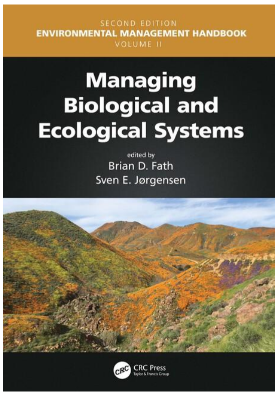

Managing Biological and Ecological Systems

Brian D. Fath, Sven E. Jørgensen, Megan Cole

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G. J. Ash, A. Wang
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Biopesticides

G. J. Ash and
A. Wang

Introduction

Inappropriate use of synthetic pesticides in agriculture can lead to environmental degradation, health risks, and loss of biodiversity. Additionally, overuse of particular pesticides may also lead to a loss in their effectiveness through pesticide resistance. Several studies have highlighted the accumulation of pesticides in soils in agricultural systems and the resultant environmental damage.[1] The short-term effects of pesticides on mammalian health are relatively well known and documented through pesticide approval processes. However, the longer-term effects are less well understood but may include cancer, neurotoxic effects, and reproductive disorders.[2] In developing countries, it is estimated that there may be 25 million cases of occupational pesticide poisoning annually.[3] Biopesticides are environmentally sensitive alternatives to the use of synthetic pesticides for the management of a wide range of agricultural pests including weeds, insects, pathogens, and other invertebrate pests. Biopesticides are a class of pesticides that contain plant, animal, or microbial products or organisms.[4] The International Union of Pure and Applied Chemistry[5] further classifies biopesticides as plant-incorporated protectants (protectants produced from genetically modified plants), biochemical pesticides (natural products that affect pests), and microbial biopesticides. Microbial biopesticides are a type of biological control in which a living organism is included as the active ingredient in an inundative application of a formulated product. A range of organisms have been used in biopesticide applications including fungi, nematodes, bacteria, and viruses for the control of weeds, insects, acarines, plant diseases, and molluscs.[6–16]

Formulations of biopesticides contain the organism, a carrier, and adjuvants, which may contain compounds such as nutrients and/or chemicals that aid in the survival of the pathogen or help in protecting the active ingredient from adverse environmental conditions.[17] The formulation of the biocontrol agents is affected by the type of biocontrol organism but must ensure that the agent is delivered in a form that is viable, virulent, and with sufficient inoculum potential to be effective in the field. Furthermore, the formulation may also dictate the means of delivery of the final product, for example, as a seed dressing or a foliar-applied formulation. To be successful, a formulation must be effective, economical, and practical to use.[18] Formulation of biopesticides can be in the form of dry products (dusts, granules, pellets, wettable powders, and encapsulated products) and liquid formulations (suspensions, emulsions, and encapsulated products).[19] The biopesticide is usually packaged, handled, stored, and applied in a similar way to traditional, synthetic pesticides.[20]

Due to the enormous costs involved in the production of synthetic pesticides, global companies have tended to focus on the registration of pesticides in the major crop/cropping systems. This has led to a number of attempts to develop biopesticides in these non-core or niche markets.[21] These markets could be of considerable size and include those that have been created by synthetic pesticide withdrawal and the organic food movement. Philosophically, the use of biopesticides is compatible with organic food production, provided the agent had not been genetically modified, the carriers and adjuvants are natural products, and the host range of the biopesticide is not considered to be too wide.[22]

The use of biopesticides as a strategy in pest management can be applied to both native and introduced pests. However, the success of this type of biocontrol revolves around the costs of production, the quality of the inoculum, and, most importantly, the field efficacy of the product.[18] Biopesticides are usually developed by collaboration with commercial companies in an expectation that they will recoup their costs through the sale of the product.

The broad range of biopesticides may be further subdivided based on the type of target pest and/or the active ingredient. For example, a bioherbicide is a biopesticide developed for weed management whereas a mycoherbicide is a bioherbicide that contains a fungus as an active ingredient. The goal of this entry is to introduce the broad range of biopesticides available and their targets.

Bioinsecticides and Bioacaricides

More than 1500 species of pathogens have been shown to attack arthropods and include representatives from bacteria, viruses, fungi, protozoa, and nematodes.[23] Diseases caused by insects have been known since the early 1800s with the first attempts at inundative applications of fungi to control insects being developed in 1884, when the Russian entomologist Elie Metchnikoff mass produced the spores of the fungus *Metarhizium anisopliae.* A mycoacaricide for the management of citrus rust mite was first registered in the United States in 1981.[24] This was based on the fungus *Hirsutella thompsonii.* Currently, the main fungal species formulated as mycoinsecticides and mycoacaricides include *M. anisopliae* (33.9%), *Beauveria bassiana* (33.9%), *Isaria fumosorosea* (5.8%), and *Beauveria brongniartii* (4.1%).[15] De Faria and Wraight[15] reported a total of 171 fungi-based products with the majority being for the control of insects (160) and mites.[28] Their review indicated that the main formulation types are fungus-colonized substrates, wettable powders, and oil dispersions containing conidia (asexual spores). In the United States, there are seven bioinsecticide products based on various strains of *B. bassiana* registered. These include products such as BotaniGard® and Mycotrol® (available as emulsifiable suspensions and wettable powders).

Bacteria that attack insects can be divided into nonspore-forming and spore-forming bacteria. The nonspore-forming bacteria include species in the Pseudomonadaceae and the Enterobacteriaceae. The spore-forming bacteria belong to the Bacillaceae and include species such as *Bacillus popilliae* and *Bacillus thuringiensis*. *B. thuringiensis* (Bt) have primarily been developed as biopesticides to control Lepidopteran larva. However, other serotypes of Bt produce toxins that kill insects in the Coleoptera and Diptera as well as nematodes. The bacterium produces δ-endotoxin, an insecticidal crystal protein, which is converted into proteolytic toxins on ingestion. Up to nine different toxins, which have different host ranges, have been described.[25] Commercial formulations of the bacteria contain living spores of the bacteria. Biopesticides based on Bt are the most widely available of the bacterially based products.[25] Up to 90% of the Microbial Pest Control Agent market is Bt or Bt-derived products. The most well known is Dipel.

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae, in conjunction with bacteria of the genus *Xenorhabdus*, have been successfully deployed as biopesticides, for example, BioVECTOR.[23] They are usually applied to control insects in cryptic and soil environments. The nematodes harbor the bacteria in their intestines. The infective third-stage larvae enter the host through natural openings and penetrate into the hemocoel. The bacteria are voided in the insect and cause septicemia, killing the insect in approximately 48 hr.

Entomopathogenic viruses have also shown promise as bioinsecticides. They were first used to control populations of *Lymantria monacha* in pine forests in Germany in 1892 (Huber, 1986, from Moscardi[26]), but the first commercial viral insecticide registered was called Viron/H for the control of *Helicoverpa (Heliothis) zea* in 1971.[27] Viruses from the family Baculoviridae have been isolated from more than 700 invertebrates, with the virus group not common outside of the Lepidoptera and Hymenoptera.[26,28] The nucleo-polyhedroviruses (NPVs) are rod-shaped, double-stranded DNA viruses that are produced in polyhedral proteinaceous occlusion bodies[28] that are ingested by the insect. Granulosis viruses (GVs) are also members of the Baculoviridae but are restricted to the Lepidoptera and have capsular proteinaceous occlusion bodies.[26] These authors[26] provide a table of products that have been developed for the control of insects using these two viral groups.

Biofungicides

Biological control of fungi that cause plant disease can be accomplished by a number of mechanisms including antibiosis, hyperparasitism, or competition. Additionally, weak pathogens may induce systemic acquired resistance in the host, giving a form of cross-protection. Biofungicides have been used in both the phylloplane and rhizosphere to suppress disease. A biological control agent for the control of foliar pathogens in the phylloplane must have a high reproductive capacity, the ability to survive unfavorable conditions, and the ability to be a strong antagonist or be very aggressive. A wide range of bacteria and fungi are known to produce antibiotics that affect other microorganisms in the infection court. Most often, these organisms are sought from a soil environment, as this environment is seen as the richest source of antibiotic-producing species. Species of *Bacillus* and *Pseudomonas* have been successfully used as seed dressings to control soil-borne plant diseases.[29] Serenade®, marketed by BASF, is a formulation of *Bacillus subtilis* (strain QST713), which has claimed activity against a wide range of plant diseases.[30] It is applied as a foliar spray to crops such as cherries, cucurbits, grapes, leafy vegetables, peppers, potatoes, tomatoes, and walnuts.[31] Fluorescent pseudomonads are also often seen as a component of suppressive soils. These bacteria may prevent the germination of fungi by the induction of iron competition through the production of siderophores (ironchelating compounds). These are effective only in those soils where the availability of iron is low. Control of foliar and fruit pathogens such as *Botrytis cinerea,* a pathogen of strawberries, has been accomplished by the foliar application of the soil-inhabiting fungus *Trichoderma viride.*[32] This fungus inhibits *Botrytis* using a combination of antibiosis and competition. On grapevines, *Trichoderma harzianum* competes with *B. cinerea* on senescent floral parts, thus preventing the infection of the ovary. It has also been shown to coil around the hyphae of the pathogen during hyperparasitism.[33] *T. harzianum* has also been reported to induce systemic resistance in plants.[34] One of the earliest commercial successes using *T. harzianum* is the product Rootshield®. Rootshield contains the T-22 strain of *T. harzianum* and is produced and marketed by Bioworks Inc. This strain of the fungus was first registered by the U.S. Environmental Protection Agency in 1993. The product is available as a granular formulation and is usually applied to soil mixtures in glasshouse situations.[35–37]

Bioherbicides

Fungi are the most important group of pathogens causing plant disease. Therefore, fungi (or oomycetes) are most commonly used as the active ingredient in bioherbicides and as such the formulated organism is referred to as a mycoherbicide.[38] However, there are examples of bacteria[7–11] and viruses being used or proposed to be used as bioherbicides.[39,40] The aim of bioherbicide development is to overcome the natural constraints of a weed–pathogen interaction, thereby creating a disease epidemic on a target host.[41] For example, the application of fungal propagules to the entire weed population overcomes the constraint of poor dissemination. After removal of the host weed, the pathogen generally returns to background levels because of natural constraints on survival and spread.

The first commercially available biopesticide for the control of weeds was DeVine®, a bioherbicide for the control of strangler vine in citrus groves in the United States. It was released in 1981.[42] In 1982, a formulation of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* was released to control northern jointvetch in soybean crops in the United States. Since then, there have been a number of products commercialized[12,21] as well as numerous examples of pathogen–weed combinations that had been reported as having potential as bioherbicides in countries including in Canada, United States, Europe, Japan, Australia, and South Africa.[18,21] Necrotrophic or hemibiotrophic fungi are usually used as the basis of mycoherbicides, as they can be readily cultured on artificial media and so lend themselves to mass production. Other desirable characteristics of fungi under consideration as mycoherbicides include the ability to sporulate freely in artificial culture, limited ability to spread from the site of application, and genetic stability. In most cases, these biopesticides are applied in a similar fashion to chemical herbicides using existing equipment, although the development of specialized application equipment and formulation may improve their efficacy and reliability. Since 2000, there have been two successful registrations for bioherbicides in Canada. In 2002, a product called Chontrol®, based on the fungus *Chondrostereum purpureum* for the control of trees and shrubs, was registered.[12] This was based on the research of Hintz and colleagues.[43–45] A more recent success in the area of bioherbicides includes the registration of Sarritor® for dandelion control by the company of the same name in Canada. This product is based on the phytopathogenic fungus *Sclerotinia minor*, which has been extensively researched by Professor Alan Watson at McGill University.[46–50]

Biomolluscicides

Biomolluscicides are a type of molluscicide derived from natural materials such as animals, plants, and microorganisms (e.g., bacterium, fungus, virus, protozoan, or nematode). They are usually used in the fields of agriculture and gardening to control pest slugs and snails. In some circumstances, biomolluscicides are also used in the health area to control molluscs acting as vectors of harmful parasites to human beings.

Currently, the most widely used biomolluscicide is Nemaslug®, a successful biomolluscicide developed by Becker Underwood (U.K.). The active ingredient of Nemaslug is *Phasmarhabditis hermaphrodita*, a nematode species from the family of Rhabditidae. The pathogenicity of *P. hermaphrodita* against slugs had not been recognized until 1994 when Wilson et al.[51] discovered that *P. hermaphrodita* could infect and kill a wide variety of pest slugs under laboratory conditions. Like entomopathogenic nematodes, *P. hermaphrodita* kills slugs by penetrating into the hemocoel of hosts through natural openings and releasing its associated bacteria, which kill the host eventually.

P. hermaphrodita was found to be associated with several different bacteria rather than one particular species, but its association with *Moraxella osloensis* proved to be highly pathogenic to gray garden slug (*Deroceras reticulatum*).[51] This bacterium was used in the mass production of *P. hermaphrodita* via monoxenic culture.[51]

The host of *P. hermaphrodita* is not restricted to only one slug species (*D. reticulatum*). It can attack and kill several species of slugs including *Arion ater*, *Arion intermedius*, *Arion distinctus, Arion silvaticus, Deroceras caruanae, Tandonia budapestensis,* and *Tandonia* sowerbyi.[51] Moreover, *P. hermaphrodita* can also parasitize several species of snails including *Cernuella virgata*, *Cochlicella acuta*, *Helix aspersa*, *Monacha cantiana*, *Lymnaea stagnalis*, and *Theba pisana*.[52]

Nemaslug is now sold in many European countries, including U.K., Ireland, France, the Netherlands, Belgium, Germany, Denmark, Norway, Finland, Poland, Spain, the Czech Republic, Italy, and Switzerland. In 2005, the retail sale of this biomolluscicide was up to £1 million in Europe and approximately 500 ha horticultural crops (e.g., lettuce and strawberries) and field crops (e.g., wheat, potatoes, and oilseed) were treated with this biomolluscicide. At the dose rate of 3×10^9/ha, *P. hermaphrodita* provides protection against slug damage similar to, if not better than, methiocarb pellets.[16]

Bacteria-based biomolluscicides are now in the process of development. *Streptomyces violaceoruber* and *Xanthobacter autotrophicus* have been examined for their molluscicidal activity against *Oncomelania hupensis* (a unique host of schistosomiasis blood fluke parasite) under laboratory conditions.[53] The results revealed that both bacteria were effective in killing *O. hupensis*, with *S. violaceoruber* causing more snail mortality than *X. autotrophicus* (90% vs. 85%).

Biomolluscicides of plant origin have also been studied extensively in recent years when the environmental pollution caused by chemical molluscicides was realized increasingly. More than 1400 plant species have been screened for their molluscicidal properties against pest snail species.[54] Several groups of compounds present in various plants have been found to be poisonous to snails at acceptable doses, ranging from <1 to 100 ppm, including saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpene lactones, and terpenoids.[54] The molluscicidal activity of the dried root latex powder of *Ferula asafoetida*, the flower-bud powder of *Syzygium aromaticum*, and the seed powder of *Carum carvi* against the snail *Lymnaea acuminata* was proved.[55] Similarly, acetogenin (extracted from the seed powder of custard apple) presented promising and stable molluscicidal activity against *L. acuminata*.[54] When sodium alginates was used as a binding matrix for the formulation of acetogenin, the release of this biomolluscicide extended over 25 days, which set up a good example for the development of biomolluscicide delivery system.[54]

The combination of bacteria-based biomolluscicides and plant-based biomolluscicides may lead to a synergistic effect between plant and microbe extracts as molluscicides. Zhang and coworkers[56] reported that higher snail mortality was produced when a mixture of *Arisaema erubescens* tuber extracts and *S. violaceoruber* dilution was applied against the snail *O. hupensis*. The mechanisms of snail toxicosis might be that the combination of *A. erubescens* tuber extract and *S. violaceoruber* dilution reduced the detoxification ability of liver and increased the oxidative damage in liver cells of snails.

Conclusion

Biopesticides are a viable alternative to synthetic pesticides in a number of crops. The development of microbial biopesticides relies on agent discovery and selection, development of methods to culture the pathogen, creation of formulations that protect the organism in storage as well as aid in its delivery, studies of field efficacy, and methods of storage. Each microbial biopesticide is unique, in that not only will the organism vary but so too will the host, the environment in which it is being applied, and economics of production and control.

There are a number of advantages of the use of biopesticides over the use of conventional pesticides, including the minimal residue levels, control of pests already showing resistance to conventional pesticides, host specificity, and the reduced chance of resistance to biopesticides. This indicates an emerging, strong role for biopesticides in any integrated pest management strategy and an important involvement in sustainable farming production systems in the future.

There have been some spectacular successes in the use of microbial biopesticides, despite the perceived constraints to their deployment.[57] In the past, biopesticides have been expected to behave in the same way as synthetic pesticides. For the ultimate success of biopesticides, microorganisms developed for biological control must be viewed by researchers, manufacturers, and end users in a biological paradigm rather than a chemical one. The business model for the commercialization of the products may also vary significantly from that used for traditional synthetic pesticides.[18]

The efficacy and reliability of many microbial biopesticides may be affected by environmental parameters as well as the aggressiveness of the pathogen. Furthermore, the narrow host range of many pathogens may restrict their commercial attractiveness. Both of these issues can be addressed by research into the use of genetic engineering and formulation.[18,58–60] As research into the molecular basis of host specificity and pathogenesis continues, it will become possible to produce more aggressive pathogens with the desired host range for biological control. The survival and efficacy of these pathogens will be enhanced through the use of novel formulations.

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