the use of surrogate species. Attempts to resolve these problems are discussed below.

Test Substance. Laboratory effects tests for insecticidal proteins generally use protein produced in transgenic microbes, such as *Escherichia coli*, not protein produced by the transgenic crop. Microbial proteins are used in effects tests because it is difficult to purify sufficient protein from transgenic plants to fulfill regulatory data requirements that require exposure to active ingredients at concentrations greatly in excess of those in the crop. Often, 60 g of protein is required to complete a set of studies for worldwide regulatory approvals, and the low concentration and poor extractability of proteins from plant tissue make it impractical to obtain this amount of purified protein from plants.

To test whether results of effects tests using microbial protein are predictive of exposure to proteins in the crop, bridging studies compare several attributes of the microbial protein and protein purified from the transgenic crop. Among other things, the bridging studies test that the proteins have similar molecular weights, amino acid sequences, glycosylations, reactivities to antibodies, and bioactivities against sensitive pest species.[24] The purpose of the bridging studies is not to show that the microbial and plant proteins are identical, but that the microbial protein is a "suitable surrogate" for the protein produced in the crop. The suitability of a particular batch of microbial protein is a judgment by the risk assessor and regulators based on a weight of evidence from the bridging studies, and it is possible that different regulators will reach different conclusions about the suitability of

a microbial protein from the same data. The different decisions may depend on the degree of confidence that each regulator places in the data or on differing acceptability criteria among regulatory authorities.

In a standard tiered assessment, if laboratory tests with high concentrations of insecticidal protein relative to predicted field concentrations do not reveal adverse effects on surrogate species, further, more realistic, tests would not be conducted because negligible risk to nontarget organisms from cultivation of the transgenic crop is established adequately.[7,17] This approach is considered by some to ignore other important sources of potential harm to nontarget organisms that may arise through interaction of the transgene products with plant metabolism or through other unintended effects of transformation and, therefore, it has been suggested that initial effects tests should include testing with plant material.[20] Proponents of the HQ approach counter that these sources of potential harm are adequately characterized by compositional analysis carried out to assess the risks from food and feed derived from the transgenic crop, and tests with plant material should only be required if compositional analysis indicates that some nutrients, antinutrients, or toxins are outside the normal range found in the crop.[6,7,17] Others go farther and argue that compositional analysis is no longer necessary for regulatory ERAs because we have sufficient experience of creating, breeding, and selecting transgenic crops to know that transgenesis is no more likely to lead to harmful unintended changes than is conventional breeding.[25] Regulatory authorities are unlikely to waive requirements for compositional analysis of transgenic crops soon; however, in theory, if evidence continues to accumulate that the composition of transgenic crops is not different from that of nontransgenic crops in ways that indicate potential harm to health or the environment, the need for compositional data should diminish. It follows that confidence in the adequacy of effects tests using purified protein for ERA should increase as this evidence builds.

Confirmation of Exposure. If no effect is seen in a study, it is possible that the organisms were not exposed to bioactive protein: the protein may have lost bioactivity or the organisms were able to avoid the protein in a heterogeneous diet. To ensure continuous or repeated exposure to bioactive protein, diet may be supplied fresh daily for the exposure part of a study. If the diet is an aqueous solution, protein is dissolved in a new quantity of solution each day; similarly, if exposure to an aquatic species is via protein dissolved in the water containing the organisms, fresh protein solution is supplied daily. If the diet is solid, a large batch of diet with protein mixed into it is prepared at the beginning of the experiment and stored frozen as aliquots that are freshly thawed daily and immediately supplied to the test organism. In each case, the old solution or diet is removed when the fresh material is supplied.[24,26] When there is reason to believe that the diet may contain proteases that will degrade or otherwise inactivate the protein, for example, when the diet contains meat or liver,[27] the diet is cooked to denature those enzymes before the addition of the insecticidal protein. Also, diets based on eggs of the moth *Ephestia* coated with insecticidal protein that were common in early regulatory studies have been replaced with diets into which the protein can be blended.[27] This change has happened because test species such as *Chrysoperla carea*, *Orius insidiosus*, and ladybird beetle larvae could eat the contents of the eggs[28,29] and not be exposed to the protein on the egg case.

Test diets other than aqueous solutions are analyzed to determine the concentration of bioactive protein; aqueous diets, such as sucrose solutions, are not analyzed because the stability of the protein in aqueous solution is known from other studies, including analyses of purity and solubility of the microbial protein preparation. Typical analysis of a diet includes estimates of protein concentration by enzyme-linked immunosorbent assay (ELISA), protein intactness by Western blotting, and bioactivity by a bioassay against a sensitive species, which is often the target pest of the protein.[24,26] For diets in which the protein is mixed into a solid matrix, several samples of diet may be analyzed by ELISA to test whether the protein was dispersed uniformly.

Determination of the concentration of bioactive protein to which an organism was exposed may be based on a weight of evidence. Earthworms are exposed to *Bt* proteins in artificial soil; however, *Bt* proteins are difficult to extract from soil, and ELISA values may be as low as 20% of the nominal concentration of protein. Nevertheless, if the Western blot shows no evidence that the protein is degraded, and if the bioassay shows a response similar to a positive control treatment at the nominal concentration, it may be decided with reasonable confidence that the worms were exposed to the nominal concentration of protein. In cases when the ELISA value is below the nominal concentration, and the bioassay response is less than in the positive control, the ELISA value is usually used as the concentration to determine the NOAEC or other measure of the effect of the protein.

Test Conditions. Since the first regulatory ERAs for the cultivation of transgenic crops, there has been a trend to increase exposure times in effects tests for insecticidal proteins. In regulatory studies, most test species are now exposed for at least 10 days, with 14−21 days being common.[24,26] Studies of this length allow the measurement of developmental end points, such as pupation and fecundity, and should be adequate to detect affects that are likely to lead to harmful effects on ecological functions (see below).

Although laboratory effects studies may achieve worst-case exposure to the insecticidal protein, they may not be worst-case with respect to environmental conditions. Effects studies are carried out to internationally recognized guidelines (for examples, see refs 24 and 26) that specify test conditions, including food availability, temperature, humidity, and light, to ensure survival and normal development of the test organism. It is possible that NOAECs measured under such conditions underestimate the effects of the protein in the field because factors such as starvation and abiotic stressors may increase sensitivity to the protein, and therefore some authors recommend introducing into studies more realistic, "less than optimal", conditions.[21]

It is not obvious that introducing more realism into tier 1 effects studies would be more protective. First, standard test conditions aim to maintain statistical power and to avoid confounding effects due to the test system; a test that introduces suboptimal conditions may fail to detect adverse effects of the insecticidal protein because they are obscured by effects of the test system, for example, by causing high control mortality. Standard test conditions also help to validate the test so that it can give repeatable results among different laboratories. Second, it is assumed that realistic conditions will always worsen any adverse effect of a protein, whereas it is possible that realistic conditions will lessen adverse effects.[14] An additional complication is that the number of abiotic factors that could be manipulated is practically unlimited, and testing in excess of worst-case exposures is designed to account for environmental variation and to remove the need to test for its effects in the laboratory.

The idea of introducing supposed suboptimal conditions into ecotoxicological effects tests is rarely discussed explicitly in the literature. Instead, there is implicit agreement that standard, "optimal" conditions are suitable for screening studies: authors who advocate increasing the ecological content of ERA of pesticides, and who stress the uncertainties inherent in extrapolating from the laboratory to the field, do not advocate introducing additional stressors into screening studies.[14,30−32] In addition, validation of HQ methods in pesticide ERA has shown that standard test conditions do not appear to systematically underestimate risk,[16] and this seems to be the case in ERA for transgenic crops producing insecticidal proteins that have been commercialized to date.[4,33]

Surrogate Species. It is not feasible to test every species potentially exposed to applications of a synthetic pesticide, or to an insecticidal protein during cultivation of a transgenic crop, for adverse effects of these substances; therefore, species are chosen for testing because they are suitable surrogates for all potentially exposed species. The best surrogates maximize our ability to extrapolate from results of effects tests to predict risks to species of value that were not tested; thus, in screening tests an important property of surrogates is that they are species likely to be at least as sensitive to the substance being tested as those potentially exposed species of value that the surrogate represents. Sensitive species may be identified from experience of testing many substances similar to those being assessed, as is the case with a variety of chemicals,[12,34] or from taxonomic relatedness to the target species in the case of insecticidal proteins that existing data suggest have narrow spectra of activity.[24]

A more ecological approach to ERA would seek to identify "keystone"[31] or "ecologically significant"[20] species that are vital for the ecological function the risk assessment is seeking to protect and test those species instead of, or as well as, the surrogate species. The idea of testing keystone or ecologically relevant species is appealing, but has a number of problems: the species may not be known; the species may not be testable; there may be different keystone species among the areas where the transgenic crop is to be cultivated; and some habitats may have no keystone species, with function being based on species diversity rather than the presence of particular species. Basing ERA on effects tests of keystone species could therefore involve the development of a large number of new test methods at considerable cost, particularly in terms of time to optimize and validate the tests. In theory, this effort could improve the ERA; however, uncertainty about species selection would remain, except that now it would relate to the ecology underlying the identification of the keystone species and its predicted ecological function, not to the ecotoxicology underlying the reliability of the surrogates. Furthermore, it is not clear that effects tests should necessarily seek to test the species that the ERA is seeking to protect. If there is good reason to suppose that the surrogate is more sensitive to the protein, perhaps from information on its mode-of-action or spectrum of activity, testing the surrogate may be more protective than testing the species of interest,[35] and under these circumstances care should be taken that the ERA is not overprotective.[14,31]

Given the need for ERAs to apply widely, the cost of regulatory packages as a limiting factor in realizing the opportunities presented by insect-resistant transgenic crops,[2] the uncertainties in the identification of keystone species in agroecosystems,[36] and the time needed to develop and validate test methods for such species, a set of effects data that can be used for ERA in different regions should be favored.[37] Under these circumstances, testing well-chosen surrogates with high concentrations of protein to allow for extrapolation is likely to give consistently better ERAs than methods based on testing local keystone or ecologically significant species.

EXPOSURE ASSESSMENTS FOR INSECTICIDAL PROTEINS

In ERAs for pesticides, lower tier assessments often assume that organisms are exposed to the maximum recommended application rate of the compound.[16] A similar approach is adopted in ERA for insect-resistant transgenic crops, whereby organisms are assumed to consume diets completely comprising the relevant transgenic crop tissue; for example, the diet of pollinators is considered to comprise 100% transgenic crop pollen and the diet of foliar nontarget arthropods to comprise 100% transgenic crop leaf tissue. The crop tissues are also considered to contain the highest measured average concentration of the insecticidal protein.[24,26] These highly unrealistic assumptions are useful for ERA because, among other things, it means it is not necessary to estimate insecticidal protein concentrations in transgenic plants from multiple locations: potential environmental variation in protein production is allowed for by the conservative nature of the exposure assessment.

Owing to the general lack of observed adverse ecotoxicological effects of insecticidal proteins, HQs based on exposures via diets comprising 100% transgenic crop tissue are usually adequate to demonstrate negligible ecological risk resulting from exposure to the insecticidal protein during cultivation of the crop: HQ = worst-case EEC/minimum estimate of NOAEC < 1 is a very conservative estimate of risk.[3,24,26] Should exposure assessments need to be refined to reflect more realistic exposures, simple empirical models are available; for example, the diets of foliar nontarget arthropods usually comprise prey that has consumed crop tissue, not crop tissue itself. Many studies have shown that the concentration of insecticidal protein in the bodies of herbivores is less than the concentration of the protein in the transgenic crop tissue on which they were feeding.[38−40] The dilution of protein varies greatly among herbivores, ranging from little dilution in spider mites[39] to no detectable protein in aphids,[40] and an average dilution to 0.2× the concentration in the leaves of the transgenic crop has been proposed as a suitable method of refining the exposure to foliar nontarget organisms.[24] Similar methods for other potentially exposed functional groups have been suggested.[26]

When simple empirical exposure models are insufficient to characterize risk adequately, pesticide ERAs have used sophisticated methods such as geographical information systems and remote sensing to estimate exposures to certain groups of organisms.[41] These methods are also useful for characterizing environmental exposures to insecticidal proteins, particularly when a threatened or endangered species is potentially exposed to harmful concentrations of protein.[42] Given the apparently narrow spectrum of insecticidal proteins in currently commercialized transgenic crops, simple worst-case exposure estimates are likely to be sufficient for most ERAs.

INTERPRETATION OF HAZARD QUOTIENTS

Laboratory effects tests often detect clear adverse effects of synthetic pesticides on one or more surrogate species at concentrations likely to result from use of the product. The question

Table 1. Parameter Values Used To Simulate the Biological Control of Herbivory by a Large- and a Medium-Sized Predator in a Transgenic Insect-Resistant Crop[a]

parameter	herbivore	medium-sized predator	large-sized predator
juvenile mortality	0.0138	0.075	0.015
adult mortality	0.009	0.01	0.007
senescent mortality	0.6	0.4	0.4
age at senescence (days)	27	75	60
foraging radius (m)	0.2	0.5	0.5
minimum dispersal distance (m)	0	0	0
maximum dispersal distance (m)	2	3	3
dispersal cost	0.001	0.001	0.001
single propagule weight (g)	6×10^{-5}	2×10^{-4}	8.4×10^{-5}
reproductive ratio	0.12	0.05	0.05
proportion free store to base	0.9	0.7	0.7
proportion free store to propagule	0.5	0.11	0.11
time as pupae (days)	0	5	8
total weight after pupae (%)	1	0.12	0.07
initial abundance (m^{-2})	1	0.05	0.05
daily food demand (% of total weight)	40.6	61.9	61.9
respiration cost (% of total weight)	2.81	2.68	2.68
daily development (%)	0.1125	0.055	0.05

[a] See refs 47 and 48 for a fuller explanation of the parameters.

is then to what extent these adverse effects will lead to ecological harm. As discussed above, part of the problem in predicting ecological effects from ecotoxicological effects is the representativeness of the surrogate species. Another important consideration is whether environmental complexity will mitigate or exacerbate the effects of the pesticide;[14,43] higher tier studies, in which more realistic exposure scenarios are evaluated, are valuable for answering this question, but even after extensive field testing of a compound, it can never be proved that some combination of environmental factors will not lead to greater than predicted effects of its use. In ERA for transgenic crops, clear adverse effects of insecticidal proteins are rare in laboratory studies, even at concentrations greater than worst-case field exposures; however, if one considers the ecological effects of transgenic crops to be inherently unpredictable from HQs owing to the complexity of ecological interactions, these observations may be unconvincing because small adverse effects that are hard to detect in the laboratory could be amplified in the field and lead to ecological harm.[44]

In pesticide ERA, predictive modeling has been suggested as a means to reduce uncertainty when laboratory data on adverse ecotoxicological effects are extrapolated to predict the likelihood of harmful ecological effects and, in particular, to determine how adverse effects on growth, survival and reproduction measured in the laboratory will be reflected in changes to the abundance of valued species and the ecological functions they perform.[45] Here we consider whether population modeling can be applied to increase confidence in ERAs for the cultivation of transgenic crops in cases when no adverse effect of the insecticidal protein is observed in the laboratory. The relevant question in this situation is not how to extrapolate from observed adverse effects in the laboratory to estimate the likelihood of harmful ecological effects, but how to identify which effects in the laboratory would indicate high likelihood of harmful effects in the field.

Functional Group Modeling and ERA of Insect-Resistant Transgenic Crops. Analyses of ecosystem dynamics can become complex quickly if the entities represented in the model are interacting species. A way to suitably simplify and represent such complexity is functional ecology, which studies the processes driving ecosystem dynamics.[46] Using the ideas of functional ecology, Caron-Lormier et al.[47,48] described a stochastic model of arable ecosystems based on the trophic interactions among organisms, and which explicitly models the flow of biomass (or energy) between different trophic groups: plants, herbivores, predators, and detritivores. Different functional types are represented within each group: plants comprise crops and weeds, and the latter may be monocotyledonous or dicotyledonous, annual or perennial; herbivores may feed on sap, leaves, or seeds; and predators may be generalists or specialists or omnivores that can also feed on plants directly. The number of these "trophic-functional types" represented in the model depends on the scenario the model is intended to simulate.

To simulate ecosystem behavior, similar species are grouped into trophic-functional types. Basic ecological rules, such as feeding, reproduction, survival, and dispersal, are implemented in the model. All functional types are subject to "natural" mortality according to a daily mortality probability, and herbivores also die as a result of predation. Individuals of the different types are then explicitly modeled and allowed to interact, and variables such as biomass and density that describe each trophic-functional type are calculated at each step of the simulation. The model accounts for management of the arable ecosystem by changes in the parameter values of particular trophic-functional types; for example, the effects of a selective herbicide could be simulated by greatly reducing the survival and growth rates of weeds while leaving the values of those parameters for the crop similar to when no herbicide is applied.

To investigate how this model could be used in ERA for the cultivation of insect-resistant transgenic crops, we used four trophic-functional types: a crop, a herbivore feeding on a hypothetical insect-resistant transgenic crop, and two predator types feeding on the herbivore. The herbivore is not the target

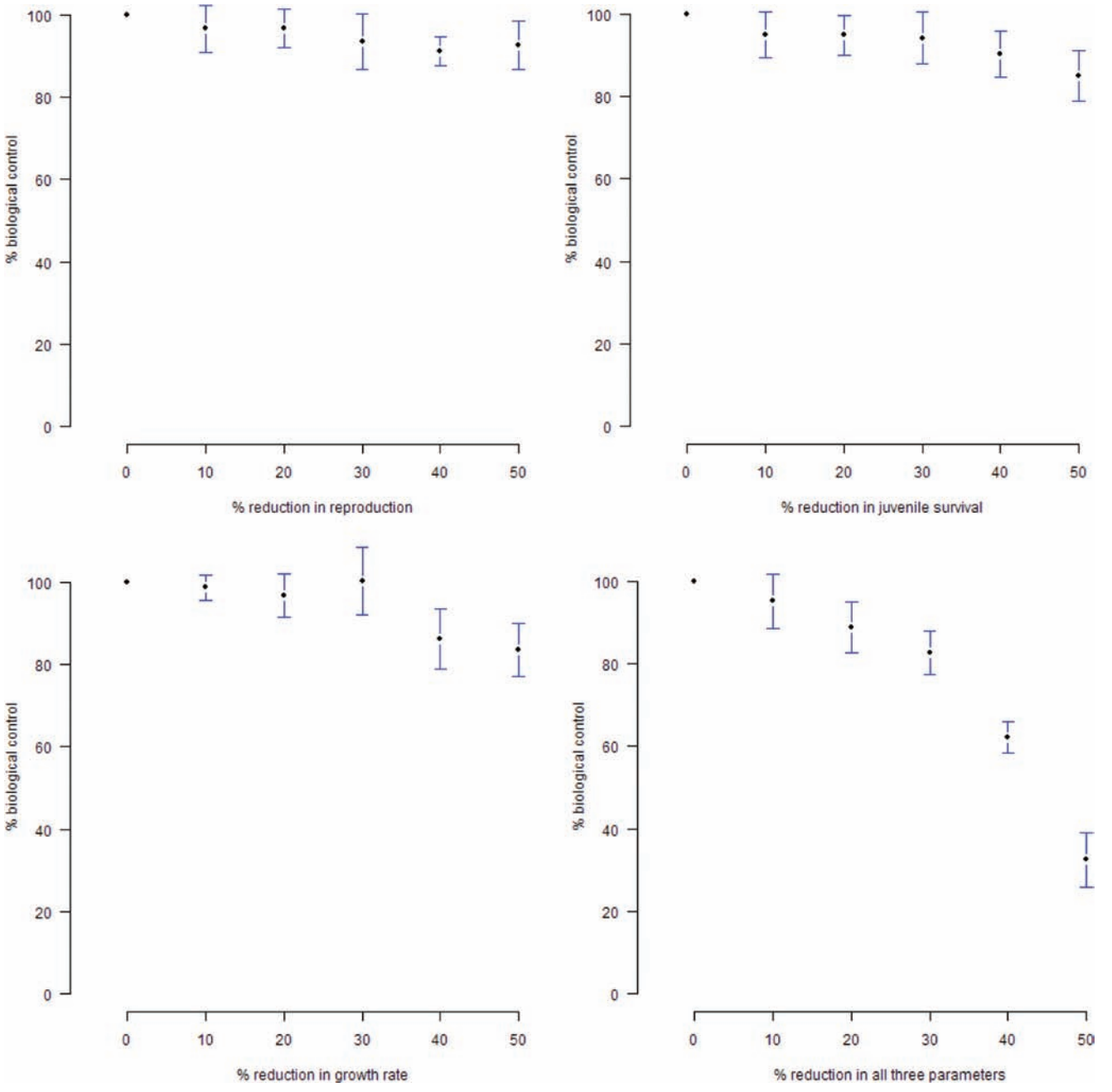

Figure 1. Means and standard deviations of 10 simulations of the amount of biological control of herbivory on a hypothetical transgenic insect-resistant crop provided by a large predator sensitive to the insecticidal protein. Reductions in life-history parameter values represent the hypothetical adverse effects of the insecticidal protein on the predator.

pest of the crop and is unaffected by the insecticidal protein. Parameter values for each invertebrate type were obtained from the published literature and are shown in Table 1. The values are intended to simulate the behavior of a small sap-feeder (e.g., an aphid), and two aphid predators (e.g., a ladybird and a lacewing). The lacewing type is represented as being potentially sensitive to the hypothetical insecticidal protein and is the species for which the simulations may be used to designate adverse effects sizes in laboratory tests (the "test predator"); the ladybird type is represented as being insensitive to the protein (the "insensitive predator").

Simulations were run with the four trophic-functional types using the default values in Table 1, and crop yield was analyzed at the end of the simulations (= CY_{def}). Simulations were also run without predators, and again crop yield was analyzed (= CY_{nopred}). To simulate the adverse effect of a transgenic insecticidal protein produced by the crop, simulations were run in which reproduction, growth, and juvenile survival of the test predator were each reduced separately by 10% decrements to a minimum of 50% of the default value; simulations were also run in which the life-history parameter values were reduced simultaneously. Two sets of simulations were run: one set with the crop, the herbivore, and the test predator; and one set with the crop, the herbivore, and both the test and the insensitive predators. Ten simulations were run for each set of parameter values. For each simulation, the crop yield was analyzed (= CY_{sim}) and was used

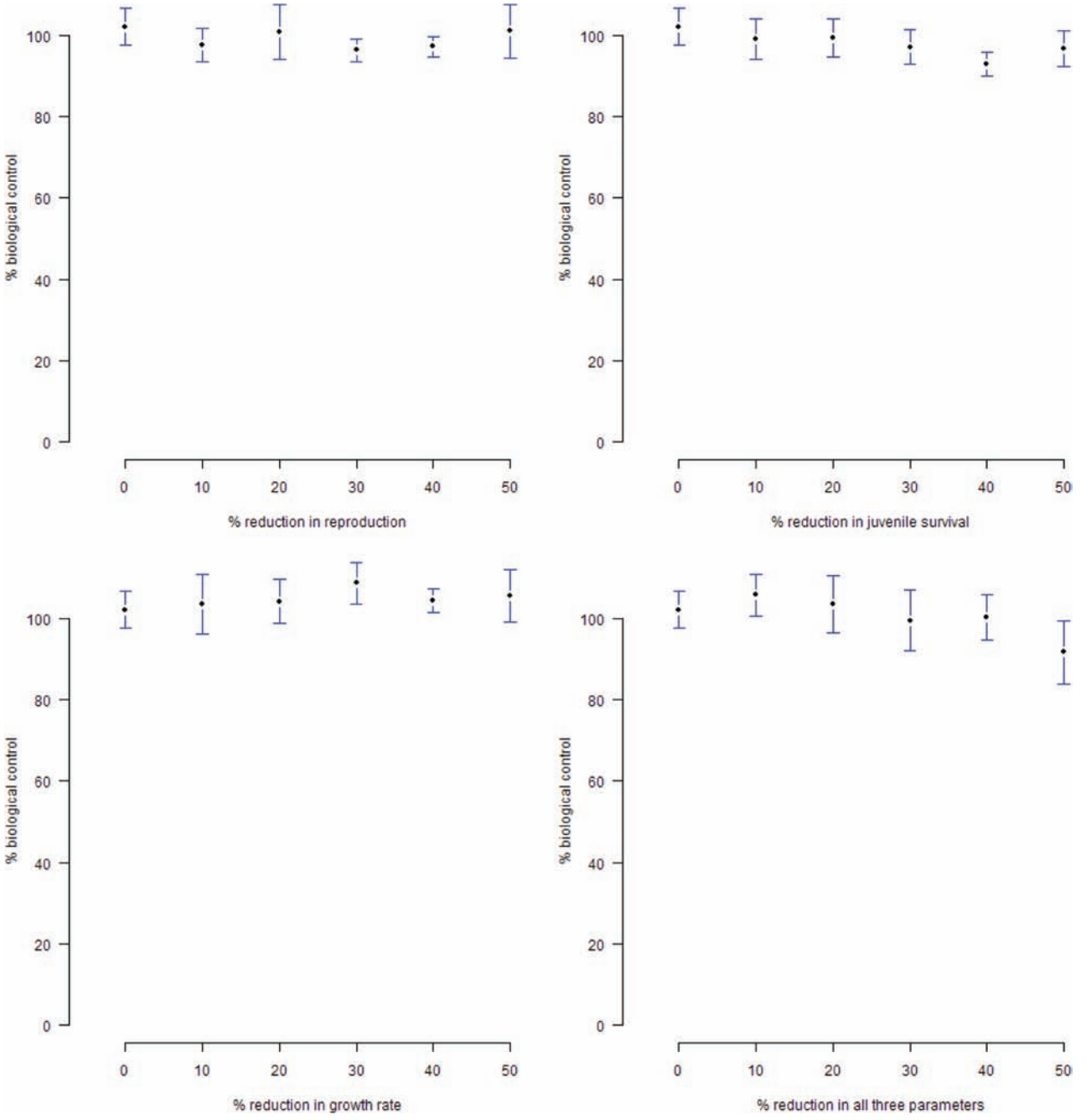

Figure 2. Means and standard deviations of 10 simulations of the amount of biological control of herbivory on a hypothetical transgenic insect-resistant crop provided by a predator sensitive to the insecticidal protein and a predator insensitive to the protein. Reductions in life-history parameter values represent the hypothetical adverse effects of the insecticidal protein on the sensitive predator.

to calculate the percent biological control provided by the predator(s):

$$\%\ \text{biological control} = \frac{(CY_{sim} - CY_{nopred})}{(CY_{def} - CY_{nopred})} \times 100$$

The loss of biological control was taken as the harmful ecological effect due to the hypothetical adverse changes in the life-history parameters caused by the insecticidal protein.

Ecological Effects of Adverse Changes in Life-History Parameters. Comparison of crop yield under default settings, with and without the predators, showed that the presence of the test predator alone increased crop yield by 12%, and the presence of the insensitive predator alone increased crop yield by 10%; the presence of both predators also increased yield by 12%. The reduction in biological control therefore represents the amount by which the 12% increase in yield due to suppression of herbivory is reduced by the hypothetical adverse effects of the insecticidal protein on the test predator.

The loss of biological control resulting from reductions in parameter values for juvenile survival, growth, and reproduction of the test predator alone are shown in Figure 1, and the results for the same parameter values with the presence of the insensitive predator are shown in Figure 2. Figure 1 shows that biological

control provided by the test predator is maintained at high levels when reproduction, growth, and juvenile survival separately are reduced by up to 30%. At 40−50% reductions in growth and juvenile survival, biological control is reduced by 15% from that under the default settings; reductions in reproduction of up to 50% appear to have little effect. When the three parameters are varied simultaneously, significant loss of biological control is seen at 20% reduction and above, with biological control reduced by 70% when all parameter values are 50% of the default setting.

Biological control is much more resilient to the adverse effects on the test predator when the insensitive predator is present: even 50% reductions in reproduction, growth, and juvenile survival separately make little difference to the level of biological control, and 50% reduction in the parameter values together only reduces biological control by about 10%.

Implications for Laboratory Effects Tests. Results of the modeling should be interpreted cautiously owing to limited parametrization and evaluation of the model.[49] Nevertheless, the simulations summarized in Figures 1 and 2 suggest how functional group modeling could be used to help design and interpret laboratory effects tests. The simulations show how the ecological implications of ecotoxicological effects can be examined under highly conservative conditions, when the potentially affected species is the only predator present, or under less conservative conditions, when another predator of the crop pest species is present. Depending on how conservative one wished to make the assessment, different simulations could be used to determine the parameters that should be assessed in laboratory effects studies and the size of an adverse effect in those studies that should trigger further evaluation in higher tier studies. A conservative assessment, using the simulations with the test predator only, might conclude that if juvenile survival were reduced by >20% in a laboratory test, higher tier studies should be required, whereas a more realistic interpretation, using the simulations with both predators, might conclude that no further studies were required unless reproduction, growth, and juvenile survival were all reduced by >50%.

The simulations also suggest how the intended effect of the insect-resistant crop could be integrated into the ERA: just as one could simulate hypothetical reductions in life-history parameter values in a predator, one could also simulate the effect of the insecticidal protein on the target pest. In this manner, one could compare the yield of a crop that controls a pest but has hypothetical (or known) adverse effects on natural enemies, with the yield of a crop that is susceptible to the pest but has no adverse effects on natural enemies. Similarly, simulations could also investigate integrated pest management options for the control of secondary pests that increase in abundance owing to removal of the primary pest and fewer insecticide applications to control that pest.[5]

CONCLUSIONS

Assessment of the ecological risks from the use of synthetic pesticides using HQs provides a pragmatic method of avoiding excessive testing of compounds that pose negligible ecological risk. HQs can also be applied to ERA for the cultivation of insect-resistant transgenic crops. Considerable progress has been made to ensure that laboratory studies, which provide the ecotoxicological effects data for HQ estimates, are robust with regard to suitably lengthy exposures to bioactive protein. Owing to the lack of detected adverse effects of insecticidal proteins in currently commercialized insect-resistant transgenic crops, simple estimates of exposure of NTOs to these proteins, which assume diets comprising transgenic crop tissue only, have been sufficient to characterize ecological risk.

Concern remains that even though laboratory studies may not detect adverse effects of insecticidal proteins on nontarget organisms, subtle effects that are difficult to detect in the laboratory may be increased by environmental interactions and result in ecological harm. Functional group modeling is a possible means to determine the size of effect in a laboratory study that would be necessary for ecological harm to occur. Preliminary investigations indicate that large (ca. 50%) simultaneous reductions in mortality, growth, and reproduction of predatory arthropods must occur before significant loss of biological control is likely. These results suggest that HQs based on laboratory no-effect concentrations and exposures estimated by assuming diets comprise crop tissue only are a highly conservative method of estimating ecological risks posed by the cultivation of insect-resistant transgenic crops.

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