

Case study

Using existing data to predict and quantify the risks of GM forage to a population of a non-target invertebrate species: A New Zealand case study

Maureen O'CALLAGHAN^{1*}, Tanya K. SOBOLEVA² and Barbara I.P. BARRATT³

¹ AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch, New Zealand

² Ministry of Agriculture and Forestry, PO Box 2526, Wellington, New Zealand

³ AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand

Determining the effects of genetically modified (GM) crops on non-target organisms is essential as many non-target species provide important ecological functions. However, it is simply not possible to collect field data on more than a few potential non-target species present in the receiving environment of a GM crop. While risk assessment must be rigorous, new approaches are necessary to improve the efficiency of the process. Utilisation of published information and existing data on the phenology and population dynamics of test species in the field can be combined with limited amounts of experimental biosafety data to predict possible outcomes on species persistence. This paper presents an example of an approach where data from laboratory experiments and field studies on phenology are combined using predictive modelling. Using the New Zealand native weevil species *Nicaeana cervina* as a case study, we could predict that oviposition rates of the weevil feeding on a GM ryegrass could be reduced by up to 30% without threat to populations of the weevil in pastoral ecosystems. In addition, an experimentally established correlation between feeding level and oviposition led to the prediction that a consistent reduction in feeding of 50% or higher indicated a significant risk to the species and could potentially lead to local extinctions. This approach to biosafety risk assessment, maximising the use of pre-existing field and laboratory data on non-target species, can make an important contribution to informed decision-making by regulatory authorities and developers of new technologies.

Keywords: risk assessment / predictive modelling / GM crops / biosafety / population dynamics

INTRODUCTION

Forage crops genetically modified (GM) for pest and disease resistance or improved forage quality have potential to play an important role in agriculture in the future. Several species of forage grasses and legumes have been genetically modified for a range of traits, including perennial ryegrass *Lolium perenne* L. (Poaceae) for improved fructan accumulation (Gadegaard et al., 2008) and tall fescue (*Festuca arundinacea*) (Poaceae) for fungal disease resistance (Dong et al., 2008). Perennial ryegrass is a key forage crop in temperate regions, where its high palatability and digestibility make it ideal for dairy and sheep production. In New Zealand, mixed grass and

legume pastures (predominantly perennial ryegrass and clover *Trifolium repens* L. (Fabaceae)) form the basis of agricultural pastoral systems. As one strategy in the drive for improved pasture and animal productivity, GM forage crops modified for a range of characteristics are currently under development including drought tolerance in perennial ryegrass, condensed tannins in clover, and grasses containing GM endophytes (Caradus, 2008).

Measuring non-target impacts is an essential part of the risk assessment process for any GM crop (Craig et al., 2008). While numerous studies have demonstrated that the environmental effects of GM annual crops (e.g. canola, wheat, potato) are typically transient and lost in subsequent growing seasons (e.g. Heuer et al., 2002), the biosafety assessment of long-lived perennial plants such as GM forage crops requires careful consideration.

* Corresponding author:
maureen.ocallaghan@agresearch.co.nz

There is a need to understand potential ecosystem effects over the longer term; pastures can typically be expected to persist for 7–10 years, at least in the New Zealand pastoral system. In addition, there is potential for GM forage crops to be grown over very extensive areas of land in the future, possibly on a scale likely to exceed that planted with the range of annual GM crops currently grown commercially. There is also a risk that GM forage plants might become established outside of the area where they were sown, including in natural ecosystems, through natural dispersal of propagules or movement of stock or hay, etc. Hence, tools and resources to assist in environmental impact assessment and prediction of long term non-target effects of GM forages are needed.

While risk assessment processes should be rigorous, there is growing concern that many data required for regulatory approval of GM crops have limited value for risk assessment (Raybould, 2006) and may be contributing to development of unnecessarily high regulatory barriers. Given that the purpose of risk assessment is to aid decision making, not to increase scientific knowledge *per se* (Hill and Sendashonga, 2003), there is a case for limiting the requirement for new risk assessment data to that required to allow a confident estimate of acceptable risk. Romeis et al. (2009) make a strong case for reducing the need for new data when assessing risk of introduction of a GM crop into a new area – in their case GM pigeonpea in India. They argue that more efficient use should be made of published information on the biology and ecology of the crop in the country where the approval is sought, combined with existing ecological and ecotoxicological regulatory data where the crop has already been approved. We suggest that there is also opportunity to utilise existing data sets on invertebrate phenology and field population dynamics, in particular where a non-target organism may have been studied previously because of its pest status or its positive contribution to ecosystem function. The range of potential non-target species that could be tested before release into most GM plant receiving environments is large and the analysis of existing data can be helpful in predicting potential impacts or indicating which species might be at risk.

Environmental non-target testing of GM plants has typically focussed on measuring impacts on “ecosystem service providers” (*e.g.* natural enemies and pollinators), and insects of cultural or aesthetic value (Romeis et al., 2008). In assessing the biosafety of GM crops, regulatory authorities will often also request data on non-target native species likely to be exposed to the GM crop. For example, before GM forage crops can be used in New Zealand, the technology must be approved for release by the New Zealand Environmental Risk Management Authority (ERMA New Zealand) under the Hazardous Substances and New Organisms Act (HSNO

Act, 1996). An application to introduce a GM forage crop must include evidence that the plants are unlikely to displace any native species within its natural habitat, or cause deterioration of natural habitats, or adversely affect New Zealand's inherent genetic diversity. Adverse impacts on beneficial species that carry out important functions in pasture must also be considered.

Biosafety studies have typically examined effects of a GM trait on a single test species under highly controlled conditions, and usually for a short period. The value of this type of testing is questionable in the biosafety assessment of perennial crops. In addition, debate still surrounds the validity of extrapolating from such laboratory experiments to predict likely impacts in the field, with many researchers concluding that both laboratory and field studies are essential (O'Callaghan et al., 2005). However, where the test species is well characterised and has been studied in the field, there is potential to exploit existing datasets to predict longer term impacts in the field, based on an impact observed in short term experiments.

Mathematical modelling is a common tool for risk assessment in a range of disciplines, and particularly in ecology. Mathematical models of population dynamics can be used to predict the fate of a test insect population under natural conditions by estimating the risk of its extinction. While the general functional structure of such models can be similar for a range of organisms, model parameters may differ substantially among species, and will often have to be estimated from experimental studies. For example, determining the threat of extinction of a non-target organism following exposure to a GM crop will also require knowledge of specific effects of the transgene-derived characteristics that can affect population dynamics of the non-target species of interest. Specific measurements obtained from the laboratory biosafety experiments, for example of the type carried out by Barratt et al. (2008), can provide such knowledge for mathematical models of population dynamics.

We describe a generic modelling approach that can be used to assist in the risk assessment of GM crops, and demonstrate its use with a New Zealand-specific case study, to estimate the long-term population survival of an endemic, non-target, herbivorous invertebrate exposed to GM forage. Existing biosafety data derived from laboratory experiments in conjunction with field population dynamics and phenology of a New Zealand native weevil, were used for parameters in a predictive model to estimate thresholds for weevil population survival. More specifically, the existing data and model were used to estimate to what extent oviposition rates could be suppressed without causing the disappearance or local extinction of a non-target weevil species from the pasture ecosystem.

CASE STUDY

The non-target invertebrate test species used in this case study was the adult stage of *Nicaeana cervina* Broun (Coleoptera: Curculionidae: Entiminae). This is one of many New Zealand native weevil species which inhabit a wide range of habitats, particularly native grassland (Barratt et al., 2000). *N. cervina* exploits key introduced grass species, including ryegrass and clover in New Zealand's developed pastures, where the weevil can be found at densities of up to 100 m⁻² (Barratt et al., 1998).

Ability to maintain field collected *N. cervina* adults in the laboratory suggested that this weevil had potential as a test insect for biosafety testing. Laboratory protocols to measure weevil survival, feeding and reproductive performance were developed by Barratt et al. (2008) in a study that aimed to identify attributes of a test species that were relevant and informative for risk-assessment studies, and develop protocols that could be used in quarantine conditions to detect impacts of GM plants on a non-target native species. Among the plants used in the laboratory assessment of impacts of GM plants on *N. cervina* were ryegrass plants infected with either of two endophytic fungi *Neotyphodium lolii* or *Epichloe festuca* and endophyte-free ryegrass. Some plants were infected with an endophyte that was genetically modified to eliminate production of the insect deterrent peramine (Barratt et al., 2008; Tanaka et al., 2005).

In addition to the biosafety study carried out by Barratt et al. (2008), a number of other studies have focused on the field ecology and phenology of this weevil, providing data that can be used in development of models to predict potential impacts in the field. Data on population dynamics of *Nicaeana* weevils were sourced from studies by Barlow et al. (2004) and Barratt et al. (2000).

RESULTS AND DISCUSSION

By applying a simple population model to assessment of risk of *N. cervina* field population survival in ryegrass pasture, we found that oviposition rates could be suppressed by up to 30% without causing local extinction of this non-target weevil species. This result does not necessarily lead to the conclusion that, if oviposition was suppressed by more than 30%, a non-target weevil population could be lost from a pasture ecosystem. However, it does suggest that additional studies may be required to define the final fate of the non-target organism. Such studies may need to consider further ecological complexities that could impact on survival of the insect of interest. For example, reduced population density of a non-target insect following exposure to a GM plant could result in further changes within the ecosystem, such as a reduction

in the number of parasitoids, which may lead to recovery of the insect population.

While estimations of reproductive potential are clearly linked to the probability of survival of the weevil population over several generations in the field, laboratory tests showed a positive relationship between the proportion of weevils feeding and those ovipositing. An analysis of the range of values recorded for feeding and fecundity of *N. cervina* suggested that a 50% reduction in feeding would result in a 28% reduction in the proportion of weevils ovipositing (Barratt et al., 2008). Reduction in feeding resulting in lower reproductive potential has implications for survival of the weevil over several generations in the field as demonstrated here. Combining this result with the population dynamics studies (modelling and phenology), we conclude that reduction in feeding of 50% or higher indicates a significant risk to the survival of the population of non-target insects.

This estimate of risk to non-target species populations can be applied to any new forage technology, or combinations of technologies. The relationship between the extent to which the fecundity of *N. cervina* can be reduced without risk to the survival of the population, and the intrinsic growth rate of that insect, is defined by the condition: $E_g = 0.7E$, where E is the average number of eggs laid by each female under natural field conditions and E_g is the average number of eggs per female exposed to GM plants.

In a more general form, this can be re-written as: $E_g/E \geq \exp(-r_m)$, where r_m is the intrinsic growth rate for *N. cervina*.

This condition connects two characteristics that influence survival of the weevil in the field, namely: changes in reproduction potential (E_g/E) and intrinsic growth rates of the weevil population in the field. Implicitly, through r_m , it accounts for a range of environmental factors that affect population dynamics of the non-target insect. The data required are values for the intrinsic rate of increase (r_m) which can be influenced by environmental or biophysical variables. For example, at lower altitudes, the intrinsic growth rate r_m for *N. cervina* is higher than it is at higher altitudes (Barlow et al., 2004) and consequently the extent of reduced oviposition caused by feeding on modified plants will be smaller.

To estimate the extent to which fecundity of a test insect fed on the GM plants can be reduced without causing local extinction or population decline, we combined laboratory studies, phenology data collected over several generations in the field, and mathematical modelling. Although testing for potentially harmful effects of GM crops on non-target organisms is generally assessed on a case-by-case basis, the main steps of the assessment procedure will be similar for a wide range of cases. For a general case, these steps are presented in Figure 1.

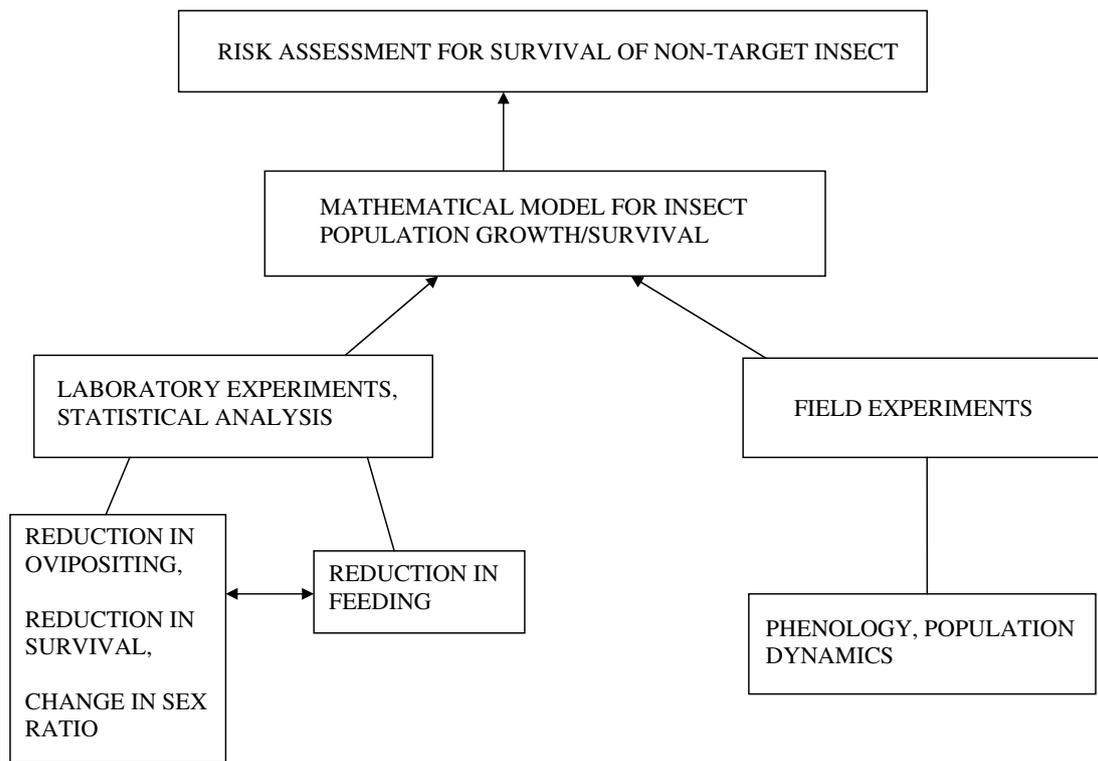


Figure 1. Generic experimental steps required for development of a mathematical model for assessing risk to non-target organisms.

Summarised at the bottom of the left hand side of Figure 1 are the main characteristics influencing survival of a population of test organisms that might be affected by feeding on a GM crop. The adverse effects of exposure to a GM crop on these characteristics can vary for different test organisms and different genetic modifications of the forage and the necessary knowledge has to come from the laboratory biosafety tests. As long as the laboratory experiments have established the range of potential adverse effects, the next step is to estimate the extent to which these effects might cause population decline, possibly leading to local extinction of the population of interest. Generally, this step requires knowledge of population dynamics, in particular the intrinsic rate of population change that can be estimated from the field observation of a tested organism over a few generations under relatively constant conditions. The part of the assessment procedure that involves field studies is shown in the right hand side of Figure 1. For the current case study we used simple modelling to predict directly from the existing data that a reduction in oviposition of greater than 30% may indicate a risk of local extinction of *N. cervina* from, in this case, a natural grassland ecosystem. Then additional information from the laboratory experiments on the correlation between feeding and reduction in oviposition

allowed us to conclude that more than 50% reduction in feeding indicates potential danger of extinction of the tested weevil.

The information in the left hand side of Figure 1 is specific to a particular test organism and to a GM technology requiring a specific set of experiments to be conducted. Field data (right hand side of the figure) will not always be available as they were in the above case study. When population dynamics data are unavailable, assumptions could be based on intrinsic rate of population change from species with similar phenology and reproductive strategies. In some cases, knowledge of population growth rates for organisms that co-exist with those of interest could be used to estimate, with the help of a mathematical model, a range of values for the required r_m , e.g. conditions for local persistence can restrict r_m by a range $r_m^{\min} \leq r_m \leq r_m^{\max}$. In this situation, using the value r_m^{\min} will represent “the worst case scenario”.

CONCLUSION

Testing for potentially harmful effects of GM crops on non-target organisms has generally been assessed

on a case-by-case basis, as most often required by regulators. However, as it is impractical to collect biosafety data on all of the large numbers of potential non-target invertebrate species in the receiving environment of each GM crop, new approaches are required. For example, Todd et al. (2008) described a screening method that can be used during the problem formulation phase of risk assessment to identify and prioritize non-target invertebrates that should be tested as part of risk analysis of any GM plant. While Todd et al. (2008) used a database of invertebrate species commonly found in New Zealand forests to demonstrate their model, a database of over 200 invertebrate species known to be found in New Zealand pastoral systems has also been compiled (L. Malone and B. Barratt, unpublished). For some of the potential non-target species listed in the pasture database, there is a wealth of existing data available on their population dynamics, in particular if they were pest species, and in some cases there is pre-existing knowledge of their tritrophic interactions with parasites, pathogens or predators. This pre-existing information, together with biosafety data collected during laboratory experiments in containment, and mathematical modelling, are valuable resources that can be used to improve efficiency of risk assessment for decision-making around the adoption of GM forages in New Zealand. The approach may also have a role to play in easing the high regulatory costs and barriers which pose a significant hurdle to adoption of imported GM crops in developing countries, where limited resources preclude the development of GM crops adapted to local conditions (Paarlberg, 2006). As several researchers have recently highlighted, if suitable data on which to base a regulatory decision are already available, further data collection should not be required (Raybould, 2007; Romeis et al., 2009). The potential of mathematical modelling and exploitation of pre-existing ecological datasets in GM plant risk assessment remains largely untapped at present.

METHODS

To estimate the risk of adverse impacts of GM forage on a non-target species in a pasture ecosystem, we considered the relationship between population densities of the exposed, non-target insect on subsequent generations. Using the New Zealand native weevil species as our example, Barratt et al. (2000) showed that the generation time of *N. cervina* is around one year, with adults emerging in winter-spring. To ensure survival of the weevil population, the population density should be above some critical level at any stage of its development, which means that we could start our estimation from any stage of the development, e.g. from the stage where most of the population are mature adults.

Let N_t be a population of mature adults at the main period of reproduction activity in spring and N_{t+1} be the subsequent generation of mature adults in the following Spring. According to Barratt et al. (2000), the sex ratio in the weevil population is close to 1:1, then:

$$N_{t+1} = P_s \cdot P_e \cdot e \cdot \left(\frac{N_t}{2}\right), \quad (1)$$

where P_e is a probability for a female to produce e viable eggs and P_s is a probability that these eggs will develop successfully through all stages to maturity. Generally, the probability of survival to reproductive age P_s can depend on population density. For example, the resource availability is often a limiting factor for the survival of the population, which means that probability of survival up to maturity decreases when the population size is close to its carrying capacity K :

$$P_s \propto \exp\left(1 - \frac{N_t}{K}\right). \quad (2)$$

Taking into account that the average number of eggs per female $E = P_e \cdot e$, equation (1) can be re-written in the equivalent form:

$$N_{t+1} = P_s \cdot E \cdot \left(\frac{N_t}{2}\right). \quad (3)$$

If environmental conditions (e.g. weevil nutrition) become less favourable, the value E could be reduced not only because of reduction in the proportion of weevils ovipositing, but also because a fertile female might produce fewer eggs. Evidence for a relationship between feeding and oviposition was presented in Barratt et al. (2008).

The weevil population can grow ($N_{t+1} > N_t$) or stay at some equilibrium level ($N_{t+1} = N_t$) providing:

$$(P_s \cdot E/2) \geq 1. \quad (4)$$

This condition is a general condition for population growth, while values of P_s and E depend on different conditions, e.g. climate, food quality.

We will use the denomination E for the average number of eggs per female in natural field conditions and use E_g for an average number of eggs per female exposed to GM plants. Then, for the condition of survival for weevils feeding on GM plants is

$$(P_s \cdot E_g/2) \geq 1. \quad (5)$$

While laboratory experiments (Barratt et al., 2008) demonstrated a significant relationship between feeding and weevil oviposition, there was little impact on weevil survival with the test plants and relatively short exposure periods used in the laboratory experiments. So, given this

scenario, and extrapolating to field conditions, we assume that the probability of survival to adult reproductive age would not be affected by feeding on the different plant lines. Thus, for estimation of P_s , we can use previously obtained field data on *N. cervina* growth rates (Barlow et al., 2004; Barratt et al., 2000). The assessment of the impact of reduced fertility on survival of *N. cervina* in the ecosystem requires knowledge of yearly rates r of change for the non-target weevil. It was shown that:

$$r = \ln(N_{t+1}/N_t) = 0.363 - 0.0864N_t. \quad (6)$$

However, from equation (3) we have:

$$\ln(N_{t+1}/N_t) = \ln(P_s \cdot E/2). \quad (7)$$

Comparing (6) and (7) we obtain:

$$\ln(P_s \cdot E/2) = 0.363 - 0.0864N_t \quad (8)$$

and the probability of survival:

$$P_s = (2/E) \cdot \exp(0.363 - 0.0864N_t). \quad (9)$$

As expected, the probability of survival through to maturity depends on population density in the manner described by equation (2). If the population size is close to carrying capacity, the reduction in fertility will not cause the extinction of the population as reduced fertility will be compensated for by an increased probability of survival. However, for populations that are present in the field at low densities, reduced fertility would indicate a higher risk of extinction. At low population densities, the probability of survival to mature age is determined by its intrinsic value, and for *N. cervina* in November, can be estimated to be equal to

$$P_s = \left(\frac{2}{E}\right) \cdot \exp(0.363). \quad (10)$$

Incorporating this probability in the growth/survival condition (equation (5)) for the weevil exposed to GM plant lines, we finally obtain:

$$\frac{E_g}{E} \geq \exp(-0.363) = 0.696, \text{ or } E_g = 0.696E \approx 0.7E. \quad (11)$$

This condition (11) estimates to what extent fecundity of *N. cervina* can be suppressed without causing disappearance of this non-target weevil from the ecosystem. An equivalent form of this condition shows the maximum allowable reduction in the rate of weevil oviposition:

$$(E - E_g)/E = 30\%. \quad (12)$$

ACKNOWLEDGEMENTS

We thank the New Zealand Foundation for Research, Science and Technology for funding under contracts C10X0601 and C06X0801.

Received October 20, 2010; accepted April 11, 2011.

REFERENCES

- Barlow ND, Barratt BIP, Ferguson C, Barron MC** (2004) Using models to estimate parasitoid impacts on non-target host abundance. *Environ. Entomol.* **33**: 941–948
- Barratt BIP, Evans AA, Ferguson CM, McNeill MR, Proffitt JR, Barker GM** (1998) Curculionioidea (Insecta: Coleoptera) of New Zealand agricultural grassland and lucerne as potential non-target hosts of the parasitoids *Microctonus aethiopoidea* Loan and *Microctonus hyperodae* Loan (Hymenoptera: Braconidae). *N. Z. J. Zool.* **27**: 47–63
- Barratt BIP, Ferguson CM, Evans AA, McNeill MR, Addison PJ** (2000) Phenology of native weevils (Coleoptera: Curculionidae) in New Zealand pastures and parasitism by the introduced braconid, *Microctonus aethiopoidea* Loan (Hymenoptera: Braconidae). *N. Z. J. Zool.* **27**: 93–110
- Barratt BIP, Crook KE, Ferguson CM, Johnstone PD, Malone LA** (2008) Developing a method for laboratory biosafety testing impacts of transgenic plants on non-target insects. *Entomol. Exp. Appl.* **128**: 437–449
- Caradus J** (2008) An opportunity lost and sorting fact from fiction. *Proc. N. Z. Grassl. Assoc.* **70**: 1–6
- Craig W, Tepfer M, Degrassi G, Ripandelli D** (2008) An overview of general features of risk assessments of genetically modified crops. *Euphytica* **164**: 853–880
- Dong S, Shew DH, Tredway LP, Lu J, Sivamani E, Miller ES, Qu R** (2008) Expression of the bacteriophage T4 lysozyme gene in tall fescue confers resistance to gray leaf spot and brown patch diseases. *Trans. Res.* **17**: 47–57
- Gadegaard G, Didion T, Folling M, Storgaard M, Andersen CH, Nielsen KK** (2008) Improved fructan accumulation in perennial ryegrass transformed with the onion fructosyltransferase genes 1-SST and 6-FFT. *J. Plant Physiol.* **165**: 1214–1225
- Heuer H, Kroppenstedt RM, Lottmann J, Berg GKS** (2002) Effects of T4-lysozyme release from transgenic potato roots on bacterial rhizosphere communities are negligible relative to natural factors. *Appl. Environ. Microbiol.* **68**: 1325–1335
- Hill RA, Sendashonga C** (2003) General principles for risk assessment of living modified organisms: lessons from chemical risk assessment. *Environ. Biosafety Res.* **2**: 81–88
- HSNO Act** (1996) Hazardous Substances and New Organisms Act. Wellington, New Zealand. www.legislation.govt.nz/act/public/1996/0030/latest/DLM381222.html

- O'Callaghan M, Glare TR, Burgess EPJ, Malone LA** (2005) Effects of plants genetically modified for insect resistance on non-target organisms. *Ann. Rev. Entomol.* **50**: 271–292
- Paarlberg R** (2006) Let them eat precaution: why GM crops are being overregulated in the developing world. In Entine J, ed, *Let them eat precaution*, AEI Press, Washington, DC, 92–112
- Raybould A** (2006) Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. *Environ. Biosafety Res.* **5**: 119–125
- Raybould A** (2007) Ecological versus ecotoxicological methods for assessing the environmental risks of transgenic crops. *Plant Sci.* **173**: 589–602
- Romeis J, Batsch D, Bigler F, Candolfi MP, Gielkens MM, Hartley SE, Hellmich RL, Huesing JE, Jepsen PC, Layton R, Quemada H, Raybould A, Rose RI, Schiemann J, Sears MK, Shelton AM, Sweet J, Vaituzis Z, Wolt JD** (2008) Assessment of risk of insect-resistant transgenic crops to non-target arthropods. *Nature Biotechnol.* **26**: 203–208
- Romeis J, Lawo NC, Raybould A** (2009) Making effective use of existing data for case-by case risk assessments of genetically engineered crops. *J. Appl. Entomol.* **133**: 571–583
- Tanaka A, Tapper BA, Popay AI, Parker EJ, Scott B** (2005) A symbiosis expressed non-ribosomal peptide synthase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory. *Mol. Microbiol.* **57**: 1036–1050
- Todd JH, Ramankutty P, Barraclough EI, Malone LA** (2008) A screening method for prioritizing non-target invertebrates for improved biosafety testing of transgenic crops. *Environ. Biosafety Res.* **7**: 35–56