Ecofriendly control of insect pests for enhancing grassland production

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ABSTRACT

Despite the increasing breadth of knowledge on the potential for using natural enemies for pest control, there still remains a heavy reliance on use of chemical pesticides to maintain productivity in grassland agriculture. Here a more knowledge-based approach that utilizes the suite of natural enemies to suppress and manage pest populations is advocated. This approach incorporates use of endophytic grasses and plant tolerance, together with classical biological control programs and conservation of the natural enemy complex including parasitoids, predators and entomopathogens. Biopesticides will also be a necessary part of ecofriendly pest management as inevitably perturbations will result in pest outbreaks. Understanding the biotic interactions within the grassland environment and aligning and integrating those with a management program that avoids disrupting and conserves biological control will provide more long term and economical pest management than can be achieved with chemical pesticides.

***Keywords*:** Biopesticides, biotic interactions, entomopathogens, fungal endophytes of grasses, parasitoids, predators.

Introduction

Insect pests in productive grasslands have direct and measurable effects on yield of crops and pastures and persistence of sown perennial species that severely impact on the anticipated economic value. Many factors are contributing to changes in the susceptibility of grazing systems to pests and their impacts. These include increasing intensification of agriculture, improved nutritive value of host plants, greater fertilizer inputs, changes in plant diversity, interactions with vertebrate grazing, management at the landscape scale and climate change. Furthermore, as movement of goods and people increase across the globe, pests are entering new territories, placing further pressure on agriculture. Pest outbreaks are an obvious result but less apparent is the year-round pressure from a range of herbivorous insect species that gradually and insidiously erodes the productivity of grazed grasslands.

Over the last 60 years the control of insect pests in agriculture has relied almost entirely on the use of insecticides which has allowed farmers to increase production to meet the burgeoning demand for food. With increasing instances of pesticide resistance, non-target effects of insecticides, contamination of soil and waterways, exposure to residues in food and risks to human health there is now more than ever a need for a more ecological approach to pest control. Such an approach enlists the armory of weapons that nature provides to deliver economical, environmentally safe and sustainable solutions to pest management.

This paper focuses on use of biological control in all its many forms, including use of naturally occurring plant endophytes, predators, parasites and entomopathogens. Many of the examples given are from New Zealand where there is a strong focus on using such methods. As a small island nation in the South Pacific, with an economy that is highly dependent on pastoral agriculture, incursions of insect pests over a long period of time have caused immeasurable losses in pasture production (Goldson *et al.,* 2005). The pasture ecosystem here comprises introduced species, predominantly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), and therefore lacks the plant diversity and trophic complexity of evolved grasslands elsewhere. The insect pests which cause damage are a combination of endemic species that have adapted well to the improved pastures, as well as invasive alien species. Research has focused on developing eco-friendly solutions for proactive and preventative management of the pest complex (Jackson *et al.,* 2002), utilizing both natural population regulators such as diseases, development of biopesticides, classical biological control methods involving introduced control agents, and fungal endophytes of grasses.

Plant tolerance and resistance

Incorporating resistance and tolerance traits in plant breeding programs that reduce or allow plants to withstand pest damage via chemical or physical means is highly advantageous in agricultural production systems, provided that can be achieved without compromising agronomic traits. In Australia, for example, subterranean clover lines (*Trifolium subterraneum*) with chemical and physical cotyledon resistance to the highly damaging redlegged earth mite (*Halytodeus destructor*) have been identified but attempts to breed accessions with these resistance factors have so far been unsuccessful because they are deficient in important agronomic traits (Nichols *et al.,* 2014).

***Fungal endophytes of grasses*:** Temperate pooid grasses including the economically important forage grasses, perennial ryegrass and tall fescue (*Festuca arundinacea*) have co-evolved with endophytic fungi belonging to the Clavicipitaceae family. Other host grasses include *Agrostis* spp., *Dactylis glomerata*, *Bromus* spp. and *Poa* spp. (Shardl, 2009). In agriculture, the focus is on the asexual *Epichloë* that form asymptomatic systemic infections in the tillers of their hosts, have no external stage and are maternally transmitted in seed. The host plant provides protection, nutrition and a means of transmission for the endophyte and in turn the endophyte can confer protection against herbivory through the production of secondary metabolites (alkaloids). Hyphae are intercellular and concentrated in the meristem and base of tillers. For recent reviews on this topic see Johnson *et al.,* (2013); Gundel *et al.,* (2013); Thom *et al.,* (2012).

A diversity of *Epichloë* species have naturally co-evolved with their grass hosts, and within those species there are strains that can be distinguished from each other genetically and by the array of alkaloids that they produce. All known alkaloids have adverse effects on certain insect pests, while two, ergovaline, and lolitrem B, are associated with livestock disorders. To minimize the risk of animal toxicity, exploitation of these endophytes for their insecticidal properties has focused on those strains that lack these two mammalian toxins.

Argentine stem weevil (ASW) (*Listronotus bonariensis*), a major pest of ryegrass in New Zealand was the first insect identified as being affected by *Epichloë* endophytes (Prestidge *et al.,* 1982). Using endophyte-free ryegrass to replace the common animal-toxic endophyte prevalent in New Zealand was not an option due to the severe damage caused by this pest. When early research identified that the alkaloid peramine was important for deterring ASW, endophyte isolates were sought in seed collections from Europe that produced this alkaloid but not the mammalian toxins. One of those, AR1, proved to be highly effective against ASW and a mealybug pest of pasture

(*Balanococcus poea*) in New Zealand-adapted cultivars (Pennell *et al.*, 2005), and gave similar increases in pasture production attributed to infection with the common toxic strain (Popay *et al.*, 1999).

Among a range of isolates investigated for their potential to control pests of ryegrass in pasture in New Zealand, AR37 produced none of the known alkaloids but protected its ryegrass hosts from five different pests (Popay and Thom, 2009). Large increases in yield of ryegrass infected with this endophyte have been attributed to the effects of AR37 on these insects. For example, yields of ryegrass infected with AR37 in a plot trial in the northern North Island were 36% greater after 3 years at one site and 17% greater after 4 years at a second site compared with the same cultivar infected with AR1 or the common toxic strain (Hume *et al.*, 2007). Increased persistence has also been demonstrated at the small paddock-scale when 3 years post-establishment and following a severe drought, significant differences in tiller densities resulted in renewal of only one of six paddocks of AR37-infected ryegrass compared with three each of paddocks infected with AR1 and the common toxic strain. All paddocks with endophyte-free ryegrass had to be resown (Thom *et al.*, 2013).

Both AR1 and AR37 in ryegrass hosts have undergone rigorous testing prior to their commercial release to measure agronomic performance, insect resistant properties and animal safety (Thom *et al.*, 2012). AR1 is completely animal-safe while AR37 can cause ryegrass staggers in sheep although episodes are on average milder, less frequent and more transient than those caused by the common toxic strain (Fletcher and Sutherland, 2009). Two other strains of endophyte have also been commercialized in New Zealand, both of which produce low concentrations of ergovaline which, while not scientifically proven, are not thought to be harmful to livestock. There has been widespread adoption of new endophyte technologies by farmers in New Zealand (Milne, 2007).

Loline alkaloids that are produced in meadow fescue by the endophyte *E. uncinata*, tall fescue by *E. coenophiala* and in *L. multiflorum* by *E. occultans*, adversely affect a wide range of insects and have no mammalian toxicity (Schardl, 2009). These alkaloids are known to reduce feeding by the native New Zealand grass grub, *Costelytra zealandica* (Popay *et al.*, 2003; Patchett *et al.*, 2011). A strain of *E. uncinatum* has been commercially released in a *Festulolium* host that affects three major pests (Barker *et al.*, 2015a, b, c) but transmission of the endophyte to seed is ineffective. Tall fescue which is widely used in the USA as a forage grass is infected by an endophyte strain toxic to livestock. A benign strain (MaxQ™) is now available to farmers in the USA and has been used to introduce endophytes (known as MaxP™) into commercially sown tall fescue pastures in Australia and New Zealand. As in ryegrass, this endophyte adversely affects a range of insects which are likely to impact on the productivity and persistence of these pastures (Popay, 2009). These fungal endophyte grass associations can also be used to reduce pest pressure in crops. In Japan for instance many small meadow areas are the source of invasions of the rice leaf bug (*Trigonotylus caelestialium*) into rice crops. It has been proposed that using endophytes in perennial or Italian ryegrass that affect this insect could be used in these grassed areas to reduce the populations of these bugs in nearby rice fields (Shiba *et al.*, 2011).

Worldwide over 40 herbivorous insect species are known to be negatively affected by endophytes including many pests of forage grasses (Kuldau and Bacon, 2008). There are almost certainly opportunities to utilize these fungal endophytes of grasses more widely in temperate grasslands to provide protection against insects. For example, the native South American grass *Bromus aulecticus* is infected with two different endophytes, one of which produces loline alkaloids that may reduce insect damage to its host (Iannone *et al.*, 2012).

***Other endophytes*:** All plants harbor endophytic organisms and there is growing interest in understanding the role and possible benefits of these organisms, including effects on phytophagous insects. For instance entomopathogenic fungi often reside in plants where they are known to have effects on insects, possibly through the production of secondary metabolites rather than initiation of disease. This is an emerging field of science with potential to add further tools to the pest management toolbox (e.g. Porras-Alfaro and Bayman, 2011).

Parasitoids and Predators

Parasitoids and predators are key components of sustainable pest management in grassland ecosystems, whether they are natural inhabitants or deliberate introductions for control of invasive alien pests. Exotic pests are often particularly troublesome as they may invade a new habitat without any of the natural enemies that suppress them in their home territories. Incursions of these invasive alien species are becoming increasingly problematic as global movement of people and goods has proliferated. Implementing a classical biological control program involving identification and release of a suitable agent (or agents) can be highly successful, but requires careful consideration of the risks and benefits of introducing organisms into a new environment (Barratt, 2011; de Clercq *et al.*, 2011). While predators may show a preference for certain prey, they are seldom host specific and their behavior in a new environment is less predictable than parasitoids. In finding a balance between the risks and benefits of importing a new organism to control another, a level of impact on non-target species may be acceptable.

In New Zealand, classical biological control programs have been successfully implemented for three major weevil pests, the lucerne weevil (*Sitona discoideus*), Argentine stem weevil (*L. bonariensis*) and most recently the clover root weevil (CRW) (*Sitona obsoletus* (formerly *S. lepidus*)). Braconid wasps (*Microctonus* spp.) which oviposit in their adult hosts, rendering females infertile, were introduced for control of each of these weevil species. A Moroccan biotype of *M. aethiopoides* was released for control of the lucerne weevil over a 3 year period between 1982 and 1985 in the South Island with the parasitoid naturally dispersing throughout New Zealand by 1998 and providing a high level of pest suppression that has continued to this day (Goldson and Gerard, 2008). In 1991 releases of *M. hyperodae* for control of ASW began (McNeill *et al.*, 2002); these were initially highly successful, but there is now evidence that parasitism levels have declined to the extent that they may no longer be effective (Goldson *et al.*, 2014). The most recent parasitoid releases have been against CRW, first found inhabiting pasture in 1995. First instar larvae damage root nodules of clover which reduces the potential for N fixation while damage to roots by later instars reduces clover yields (Gerard *et al.*, 2007). In this case an Irish biotype of *M. aethiopoides* has been released with spectacular success (Gerard *et al.*, 2011). Beginning in 2006, the parasitoid was widely distributed throughout the North Island using nursery sites and many ‘mini-releases’ of parasitized weevils to farmers (Gerard *et al.*, 2011). After CRW reached the South Island in 2006, strategic releases were made based on knowledge of dispersal of both the weevil and the parasitoid (Phillips *et al.*, 2007). This project culminated in a mass release program in two regions in the southern South Island after very high populations of CRW caused clover to largely disappear from the area. Over 2 years an estimated 900,000 parasitized weevils have been distributed to farms across these regions by a small group of scientists and technical staff in conjunction with industry representatives. Already clover is returning to pasture with a cost benefit analysis in this region alone estimating a high return on investment in dollar terms to sheep, beef and dairy farmers (Basse *et al.,* 2015).

Both natural and classical biological control programs in agricultural systems may also benefit from conservation measures that preserve and enhance their populations in order to achieve economic pest suppression. Increased plant diversity, for example, can enhance survival, longevity and reproductive capacity of both parasitoids and predators by providing suitable food such as nectar or alternate hosts as a resource when pest populations are low or not in the appropriate life stage.

Entomopathogens

The majority of endemic and native insects are likely to be associated with entomopathogens, including fungi, bacteria, viruses, microsporidia and protozoa, that have evolved with them as a means of population regulation and indeed as part of the trophic web within ecosystems (Peyretaillade *et al.,* 2015). Understanding the role they play in the population regulation of their hosts is a critical first step towards determining their potential value in insect pest management.

Clearly entomopathogens that function as natural population regulators without human intervention are likely to be very useful in perennial grassland systems. A key to their success is an ability to exist in the environment in the absence of their hosts if they are only or mainly horizontally transmitted. Despite environmental conditions that are often detrimental to their survival, entomopathogenic organisms can exert useful control of insects that feed above-ground. For example the microsporidian *Nosema pyrausta*, which is both horizontally and vertically transmitted, can regulate populations of the European cornborer, *Ostrinia nubilalis* (Lewis *et al.,* 2009). A thorough understanding has been gained of the dynamics of *N. pyrausta* in its host population and how it interacts with other management options such as plant resistance, insecticides, parasitoids and predators (Lewis *et al.,* 2009). Such knowledge is important for designing Integrated Pest Management (IPM) strategies.

Unlike pathogens of above-ground pests, soil-borne entomopathogens attacking soil-dwelling pests have the advantage of an environment that provides some protection for the infectious agent outside its host. Larvae of the New Zealand grass grub are susceptible to a range of diseases which cause populations to show a pattern of delayed density dependence (Popay, 1992). Using a simple predictive model, Barlow *et al.,* (1985) concluded that the pathogens responsible were having a highly significant effect on grass grub populations in the area where they were monitored. Climatic and farm management interventions, such as drought and cultivation, can break the cycle of disease through successive generations by reducing both the host population as well as viability of microsporidian and protozoan spores (Popay 1992; Jackson *et al.,* 2002). Insects infected by disease are more susceptible to effects of insecticides, both chemical and biological (Popay, 1992; Pierce *et al.,* 2001), resulting in many more diseased individuals being killed than healthy ones. Premature death of diseased individuals results in a reduction in seasonal disease incidence with consequences for reduced levels of disease in the next generation.

A reliance on natural disease cycles to maintain populations below economic injury levels is risky, however, particularly under intensive farming regimes where there is a low tolerance of yield reductions. Nevertheless, knowledge of the natural pathogens and their impact on population regulation of target pests should be given consideration in any IPM system (Jackson *et al.,* 2002).

Biopesticides

In the context of this paper, the term biopesticides is applied to the mass production and application of microbes such as bacteria, fungi, microsporidia, viruses, protozoa or nematodes for the management of pests. Biopesticides are not self-sustaining and repeat applications are required to suppress populations. Despite a long research history, biopesticides excluding *Bacillus thuringiensis* have captured only a small market share in the pest control industry, although there is now increasing commercial interest in their use (Glare *et al.,* 2012). Factors that limit their use are mass production of effective organisms, shelf-life and stability, consistent efficacy in the environment, rapidity of effects and ease of application. Yet they rate highly in environmental friendliness compared with traditional insecticides due to a narrower host range, less risk to humans and low environmental contamination.

Entomopathogenic fungi in the genera *Beauvaria, Metarhizium, Lecanicillium* and *Isara* are amenable to mass production and have been identified as potential biopesticides (Vega *et al.,* 2009). Isolates of *Metarhizium* have been successfully commercialized for control of grasshoppers and locusts in several countries (Lomer *et al.,* 2001) and have potential to control other grassland insect pests.

Bacteria also offer potential as biopesticides. *Paenobacillus popilliae*, the causative agent of milky disease, was the first microbial pesticide registered in the USA where it was used for control of Japanese beetle (*Popillia japonica*). A spore powder formulation was developed and used in a colonization program to establish milky disease in pastures and amenity turf areas throughout the north eastern United States (Klein, 1992). Over time, however, the effectiveness of the bacteria in commercial preparations appeared to wane (Klein, 1992).

Two species of bacteria with specific application to grassland pests are *Serratia entomophila* and *Yersinia entomophaga*. Pathogenic plasmid-bearing strains of *Serratia entomophila* cause 'amber disease' in New Zealand grass grub resulting in rapid cessation of feeding once ingested although infected individuals take some time to die (Jackson *et al.,* 2001). Natural epizootics are known to occur but the bacteria are amenable to fermentation and mass production and have been formulated for commercial application as a bioinsecticide (Jackson *et al.,* 1992). Farmers are encouraged to apply *S. entomophila* prophylactically to reduce densities of grass grub before they reach damaging levels and to establish a source of inoculum that will continue to naturally infect grass grub larvae in the next generation (Jackson *et al.,*. 2002).

A promising insecticidal toxin-producing bacterium, *Y. entomophaga*, affects a range of major pasture pests in New Zealand, including grass grub and porina larvae (*Wiseana* spp.) (Hurst *et al.,* 2011; Ferguson *et al.,* 2012). Its use as a biopesticide is still in the experimental stage but it can be applied as a spray, in granules or incorporated into bait. The activity of *Y. entomophaga* is specific to insects and although it may affect other plant feeders, beneficials such as predatory staphylinids and earthworms are unharmed (Ferguson *et al.,* 2012).

Conclusions

Ecofriendly pest management requires a paradigm shift in thinking from one where the emphasis is on silver bullet control achieved through pesticide use to a knowledge-based management that makes use of all resources that nature provides with the aim of achieving long term suppression of pest populations. This ecological approach as advocated by others (e.g. Lewis *et al.,* 1997; Vega *et al.,* 2009) brings together a much more complete understanding of the biotic interactions that keep an agricultural system in a balanced state. These authors argue that use of biopesticides and transgenic plants, although relatively more environmentally friendly than chemical insecticides, still represent the same 'silver bullet' thinking. While this is indeed an ideal system to aim for it is very difficult to achieve in practice within the requirements of meeting productivity targets in grassland agriculture. The disturbances created by pasture renewal, cropping, grazing impacts and influences of an unpredictable climate inevitably change the biotic interactions that can lead to pest outbreaks. Nevertheless the basic underlying principles of utilizing the natural ecology of the environment are worth pursuing.

With a better understanding of all components within farm systems that impact on pests, a best practice management system can be achieved. Considerable research effort is needed to determine populations of insects needed to maintain an effective natural enemy complex capable of suppressing pests. This knowledge may alter the thinking that demands a very high rate of mortality from a single control measure to one which maintains a pest population below an economic threshold and is thus more likely to be successful in the longer term. Thus farmer's expectations may need to focus more on achieving long term suppression of pest populations, while accepting that there will be times when outbreaks will occur. Understanding the interactions between composition, diversity, and management at the paddock, farm and landscape scale and the natural enemy complex of the pests in order to reduce disruption of these important population regulators is essential. Prediction and monitoring can indicate when populations are increasing towards damaging levels, and interventions such as an application of an appropriate biopesticide can then be implemented to reduce populations with minimum impact on beneficials. Alien invasive species pose particular problems where there is an absence of a specialist natural enemy. In such cases introducing a biological control agent offers a solution, provided adequate consideration is given to biosafety.

In temperate areas, pest-proofing pastures by sowing appropriate endophytic grasses provides an important foundation on which to develop a long term pest management program. Endophytes that affect a range of insects have been proven to be most effective at increasing pasture production and persistence. Further developments in this field have the potential to utilize the loline alkaloids in ryegrass and tall fescue to reduce damage by a wide range of insects, including very damaging root-feeding scarab grubs that cause problems in many countries. Not all pests are affected by endophytes, however, and the presence of other endophyte-free pasture components will also reduce the effectiveness of endophytic grasses. Thus an integrated pest management program that builds on this foundation is also required.

Environmentally-friendly pest management in agriculture is an achievable goal and one that all pest management practitioners should be working towards. It will deliver high returns on investment, although too often the value is not measured either in monetary terms or in productivity gains (Naranjo *et al.,* 2015). Implemented by proactive conservation and utilization of a suite of natural tools, however, it will provide longer term pest suppression at a lower cost to farmers and to the environment than current reliance on short term reactive solutions.

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