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Microbial biopesticides for control of invertebrates: Progress from New Zealand

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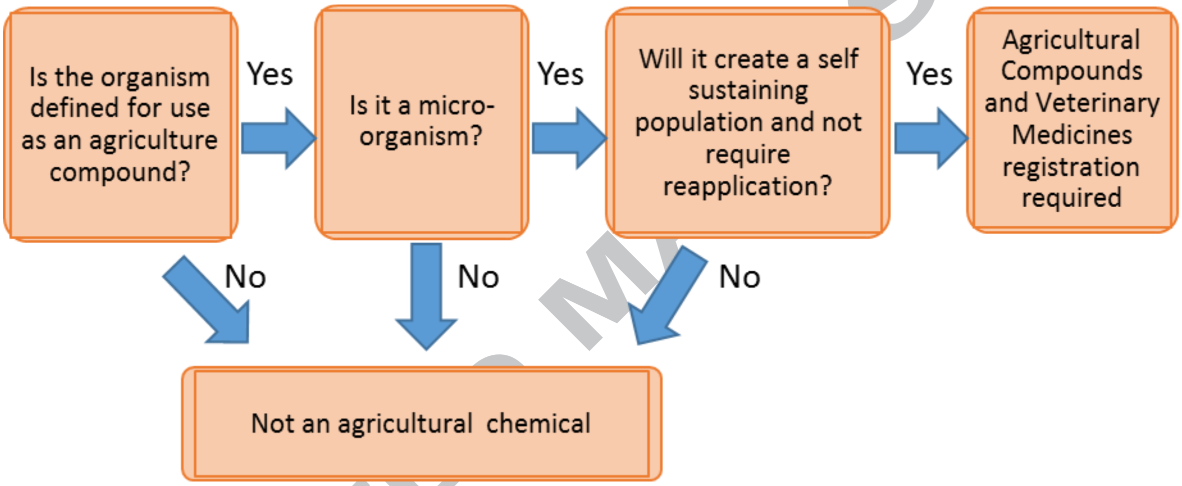
Abstract

Biopesticides are needed for control of endemic and invasive pests impacting New Zealand's primary sectors including pests that are emerging as a result of climate change and farming intensification. Products developed in New Zealand are usually based on endemic strains of microorganisms, including new species/strains with novel modes of action. For example, Invade and BioShield were developed using endemic strains of the bacterium *Serratia entomophila*, for use in New Zealand only. To date, most of these home-grown products have either struggled for market share or have remained in small niche markets. However, the number of products registered for use has been steadily increasing in response to consumer demand. Factors limiting past use of biopesticides in New Zealand include market size, registration costs and limited efficacy over a range of climatic zones. Many promising new agents are currently under development as biopesticides with international applications and the launch of several new start-up companies suggests a brighter future for biopesticide use in New Zealand.

Keywords: Microbial control, bacteria, fungi, nematodes, regulation, New Zealand, biological control

Graphical abstract



1. Introduction

New Zealand's economy and reputation are closely linked to its agricultural and natural landscapes, with 85% of overseas earnings derived from agriculture, forestry and tourism. The productive capacity of the pastoral, horticultural, arable and forestry sectors relies on numerous introduced plant species. These exotic plant species are subject to endemic pest species that have adapted to feeding on introduced crops and a wide range of invasive species that have established successfully in New Zealand.

New Zealand is very prone to pest incursions (e.g. O'Callaghan et al. 2002, Goldson et al. 2016) which have sometimes led to devastating pest outbreaks. In some cases, the introduced species are not pests in their native ranges, for example *Sitona obsoletus* (= *S. lepidus*), the clover root weevil, which is native to Europe, has adversely affected clover based pastures in New Zealand since its introduction in the 1990s (Gerard et al. 2007). External factors, such as globalisation and free trade, mean that the rate of pest incursions is only likely to increase and place further pressure on existing methods for pest control (Kriticos et al. 2005). In addition, some endemic pests are becoming more problematic as a result of intensification of farming systems, land use changes and changing climatic conditions. For example, populations of the endemic pasture pest the New Zealand grass grub (*Costelytra zealandica*) rapidly increased to economically damaging levels following installation of irrigation schemes on previous dryland pasture (Jackson et al. 2012). The distribution of the exotic pest South African black beetle *Heteronychus arator* is also likely to increase as a result of hotter summers in New Zealand (Bell et al. 2011).

To reduce the impact of insect pests, pastoral farmers and horticultural and arable growers have relied heavily on pesticides, biological control and, in some situations, integrated pest management (IPM) systems (Manktelow et al. 2005). For some pests, such as several weevil pests, implementation of biological control strategies based on carefully selected parasitoid wasps have been extremely successful (Goldson et al. 2004, Gerard et al. 2007). Similarly, long-standing IPM systems underpin New Zealand's highly competitive apple and kiwifruit export industries (e.g. Stephens and McKenna 1999). Despite these approaches, there is still considerable reliance on synthetic pesticides in New Zealand. A review in 2004 found that pesticide imports constituted approximately 1% of the value of New Zealand's agricultural exports at that time, which was valued at US$8 billion (Manktelow et al. 2005). However, several synthetic pesticides have been banned recently (e.g. fenitrothion or phorate; Environmental Protection Agency 2017) or will be de-registered in the future (e.g. diazinon will be restricted for pasture use by 2028; Environmental Protection Agency 2016). The threat of further de-registration of pesticides, declining social acceptability of these products, increasing incidence of resistance, and ongoing imposition of phytosanitary requirements by off-shore markets is driving increased interest in alternative solutions, in particular microbial biopesticides (Glare et al. 2012). An additional driver in New Zealand is the high likelihood of new pest incursions which further highlights the need for safe control options as incursions most often occur around ports and in heavily populated areas. Microbial biopesticides are also an attractive option with their low non-target impacts and they fit well with the many finely tuned IPM systems mentioned above that underpin several key export crop production systems.

Sustainable invertebrate pest control measures in New Zealand have been investigated for over 100 years. Many introductions of exotic invertebrates for classical biological control of pests and weeds have been undertaken, but herein we limit our discussion to microbial insect pathogens, as the active agents for biopesticides. Records of attempts at biological control, including with microbial pathogens of insects, date back to the 19th century. For example, a report in the Bee and Poultry Journal of 1893 (Anon. 1893) details the importation and use of *Beauveria brongniartii* from France for management of codling moth and other insects. Most studies used microorganisms naturally occurring in New Zealand as potential insect control agents, rather than imported species. At various times in New Zealand, viruses, protozoa, nematodes, bacterial and fungal agents have all been examined as potential agents (listed in Glare et al. 1993). Despite a long history of microbial control research, there are currently few products based on microorganisms registered for invertebrate pest control in New Zealand (Table 1).

2. Registered microbial biopesticides

Out of 317 insecticides registered since 1962 under the Agricultural Compounds and Veterinary Medicines (ACVM) regulations of the Ministry for Primary Industries, only 17 are based on microbial agents (Ministry for Primary Industries 2017) (Table 1). *Bacillus thuringiensis* (Bt) based products used to control Lepidoptera have been the most commonly registered insecticidal biopesticides in New Zealand, as is the case in most countries (Jackson et al. 2010). There are no locally produced products based on Bt as it has been relatively easy to import well evaluated Bt products from overseas and Bt is ubiquitous in its distribution. The major use of Bt-based products has been in apples and kiwifruit, where they form part of ongoing IPM programmes. New Zealand's kiwifruit production systems remain based on the "Kiwigreen" IPM system developed in the 1990s, with late season leafrollers managed using Bt (Stephens and McKenna 1999, Aitken et al. 2004). The relative specificity of Bt products has also led to their use in environmentally sensitive areas such as natural ecosystems and urban settings (O'Callaghan and Brownbridge 2009). Bt products have been registered and used in urban eradications of invasive moths in New Zealand. For example, Foray 48B was used extensively in the eradication of painted apple moth and white spotted tussock moth (O'Callaghan et al. 2002; Glare 2009) from two of New Zealand's largest cities, Auckland and Hamilton. Similarly, *B. thuringiensis israeliensis* (Bti) was used along with methoprene during mosquito eradication projects (Gear et al. 2013).

A review of the ACVM database in 2015 (O'Callaghan et al. 2015) included additional microbial biopesticides that are no longer listed, possibly because some products have been deregistered. Products no longer listed include several based on Bt (Vectobac, Biocrystal kurstaki), as well as on the fungus *Lecanicillium lecanii* (ENtocide L), and nematode products *Heterorhabditis bacteriophora* (Nematop), and *Steinernema* spp. (Nemastar, Nemacyt, Nemaplus). Currently registered nematode products are offered for sale by a local company, Bioforce (http://www.bioforce.co.nz/products.html). *Steinernema carpocapsae* is used for cutworm control in pasture, brassicas, cereals and maize, *S. feltiae* for fungus gnats (Sciaroidea) in glasshouses and *H. bacteriophora* for black vine weevil (*Otiorhynchus sulcatus*) control, primarily in pasture and turf. Although nematodes are often included with microbial pesticides, in the most recent guidelines by the New Zealand ACVM, they are excluded as called macro-organisms (Ministry for Primary Industries 2016).

Importation of biopesticides containing microorganisms not known to be present in New Zealand requires approval from the Environmental Protection Agency (EPA) and Ministry for

Primary Industries (MPI). The New Organisms Act of 1998 determined that an organism not recorded as present in the country before 1996 requires specific clearance from the EPA before it can be released in New Zealand. Recent efforts to import the nematode *Phasmarhabditis hermaphrodita* for slug control required a countrywide survey to demonstrate that the nematode was naturally present in New Zealand (Wilson et al. 2016, reviewed below). The effort and costs required to demonstrate presence in New Zealand will only be justified where there is sufficient market size and high demand for the product. There are obvious benefits in importing commercially available biopesticides from off-shore. The research and development and safety testing has already been completed and effective formulations developed. New Zealand regulators are generally accepting of EU and US EPA data packages and this can reduce the costs of registration in New Zealand significantly. However, further work may be needed to ensure that the imported products will be effective and safe under New Zealand environmental conditions and within the country's cropping systems. This will require research within New Zealand, testing imported products against endemic non-target organisms under local conditions. Generally such work is conducted by independent researchers for the importing companies. It may be necessary to evaluate activity against a range of New Zealand-specific non-target endemic and/or iconic species which can be problematic if laboratory bioassay techniques have not already been developed.

3. Examples of products developed in New Zealand

3.1 Bioshield® (previously Invade®)

One of the more interesting biopesticides registered in New Zealand is based on the indigenous insect pathogenic bacterium *Serratia entomophila* (recently transferred to the

Yersinaceae, Adeolu et al. 2016). This bacterium was discovered as a causal agent of "amber disease" in the New Zealand grass grub, the most damaging scarab pest of pastures in New Zealand (Trough et al. 1982), and was developed into a biopesticide sold as Invade™ (Jackson et al. 1992). However, while the high host specificity of *S. entomophila* means the product has no non-target effects, it also limits the market size for Invade. In addition, this product was initially produced as a liquid formulation that required refrigerated storage and distribution. These limitations were overcome with the development of a stable granular formulation of this non spore-forming bacterium - BioShield® - based on biopolymer technology (Johnson et al. 2001; Glare et al. 2016). Bioshield Grass Grub remains one of the very few biopesticide products worldwide to be based on a non-sporeforming bacterium. Various formulations of the BioShield product have performed well in the field (Townsend et al. 2004; Zydenbos et al. 2016). However, despite *C. zealandica* being a widespread pest in New Zealand, the small market size has meant that the product has not been continuously produced and marketed to farmers. At the time of writing (2017), a New Zealand company Biostart has registered both a liquid and granular formulation as Bioshield Grass Grub®**.** The liquid formulation has Biogro (organic) certification and has attracted interest as a control measure for grass grub in vineyards. Grass grub remains a significant problem not only in pastures but also in a range of arable and horticultural crops and Bioshield Grass Grub® is expected to play a key role in management of this pest following the anticipated withdrawal of diazinon (Environmental Protection Agency 2017).

3.2 Beaublast®, based on **Beauveria bassiana and related products**

A start-up company, Biotelliga Ltd (Auckland, New Zealand), developed and registered (between 2004 and 2008) several mycoinsecticides based on *Beauveria bassiana*

(Beaugenic®, Beaublast®) and *Lecanicillium lecanii* (eNTokill®, eNtoblast®), insect toxic metabolites from *B. bassiana* and formulation ingredients such as oils. These components were sold either formulated together, or the oil additives sold separately and were all registered as insecticidal. The products were mainly sold to the glasshouse vegetable industries in the northern regions of New Zealand. The company claimed that the combination of highly efficacious toxic metabolites combined with repellency effects and infection by the entomopathogenic conidia led to excellent control of pests such as aphids, whiteflies and thrips. The products were sold for around 4-5 years and were successfully used on over 1,000 hectares (Stephen Ford, pers. comm.) but production has ceased in recent years as the company has looked to license the technologies internationally.

3.3 Fungal endophytes

One of the success stories for biopesticides (using the definition of biopesticides which includes endophytes; Glare et al. 2012) in New Zealand is not a registered microbial product, but carefully selected plant-fungal endophyte combinations, sold as fungus-colonised seed, and covered by plant variety rights protection. The ability of naturally occurring endophytes from the genera *Epichloë* (Ascomycetes; Clavicipitaceae) to provide resistance to pests and diseases of grasses has been developed into a thriving industry, both in New Zealand and internationally (Johnson et al. 2013). Some asexual *Epichloë* species, such as *E. festucae* var. *lolii* that infects perennial ryegrass and *E. coenophiala* in tall fescue, were commercialised in New Zealand from the early 2000s, and now almost all ryegrass seed sold contains a beneficial endophyte. One of the main companies involved is the New Zealand company, Grasslanz Technology Ltd (Johnson et al. 2013). Through selection, several novel endophytes that produce predominantly insecticidal bioactives (active against weevils, aphids and beetles) have now been successfully commercialized and are grown in many temperate grassland areas in New Zealand, Australia, USA, and South America under trade names such as AR1™ and AR37™ in ryegrass and MaxQ in tall fescue (Card et al. 2016).

4. Registration process in New Zealand

In New Zealand registration of pesticides, including those based on microorganisms, is through the ACVM, a part of New Zealand's Ministry for Primary Industries. If the organism is new to New Zealand (including isolated in New Zealand but not previously documented), or modified in any way, approvals are also needed from the EPA. An overview of the registration process is shown in Figure 1.

Guidelines for the registration of biopesticides - referred to as "Microbial Agricultural Chemicals" (MAC) - were established for the first time in August 2016. Microbial Agricultural Chemicals are trade name products that contain micro-organisms in the product formulation and are used as agricultural chemicals (Ministry for Primary Industries, 2016). The micro-organisms that constitute the active ingredients in the formulation, referred to as microbial active ingredients (MAIs), are defined as any micro-organism, genetically modified organism or naturally occurring mutants of micro-organisms, intended for sale to manage pests. This includes whole organisms (viable or non-viable), organelles, metabolites, spores or occlusion bodies used for the control or management of invertebrate pests, weeds or microbial pathogens of crops.

As with most countries, application for registration requires technical data and/or scientifically sound arguments to support the application. The ACVM guidelines (2016) list the following data requirements:

1. Quality, purity and stability of the product;

2. Product's efficacy for all label claims;
3. Crop safety
4. Maximum residue levels (MRLs), including the establishment of an MRL resulting from trial work that adheres to the residue guidelines or an argument or data to show that the MAI fits an existing exception in the MRL Notice (Schedule 2) or to promulgate a new exception;
5. Any possible impact on trade resulting from the use of the MAC in crops, and/or carry over residues as a result feed crops in food-producing animals, if applicable.

Note that these changes have occurred within wider context of significant proposed revision of the Hazardous Substances regulation by MPI.

5. Future directions

Given the limited size of the New Zealand market, to date few companies have been inclined to invest in development of local products or importation and registration of products developed overseas. However, interest and investment is growing with several start-up companies appearing in recent years and more traditional agrochemical companies are investigating microbial biopesticides as part of their portfolio of crop protection products, primarily in response to increasing levels of pesticide resistance in key pest species. As discussed above, BioStart (http://biostart.co.nz) has begun producing and selling Bioshield Grass Grub® based on *Serratia entomophila.* A New Zealand company which targeted discovery of novel biological agents, Biodiscovery, joined with the USA company Bioconsortia and are investigating developing microbial consortia for pest management and plant protection (https://bioconsortia.com). Several of the larger international pest control and life sciences companies, such as Bayer and BASF, are active in the New Zealand market. Biotelliga Ltd (http://www.biotelliga.com) has been developing biopesticides since the early 2000s and has several registered products, although the company is not currently selling these products (Table 1). Another company, Bioforce (www.bioforce.co.nz), specialises in predators and parasitoids, but also sells some insecticidal nematodes and a new company, Ecolibrium Ltd was recently registered and intends to produce biopesticides. Several other companies specialise in importation of microbial biopesticides (Table 1).

New Zealand agriculture has traditionally been strongly reliant on use of synthetic pesticides but as pesticides are de-registered overseas, they are becoming unavailable to New Zealand growers and companies are being forced to source alternatives. Biopesticide registrations are finally increasing in New Zealand in response to this need. Growth in the biopesticide market, both through development of local products and increased importation of products from overseas, is expected to increase as New Zealand growers press for alternative solutions for pest problems. Progress will come through direction of effort and resources to several key areas.

Exciting new opportunities are presented by tapping into New Zealand's unique and relatively under-explored soil and insect-associated microbiota. The potential of further biodiscovery efforts is illustrated by the prior identification of two new insecticidal bacteria (*Serratia entomophila* and *Yersinia entomophaga*) from a single endemic scarab pest. Not only were new species described, but their insecticidal toxins (Busby et al. 2013) and toxin delivery systems (Heymann et al. 2013) also proved to be novel. Further biodiscovery research is warranted and should be fast-tracked to expedite the development of additional novel microorganisms and transfer to industry. Microorganisms with novel modes of action, combinations of agents and new formulations and delivery systems are all areas being explored to bring the next generation of biopesticides to market in New Zealand. Biopesticides with wider host range (and hence larger markets) are required to replace synthetics in mainstream agriculture. Cost of goods is another key issue under consideration during biopesticide development, as some agents are known to be effective but cannot be produced cheaply. As outlined in Glare et al. (2012), the desired characteristics of biopesticides include shelf life at room temperature for over 12 months, persistence in the phylloplane for 3 weeks for foliar applied products and compatibility with current farming practice. Some examples of promising new agents under development are briefly discussed below.

5.1 Yersinia entomophaga

The bacterium *Y. entomophaga* (Yersiniaceae) was discovered in New Zealand in the 1990s (Hurst et al. 2011a) and has considerable potential as a biopesticide as it is active against a reasonably wide range of pests including Lepidoptera, Coleoptera and Orthoptera. Insecticidal activity is through oral ingestion, followed by deterioration and loss of peritrophic membrane in mid-gut that allows the bacteria to enter the haemocoel, and death follows rapidly (Hurst et al., 2011b, 2014, 2015; Marshall et al., 2012). The bacterium can kill in only a few days, making it an effective control agent if it can be applied or delivered to the target insect pest. Several delivery systems are being evaluated including incorporation into baits for controlling caterpillars (Brownbridge et al. 2008) and sprays for foliar feeding pests (Jones et al. 2015). The bacterium is safe for a wide range of non-target species including earthworms and bees and has no activity against small mammals, birds or fish.

5.2 Brevibacillus laterosporus

Local strains of the insecticidal bacterium *Brevibacillus laterosporus* are under development as the active agent in New Zealand-developed products (van Zijll de Jong et al. 2016). *B. laterosporus* has been described from many countries and is active against a number of insect species, including Coleoptera, Lepidoptera and Diptera (Ruiu 2013). Strains of this species were isolated from seeds of brassica and found to be active against insects, including diamondback moth larvae, *Plutella xylostella* (van Zijll de Jong et al. 2016). The toxin profiles of the New Zealand strains differed from those overseas (T. Glare and M. Ormskirk, unpubl. data) and, given the potential value, research is underway to develop a commercial product through a local company. An issue hindering commercial development is that the cause of toxicity to insects is not fully understood. As discussed above, importation and development of exotic strains can be problematic, so substituting locally isolated strains of insect pathogens is useful approach to develop biopesticides for New Zealand.

5.3 Phasmarhabditis hermaphrodita

Issues with the importation and implementation of biopesticides developed outside of New Zealand are illustrated by recent experiences with the nematode *Phasmarhabditis hermaphrodita* which has been sold as biological molluscicide in Europe since 1994 (De Ley et al. 2014). Slugs are a major issue in cropping in New Zealand, so importation of the product from the Northern Hemisphere has been investigated. However, there were no published records of the nematode in New Zealand so a nationwide sampling survey of endemic slug populations was required. The survey found the nematode at three sites throughout New Zealand (Wilson et al. 2016) meaning this it is possible to import the product. However, the small market size has not yet attracted company interest in funding the registration process, when larger markets such as California are being explored. To be clear, under the current guidelines nematodes are required to be registered but not as microbials.

6. Recommendations for increasing adoption of biopesticides in New Zealand

6.1 Focus on strategic pest issues

Focus needs to be placed on identifying microorganisms in New Zealand that target both New Zealand's key insect pests and economically significant global pests. This would take advantage of the often unique microbial pathogens found in New Zealand, and as the targets are sufficiently large international markets have a higher chance of attracting investment for research and development. Biopesticides could be developed in cooperation with multinational companies with more resources and expertise in development and registration in other jurisdictions. This approach is not necessarily straightforward as each jurisdiction needs to ensure that exotic microorganisms (from New Zealand) pose no issues in terms of environmental safety. In the future, metagenomics techniques may provide sufficient evidence that microorganisms of interest have a cosmopolitan distribution and this would help to reduce barriers to importation.

6.2 Streamline regulatory environment

Further changes to regulatory procedures, such as streamlining testing for the specific nature of each biopesticide, and additional resources to ease the progression of new biopesticides through the registration process would be very advantageous. In some jurisdictions, there are strong Government directives which incentivise industry to focus on development of biopesticides as opposed to synthetic pesticides, in part through simplification and cost reduction in biopesticide registration. New Zealand has recently made a good start in issuing specific guidelines for microbial-based products (as described above), but cost will remain an issue for development of products for small markets. With a relatively small research community, there is also a lack of specialists to evaluate new products, which can delay the process and lead to unnecessary costs.

6.3 Use proven organisational models for commercial development and uptake

Driven by the need to meet minimum residue limits on export products, New Zealand has a history of development of successful IPM programmes that are underpinned by biopesticides and biological control, such as in apples and kiwifruit. A key factor in the success of these programmes has been the effectiveness of industry bodies such as Zespri, a levy-funded organisation that funds research that underpins the competitive advantage of the kiwifruit sector and is involved in evaluation of new products, including biopesticides. Products with demonstrated efficacy are endorsed by Zespri and the organisation works closely with growers to achieve best practice in kiwifruit production, including educating growers about implementation of new products. Such industry organisations have a key role in increasing the development and uptake of biopesticides into mainstream agriculture, especially in a small economy country such as New Zealand.

Historically, biopesticide research and development has been undertaken within Universities and research organisations, rather than by industry. More recently there has been a move to partner early with companies with required expertise in scale-up of production and formulation and full commercialisation. Partnering early with companies is a useful strategy for researchers in New Zealand, where research and development funding is limited.

6.4 Invest in infrastructure

Some strategic investment in infrastructure may also be required. There is currently limited capacity for large scale production of microbial biomass in New Zealand, which has hampered product development. There has been interest for a number of years in investment in production facilities, but few advancements. A recommendation is that such infrastructure is developed, which may require direct government investment.

7. Conclusions

New approaches, such as trait-led biodiscovery, novel formulations and the use of genomic tools to find useful strains, are leading to new products with potential international applications. The launch of several new start-up companies suggests a brighter future for biopesticide use in New Zealand. The issue of small market size can be addressed through international partnerships or special registration assistance programmes and government co-development investment. The very strong drivers for the move away from synthetic pesticide use to more sustainable production systems to maintain market access for New Zealand's exports are expected to lead to further investment in the biopesticide industry.

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References

Adeolu M., Alnajar S., Naushad, S., Gupta R. 2016. Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. Int. J. Syst. Evol. Microbiol. 66, 5575-5599.

Aitken A.G., Kerr J.P., Nixon C., Hewett E.W., Hale C.N. 2004. Zespri's KiwiGreen programme - world firsts in this vital crop management system. Growing futures case study series #2. http://www.martech.co.nz/images/02kiwi.pdf (accessed July 2017).

Anonymous 1893. White grub culture. *New Zealand Farmer, Bee and Poultry Journal,* February, 1893, p 45.

Bell, N.L., Townsend R.J., Popay A.J., Mercer, C., Jackson, T.A. 2011. Black beetle: lessons from the past and options for the future. Pasture Persistence – Grassland Research and Practice Series 15, 119-124. https://www.grassland.org.nz/publications/nzgrassland_publication_2243.pdf

Brownbridge, M., Ferguson, C., Saville D., Swaminathan J, Hurst, M.R.H., Jackson TA. 2008. Potential for biological control of porina (*Wiseana* spp.) with a novel insecticidal bacterium *Yersinia* n. sp. (MH96) EN65 strain. N. Z. Plant Prot. 61, 229-235.

Busby, J.N., Panjikar, S., Landsberg, M.J., Hurst, M.R., Lott, J.S. 2013. The BC component of ABC toxins is an RHS-repeat-containing protein encapsulation device. Nature 501, 547-50.

Card, S., Johnson, L., Teasdale, S., Caradus, J. 2016. Deciphering endophyte behaviour: the link between endophyte biology and efficacious biological control agents. FEMS Microbiol. Ecol. 92, fiw114

De Ley, I.T., McDonnell, R.D., Lopez, S., Paine, T.D., De Ley, P. 2014. *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae), a potential biocontrol agent isolated for the first time from invasive slugs in North America. Nematol. 16, 1129-1138.

Environmental Protection Authority 2016. Hazardous Substances Update: April 2016 http://www.epa.govt.nz/news/news/Pages/Read-the-Hazardous-Substances-Update-April-2016.aspx (accessed 12 May 2016).

Environmental Protection Authority 2017. http://www.epa.govt.nz/news/news/Pages/Plant-insecticides-containing-fenitrothion-or-phorate-banned.aspx. (accessed 3 August 2017).

Gerard, P.J., Hackell, D.L., Bell, N.L. 2007. Impact of clover root weevil *Sitona lepidus* (Coleoptera: Curculionidae) larvae on herbage yield and species composition in a ryegrass-white clover sward. N. Z. J. Agric. Research 50, 381-392.

Gear, H., Glare, T.R., O Callaghan, M, 2013. Maintaining environmental integrity in invasion areas, in Kay, B.H, Russell, R.C. (Eds.) Mosquito eradication: the story of killing Campto. CSIRO Publishing, pp. 197-214.

Glare, T.R. 2009. Use of pathogens for eradication of exotic lepidopteran pests in New Zealand, in Hajek, A.E., Glare, T.R., O'Callaghan, M. (Eds.) Use of Microbes for Control and Eradication of Invasive Arthropods. Progress in Biological Control Vol. 6, Springer pp. 49-70.

Glare, T.R., Jurat-Fuentes J-L., O'Callaghan M. 2016. Basic and applied research: entomopathogenic bacteria, in Lacey, L.A. (Ed.). Microbial Control of Insect and Mite Pests: from Theory to Practice. Academic Press, New York. pp. 47-67.

Glare, T.R., Caradus, J., Gelernter, W.D., Jackson, T.A., Keyhani, N.O., Köhl, J., Marrone, P.G., Morin, L., Stewart, A. 2012. Have biopesticides come of age? Trends Biotechnol. 30, 250-258.

Glare, T.R., O'Callaghan, M., Wigley, P.J. 1993. Checklist of naturally-occurring entomopathogenic microbes and nematodes in New Zealand. N. Z. J. Zool. 20, 95-120.

Goldson, S.L., McNeill, M.R, Gerard, P.J., Proffitt, J.R, Phillips, C.B, Cane, R.P, Murray, P.J. 2004. British-based search for natural enemies of the clover root weevil, *Sitona lepidus* in Europe. N.Z. J. Zool. 31, 233-240.

Goldson, S.L., Barratt, B.I., Armstrong, K.F. 2016. Invertebrate biosecurity challenges in high-productivity grassland: The New Zealand example. Front. Plant Sci. 7, 1670.

Heymann, J.B., Bartho, J.D., Rybakova, D., Venugopal, H.P., Winkler, D.C., Sen, A., Hurst, M.R., Mitra, A.K. 2013. Three-dimensional structure of the toxin-delivery particle antifeeding prophage of *Serratia entomophila*. J. Biol. Chem. 288, 25276-84.

Hurst, M.R.H., Becher, S.A., Young, S.D., Nelson, T.L., Glare, T.R. 2011a. *Yersinia entomophaga* sp. nov. isolated from the New Zealand grass grub *Costelytra zealandica*. Int. J. Syst. Evol. Microbiol. 61, 844-849.

Hurst, M.R.H., Beattie, A.K., Jones, S.A., Hsu, P.C., Calder, J., van Koten, C., 2015. Temperature-dependent *Galleria mellonella* mortality as a result of *Yersinia entomophaga* infection. Appl. Environ. Microbiol. 81, 6404-6414.

Hurst, M.R.H., Jones, S. A., Binglin, T., Harper, L.A., Jackson, T.A., Glare, T.R. 2011b. The main virulence determinant of *Yersinia entomophaga* MH96 is a broad-host-range toxin complex active against insects. J. Bacteriol. 193, 1966-1980.

Hurst, M. R. H., van Koten, C., Jackson, T. A. 2014. Pathology of *Yersinia entomophaga* MH96 towards *Costelytra zealandica* (Coleoptera; Scarabaeidae) larvae. J. Invertebr. Pathol. 115, 102-107.

Jackson, T.A., Brownbridge, M., Glare, T.R. 2010. Registration and use of microbial biopesticides in New Zealand, in Kabaluk, J.T., Svircev, A.M., Goettel, M.S., Woo S.G. (Eds.) Use and Regulation of Microbial Pesticides in Representative Jurisdictions Worldwide, with an Elaboration and Analysis of Canada. IOBC Global, pp. 89-93.

Jackson, T.A., Pearson, J.F., O'Callaghan, M., Mahanty, H.K., Willocks, M. 1992. Pathogen to product - development of *Serratia entomophila* (Enterobacteriacae) as a commercial biological control agent for the New Zealand grass grub (*Costelytra zealandica*), in Jackson, T.A., Glare T.R. (Eds.), Use of Pathogens in Scarab Pest Management. Intercept, Andover, UK. pp. 191-198.

Jackson, T.A., Townsend, R.J., Dunbar, J.E., Ferguson, C.M., Marshall, S., Zydenbos, S.M. 2012. Anticipating the unexpected - managing pasture pest outbreaks after large-scale land conversion. Proc. N. Z. Grassl. Assoc. 74, 153-158.

Johnson V.W., Pearson J.F., Jackson T.A. 2001. Formulation of *Serratia entomophila* for biological control of grass grub. N. Z. Plant Prot. 54, 125-127.

Johnson, L.J., de Bonth, A.C.M., Briggs, L.R., Caradus, J.R., Finch, S.C., Fleetwood, D.J., Fletcher, L.R., Hume, D.E., Johnson, R.D., Popay, A.J., Tapper, B.A., Simpson, W.R., Voisey, C.R., Card, S.D. 2013. The exploitation of *Epichloë* endophytes for agricultural benefit. Fungal Divers. 60, 171-188

Jones, S.A., Ferguson, C.M., Philip, B.A. van Koten, C., Hurst, M.R.H. 2015. Assessing the potential of *Yersinia entomophaga* to control plantain moth in a laboratory assay. N.Z. Plant Prot. 68, 46-150.

Kriticos, D.J., Phillips, C.B., Suckling, D.M. 2005. Improving border biosecurity: potential economic benefits to New Zealand. N. Z. Plant Prot. 58, 1-6.

Manktelow, D., Stevens, P., Walker, J., Gurnsey, S., Park, N., Zabkiewicz, J., Teulon, D., Rahman, A. 2005. Trends in Pesticide Use in New Zealand, 2004. Report to the New Zealand Ministry for the Environment, Project SMF4193.

Marshall, S.D.G., Hares, M.C., Jones, S.A., Harper, L.A., Vernon, J.R., Harland, D.P., Jackson, T.A., Hurst, M.R.H. 2012. Histopathological effects of the yen-tc toxin complex from *Yersinia entomophaga* MH96 (Enterobacteriaceae) on the *Costelytra zealandica* (Coleoptera: Scarabaeidae) larval midgut. Appl. Environ. Microbiol. 78, 4835-4847.

Ministry for Primary Industries 2016. http://www.foodsafety.govt.nz/elibrary/industry/ microbial-agricultural-chemicals-information.pdf.

Ministry for Primary Industries 2017. www.foodsafety.govt.nz/industry/acvm/ (accessed 3 August 2017)

Ministry for Primary Industries 2016. Microbial Agricultural Chemicals Guidelines. http://www.foodsafety.govt.nz/elibrary/industry/microbial-agricultural-chemicals-information.pdf (accessed 27 November 2017)

O'Callaghan M., Brownbridge M. 2009. Environmental impacts of microbial control agents used for control of invasive pests, in Hajek, A., Glare, T.R., O'Callaghan, M. (Eds). Use of Microbes for Control and Eradication of Invasive Arthropods. Springer. pp. 305-330.

O'Callaghan, M., Glare, T.R., Jackson, T.A. 2002. Biopesticides for control of insect pest incursions in New Zealand, in Goldson, S.L., Suckling, D.M. (Eds.) Defending the green oasis: New Zealand Biosecurity and Science. New Zealand Plant Protection Society Inc. Christchurch New Zealand, pp. 137-152.

O'Callaghan, M., Wilson, M.J., Zydenbos, S.M. 2015. Biopesticides for New Zealand's pests - opportunities and challenges. N. Z. Plant Prot. 68, 443.

Ruiu, L., 2013. *Brevibacillus laterosporus*, a pathogen of invertebrates and a broad spectrum antimicrobial species. Insects 4, 476–492.

Stevens, P.S., McKenna, C.E. 1999. Factors affecting the efficacy of *Bacillus thuringiensis* against *Cnephasia jactatana* in kiwifruit . Proceed. N.Z. Plant Prot. Conf. 52, 89-93.

Townsend, R.J., Fergusson, C., Proffitt, J., Slay, M., Swaminathan, J., Day, S., Gerard, E., O'Callaghan, M., Johnson, V., Jackson, T. 2004. Establishment of *Serratia entomophila* after application of a new formulation for grass grub control. N. Z. Plant Prot. 57, 310-313.

Trought, T.E.T., Jackson, T.A., French, R.A. 1982. Incidence and transmission of a disease of grass grub (*Costetytra zealandica*) in Canterbury. N. Z. J. Exp. Agricul. 10, 79-82.

van Zijll de Jong, E., Roush, T.L., Glare, T.R., Hampton, J.G. 2016. Discovery of two *Brevibacillus laterosporus* isolates from brassica with insecticidal properties against diamondback moth. Biocon. Sci. Tech. 26, 426-431.

Wilson, M.J., Wilson, D.J., Aalders, L., Tourna, M. 2016. Testing a new low-labour method for detecting the presence of *Phasmarhabditis* spp. in slugs in New Zealand. Nematol. 18, 925-931.

Zydenbos, S.M., Townsend, R.J., Lane, P.M.S., Mansfield, S., O'Callaghan, M., van Koten, C., Jackson, T.A. 2016. Effect of *Serratia entomophila* and diazinon applied with seed against grass grub populations on the North Island volcanic plateau. N. Z. Plant Prot. 69, 86-93.

Table 1: Currently registered microbial biopesticides for invertebrate control in New Zealand (Source: Agricultural Compounds & Veterinary Medicines, New Zealand, Ministry for Primary Industries website)

Active agent	**Type of agent**	**Trade Name**	**Registrant**	**Label claim targets**
Bacillus thuringiensis (Bt)	Bacterium	Delfin	Hilado Pty Ltd	Caterpillars on apples, kiwifruit, avocados, grapes, citrus, vegetables, berry fruits, brassicas and tomatoes
		Yates Nature's Way Caterpillar Killer	Dulux Pty Ltd	Caterpillars on fruits, vegetables, vines, herbs, flowers and ornamentals
		Bactericide WG	Agrinova NZ Limited	Caterpillars on apples, kiwifruit, avocados, grapes, citrus, vegetables, berry fruits, brassicas and tomatoes
		Organic Caterpillar	Kiwicare Corporation Ltd	Caterpillars on fruits, vegetables and ornamentals
		Biobit DF Biological Insecticide	Valent BioSciences	Caterpillars on fruits, vegetables and ornamentals

		XENTARI WG	Valent BioSciences	Caterpillars on brassica
		Foray 48B	Valent BioSciences	Caterpillars, forests parks and shrubs
		DIPEL ES	Valent BioSciences	Caterpillars on kiwifruit, avocados, vegetables and ornamentals
		DIPEL DF	Valent BioSciences	Caterpillars on fruit, vegetables and ornamentals
		Bactur	Grosafe Chemicals Ltd	Caterpillars on fruits and vegetables
		Agree	Hilado Pty Ltd	Leafrollers on kiwifruit, white butterfly, soyabean looper and diamondback moth on brassica and horticulture
		BMP- 48 LC	Agrenz Limited	Gypsy moth, fall webworm, tussock moth, painted apple moth
Bacillus firmus + clothianidin	Bacterium	Poncho Votivo	Bayer New Zealand Limited	Argentine stem weevil, black beetle, greasy cutworm and nematodes in maize and corn
Serratia entomophila	Bacterium	Invade	Biostart	*Costelytra givenii* (Coleoptera)
Serratia entomophila		Bioshield Grass-Grub	Biostart	*Costelytra givenii* (Coleoptera)
Beauveria bassiana	Fungus	Beaugenic	Biotelliga Limited	Thrips

Beauveria bassiana		Beaublast	Biotelliga Limited	Aphids, psyllids, thrips and whitefly
Beauveria bassiana		CONTEGO BB	Biological Solutions Ltd	Aphids, mites, thrips and whitefly
Lecanicillium lecanii	Fungus	eNTokill	Biotelliga	Aphids, psyllids, thrips, whitefly, mealy bug, passion vine hopper
Lecanicillium lecanii		eNtoblast	Biotelliga	Aphids, psyllids, thrips, whitefly, mealy bug, passion vine hopper
Cydia pomonellla granulosis virus	Virus	MADEX 3	Key Industries Ltd	Codling moth in pipfruit
Cydia pomonellla granulosis virus		VIREX	Agrinova NZ Limited	Codling moth in pipfruit
Cydia pomonellla granulosis virus		CYD-X Biological Insecticide	Hilado Pty Ltd	Codling moth in pipfruit

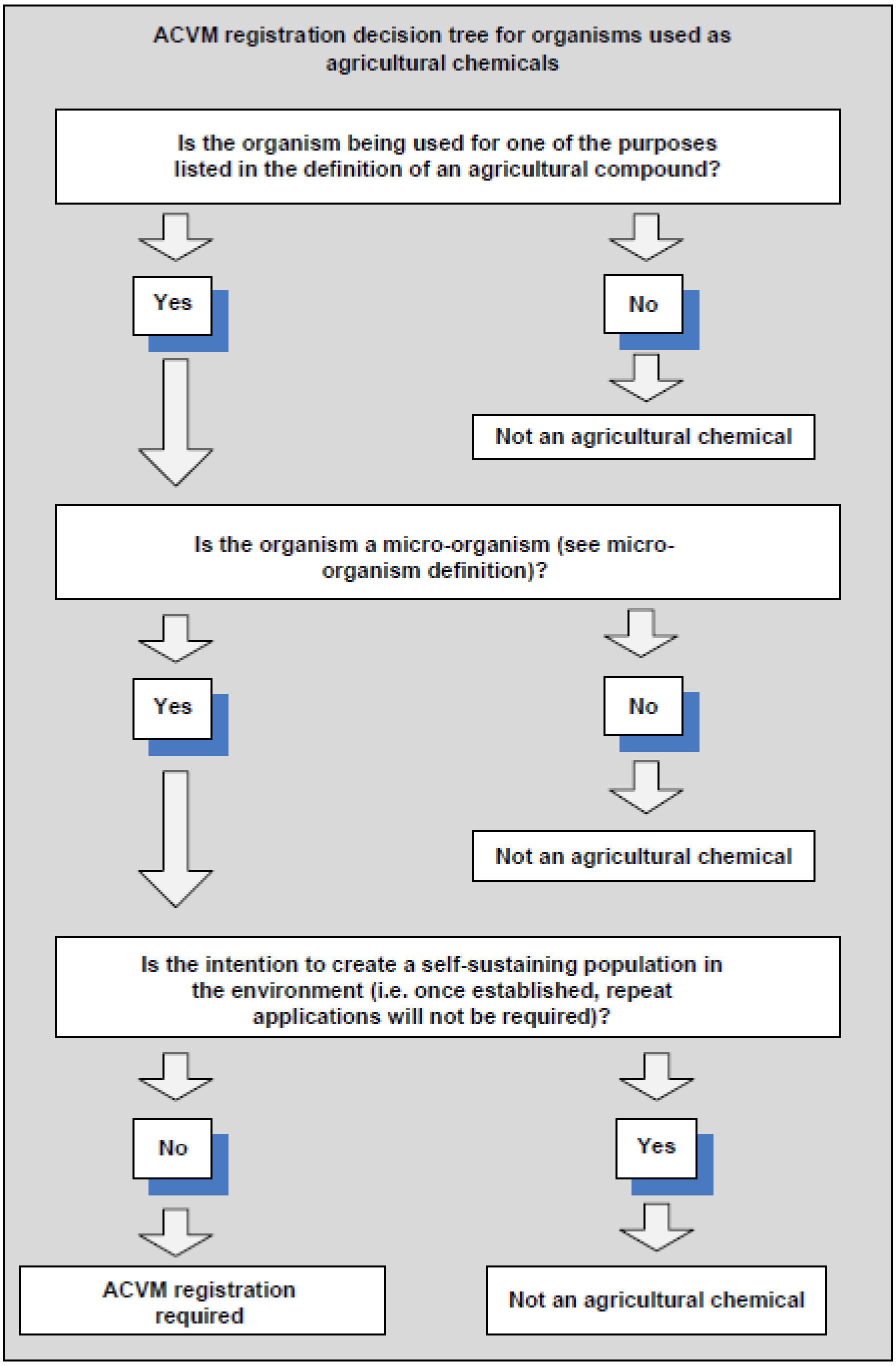

Figure 1. Flow diagram of the registration process for microbial products in New Zealand. Agricultural compounds are defined as "any substance, mixture of substances, or biological compound, used or intended for use in the direct management of plants and animals." (Source. Microbial Agricultural Chemicals Guidelines, Ministry for Primary Industries, 11 August 2016)

Highlights

Microbial biopesticides for control of invertebrates: Progress from New Zealand

1. New Zealand's primary sectors are facing constant threat from pest invasions.

2. Widespread use of biopesticides is limited by availability of products.

3. Novel products based on endemic microorganisms is underway.

4. Regulatory agencies are implementing new registration processes.

5. Industry co-investment and infrastructure development is needed.