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Case study

Developing biosafety risk hypotheses for invertebrates exposed to GM plants using conceptual food webs: A case study with elevated triacylglyceride levels in ryegrass

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Regulators are acutely aware of the need for meaningful risk assessments to support decisions on the safety of GM crops to non-target invertebrates in determining their suitability for field release. We describe a process for developing appropriate, testable risk hypotheses for invertebrates in agroecosystems that might be exposed to plants developed by GM and future novel technologies. An existing model (PRONTI) generates a ranked list of invertebrate species for biosafety testing by accessing a database of biological, ecological and food web information about species which occur in cropping environments and their potential interactions with a particular stressor (Eco Invertebase). Our objective in this contribution is to explore and further utilise these resources to assist in the process of problem formulation by identifying potentially significant effects of the stressor on the invertebrate community and the ecosystem services they provide. We propose that for high ranking species, a conceptual food web using information in Eco Invertebase is constructed, and using an accepted regulatory risk analysis framework, the likelihood of risk, and magnitude of impact for each link in the food web is evaluated. Using as filters only those risks evaluated as likely to extremely likely, and the magnitude of an effect being considered as moderate to massive, the most significant potential effects can be identified. A stepwise approach is suggested to develop a sequence of appropriate tests. The GM ryegrass plant used as the "stressor" in this study has been modified to increase triacylglyceride levels in foliage by 100% to increase the metabolisable energy content of forage for grazing animals. The high-ranking "test" species chosen to illustrate the concept are New Zealand native species *Wiseana cervinata* (Walker) (Lepidoptera: Hepialidae), *Persectania aversa* (Walker) (Lepidoptera: Noctuidae), and the self-introduced grey field slug, *Deroceras reticulatum* (Müller).

Keywords: risk analysis / biosafety / genetically modified plants / food webs / non-target species

INTRODUCTION

Crops produced by genetic modification (GM) or by other novel technologies which confer pest or disease resistance, drought tolerance or have other characteristics which improve product quality or productivity have the potential to play an important role in crop production in the future. In many countries, the introduction of such crops into agricultural systems requires regulatory approval. In New Zealand, GM plants are considered "new organisms" and are subject to approval under the Hazardous Substances and New Organisms Act 1996 (HSNO). The Environmental Risk Management Authority (ERMA New Zealand) has responsibility to consider applications under HSNO and applicants need to carry out a risk assessment to demonstrate that the new plants are unlikely to displace any native species within its natural habitat, cause deterioration of natural habitats, or adversely affect New Zealand's inherent genetic diversity. Adverse impacts on beneficial species which carry out important functions in agroecosystems must also be considered. These are amongst the public policy goals that applicants need to address so that the regulator is able to make informed decisions on the biosafety of the plants.

Measuring non-target impacts is an essential part of the risk assessment process for any GM crop. It can be challenging to decide which non-target species should be tested, and what tests to carry out in order to produce

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a meaningful risk assessment that is useful to regulators. Raybould (2006) suggested a procedure by which assessment endpoints, such as valued species, processes, etc., that might be at risk are evaluated against the new technology to determine how they could be subject to harm by developing a testable risk hypothesis. Determination of the most relevant risk hypotheses and a plan to test them are known as problem formulation. Following this rationale, the likelihood of collecting information which does not inform decision-making is reduced. Clearly problem formulation requires case-by-case consideration depending upon the nature of the plant modification, the scale of production and characteristics of the crop itself, the likelihood that the plants will move out of the agricultural environment into natural ecosystems, etc., Romeis et al. (2008a) noted that problem formulation should result in a research plan that determines the relationship between the stressor and the ecological impacts of concern, taking into account ecological considerations that might affect the nature and extent of possible environmental impacts, including the intended scale of cultivation of the GM crop. This approach accords with that of the U.S. Environmental Protection Agency's ecological risk assessment framework which is partitioned into problem formulation, analysis and risk characterization (USEPA, 1998). The output from problem formulation is an analysis plan. The analysis phase investigates aspects of exposure to, and impact from, the stressor and provides the information required to predict ecological consequences. Risk characterisation culminates in information which facilitates decision making, noting assumptions and areas of scientific uncertainty (USEPA, 1998). The EPA guidelines also point out that once a risk assessment is complete, risk management/mitigation measures and risk communication need to be considered.

Todd et al. (2008) have developed a screening method to identify and prioritize non-target invertebrates for risk analysis with GM plants. The method uses a comprehensive body of published information on biological and ecological information, trophic relationships of individual invertebrate species known to be found in the target environment, and information about the transgenic plant being considered, which is entered into a database (Eco Invertebase). This database includes information on the potential hazards posed by the plant, the exposure of each species to the plant, as well as environmental aspects of risk, economic, cultural and social values of species at risk, and the ability to carry out tests with the species. This information is then scored against each of the criteria being measured such that information for a species that is positively correlated with a particular criterion receives a high score for that criterion (*e.g.* rare native species receive high scores for the value criterion, species likely to be susceptible to a toxin expressed by a GM plant receive high scores for the hazard criterion, etc.). A mathematical model (PRONTI – priority ranking of non-target invertebrates) combines the criterion scores to produce an overall score for each species, allowing the species to be more objectively and transparently prioritized for risk assessment testing according to a set of pre-determined selection criteria. The GM plant we have used as a case study is ryegrass (*Lolium perenne* L.), a forage plant which has been modified to produce higher levels of the lipid triacylglyceride (TAG). TAG, consisting of three fatty acids and one glycerol molecule, is a relatively minor lipid in plant leaves and the intention is to increase the normal level of about 3.5% dry weight to 8–9%. The "high-lipid ryegrass" (HLR) plants are currently being developed *via* insertion of the gene controlling the diacylglycerol O-acyltransferase (DGAT) enzyme from *Arabidopsis,* the enzyme involved in the final step in the TAG biosynthesis pathway where DAG (diacylglyceride) is converted to TAG. The transgenic plants will be homozygous, and have increased lipid levels restricted to foliage by means of encapsulation of the TAG by polyoleosins (Roberts et al., 2008). The application of this technology is to increase expression of DGAT in ryegrass leaves and hence to elevate lipid content which will serve to increase the metabolisable energy content of forage for grazing animals. Grazing animals would consequently need to consume less to achieve the same live-weight gains, with associated environmental benefits. Furthermore, encapsulation of lipids would provide consumer health benefits by increasing the proportion of unsaturated fats in dairy and meat products.

Gilbert and Chino (1974) made the observation "To a great extent, the obvious success of insects on this planet has been their ability to utilize lipids efficiently as substrates for reproduction, embryogenesis, metamorphosis and flight". The fat body, an organ which is unique to insects, plays a major role in storage and release of energy in response to demands of the insect. Mobilisation of lipid reserves from the fat body is essential to supply energy for metabolism, growth, activity, flight, embryogenesis (lipids are the main component of insect oocytes and energy source for the developing embryo) and immune response (lipids are mobilised to the haemolymph in response to challenges to the immune system). The fat body also serves as an endocrine organ producing antimicrobial peptides and assists in detoxification during nitrogen metabolism. Fat body cells are known as adipocytes and they contain lipid droplets composed mainly of TAG. Herbivorous insects consuming foliage hydrolyse the TAG by means of midgut lipases (Turunen and Crailsheim, 1996). The products of digestion are absorbed by the midgut epithelium and used for synthesis of TAGs, DAGs and phospholipids. Insect lipid metabolism and fat body function in insects has been reviewed by Gilby (1965), Gilbert and Chino (1974), Canovoso et al. (2001), Van der Horst (1983), and Arrese and Soulages (2010). While most research on invertebrate TAG metabolism has been carried out on insects, Garin et al. (1996) found significant quantities of TAG in the eggs of the snail *Pomacea canaliculata* Lamm.

Intuitively, of the invertebrates including insects, the herbivores are likely to be impacted most by an increase in plant lipid levels. However, parasitoids, especially koinobiont endoparasitoids have a close physiological relationship with their host, and host quality influences parasitoid development and survival. Parasitism has been shown to enhance metabolism of fatbody TAGs and increase the level of fatty acids in the haemolymph (Visser and Ellers, 2008). Early instar parasitoids usually feed on haemolymph of the host, but later stages often feed on fat body directly, or on teratocytes which are derived from epithelial cells surrounding the parasitoid egg. These dissociate once the egg hatches and absorb hydrolysed host lipids from the fat body. Some parasitoid venoms disrupt the host fat body and release lipid to the haemolymph elevating lipid levels. These various mechanisms allow parasitoid larvae to manipulate the lipid reserves of the host so that they do not need to independently synthesise lipids. This is thought to have led to a loss of the ability of many parasitoids to synthesise lipids, a trait that has transferred to the adult stage in the orders Hymenoptera and Diptera which do not synthesise or store fatty acids (Visser and Ellers, 2008). Many adult parasitoids are therefore dependent for their survival and reproduction upon the fat reserves accumulated from the host by the larval stage and are hence influenced by host quality.

While parasitoids are likely to be exposed to a single host throughout their larval development, predators that generally feed on a number of prey organisms also have the opportunity to accumulate TAGs. Mayntz and Toft (2001) showed that the nutrient composition of the food of a prey species can have a beneficial impact on the predator. This was tested in a study using spiders feeding on fruit flies that showed that the main nutrient groups, amino acids and lipids, transferred benefits through two trophic levels. Mayntz et al. (2005) found that predators were able to "balance" their diet to compensate for previous imbalance. Beetles and spiders pre-fed with a lipid-rich diet tended to select more protein-rich food and *vice versa*. This study also demonstrated that, contrary to general belief prey animals were consistent in body composition despite a variable diet, they do in fact vary in their body composition when provided with variable diets.

Ecosystems are extremely complex, and our understanding of the myriad interactions between organisms in even relatively "simplified" agricultural systems is rudimentary. Consequently, uncertainty in predicting risk will inevitably remain, and the limitations arising from this need to be clearly acknowledged in a risk assessment. However, in an attempt to develop more realistic, testable risk hypotheses for GM plants, as called for by Raybould (2006) and others, it is helpful to understand some of the most significant interactions that occur within the biotic community of which the GM plant in question will become a member. In the case of transgenic pasture species such as HLR, clearly the intention is for the GM plant to become a dominant and durable component of the sward. Hagvar and Aasen (2004) presented a number of possible mechanisms by which Bt plants might impact on insects from a range of food web levels, noting examples where evidence is available for such effects. Mulder and Lotz (2009), also with reference to Bt plants, noted the paucity of published research on the impact of Bt corn (*Zea mays* (L.)) on "ecological networks" and impacts on ecosystem services.

Our aim in this paper is to contribute to the theory and process of meaningful risk hypothesis development and problem formulation to better meet the information requirements of GM biosafety regulation by identifying what may be the most significant effects of a proposed GM plant on the invertebrate community and potentially on ecosystem services. Specifically this contributes to the "problem formulation" aspect of the risk assessment framework as defined by the EPA (USEPA, 1998), where risk hypotheses are developed, assessment endpoints identified and risk analysis plans are made. The process employs a stepwise analysis of the trophic relationships within the community, informed by a risk assessment process employed by many regulatory agencies, to define the species within the community that might be most at risk. To illustrate our process, we used three invertebrate species that had scored highly as potential assessment endpoints when Todd et al.'s (2008) PRONTI method was applied to the case of HLR in New Zealand pastures (see Methods for details): *Wiseana cervinata* (Walker) (Lepidoptera: Hepialidae), a New Zealand native species which has become a pest following the introduction of exotic pasture species; *Persectania aversa* (Walker) (Lepidoptera: Noctuidae), the Southern Armyworm, another native species which has become a minor pest in cereals and corn; and the grey field slug, *Deroceras reticulatum* (Müller), an introduced slug which damages seedlings and established plants of a wide range of plant species. Unlike most studies on risk assessment for GM plants, where the stressor is likely to represent an obvious hazard to non-target invertebrates, the transgenic perennial HLR crop used as a stressor in this case study might provide an individual or population benefit to some herbivorous invertebrates, while possibly being a hazard to others, presenting a novel challenge to the process of risk hypothesis development and problem formulation.

Table 1. Risk hypotheses developed for *Wiseana cervina* and *Persectania aversa* larvae feeding on HLR.

Level 1: Larvae feeding on HLR exhibit some/all of the following characteristics:	
Physical/physiological change	Improved survival Larvae grow more rapidly Larvae have increased biomass Larvae have increased TAG levels Pupae have higher biomass Adults have higher biomass Adult females have higher fecundity, produce more eggs
Phenological change	Larvae develop more rapidly through larval instars Larvae pupate earlier Adults emerge earlier in season Eggs produced earlier in season More generations per year
Behavioural change	Larger larvae consume more vegetation Adults have increased mobility
Level 2: Some/all of the Level 1 effects are demonstrated, so consider:	
Population effect	Species has increased fitness, density, competitive ability, resistance to starvation Species becomes more dominant in the pastoral invertebrate community
Tritrophic effect	Natural enemies experience increased fitness, density, competitive ability Natural enemies benefit/disadvantaged by changes in host phenology
Effect on vegetation	Food plants under increased pressure from herbivores
Level 3: Some/all of the Level 2 effects are demonstrated, so consider:	
Trophic cascade effect	Other hosts of parasitoids/predators at increased risk from fitter and more abundant natural enemies Reduced impact on plants from herbivores which are under increased natural enemy pressure

RESULTS AND DISCUSSION

The potential impact of HLR on *W. cervinata*, *P. aversa* and *D. reticulatum* (Müller) is shown in Tables 1 and 2. The direct effects of the stressor on herbivores can be divided into aspects of increased growth rates, biomass, and TAG level; changes in developmental rate, generation time and seasonality; and changes in feeding and activity (dispersal capability, etc.). If some or all of these potential effects are demonstrated in laboratory feeding tests that compare HLR with an equivalent control then the impacts of these changes at Level 2 could be considered. These could include population effects which could be predicted from increased survival and fitness of individuals, increased ability to compete, resist diseases and avoid starvation, and tritrophic effects on natural enemies. The magnitude of population effects that would be of concern would be easy to determine for pest species where damage thresholds are well known. Further trophic cascade effects that could be considered are indicated in Tables 2 and 3 if evidence for Level 2 effects is demonstrated. While Tables 1 and 2 list measurements that can be made in order to accept or reject the hypothesis that a stressor with enhanced nutrient qualities will be beneficial to aspects of invertebrate growth and development, if experimental data suggested a detrimental effect, then a new hypothesis would need to be constructed.

Wiseana cervinata

Using information from Eco Invertebase, a food web for *W. cervinata* is shown in Figure 1. This New Zealand native species has exploited exotic pasture species introduced for pastoral farming and become a major pasture pest in New Zealand. Considerable research has been carried out on this species and hence information on food plants and natural enemies is readily available (*e.g.*

Table 2. Risk hypotheses developed for *Deroceras reticulatum* feeding on HLR.

Level 1: Slugs feeding on HLR may exhibit some/all of the following characteristics:	
Physical/physiological change	Immature stages grow more rapidly All stages have increased biomass All stages have increased lipid levels Females have increased fecundity, produce more eggs
Phenological change	Immature stages reach maturity more rapidly Slugs complete more generations per year Eggs produced earlier in season
Behavioural change	Larger slugs consume more vegetation
Level 2: Some/all of the Level 1 effects are demonstrated, so consider:	
Population effect	Species has increased fitness, density, competitive ability, resistance to starvation Species becomes more dominant in the pastoral invertebrate community
Tritrophic effect	Natural enemies experience increased fitness, density, competitive ability Natural enemies benefit/disadvantaged by changes in host phenology
Effect on vegetation	Food plants under increased pressure from herbivores
Level 3: Some/all of the Level 2 effects are demonstrated, so consider:	
Trophic cascade effect	Other hosts of parasitoids/predators at increased risk from fitter and more abundant natural enemies Reduced impact on plants from herbivores which are under increased natural enemy pressure

Cameron et al., 1989; Eyles, 1966), although no biological control agents specifically for *W. cervinata* have successfully established in New Zealand (Ferguson et al., 2007). Several vertebrate predators are listed in Eco Invertebase such as birds, rat, cat and hedgehog, all of which are large, mobile and general feeders. These were considered unlikely to be affected by a change in the nutritional status of a single prey item (Tab. 3). However *Thyrocephalus chloropterus* (Erichson) (Coleoptera: Staphylinidae) is a predator feeding on a range of invertebrates which is more likely to remain in the HLR pasture and feed on Lepidoptera larvae. Eyles (1973) showed that *Wiseana* spp. are consumed by *T. chloropterus* and that consumption rates are potentially high. Thus, an increase in the nutritional value of *W. cervinata* could have a significant effect on *T. chloropterus* populations. Parasitoids with a wide host range such as *Plagiomyia* sp. and *Lissopimpla excelsa* (Costa), which were likely to benefit from potentially larger, more abundant and energy-rich hosts, were therefore considered to potentially pose a greater threat to populations of other species in the environment as opposed to more host-specific natural enemies (Tab. 3; Fig. 1). In contrast, generalist herbivors exposed to GM plants, such as insect resistant plants, would be expected to be less affected because they could switch to alternative hosts (Romeis et al., 2008b). *Wiseana cervinata* feeds on a wide range of plant species (Fig. 1), which would be disadvantaged by higher densities of larger, fitter herbivores in the environment. Those plants likely to be in pastures with HLR, such as other grasses and clover, were considered to be most at risk (Fig. 1).

Persectania aversa

As for *W. cervinata*, a food web was constructed for *P. aversa* (Fig. 2). A similar logic to that outlined for *W. cervinata* above was followed for this species. Birds known to feed on *P. aversa* such as magpies, gulls and starlings were not considered to be susceptible to a change in the nutritional status of herbivores in a pasture because of their mobility and wide food range (Tab. 4). In this case impacts of increased fitness of *P. aversa* populations in pasture were considered most significant for two predatory Hemiptera and three parasitoids. *Nabis kinbergii* Reuter (Hemiptera: Nabidae) is a polyphagous predator of invertebrates, capable of consuming large numbers of lepidopteran larvae (Ma et al., 2005). A population of this species was considered likely to benefit from the presence of a higher density of nutritionally enhanced prey. Similarly, *Cermatulus*

Table 3. Level 2 predicted risk assessment for *Wiseana cervinata* feeding on HLR. Shaded rows are those where risks are considered likely (or more) to occur with moderate (or higher) magnitude.

Risk: Predators experience increased fitness, density, competitive ability				
Predator	**Likelihood**	**Magnitude**	**Reason**	**Assessment of potential impact**
Gymnorhina tibicen hypoleuca (Gould) (magpie)	Very unlikely	Minimal	Wide range of prey, mobile, prey probably not limiting, feed during day when *W. cervinata* not on surface	None
Erinaceus europaeus L. (hedgehog)	Unlikely	Minor	Insectivorous, wide range of prey, mobile, prey probably not limiting, but feed at night when *W. cervinata* active	Population could benefit from abundant, nutrient rich food source
Rattus exulans (Peale) (rat)	Very unlikely	Minor	Omnivorous, very wide food range, mobile, prey probably not limiting, but feed at night when *W. cervinata* active	None
Felis catus L. (cat)	Very unlikely	Minimal	Wide range prey, mobile, prey probably not limiting, but probably has preference for vertebrate prey. Feed at night when *W. cervinata* active	None
Sturnus vulgaris L. (starling)	Unlikely	Minor	Wide range prey, mobile, but feed in flocks and known to select available abundant prey-type; feed during day when *W. cervinata* not on surface, but recorded feeding on this species	Population could benefit from abundant, nutrient rich food source, available earlier for feeding young
Thyreocephalus chloropterus (Erichson) (rove beetle)	Likely	Moderate	Likely to have wide range prey, but relatively immobile, prey could be more limiting in pasture. Demonstrated that *Wiseana* very acceptable prey and consumption rates potentially high (Eyles, 1973)	Individual and population benefit from abundant, nutrient rich food source
Risk: Parasitoids experience increased fitness, density, competitive ability				
Parasitoid	**Likelihood**	**Magnitude**	**Reason**	**Assessment of potential impact**
Pales usitata Hutton	Likely	Minor	*Wiseana* spp. are only known hosts, solitary parasitoid	Benefit from energy-rich host
Hexamera alcis (Walker)	Likely	Minor	*Wiseana* spp. are only known hosts, solitary parasitoid	Benefit from energy-rich host
Ichneumon lotatorius F.	Likely	?	No information available on host range, biology	Possible benefit from energy-rich host
Occisor versutus Hutton	Likely	?	No information available on host range, biology	Possible benefit from energy-rich host

Table 3. Continued.

Risk: Parasitoids experience increased fitness, density, competitive ability

Parasitoid	Likelihood	Magnitude	Reason	Assessment of potential impact
Plagiomyia sp.	Likely	Moderate	Also attacks *Agrotis ypsilon anietuma* Walker, *Graphania* (*Melanchra*) *ustistriga* Walker, gregarious parasitoid	Benefit from energy rich host, increase number parasitoids per host, increased impact on other native hosts (some also minor pests)
Lissopimpla excelsa (Costa)	Likely	Moderate	Solitary parasitoid but wide host range not limited to Hepialidae: *Mythimna separata*, *Wiseana umbraculata*, *Orthoclydon praefectata*, *Laphygma exempta*, *Tiracola plagiata*, *Herpetogramma licarsisalis*, *Pseudocoremia suavis*, *Epiphyas postvittana*	Benefit from energy rich host, increased impact on other native hosts (some also minor pests) but likely to be wider
Degithina decepta (Smith)	Likely	Minor	*Wiseana* spp. are only known hosts	Benefit from energy-rich host

Risk: Food plants under increased pressure from herbivore feeding on HLR

Plant	Likelihood	Magnitude	Reason	Assessment of potential impact
HLR (GM)	Extremely likely	Major	Amongst preferred plants in diet so increased fitness and abundance of *W. cervinata* could increase feeding intensity on HLR	Disadvantaged by increased pest pressure
Cortaderia sp. (pampas grass)	Highly improbable	Minimal	Plant could be at margins of HLR crop, but unlikely to feed preferentially on pampas grass. Offspring of HLR-fed *W. cervinata* unlikely to cause anything other than superficial damage except perhaps to seedling plants	None
Tussock spp.	Very unlikely	Minor	Plant could be in same environment as HLR, but unlikely to be fed on preferentially. Offspring of HLR-fed *W. cervinata* unlikely to reach sufficient population density to have a major impact on tussock species	None
Trifolium repens L.	Very likely	Major	Plant in same environment as HLR, and is amongst preferred plants in diet so increased fitness and abundance of *W. cervinata* could increase pressure on white clover	Disadvantaged by increased pest pressure
Medicago sativa	Unlikely	Minor	Plant not usually in the same environment as HLR although could be adjacent and so increased pest density could be have a small impact. Not a particularly preferred food plant.	Minor
Other pasture spp.	Likely	Moderate	Other pasture species in sward with HLR likely to be subject to increased feeding pressure.	Minor
Barley, potato, brassicas	Extremely unlikely	Minimal	Damage recorded as rare	None

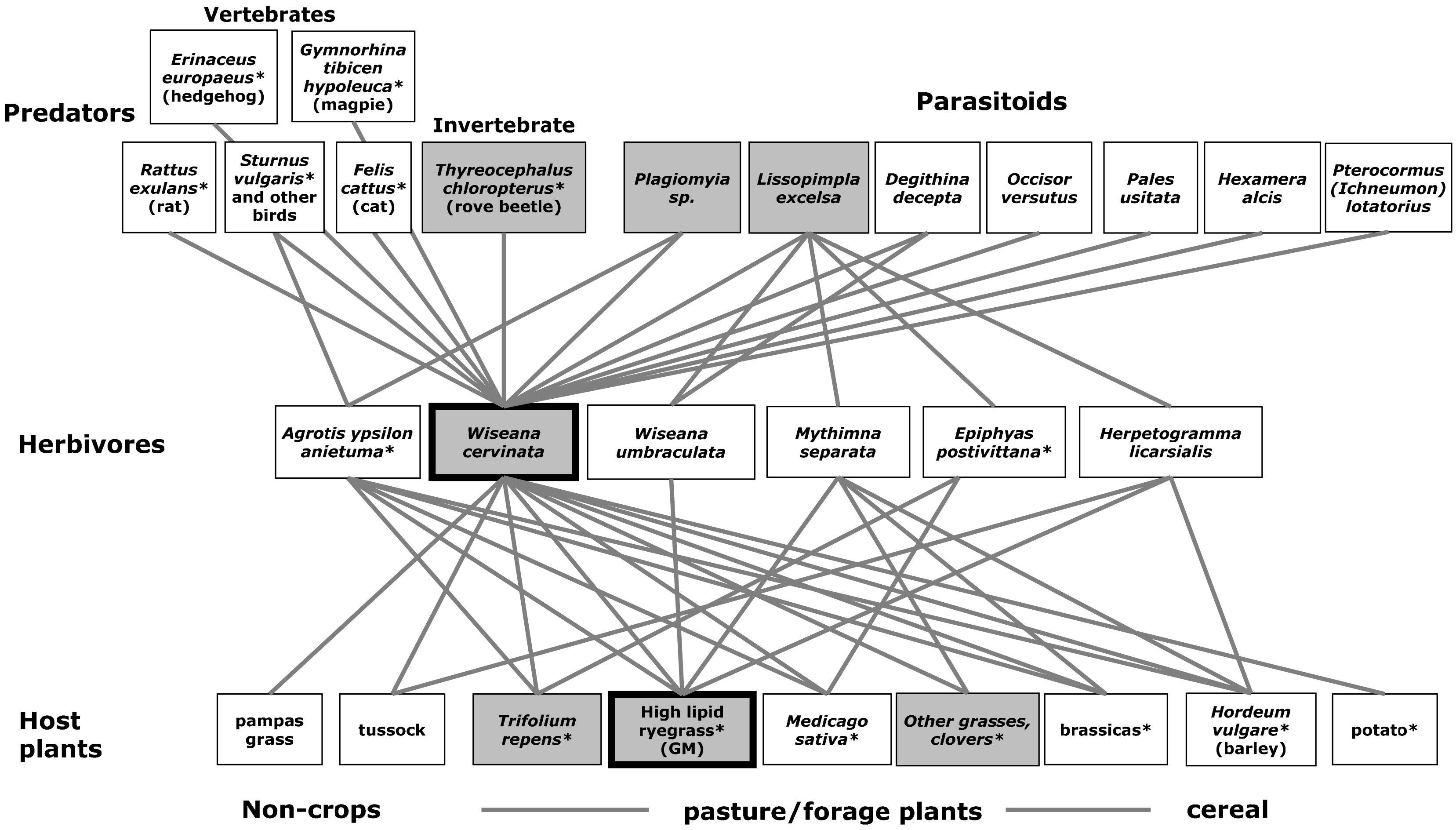

Figure 1. Food web for *Wiseana cervinata* including all records in Eco Invertebase. Boxes with thick borders contain the test species and stressor; species followed by an asterisk are exotic to New Zealand. Shaded boxes indicate species for which the risk assessment suggested that the stressor would have a "likely" or higher impact with "moderate" or higher severity of impact (see Tab. 4).

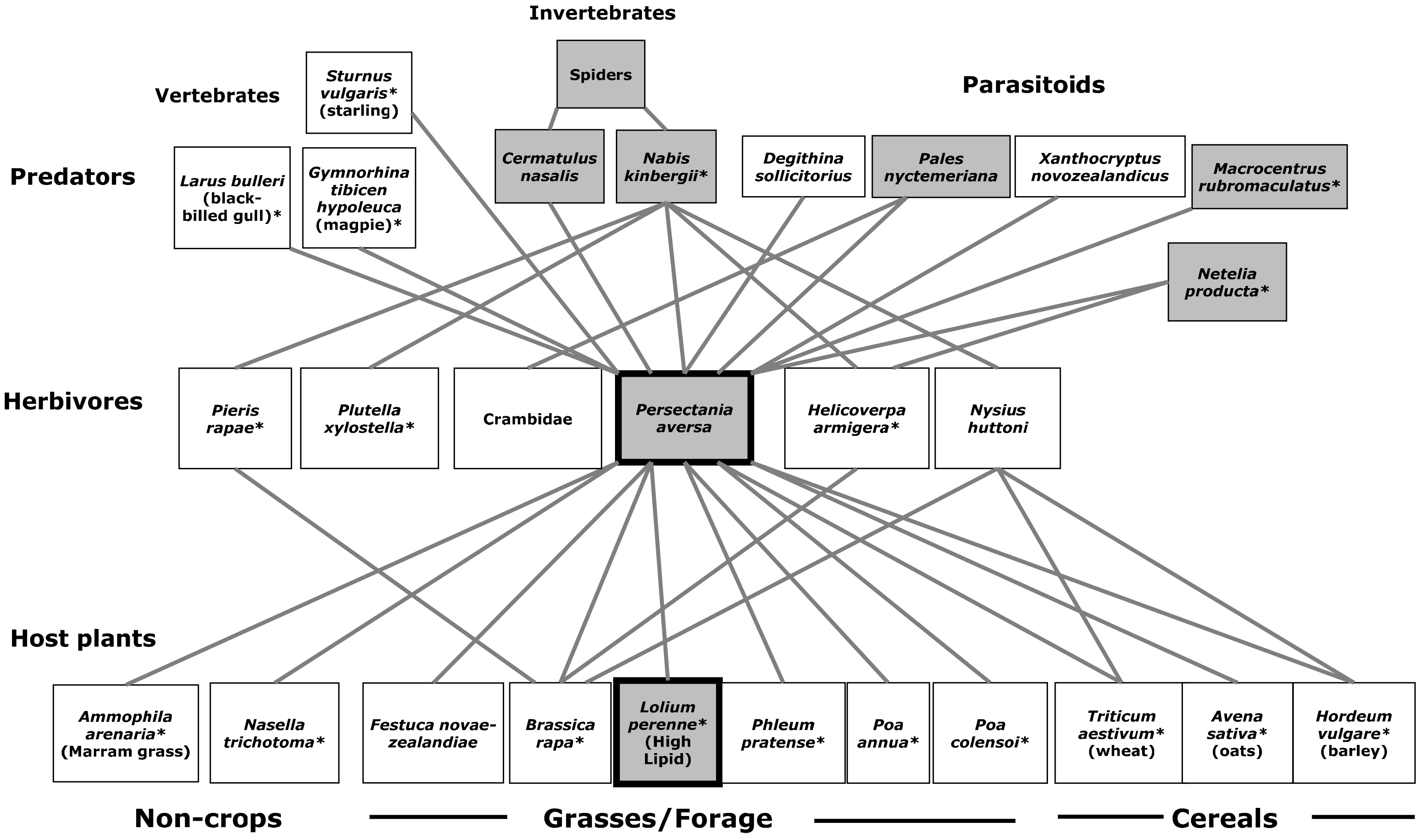

Figure 2. Food web for *Persectania aversa* including all records in Eco Invertebase. Boxes with thick borders contain the test species and stressor; species followed by an asterisk are exotic to New Zealand. Shaded boxes indicate species for which the risk assessment suggested that the stressor would have a "likely" or higher impact with "moderate" or higher severity of impact (see Tab. 5).

Table 4. Level 2 predicted risk assessment for *Persectania aversa* feeding on HLR. Shaded rows are those where risks are considered likely (or more) to occur with moderate (or higher) magnitude.

Risk: Predators experience increased fitness, density, competitive ability				
Predator	**Likelihood**	**Magnitude**	**Reason**	**Assessment of potential impact**
Larus bulleri Hutton (black-billed gull)	Very unlikely	Minimal	Wide range prey, mobile, prey probably not limiting, *P. aversa* cryptically coloured	None
Magpie	Very unlikely	Minimal	Wide range prey, mobile, prey probably not limiting, *P. aversa* cryptically coloured	None
Starling	Unlikely	Minor	Wide range prey, mobile, but feed in flocks and known to selectively feed on available prey-type; *P. aversa* cryptically coloured	Population could benefit from abundant, nutrient rich food source, available earlier for feeding young
Nabis kinbergii Reuter	Likely	Moderate	Polyphagous, moderately mobile predator, feeds on wide range of Lepidoptera, Hemiptera, Diptera (Syrphidae), mites. Known to consume large numbers of *Plutella xylostella* (Lepidoptera: Plutellidae) (Ma et al., 2005)	Population and individual benefit from energy rich host, increased impact on wide range of hosts (some are pests)
Cermatulus nasalis (Westwood)	Likely	Moderate	Polyphagous, mobile predator, feeds on Lepidoptera (incl. red admiral), Coleoptera, Hymenoptera, Hemiptera (cicadas). Higher feeding levels produced heavier adults. Considered beneficial.	Population and individual benefit from energy rich host, increased impact on other native hosts (some also minor pests) but possibly be wider
Risk: Parasitoids experience increased fitness, density, competitive ability				
Parasitoid	**Likelihood**	**Magnitude**	**Reason**	**Assessment of potential impact**
Xanthocryptus novozealandicus Dalla Torre	Highly improbable	Minimal	Native parasitoid of wood-boring Coleoptera and records on noctuids considered doubtful (Parrott, 1952). If attacked development would probably fail	None
Macrocentrus rubromaculatus (Cameron)	Likely	Moderate	Polyembryonic so improved nutritional resource in host could result in higher numbers, known to attack several noctuid spp.	Benefit from energy rich host, increased impact on other native hosts (some also minor pests) but possibly be wider
Degithina sollicitorius (F.)	Likely?	Minor-mod??	Not much information on this parasitoid. Pupal parasitoid so not developing in feeding host but pupal resources could be improved	Possibly minor – moderate but more info required
Netelia producta (Brulle)	Likely	Moderate	Solitary parasite but wide host range	Benefit from energy rich host, increased impact on other native hosts (some also minor pests) but possibly be wider

Table 4. Continued.

Risk: Parasitoids experience increased fitness, density, competitive ability				
Parasitoid	**Likelihood**	**Magnitude**	**Reason**	**Assessment of potential impact**
Pales nyctemeriana Hudson	Likely	Moderate	Solitary parasitoid but host range includes sod webworms, *Nyctemera annulata* (Hypsidae), *Melanchra* spp., *Ariathisa comma* (Noct), *Selidosema* sp. (Geometridae)	Benefit from energy rich host, increased impact on other native hosts (some also minor pests) but possibly be wider
Risk: Food plants under increased pressure from herbivore feeding on HLR				
Plant	**Likelihood**	**Magnitude**	**Reason**	**Assessment of potential impact**
HLR (GM)	Extremely likely	Major	Amongst preferred plants in diet so increased fitness and abundance of *P. aversa* could increase feeding intensity on HLR	Disadvantaged by increased pest pressure
Ammpohila arenaria (L.) Link	Highly improbable	Minimal	Marram grass not found in same environment as HLR, although could be in vicinity, but probably not highly preferred species. Population density unlikely to be high enough in this environment to have an impact on the plant	Insignificant
Nasella trichotoma (Nees) Hack.	Unlikely	Minimal	Considered an important weed. Could occur in same environment as HLR but unlikely to be a preferred species. Weed status suggests impact of no concern	Insignificant
Festuca novaezelandiae (Hack.) Cockayne	Unlikely	Minor	Fescue tussock found in mid-altitude tussock grasslands. Not found in same environment as HLR, although could be in vicinity, but probably not highly preferred species. Population density unlikely to be high enough in this environment to have an impact on the plant	Insignificant
Phleum pratense L.	Likely	Minor	Plant in the same environment as HLR, possibly not a preferred plant species, and not a critically important component of pastures	Insignificant
Poa annua L.	Likely	Minimal	Plant often in the same environment as HLR, but considered a weed grass. Consequently damage is not likely to be of concern	Insignificant
Poa colensoi Hook.f.	Unlikely	Minimal	Blue tussock found in mid-altitude tussock grasslands. Not found in same environment as HLR, and probably not highly preferred species. Population density unlikely to be high enough in this environment to have an impact on the plant	Insignificant
Brassica rapa L., wheat, barley, potato	Unlikely	Minimal	Not found in same environment as HLR, and probably not highly preferred species. Population density unlikely to be high enough in this environment to have an impact on the plant	Insignificant

nasalis (Westwood) (Hemiptera: Pentatomidae), is another polyphagous predator. Edwards and Suckling (1980) found that when provided with coleopteran larvae, higher feeding levels of *C. nasalis* resulted in larger, heavier adults with shorter developmental times. The parasitoid *Macrocentrus rubromaculatus* (Cameron) (Hymenoptera: Braconidae) is gregarious and known to parasitise a number of noctuid species (Parrott, 1954). An improved nutritional resource could result in higher numbers of parasitoids per host. *Netelia producta* (Brulle) is a solitary ichneumonid, with a wide lepidopteran host range, and *Pales nyctemeriana* Hudson is a tachinid parasitoid also known from a number of hosts in the lepidopteran families Noctuidae and Geometridae (Valentine, 1967). Although *P. aversa* was considered likely to feed on the grasses *Phleum pratense* and *Poa annua*, these are less productive species in pasture and hence the magnitude of impact on these species was considered minor and minimal, respectively.

Deroceras reticulatum

The food web for *D. reticulatum* constructed using information in Eco Invertebase is shown in Figure 3. This species has a very wide host plant range including pasture, cereals, vegetable and ornamental crops (Fig. 3). In contrast to the two Lepidoptera, it can also be predatory (and cannibalistic) under some circumstances. Natural enemies of *D. reticulatum* recorded in the literature are mainly generalist vertebrates which were not considered likely to be significantly advantaged by energy-rich prey items. However, invertebrate predators such as Carabidae, for which there are some data on slug predation, but which also feed on a wide range of prey were considered likely to experience benefits, and hence potentially impact negatively on other invertebrates. Because of the limited mobility of slugs, widespread impacts on crops outside of the pasture environment were not considered likely. As for the previous case studies, avian predators were not considered likely to be subject to anything more than a minor impact from an increase in the abundance or nutritional status of slugs (Tab. 5). However, species of Carabidae, including *Megadromus antarcticus* (Chaudoir), which would be more restricted to feeding within a pasture have been reported feeding on *D. reticulatum*. *Megadromus antarcticus*, while not widely distributed in New Zealand pastures, was found to consume 0.55 slugs/beetle per day (Chapman et al., 1997), which might be indicative of consumption rates for other species in the genus. Carabidae usually feed nocturnally, which coincides with the main activity period for slugs and so it was considered likely that if slugs benefited from feeding on HLR, then there would be a moderate impact on these predators. *Deroceras reticulatum* is known to feed on pasture grasses and legumes, and an increase in population density and growth rates was considered extremely likely to impact on these species with potentially major magnitude.

It should be taken into account that the information used to construct the food webs was only that which has been entered into Eco Invertebase. Clearly there are other trophic relationships that could be added with current knowledge, both published and unpublished. Consequently the more comprehensive the database becomes, the more complex and hence informative the food webs can become, and the more relevant the risk hypotheses developed from them. In addition, it is likely that it will always be necessary to consult literature further to find more detailed information about natural enemies in order to make judgements about the likelihood and magnitude of potential impacts. Needless to say, high quality information is not always available.

GM plants such as HLR present a novel challenge for risk assessment. Consideration of comparative risk from alternative management strategies is vital in presenting a balanced assessment of risk (*e.g.* use of pesticides *cf.* insect resistant GM plants). In this case study there is no equivalent non-GM technology for elevating ryegrass lipids, although environmental benefits from changed farm management practices (*e.g.* reduced application of fertilisers) are likely to accrue from the use of HLR for grazing stock, and these could be brought into the risk assessment equation. A plant which might benefit invertebrate herbivores in the environment, at face-value, might be dismissed as of no consequence. However, given the complexity of ecosystems, a benefit to one species may represent a hazard to another species within the same community. Clearly an understanding of the potential impact of the modified plant on the pest status of its herbivores is desirable. Furthermore, the danger of an innocuous herbivore or minor pest acquiring pest prominence in a crop with altered nutritional status would also represent an undesirable outcome. Eco Invertebase and PRONTI are well equipped to identify such (often non-intuitive) potential, and to serve as a repository for food web information that can be utilised to develop risk hypotheses.

Several studies have recommended methods for risk hypothesis development and test species selection for GM plants (*e.g.* Romeis et al., 2008a, 2011; Wolt et al., 2010) largely based on principles and guidelines of USEPA (1998) and EFSA (2010). Our approach employs a database of published information on species in the receiving environment encompassing hazard, exposure, cultural, economic and environmental values as well as biological, phenological and ecological information. This allows trophic interactions between invertebrates present in the environment to be identified and significant potential impacts from the stressor recognized. Hence the

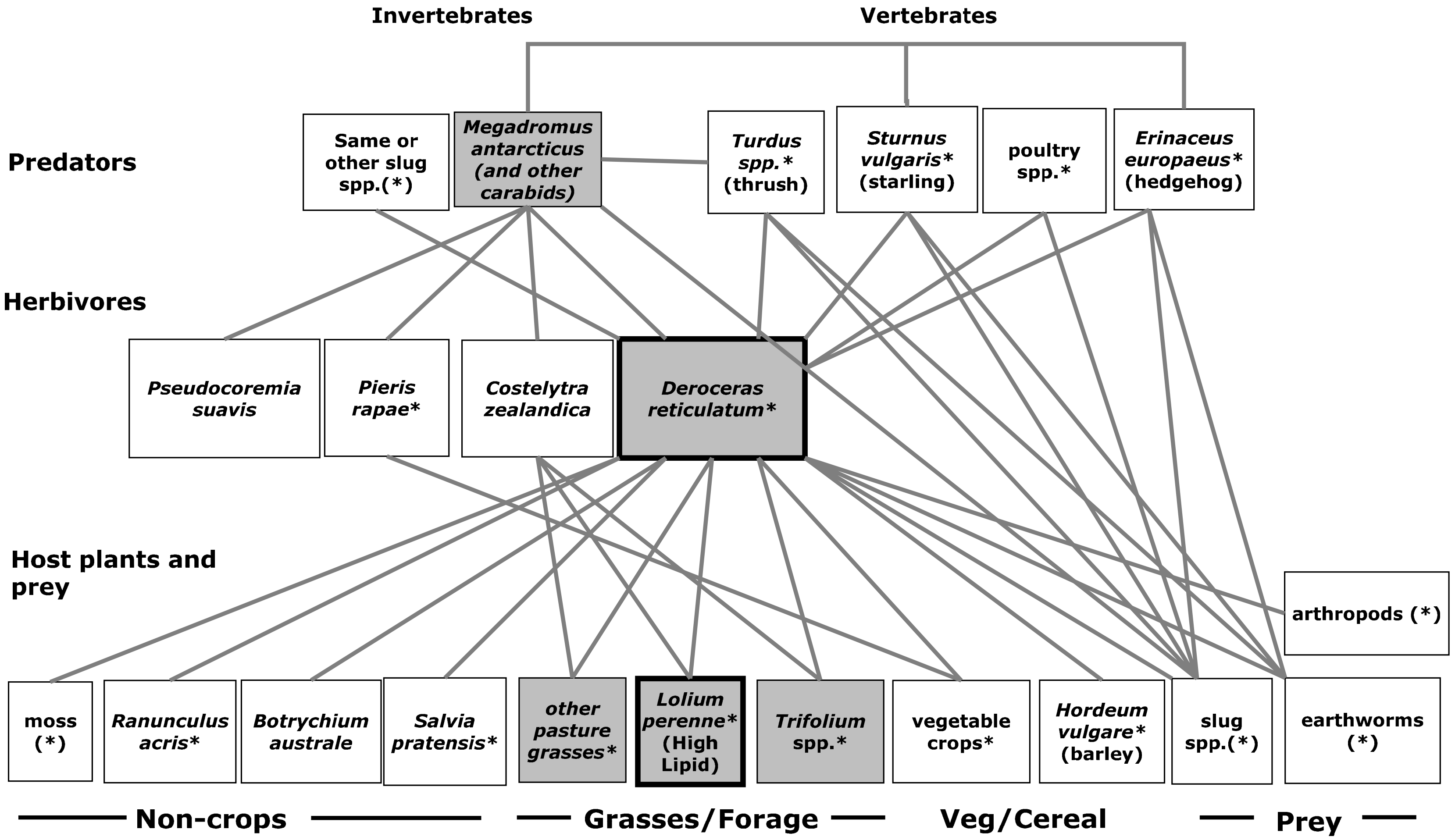

Figure 3. Food web for *Deroceras reticulatum* including all records in Eco Invertebase. Boxes with thick borders contain the test species and stressor; species followed by an asterisk are exotic to New Zealand, an asterisk in brackets shows groups in which some species might be exotic. Shaded boxes indicate species for which the risk assessment suggested that the stressor would have a "likely" or higher impact with "moderate" or higher severity of impact (see Tab. 6).

Table 5. Level 2 predicted risk assessment for *Deroceras reticulatum* feeding on HLR. Shaded rows are those where risks are considered likely (or more) to occur with moderate (or higher) magnitude.

Risk: Predators experience increased fitness, density, competitive ability

Predator	Likelihood	Magnitude	Reason	Assessment of potential impact
Hedgehog	Likely	Minor	Wide range prey, mobile, feed at night when slugs active; probably some benefit from abundant nutrient-rich food source	Minor benefit
Turdus philomelos Br. (thrush)	Unlikely	Minor	Wide range prey, mobile; feed during day when slugs less active, but can still find them successfully; probably some benefit from abundant nutrient-rich food source	Minor benefit
Starling	Unlikely	Minor	Wide range prey, mobile, but feed in flocks and known to select available prey-type; feed during day when slugs not active, but recorded on this species; probably some benefit from abundant nutrient-rich food source	Minor benefit
Poultry	Unlikely	Minor	Wide range prey, mobile, feed during day when slugs less active; probably some benefit from abundant nutrient-rich food source	Minor benefit
Megadromus antarcticus (Chaudoir)	Likely	Moderate	Distribution limited to Canterbury, but capable of reducing slug populations, consume mean of 0.25 slugs/beetle/day in the field (Chapman et al., 1997)	Moderate benefit
Other Carabidae	Likely	Moderate	Wide range prey, mobile, feed at night when slugs active. Large carabid species known to be slug predators; probably benefit from abundant nutrient-rich food source	Moderate benefit

Risk: Food plants under increased pressure from herbivore feeding on HLR

Plant	Likelihood	Magnitude	Reason	Assessment of potential impact
HLR (GM)	Extremely likely	Major	Amongst preferred plants in diet so increased fitness and abundance of slugs could increase feeding intensity on HLR	Disadvantaged by increased pest pressure
Other pasture grasses	Extremely likely	Major	Amongst preferred plants in diet so increased fitness and abundance of slugs could increase feeding intensity on pasture grasses	Disadvantaged by increased pest pressure
Pasture legumes	Extremely likely	Major	Amongst preferred plants in diet so increased fitness and abundance of slugs could increase feeding intensity on clovers	Disadvantaged by increased pest pressure
Vegetable crops	Unlikely	Minor	Many are preferred plants in diet, but not usually growing in same environment as HLR, but could be in close proximity. However, slugs not highly mobile	Minor risk to crop
Barley	Unlikely	Minor	Acceptable food plant for slugs but not found in same environment as HLR, but could be in close proximity. However, slugs not highly mobile	Minor risk to crop

Table 6. Risk framework: for each risk identified, the likelihood of occurrence, and if it did occur, the magnitude of the adverse effect are estimated. The shaded areas represent the threshold used to determine significant risk in the case study examples.

Likelihood of an adverse effect	**Magnitude of adverse effect**
Highly improbable	Minimal (short term, localised)
Improbable (remote)	Minor
Very unlikely	Moderate
Unlikely	Major
Likely	Massive (irreversible ecosystem damage incl. species loss)
Very likely	
Extremely likely	

methodology has the ability to incorporate and synthesise a large body of information about the particular ecosystem into the process of risk hypothesis development.

METHODS

The information in Eco Invertebase was used to construct a conceptual trophic food web for three pasture-dwelling invertebrates which ranked highly in PRONTI. The PRONTI model (Todd et al., 2006) had been modified to allow for stressors that might represent a benefit to potential test species rather than a hazard. The model used was essentially the same as that described by Todd et al. (2008) except for two changes. Firstly, the interaction between each receptor species and the stressor was assessed for the likelihood of the receptor benefiting from the stressor (either directly from feeding on the stressor or indirectly through an intermediary species) rather than assessing the potential hazard posed by the stressor. Secondly, a new resilience parameter (replacing parameter I4 in Todd et al. (2008)) was calculated using a measure of the receptor's attributes that could either constrain its ability to benefit from the stressor or reduce its level of exposure to the stressor (*e.g.*, low reproductive rate, large number of predators, presence of disease, other resource limitations, low receptor density in the stressor's target ecosystem, stressor forms only a small portion of the diet). These new benefit and resilience measures directly replaced the hazard and resilience parameters in the PRONTI model, which was otherwise unchanged.

The two Lepidoptera species, *W. cervinata* and *P. aversa* were chosen because they were two of the species considered to be at greatest risk from the stressor according to the output from the PRONTI model. As pests they are important in this situation where the stressor may be of benefit to the assessment endpoints. *Deroceras reticulatum*, the introduced slug (also a pest) ranked less highly by the PRONTI model, was chosen as a contrasting example of a non-arthropod invertebrate, which can also be predatory. The food web was constructed not only using species recorded as natural enemies or host plants of the test species, but also the trophic relationships of the natural enemies themselves, their hosts, food plants, etc.

For each of the three test species chosen for this case study, all predators, parasitoids and food plants recorded in Eco Invertebase were listed, and for each trophic link in the food web an assessment was made of the likelihood of an effect, and the potential magnitude of an effect if it was to occur. The method used to assess risk at each level was based on the framework used by ERMA New Zealand (Tab. 6) to assess each risk identified by the applicant to develop or introduce a new organism and those submitting views on such applications (ERMA New Zealand, 1998).

Risk hypotheses were constructed at three levels for which tests could be carried out sequentially. Firstly, the potential impact(s) of the stressor on the test species ranked by PRONTI were considered (Level 1). Laboratory tests would need to be carried out to test these hypotheses. The assumption was then made that some or all of these impacts were realised, and Level 2 risk hypotheses were then constructed around potential impact at lower and higher trophic levels. Each of these was categorised using the risk framework rankings (Tab. 1) and justifiable reasons for the ranking given, using information available about the species concerned from references recorded in Eco Invertebase, and other information available in the literature. Those species considered to be at "moderate" risk (or above), with a likelihood of "likely" (or above), were then used to construct a "main effects" food web.

This simplified food web could then be used both to guide the design of experiments to test the Level 2 hypotheses and to support decisions on further testing that could be carried out if some or all of the Level 1 risk hypotheses were proven. Based on the results from Level 2 testing, impacts beyond this could be considered (Level 3), however, comprehensive data for this would probably not be available from Eco Invertebase, but would have to come from further literature searching or experimentation. If the Level 1 risk hypotheses were not or only partially proven, then it may not be necessary to proceed to Level 2, or the rationale for Level 2 risk hypotheses could be modified.

For an application to ERMA NZ, risk hypotheses appropriate to the type of application (*e.g.* application for field trial *vs.* application for release) would need to be developed. Those which can be tested in the laboratory are clearly unlikely to provide information on population impacts and changes in community species composition. In an application for a field trial, decisions would be made on the basis of laboratory containment tests, and would need to express the intention to examine population effects of the stressor in the field trial. An application for a field release would require that such data are available.

CONCLUSION

The number of invertebrate species that potentially could be exposed to transgenic or "novel" pasture plants is vast. Clearly they cannot all be included in biosafety testing. However, selection of species from a ranked list achieved using the PRONTI ranking system is likely to be attractive to regulators because it is relatively objective, transparent, and based on a comprehensive body of information derived from published sources. The case studies in this paper have illustrated the potential for Eco Invertebase to be further used to construct simple trophic food webs, and we have demonstrated how food webs can be used to generate testable hypotheses relating to indirect interactions between invertebrates and transgenic plants. We need to acknowledge that the food webs are limited to three trophic levels and at this stage do not incorporate decomposers and soil micro-organisms, which are also major drivers of ecosystem function and services, and should be incorporated to advance this approach further. We have demonstrated a process which has a tiered structure so that if, after exposing a test insect to the stressor in robust laboratory tests, Level 1 risk hypotheses are rejected, then higher tier tests (Levels 2 and 3) would in most cases be unnecessary. The process allows for assessment endpoints to be specifically identified in advance of tests being conducted using a conventional risk assessment approach, such as that outlined in the U.S. Environmental Protection Agency Guidelines (USEPA, 1998), that is meaningful to regulators. Determining when sufficient information has been collected in order for regulators to make a robust decision will always be challenging, especially in the face of uncertainty that will inevitably remain. Clearly, long-term impacts and the potential for irreversible ecological change also need to be considered.

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