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Formulations of Biopesticides

Susan Boyetchko, Eric Pedersen, Zamir Punja, and Munagala Reddy

1. Introduction

A large number of factors can potentially affect the economic feasibility of any given biological control product. These include the impact on the target pest, market size and spectrum of pests affected by the biocontrol agent, variability of field performance, costs of production, and a number of technological challenges, including fermentation, formulation, and delivery systems *(1–4)*. Selection of the appropriate formulations that can improve product stability and viability may reduce inconsistency of field performance of many potential biological control agents *(2,5,6)*. It has been indicated that slow progress in research on formulation and delivery systems is a major hurdle to the development of biopesticide products *(1,7)*. This chapter summarizes the efforts and successes toward formulation of biocontrol products for use against diseases (biofungicides), weeds (bioherbicides), and insect pests (bioinsecticides). The discussion emphasizes the use of bacteria, fungi, and viruses as the agents. Information on formulation of other important biocontrol agents, such as nematodes, can be found elsewhere *(8)*.

Since growers may not be willing to invest in new equipment to apply biological control products, microbial agents must be sold in a product form that is compatible with existing equipment and farm management practices. Compatibility with cultural and chemical control methods, as well as field application systems, are important requirements for the success of biocontrol products *(3,7)*, as well as the need for the agricultural industry to accept and adopt the new technology. The establishment of a modest market share for biopesticide products in the future

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should prompt the development of new and innovative technologies in formulation and delivery in addition to those already available *(9)*.

A number of challenges are encountered in the formulation of biocontrol agents, including good market potential, ease of production and application, adequate product stability and shelf life during transportation as well as in storage, and guaranteed propagule viability and efficacy over the long term *(7)*. Some reasons why biocontrol agents have met with limited commercial success are difficulty of production, sensitivity to UV light and desiccation, requirement of high humidity for infection, insufficient performance over a wide range of environmental conditions, and lack of appropriate formulation *(10)*. Formulations should be used to alter the microbial product to improve product stability, bioactivity, and delivery (i.e., ability to mix and spray the product) as well as to integrate the biopesticide into a pest management system *(11)*. Other important characteristics of a successful formulation are convenience of use, compatibility with end-user equipment and practices, and effectiveness at rates consistent with agricultural practices *(12)*. For foliar biocontrol agents, environmental factors that influence plant infection and disease development are temperature, free moisture or dew period, and protection against UV irradiation and desiccation *(11,13)*. For soil-applied biocontrol agents, physical and chemical characteristics of soil, moisture, and temperature regimens, as well as microbial competition can all influence efficacy. All of these parameters need to be taken into consideration when developing an appropriate formulation.

2. Formulation of Bacterial Biopesticides

2.1. General Requirements

Bacteria are generally mass-produced using a deep-tank liquid fermentation process, although in some cases they may be more amenable to semisolid or solid-state fermentation. Nutrient components of the fermentation medium and growth conditions are critical to both biomass and secondary metabolite production *(14)*. Components of the growth medium should be inexpensive and readily available. Developing the final formulation usually requires processing of the ferment and addition of further components. The end-product can be a solid, liquid, slurry, powder, or granular. The formulation should maintain bacterial viability during transit from manufacturer to retailer and long-term storage (a minimum of 4 mo) *(14)*. Formulation of the biopesticide also plays a major role in consistency of performance by improving or maintaining bacterial survival following application. A suitable formulation should provide a protective habitat for the introduced bacteria, thereby increasing their potential for survival and successful colonization *(15)*.

Bacteria may be formulated in either a dormant or a metabolically active state *(14)*; the former tend to have a longer shelf-life and are more tolerant to temperature fluctuations and chemical pesticides. However, these formulations may be more expensive to produce and require a lag period before they become metabolically active and express beneficial effects. On the other hand, formulations containing active cells may be less tolerant to temperature fluctuations, less compatible with chemical pesticides, have a shorter shelf-life, and require specific packaging for gas and moisture exchange, but the bacteria are active at the time of application.

2.2. Formulations Developed

Formulations for bacterial biopesticides may be either liquid or dry. Liquid formulations include those that are oil-based, aqueous-based, polymer-based, or combinations thereof. Aqueous-based formulations require few steps other than fermenting bacteria in a liquid medium and adding components, such as stabilizers, stickers, surfactants, coloring agents, antifreeze compounds, and additional nutrients *(9,16–18)* (**Table 1**). Alternatively, the ferment can be processed (e.g., concentrated or dried) and then resuspended in a liquid medium. The fluid properties of the formulation can be altered by the addition of polymers (e.g., polysaccharides or derivatives of polyalcohols). Oil-based formulations typically involve blending a processed ferment with a mineral or vegetable-based oil carrier and emulsifiers to allow dilution in water. Oil-based formulations reduce evaporation of droplets and allow for ultra-low-volume aerial application. Dormant propagules are generally formulated in oil-based and polymer-based liquids, whereas dormant or metabolically active propagules can be formulated in aqueous-based liquids.

Dry formulations, wettable powders, dry flowables, and granulars (including wettable granules) can be produced through such processes as spray drying, freeze-drying, or air drying either with or without the use of a fluidized bed. Wettable and dry granulars are produced by adding binder, dispersant, wetting agents, and water to the dry powdered ferment in a granulator. The extra processing steps in producing a dry formulation increase manufacturing cost, but reduce shipping cost because of the reduced weight.

Most dry formulations include an inert carrier, such as fine clay, peat, vermiculite, alginate, or polyacrylamide beads. The carrier facilitates delivery of the necessary concentration of viable cells in the correct physiological state. Among all of the components that make up a formulation, the carrier occupies the greatest volume and, therefore, often functions as an extender. Effective carriers are inexpensive, easily sterilized, nontoxic, and consistent in physical characteristics. Moreover, the carrier must ensure both adequate dispersal of the bacteria and performance by protecting the bacteria from adverse environmental conditions.

Table 1

Examples of Registered Bacterial Biofungicides and Methods of Formulation and Delivery

Biocontrol agent	Trade name	Formulation	Delivery	Company
Agrobacterium radiobacter K84	Galltrol-A	Petri plate with pure culture	Root dips, drench	AgBioChem, Inc., Orinda, CA
A. radiobacter	Nogall, Diegall	Washed plates, culture suspension	Root dips, drench	Bio-Care Technology Pty, Ltd., Somersby, NSW, Australia
Pseudomonas fluorescens A506	BlightBan A506	Wettable powder	Spray, drench	Plant Health Tech, Boise, ID
P. fluorescens NCIB	Conquer	NA	Spray	Mauri Foods, North Ryde, Australia
P. fluorescens NCIB 12089	Victus	Aqueous suspension of fermentation broth	Spray	Sylvan Spawn, Kittanning, PA
P. syringae ESC 10	Bio-save 100/1000	Wettable powder	Post harvest drench, dip or soil applied	EcoScience Corp., Orlando, MA
P. syringae ESC 11	Bio-save 110			
Bacillus subtilis	Epic	Dry powder	Added to a slurry, mix	Gustafson, Inc., Dallas, TX
B. subtilis GB03	Kodiak, Kodiak HB	Dry powder	Added to a slurry, mix	Gustafson, Inc.
B. subtilis GB03	System 3	Dust	Seed treatment in planter box	Helena Chemical Co., Memphis, TN
Burkholderia cepacia type Wisconsin M36	Blue Circle	Peat carrier or liquid	Seed treatment or drip irrigation	CCT Corp., Carlsbad, CA
Streptomyces griseoviridis K61	Mycostop	Powder or spray	Drench, dip, or spray	Kemira Agro Oy, Pokkalankatu, Finland

Other materials that have been added with the bacteria are diatomaceous earth, adhesive clays, such as talc and vermiculite, cellulose derivatives (e.g., carboxy-methyl-cellulose), and other polymers, such as xanthan gum ***(19)***. Techniques for the immobilization of bacteria with polymers such as polyacrylamide and sodium alginate, are available ***(5,19,20)***. However, slow hydration and release of the active ingredient are major impediments to this technology. Alginate has been successfully used to formulate a variety of bacteria, including *Pseudomonas* spp. ***(7)***. Carriers, such as Pyrax or powdered wheat bran, that provide a food base have been incorporated with the bacterial biomass and alginate. Digat ***(21)*** described a new encapsulation method for bacterial inoculants that resulted in a high concentration of bacteria (10^7 cfu) in a 6 mm granule. The bacteria were suspended in a nutrient broth that caused less nutritional stress, and it was suggested that the system enabled the formulation of several microbial agents or strains (i.e., a cocktail mix).

Stringent quality control at all stages of manufacturing is necessary to produce a high-quality product. Any variability in the manufacturing process, whether the result of contamination or inconsistent procedures, can reduce the reliability of the end-product. For example, *Bacillus thuringiensis* is easily produced in liquid fermenters, but production conditions strongly influence potency of the final product ***(22)***.

2.3. Formulation of Bacterial Bioherbicides

One of the challenges confronting the use of phytopathogenic bacteria for biological weed control is the requirement of free water for dispersal and the need for wounds or natural openings for entry of the bacteria into the plant ***(23,24)***. Researchers investigating *Xanthomonas campestris* pv. *poae* for control of annual bluegrass (*Poa annua* L.) have demonstrated that cutting or mowing of turfgrass will permit the bacteria to enter into the plant ***(25)***. In addition, bacteria applied at a rate of 10^9 cfu/mL at high water volumes (400 mL/m^2) showed over 90% disease severity in the annual bluegrass.

One formulation that has facilitated the penetration and entry of bacteria into plant stomata and hydathodes is the organosilicone surfactant Silwet L-77 (0.2%) ***(24)***. To deliver liquid into the stomata of a leaf, a low surface tension of 30 dynes/cm or lower is required; Silwet reduces the water surface tension to 20 dynes/cm. Application of *Pseudomonas syringae* pv. *tagetis* with this surfactant facilitated the penetration and entry of the bacteria into stomata and hydathodes, resulting in significant increases in disease severity and incidence in Canada thistle when compared to plants sprayed with the bacteria minus the surfactant ***(23)***. It has also been suggested that delivery of the bacteria into these natural openings protects them from UV irradiation and desiccation. Research on *P. syringae* pv. *phaseolicola* (Psp) for biocontrol of kudzu

(*Pueraria lobata* [Willd.] Ohwi) has also demonstrated that formulation with Silwet L-77 led to higher disease severity in the field ***(26)***. Bacteria may be delivered to the soil in a granular form and banded at planting or applied as a liquid, but the type of delivery system will depend on the type of crop and farming practice in use (**Table 2**).

2.4. Formulation of Bacterial Biofungicides

Bacteria that are registered for use as biofungicides have been recently reviewed by several authors ***(7,27,28)*** (**Table 3**). Several *Bacillus*-based products are currently being used for disease control and yield enhancement. In China, *Bacillus* spp. are used to enhance yield of wheat, rice, corn, sugarbeet, rapeseed, turnip, and Chinese cabbage ***(29)***. In the United States, the products Epic®, Kodiak®, and Kodiak HB (*Bacillus subtilis* GBO3) are available for use on cotton, legumes, vegetables, and ornamentals to control diseases caused by *Rhizoctonia* and *Fusarium* species. The products are formulated as wettable powders and are compatible with several seed treatment fungicides (**Table 1**).

Agrobacterium tumefaciens is commercially available in Australia, the United States, and New Zealand, and is formulated as a concentrated liquid or a moist peat-based product, or is supplied as a nonformulated agar culture ***(28,30)***. Following suspension in water, the bacteria can be applied to seeds, cuttings, roots or root wounds of susceptible orchard and ornamental plants as a dip, spray, or drench.

Mycostop® is a biofungicide based on the bacterium *Streptomyces griseoviridis* K61, which is formulated as a wettable powder and is registered in various countries for control of damping-off and root and basal rot diseases of ornamentals and vegetables caused by *Fusarium*, *Phomopsis*, and *Pythium*. The product contains mycelium and spores and can be applied to seed as a dry powder or suspended in water and used as a dip, spray, or drench, and is compatible with a range of insecticides, fungicides, and herbicides.

Three products based on *Burkholderia cepacia* strains are Blue Circle® (type Wisconsin M36), Deny® (type Wisconsin Iso J82), and Intercept. They are formulated as either liquids or peat-based products for control of the fungi *Fusarium*, *Phytophthora*, and *Pythium*, and the nematodes *Globodera rostochiensis*, *Heterodera glycines*, and *Hoplolaimus columbus*. Other bacterial-based biofungicide products are listed in **Table 1**.

2.5. Formulation of Bacterial Bioinsecticides

Most of the commercial bioinsecticides in use today are based on formulations of *B. thuringiensis* Berliner (Bt), an aerobic gram-positive spore-forming bacterium ***(31,32)*** (**Table 3**). The principle mode of action of Bt biopesticides is based on target insect ingestion of the toxic delta-endotoxin protein, which causes feeding inhibition and eventual toxemia to the mid-gut of susceptible

Table 2
Examples of Registered and Unregistered Fungal and Bacterial Bioherbicides and Methods of Formulation

Biocontrol agent	Trade name	Target weed	Formulation	Company
Phytophthora palmivora MWV	DeVine	Stranglervine	Liquid	Abbott Labs, Chicago, IL
Colletotrichum gloeosporioides f.sp. *aeschynomene*	Collego	Northern jointvetch	Dry powder	Encore Technologies, Minnetonka, MN
C. gloeosporioides f.sp. *malvae*	BioMal	Round-leaved mallow	Wettable powder	Philom Bios, Sasaktoon, Canada
Puccinia canaliculata ATCC 40199	Dr. BioSedge	Yellow nutsedge	NA	NA
C. gloeosporioides	LUBOA II	Dodder	Granular mixture	Ningxia Region, China
	NA	Silky hakea	Granular, wheat bran	Plant Protection Reasearch Institute, Stellenbosch, South Africa
C. coccodes	Velgo	Velvetleaf	Water + sorbitol (0.75%)	
C. truncatum	COLTRU	Hemp sesbania	Fungus-infest wheat gluten (PESTA)	
Alternaria cassia	CASST	Sicklepod	Water + nonoxynol surfactant, paraffin wax, mineral oil, soybean oil, lecithin	Mycogen Corp., San Diego, CA
Pseudomonas syringae pv. *tagetis*	NA	Canada thistle	Silwet L-77, Silwet 408	Encore Technologies
Xanthomonas campestris pv *poaea*	Camperico	Annual bluegrass	NA	Japan Tobacco, Kanagawa, Japan

Table 3
Examples of *Bacillus thuringiensis* Bioinsecticides

Insect target	Pathotype of *B. thuringiensis*	Example of commericial product	Company
Lepidopteran	*B. thuringiensis* var. *kurstaki*	Dipel	Abbott Labs
		Javelin, Thuricide	Ecogen, Langhorne, PA
		Foray, Novo Biobit, Bactospeine	Thermo Trilogy, Baltimore, MD
		MVP	Mycogen
Dipteran	*B. thuringiensis* var. *israelensis*	Teknar	Ecogen
		Skeetal	
		Vectobac	Abbott Labs
Coleopteran	*B. thuringiensis* var. *san diego*	M-Track	Mycogen
	B. thuringiensis var. *tenebrionis*	Trident	Ecogen
		Novodor	Thermo Trilogy

larva. Only a few species of insects in the families Lepidoptera, Coleoptera, and Diptera are susceptible to the protein. Thus, these biopesticides have a relatively narrow insecticidal spectrum. Examples of commercially available Bt-based biopesticides are listed in **Table 3**. These products are formulated as concentrated liquids, oil-based flowables, wettable powders, water dispersible granules, and dusts.

Several new Bt-based products have been developed using recombinant DNA technology. Two products currently available in the United States, MVP™ and M-Track™, have been developed using Mycogen Corporation's CellCap® encapsulation process. This is a process whereby a gene encoding the delta-endotoxin protein is removed from Bt, incorporated into a plasmid, and inserted into an isolate of *Pseudomonas fluorescens* ***(33,34)***. The recombinant cells are grown in aerobic culture and induced to express the delta-endotoxin before being killed through heat and chemical treatment. The dead bacterial cells in the aqueous formulation serve as microcapsules that protect the fragile Bt toxin from environmental degradation.

3. Formulation of Fungal Biopesticides

3.1. Mycoherbicides

Environmental conditions, such as temperature and moisture regimes, are major limitations to the efficacy of mycoherbicides. Moisture required for disease development is often dependent on the amount of dew period. "DeVine," the first registered mycoherbicide, is a liquid formulation of chlamydospores of *Phytophthora palmivora* for control of stranglervine ***(35,36)***. The product is not very stable and there is only 6 wk of shelf life when the product is refrigerated. "Collego" (*Colletotrichum gloeosporioides* f.sp. *aeschynomene*) is formulated as dried spores in a wettable powder ***(37)***. For control of sicklepod, "CASST" is formulated as spores of *Alternaria cassiae* in emulsifiable paraffinic oil ***(11)*** (**Table 2**).

Several adjuvants and other amendments can be used to enhance spore germination, improve pathogen stability, and modify the environmental requirements or expand the host-range of various mycoherbicides ***(11)***. For example, *Colletotrichum truncatum* is a host-specific and highly virulent pathogen on hemp sesbania, but requirement for free moisture has limited its bioherbicidal potential ***(37)***. Formulating the biocontrol agent using unrefined corn oil as an adjuvant significantly enhanced its bioactivity and reduced its dew period requirement from 12 to 2 h and reduced spray volume requirements from 500 to 5 L/ha ***(37)***.

Surfactants have been explored as ingredients in formulations because they help to wet the plants by reducing surface tension and they may improve dispersal of the fungal spores in the spray droplet mix. Several surfactants that have been used are Tween 20 with *Fusarium lateritum*, nonoxynol with *Alternaria macrospora*, and *A. cassiae* and sorbitol with *Colletotrichum coccodes* ***(11)***. Selection of appropriate surfactants must first include an evaluation of their inhibitory or stimulatory effects on spore germination, infection, and other aspects of disease development.

Use of invert emulsions (water-in-oil) with foliar fungal biocontrol agents has provided a favorable environment for germination and infection ***(1,11,38,39)***. The efficacy of *C. truncatum* was significantly improved when applied with an invert emulsion ***(40)***. Research with *Alternaria cassiae* indicated that the level of spore inoculum per droplet could be dramatically reduced from 10–100 to 1 spore per droplet to achieve effective control of sicklepod when formulated with an invert emulsion ***(41–43)***. However, invert emulsions are very viscous and may demonstrate phytotoxicity in some target plants. Connick et al. ***(44)*** developed an invert emulsion with improved water-retention properties that was less viscous. Also, vegetable oils can be used to enhance efficacy of mycoherbicides, such as *Colletotrichum orbiculare*, for control of spiny cocklebur ***(45)***. No phytotoxicity and improvements in spread of the invert emulsion were observed.

Although liquid formulations have been primarily used for post-emergence mycoherbicides, solid-based formulations have been developed for those mycoherbicides that infect the weeds at or below the soil surface, a system more appropriate for preemergence mycoherbicides ***(11,46)***. These formulations can provide a food-base for the pathogen, act as a buffer in environmental extremes, and retain inoculum so it may not be easily washed away. A wheat-gluten matrix (liquid inoculum, semolina wheat flour, and kaolin) has been used to formulate fungal agents, such as *C. truncatum, A. crassa,* and *Fusarium lateritium* ***(47)***. This formulation has been termed "PESTA" and can be applied as preemergent and soil-incorporated treatments. Shelf-life of the product can be improved by manipulating the water activity (moisture content of the granule) and sucrose content ***(48)***. Other solid substrates used to formulate mycoherbicides are bran, wheat kernels, cornmeal/sand, and vermiculite ***(1,11)***. For example, mycelium, micro- and macroconidia and chlamydospores of *Fusarium solani* f.sp. *cucurbitae* were formulated in cornmeal-sand for control of Texas gourd ***(49)***. This pre-emergent granular formulation provided 96% control of the weed.

3.2. Formulations of Fungal Biofungicides

The environmental conditions discussed previously that limit the efficacy of mycoherbicides, namely temperature and moisture, also affect growth and survival of fungal biofungicides. The organisms researched as biocontrol agents are primarily filamentous fungi, e.g., *Gliocladium virens* and *Trichoderma harzianum*, but there are also examples of some yeast-like fungi, e.g., *Pseudozyma flocculosa* ***(50)*** and *Tilletiopsis pallescens* ***(51)***. The applications of these biofungicides are for control of root-infecting pathogens, e.g., *Pythium, Rhizoctonia*, and foliar fungal pathogens, e.g., powdery mildew ***(50–52)*** and *Botrytis* ***(28)***. The formulations that have been developed include granules, pellets, dusts or wettable powders containing spore inocula that are applied directly or as a suspension in water (**Table 4**). The granular formulations protect against desiccation as well as provide a food base for the fungus, whereas the powders are easily amenable to spraying and provide coverage of large areas. Treatment of seeds with liquids or dusts is an alternative method of application of these biocontrol agents. In addition, formulations of spores in invert emulsions have been tested for yeasts, such as *Tilletiopsis* ***(51)***.

The use of alginate prill was successfully developed to formulate *Gliocladium virens* (Soil Gard) as a granular formulation for control of root-infecting fungi in potting media ***(7)***. Similarly, powder or dust formulations containing *Trichoderma* with pyrophyllitte clay (Pyrax) have been successfully deployed. Biomass production is generally achieved in large-scale deep tank fermenters containing appropriate nutrient substrates, and then either used wet or dried prior to formulation ***(7)***. Most of the factors that affect product development are similar to those discussed under **Subheading 2.2.** for bacteria.

3.3. Formulations of Fungal Bioinsecticides

Several fungi have been studied as potential biological control agents of insects, and the most well researched include *Verticillium lecanii* for control of

Table 4
Examples of Registered Fungal Biofungicides with Methods of Formulation and Delivery

Biocontrol agent	Trade name	Formulation	Delivery	Company
Ampelomyces quisqualis M10	AG10 Biofungicide	Water-dispersible granule	Spray	Ecogen
Fusarium oxysporum (nonpathogenic)	Fusaclean	Spores, microgranule	Drip to rock wool	Natural Plant Protection, Nagueres, France
Pythium oligandron	Polygandron	Granule or powder	Seed treatment or soil incorporated	Vyskumny ustav rastlinnej, Piestany, Slovak Republic
Phlebia gigantea	Rotstop	Spores in inert powder	Spray, chain saw oil	Kemira Agro Oy, Finland
Trichoderma spp.	Bio-Fungus	Granular, wettable powder	Spray or injected	Grondortsmettingen, St.-Katelijne-Waver, Belgium
T. harzianum	Trichodex	Wettable powder	Spray	Makhteshim Chemical Works Ltd., Beer Sheva, Israel
T. harzianum Rifai KRL-AG2	T-22G, T-22 HB	Granules or dry powder	Granules added in furrow broadcast	Bioworks, Inc., Geneva, NY
T. harzianum ATCC 20476 *T. polysporum* ATCC 20475	Binab T	Wettable powder and pellets	Spray, mixing with potting medium	Bio-Innovation AB, Algaras, Sweden
Gliocladium virens GL-21	SoilGard, GlioGard	Granules	Incorporated in soil, soilless mix	Thermo Triology
Coniothyrium minitans	Contans	Granules	Soil application	Prophyta, Malchow-Poel, Germany
Candida oleophila I-182	Aspire	Wettable powder	Drench, dip or spray	Ecogen

aphids, *Beauvaria bassiana* for whiteflies, locusts and beetles, *Metarhizium flavoviride* and *M. anisopliae* for locusts, and *Lagenidium gigantem* for mosquito larvae control ***(53,54)***. The fungi may be applied directly to the insect as wettable powders, emulsions or dusts, amended into baits or traps, or added to soil ***(55–59)***. Formulations are essential to protect against environmental extremes of moisture and temperature, as well as to provide protection from UV damage and desiccation. For example, sunlight, especially the UV-B component (280–320 nm), was one of the most important factors limiting survival of *B. bassiana* conidia on foliage ***(60)***. Entomopathogens can be applied under field conditions in oil at ultralow volumes to increase their efficacy and to protect against UV damage ***(57,61–63)***. Sunlight blockers (clay) and UV-B-absorbing compounds (Tinopal) can be added to inoculum formulations or starch encapsulation ***(64–66)*** to increase survival and shelf-life.

4. Formulation of Viruses

4.1. General Requirements

Baculoviruses have been investigated for control of insect pests belonging to the Lepidoptera, Hymenoptera, and Coleoptera ***(67)***. Their advantages are that they are highly specific, do not attack beneficial insects, and can persist in the environment, making long-term control of insect pests possible. Examples of baculoviruses are the nuclear polyhedrosis viruses (NPVs) and granulosis viruses (GVs). Some limitations of these biocontrol agents are the slow speed of biological activity, their low stability under UV light, and difficulties of production ***(10)***. Stability of the baculoviruses, which is often a function of their viability, is not a significant problem for small-scale field trials since the viruses are collected from macerated larvae and mixed with water and can be stored for short periods through refrigeration ***(67)***. However, these systems do not lend themselves to large-scale production and application. Formulation of these viruses is an important aspect of product development but has not received as much attention by researchers as the bacteria and fungi.

4.2. Formulations Developed

The majority of the baculoviruses are formulated as concentrated wettable powders ***(67)***. The corn earworm (*Helicoverpa zea)* NPV biocontrol product, "Elcar," is either spray- or air-dried after being diluted with an inert carrier. Such products as the gypsy moth (*Lymantria dispar* L.) NPV are freeze-dried either with a carbohydrate or by acetone precipitation. Such factors as UV irradiation, particularly wavelengths of 290–320 nm, can inactivate the virus. Some UV protectants, either reflectants or absorbers, can be added to formulations to stabilize the baculoviruses. Several effective dyes, such as lissamine green, acridine yellow, alkali blue, and mercurochrome have been used as UV protectants, especially to absorb UV-A irradiation ***(68)***. Optical brighteners (fluorescent brighteners), such as those commonly used in soaps, detergents, and fabric softeners, also absorb UV light and have been shown to significantly reduce photodegradation of NPVs and enhance viral activity ***(68–72)***. The precise mode of action of optical brighteners is not known, but research suggests that they interfere with the chitin microfibrils in the paratropic membrane lining of the midgut of insects, which aids in the protection of invasion by microbes, such as baculoviruses in insects. The brighteners act in the insect midgut and thus affect the host susceptibility to the baculovirus. Optical brighteners may therefore increase the host-range spectrum of baculoviruses.

5. Methods for Delivery of Formulated Biocontrol Products

Delivery of products must be easy, economical, effective, timely to the appropriate site of action, and compatible with current agronomic practices and equipment. Formulated microbes can be delivered to seed, seed pieces, tubers, cuttings, seedlings, transplants, mature plants, or soil; these delivery methods are discussed in more detail below in **Subheadings 5.1.–5.3.**

5.1. Seed Treatment

For optimal protection of germinating seeds and seedlings against disease, the biofungicides need to be delivered in a manner that allows the organism(s) to colonize the spermosphere and the developing rhizosphere at a density that is high enough to suppress the pathogen ***(73)***. Biocontrol agents can be precoated or encapsulated onto the seed, mixed with the seed at planting, applied in-furrow, or incorporated into the soil-mix or seed bed ***(74–76)***.

Precoating of seed usually involves formulations of dry powders or oil- and polymer-based liquids with dormant microbes that are capable of surviving a period of desiccation ***(14)***. Additives, such as xantham gum and gum arabic, are sometimes used to increase adhesion of the microbial product to the seed. A specialized seed-coating process, termed seed encapsulation, involves enveloping the seed, microbe, and possibly other components, such as pesticides or micronutrients, in a gelatinous or polymer gel-matrix, thereby prolonging survival of microbial agents on seed. An example of a seed encapsulation product is GEL-COAT™, which is an alginate hydrogel preparation patented as a delivery system for entomopathogenic nematodes. The seed encapsulation method of delivery has the distinct advantage of user safety and reduced environmental hazard, since the active ingredients are effectively sealed until they are released during seed germination. Factors to consider in selecting a formulation for coating seeds include inoculum density achievable on the seed, stability of the coating, both for microbe viability and coat integrity, and the feasibility and cost of production ***(12)***.

Formulations consisting of fine dusts or powders, wettable powders, or liquids can be applied to seed with or without sticker materials at the time of planting. Delivery at the time of planting usually ensures a high number of viable microbes and may allow growers to apply the product directly into the planter box. Drawbacks to this delivery method include possible variability in efficacy resulting from a reliance on the grower's ability to apply the seed treatment correctly and the extra task for growers.

5.2. Soil Treatment

If seed treatment is not a practical option, e.g., if direct inoculation onto seed is harmful to the microbe due to desiccation, or presence of inhibiting compounds *(77)*, biocontrol agents can be applied to soil. Soil treatment is most effective when the agents are applied as a post-fumigation treatment or at time of planting. In sterile soil or growth mixes, colonization by pathogens may be reduced by establishing a high population of the biocontrol agent. This creates a "suppressive soil," making subsequent colonization by other less beneficial organisms difficult *(7)*. Dust, powder, and granular formulations can be broadcast and incorporated into soil, whereas wettable powder, water-dispersible granular, and liquid formulations can be delivered in furrow *(14,74,75)*.

Soil application may also be a useful method for controlling overwintering pathogen propagules in soil. For example, the product CONTANS®, a water dispersible granular formulation of the hyperparasite *Coniothyrium minitans*, can be incorporated into soil to reduce the number of sclerotia of *Sclerotinia sclerotiorum*.

In greenhouse crops, a simple yet effective method of delivering biocontrol agents to soil or growth medium is by direct injection into an irrigation system, such as overhead boom or spaghetti systems. This type of delivery is advantageous in that it allows precise control of the concentration and total volume of microbial suspension being applied, and requires minimal labor to treat large numbers of plants. Multiple treatments of a crop are also possible when existing irrigation equipment is utilized. The one drawback to this type of delivery system is that it requires specialized injection equipment and therefore is not cost-effective unless the grower has equipment for injecting liquid fertilizers.

Root-colonizing fluorescent pseudomonad bacteria have been demonstrated to grow on wheat and barley straw, suggesting the possibility of using crop residues retained under minimum and zero-tillage as a method for delivering them as microbial inoculants *(78–80)*. Populations of 10^6 cfu/g straw applied onto barley residues were recovered the following year and were capable of colonizing roots of winter wheat in the year of application *(80)*. Bacterial populations were greater in no-till seeded crops than in conventionally seeded crops, indicating that cropping systems can influence the activity and survival of the soil microbial inoculants. Some factors that should be considered if application onto crop residues is pursued are addition of UV protectants and antidesiccants to the formulation, and application of the inoculum into the crop residue to maximize the benefits of these residues, which would protect the bacteria from extremes in temperature and moisture.

5.3. Treatment of Plants

Biocontrol products can also be applied to plant roots, wounds, and foliage by drenching, dipping, or spraying. Formulated bacteria can be applied directly to roots as a dip or drench *(81)*. Spores of the biofungicide *Phelbia gigantea* in an aqueous suspension can be brushed onto freshly cut stumps of pine to prevent entry of *Heterobasidion annosum* *(82)*, thereby protecting exposed wounds. Alternatively, spores can be incorporated into chain saw oil so that they are delivered at the same time the tree is harvested.

Formulations of bacteria or fungi used as foliar sprays vary according to the crop to be treated, the pest to be controlled, and the anticipated delivery system. The two formulations most commonly used for foliar sprays are liquids and slurries, with the slurries usually reconstituted from either dry or moist carrier-based formulations. Emulsifiers, stickers, spreaders, and other adjuvants and additives aid in application, dispersal and adhesion of the microbes on plant surfaces, and protect the microbes from adverse environmental conditions, such as desiccation, unfavorable pH, and UV radiation *(32,83)*. A broad range of spray application equipment and techniques is available for applying chemical pesticides, including high volume (1000 L/ha), medium volume (350 L/ha), low to very low volume (3–150 L/ha), and ultra low volumes (0.5–3.1 L/ha), controlled droplet application, and electrostatic spraying *(58)*. If biocontrol agents are to be applied using the same techniques, formulations must have the necessary physical properties. Steinke and Akesson *(84)* found that surface tension and viscosity of the suspension to be sprayed are important factors in reducing droplet size and maintaining the necessary dispersion and control of droplets. Density of the suspension was not an important factor. Successful application of biocontrol agents using different spray techniques has been achieved. For example, Bt-based products have been applied to numerous crops using conventional ground or aerial spraying methods. Highly concentrated ultra-low volume liquid formulations of Bt-based products have also been used to control insect pests on such crops as cotton and banana *(32)* and to control spruce bud worm over large areas of coniferous forests *(18,32)*. A low-volume electrostatic rotary atomizer has been used to apply *Verticillium lecanii*, an entomopathogenic fungus, to successfully control the aphid *Aphis gossypii*. In addition, ultralow-volume equipment, such as spinning disk sprayers, are now commonly used for application of baculoviruses in forests *(67)*.

6. Conclusions and Future Research

Although extensive research has led to the identification of numerous strains of bacteria, fungi, and viruses that can act as potential biocontrol agents, one of the major factors that has limited their commercial success is adequate biomass scale-up and formulation technology. With each microbial agent, there are inherent obstacles to formulation that need to be addressed, including the effect on viability of propagules, microbial stability, competitive ability after application, and activity under various environmental conditions. The formulations developed should also be compatible with crop production practices and equipment.

Future research efforts in formulation technology should emphasize processes that will yield optimal infectivity of the agent and bioactivity, as well as achieving viable and stable biological products. Little research has been conducted on methods to promote efficacy of the product after it has been applied to the target pest. In addition, delivery and application technology of the formulated product need to be addressed. Timing of application as well as placement of the formulated product onto the target pest must be considered to obtain a highly effective biopesticide product. Future research should attempt to develop systematic approaches for selecting formulations based on the characteristics of the biocontrol agent. The potential success of a biocontrol product during the discovery phase should include an evaluation of the ability to mass-produce and formulate the agent. Screening of all formulations currently available to select the most effective one should give way to developing criteria that enable researchers to rapidly select classes of formulations based on their determined characteristics and the desired characteristics of the biocontrol agent. Adequate funding of research by the private and public sectors to develop new formulation technologies and the dissemination of research findings should considerably enhance the rate at which future developments are made in this area. Collaborations between biologists and chemists, particularly in the area of food chemistry and preservation, would facilitate development of new formulations and applications.

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Delivery Systems and Protocols for Biopesticides

Roy Bateman

1. Introduction

1.1. Conditions for Successful Biopesticide Development

Biopesticides have little *raison d'être* unless they are biologically specific. Their perceived advantage of mammalian safety over chemicals has been eroded by new developments in pesticide chemistry ***(1)*** and with possible rare exceptions, biopesticides will be targeted at "niche markets." Research and development, therefore, will be reliant to a greater or lesser extent on public support, but unfortunately microbial research is usually funded piecemeal. Multidisciplinary teams are uncommon, but where opportunities have arisen to form such teams the results have often been rewarded with success, where "success" could be defined as: scientific novelty or elegance (with outputs in the scientific literature) or implementation with commercial products available for use. Although a successful outcome will be biology driven and must depend on sound science, the ultimate test must be the latter: we are in the business of providing technical solutions, not the production of "better mousetraps."

The opportunities for biopesticide development will be described in detail in this section of the volume; the greatest scope appears to be in the following broad categories ***(2)***:

1. Treatment of natural and seminatural habitats in which conservation of biodiversity is important (e.g., pest management in forests and rangelands);
2. Crops subject to public pressure for high ecological and toxicological standards (e.g., organic food crops, reduction of pesticide residues);
3. Substitution for chemical applications deemed to be unsatisfactory (e.g., insecticide resistance management strategies);
4. Situations in which very low mammalian toxicity is crucial (e.g., storage pests);

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