Formulation Development and Delivery of Biopesticides

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Abstract

Biopesticide formulation development is integral for end product development and risk reduction associated with commercialization and acceptance by the end user. Development of robust formulations for biopesticides is a key step towards advancing this technology into integrated pest management systems. A granular formulation protocol using extrusion-spheronization-fluidized bed drying for biopesticidal bacteria and fungal hypha and spores is described. Establishing low granule water activity (a_w, 0.2-0.3) is a key factor in extending the shelf-life of the product. Starch type and amount provided controlled release attributes to the biopesticide granules. Microencapsulation of bioherbicide, *Colletotrichum truncatum* 00-003B1 (*Ct*), conidia and bioinsecticide nucleopolyhedrovirus (NPV), by complex coacervation is described for foliar application of biocontrol agents.

Introduction

Microbial-based biocontrol is a promising strategy for pest management in crop production. Biopesticide formulation development and delivery are key steps towards advancing this technology into integrated pest management systems. The inundative approach to pest management employs massive doses of a biocontrol agent (Menaria 2007; Yandoc-Ables et al. 2006). The biocontrol agents are strategically applied to capitalize on the optimum parasite-host interaction for pest growth suppression or death. The decision on formulation type is dependant on the mode of action of the biocontrol agent and the stage at which the host plant is most susceptible to the agent. In the case of weed management, granular formulations usually are best suited for pre-emergent application to weeds while water-oil, water-oil-water emulsions or wettable powder formulations are most appropriate for spray application for post-emergent weed and plant disease management. For soil-borne plant pathogens, soil-applied granules and seed-applied biocontrol agents are appropriate. Foliar application of bioinsecticidal nucleopolyhedroviruses is suitable for aboveground feeding insect pests.

The biopesticide formulation must shelter, maintain population stability and efficacy of the biocontrol agent, be inexpensive and applied using conventional farm equipment. Table 1 shows an abbreviated list of formulation components and their functions used for the preparation of granules, wettable powders, invert (water in oil) and water-oil-water emulsions. A comprehensive review of this subject is provided by Burges (1998).

Table 1. Formulation type, ingredients and functions (Burges 1998).

Formulation	Formulation Ingredients	Function
Liquid, spray application	Vegetable, Mineral oil	Carrier, spreader
	Clay, Tinopal	Sunscreen UV protection
	Bran, citrus pulp	Feeding stimulant
	Glycerol	Humectant, osmotic protection
	Lecithin, SPAN's	Emulsifiers
	Xanthan gum	Thickner
	Gelatin	Sticker, Thickner
Granules, soil application	Kaolin, clay	Carrier, dispersant
	Cereal flour	Nutrient
	Diatomaceous earth	Free-flow agent
	Microcrystalline cellulose	Dispersant
	Vegetable gum, corn syrup, PVP	Binder
	Methyl cellulose	Humectant

Biopesticide formulations are composed of the active, carrier and adjuvant ingredients. Granular formulations for biopesticides are composed of matrices of natural products and formed into a flowable product. Examples of bioherbicidal formulations including granular, seed treatment and emulsion formulations are reported in Table 2. These formulations provide protection for bioherbicidal microorganisms from harsh environmental conditions and anthropogenic sources (Walker and Connick 1983; Connick et al. 1991; Quimby et al. 1999). For example, the pesta formulation is a matrix of durum wheat, kaolin, sucrose and the bioherbicide culture and has been adapted by many researchers for studying weed management in laboratory and field experiments (Connick et al. 1996; Daigle et al. 2002). Granular and microencapsulation formulation research for biopesticides currently under development at Agriculture and Agri-Food, Saskatoon Research Centre is described in this report.

Table 2. Formulations for bioherbicidal microorganisms.

Formulation	Biopesticide	Host	Reference
Granule			
Pesta	*Alternaria cassiae*	Sicklepod (*Senna obtusifolia*)	Connick et al. 1991
Pesta	*F. oxysporium* Foxy 2	*Striga* spp.	Elzein et al. 2004
Pesta	*Colletotrichum truncatum*	Hemp sesbania	Boyette et al. 2007
Pesta	*Pseudomonas fluorescens* BRG100	Green foxtail (*Setaria viridis*), wild oat (*Avena fatua*)	Daigle et al. 2002
Pesta	*P. fluorescens* G2-11	Green foxtail, velvet leaf	Zdor et al. 2005
Alginate	*A. cassiae, A. macrospora, F. lateritum, C. malvarum, Phyllosticta* sp	None indicated	Walker and Connick 1983
Pesta, Rice-alginate prill	*F. oxysporum* f.sp. *erythroxyli*	Coca, (*Erythroxylum coca*)	Bailey et al. 1998
Barley granules	*Phomopsis convolvulus*	Bindweed *Convolvulus arvensis* L	Vogelgsang *et al.* 1998
Alginate microencapsulate - gelatine or agar	*F. avenaceum*	Marsh reed grass *Calamagrostis canadensis*	Winder et al. 2003
Starch (Stabileze process)	*C. gloeosporioides, F. oxysporum*	None indicated	Quimby et al. 1999
Seed coating			
	Fusarium nygamai	*Striga*	Zahran et al. 2008
Liquid			
Invert emulsion	*C. truncatum*	Hemp sesbania	Boyette et al. 1991, 1993
Invert emulsion	*Alternaria cassiae*	Sicklepod	Walker and Boyette 1985
Invert emulsion	*Ascochyta pteridis*	Bracken *Pteridium*	Womack et al. 1996
Water/oil/water (WOW)	*C. orbiculare*	Bathurst burr *Xanthium spinosum*	Auld et al. 2003
Water, 0.1% Tween 80	*C. truncatum*	Scentless chamomile	Graham et al. 2006
Water, surfactants	*Myrothecium verrucaria*	Sicklepod	Weaver et al. 2009

Materials and methods

Granular formulation preparation

The procedure of Hynes and Boyetchko (2011) was used. Briefly, 85 ml of a stationary phase culture of *Pseudomonas fluorescens* BRG100 (*Pf*) (Boyetchko 1997), 60 hrs old from M9 medium was blended into 150 g oat flour, 40 g maltose, and 10 g peat to prepare the pesta granular formulation dough (Daigle *et al.* 2002). The dough containing 9 $\log_{10}$ cells/g was extruded through a 1.2 mm, 25%, and 0.7 mm, 22.5%, dome die using a single screw Fuji Paudal MG55 granulator (LCI Corp., Charlotte, NC, USA). Extrudate was transferred into a Fuji Paudal Marumerizer (spheronizer), model QJ-230 (LCI Corp., Charlotte, NC, USA) and spheronized producing pesta granules approximately 1.2 and 0.7 mm in diameter (Figure 1). Pesta granules were transferred to a Sherwood Scientific fluidized bed drier (Sherwood Scientific Ltd., Cambridge, UK) and dried for 20 min. Water activity (a_w) was determined following drying (Aqua Lab, Decagon Devices, Pullman, WA, USA). Solid state fermented *Phoma macrostoma* was milled prior to

single screw extrusion and spheronization as described above. A flowable granular product with minimal dust was produced (Bailey et al. 2009).

Addition of starch to biopesticide granules
Corn, pea, potato and rice starches at 13 and 26% wgt/wgt were blended individually into the granule recipe described above. Biopesticide extrudate was developed into granules as described above.

Granule disintegration and size determination
Granule disintegration was determined by particle size analysis using laser diffractometry (Mastersizer 2000, Malvern Instruments, Worcestershire, UK) and reported as volume mean diameter (VMD) or De Brouckere mean diameter, $D[4,3] = \Sigma d^4/\Sigma d^3$, where volume has a d^4 dependence and surface area a d^3 dependence (Rawle 2009). VMD was reported in μm (for assay details see Hynes and Boyetchko 2011).

Microencapsulation of biopesticides by complex coacervation
The preparation of the complex coacervate formulation was described by Hynes et al. (2010). Briefly, an invert emulsion (water in oil) was prepared by dispersing 3 g of an aqueous suspension of *Colletotrichum truncatum* (*Ct*) conidia, (7.6 $\log_{10}$ conidia/ml), into 7.3 g of non-refined canola oil and 300 mg of soya lecithin. This is the "core" of the formulation. Individual gelatine (1%) and gum Arabic (2%) aqueous solutions adjusted to pH 7.0 were prepared. Gelatine and gum Arabic is the protein-polysaccharide pair for complex coacervate wall development and encapsulation "shell" of the core containing the biocontrol agent. The *Ct* conidia oil suspension was slowly added to the gelatine solution while being stirred at 300 rpm using a paddle stirrer. Fifty ml of gum Arabic was pumped, 5 ml/min, into the emulsion while stirring at 300 rpm. The pH was adjusted to 4.0 with 1N HCl. *Ct* conidia retention by the invert emulsion and the microencapsulated emulsion droplets by the complex coacervate were monitored by enumeration using a microscope.

Nucleopolyhedrovirus rearing was described in Erlandson et al. (2007). Baculovirus, suspended in dH_2O, 9 $\log_{10}$ NPV/ml, were added to the gelatine solution with mixing using a paddle stirrer as described above. Addition of gum Arabic and adjustment of pH was carried out as described above. The complex coacervate suspension was prepared for freeze drying.

Results

Granular formulation preparation
Granular formulations of biopesticides were produced by i) blending the microbial agent, bacteria or fungi, with carrier and adjuvants and ii) single screw extrusion of the biopesticide-laden dough followed by spheronization and fluidized bed drying (Figure 1). Granule size was dependant on the extrusion domes used, e.g. 0.7, 0.8, 1.0, 1.2 and 2.0 mm diameter holes. Starch, corn, pea, potato and rice, amendments to the bacterial and fungal granular formulation recipes did not affect the extrusion characteristics but did affect the disintegration properties of the granular product.

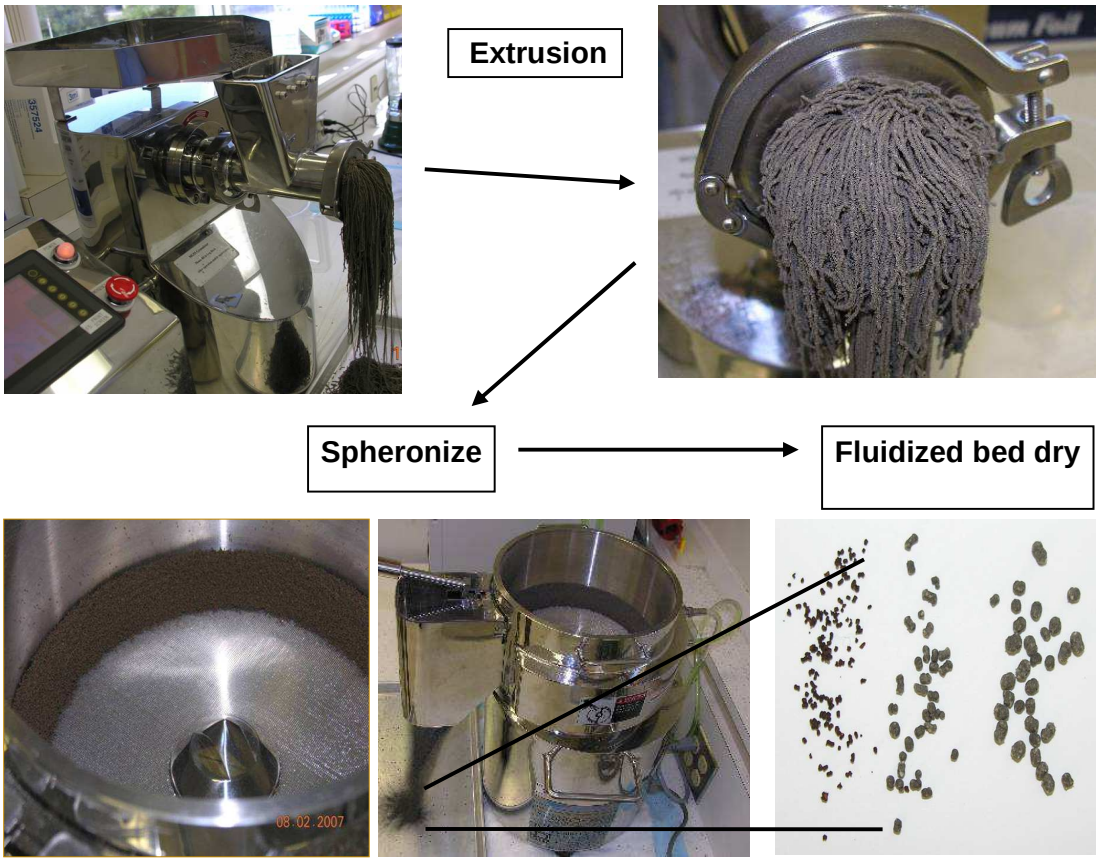

Figure 1. Granule formulation protocol - single screw extrusion, spheronization, fluidized bed drying.

Microencapsulation of biopesticides by complex coacervation

A two step process to formulate *Ct* conidia by microencapsulation using complex coacervation was developed and included: i) emulsification of an aqueous suspension of *Ct* conidia in non-refined vegetable oil with the aid of surfactant, soya lecthin and ii) encapsulation of *Ct* conidia-invert emulsion within a gelatine-gum Arabic shell. Soya lecithin promoted retention of *Ct* conidia in oil and stability to the invert emulsion. The efficacy of the *Ct* on the host weed, scentless chamomile, in greenhouse and field studies was not changed following the microencapsulation process.

A one step process was developed to microencapsulate baculovirus and included: encapsulation of the baculovirus within a gelatine-gum Arabic shell as it formed followed by freeze drying. The dry gelatine-gum Arabic shell containing baculovirus formed an elaborate branched structure when viewed under the dissecting microscope (Figure 2). The efficacy of the baculovirus on the insect pest, cabbage looper, *Trichoplusia ni*, was not changed following the complex coacervation process.

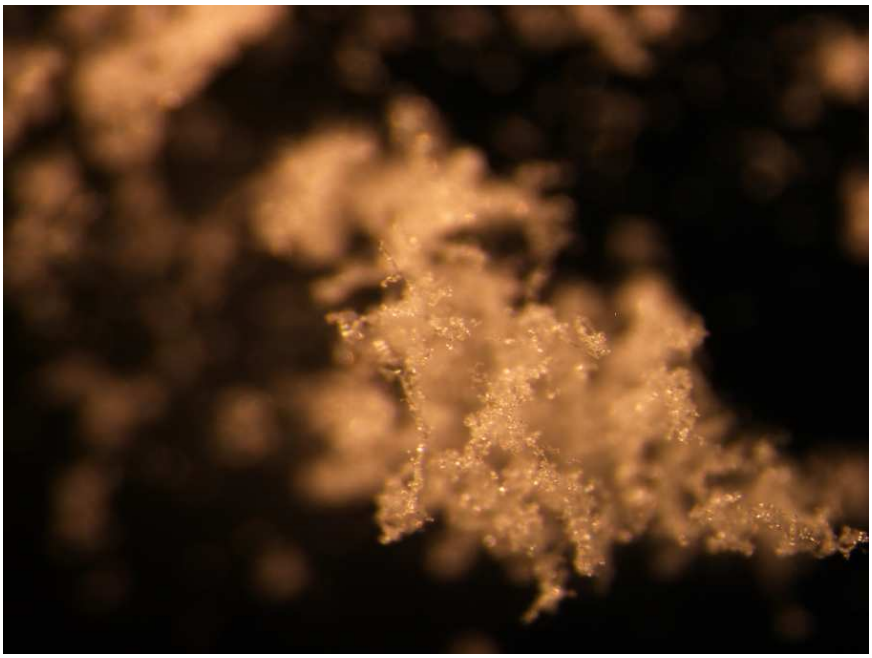

Figure 2. Complex coacervate containing bioinsecticidal baculovirus.

Discussion

Granular formulation

Granular formulations must be capable of sustaining large populations (about 1 billion per gram) of biopesticidal microorganism over the shelf-life of the product. Granule size and water activity (a_w) affect biopesticide efficacy. Biopesticide granules containing *Phoma macrostoma*, a bioherbicidal fungus for control of dandelion and some other broadleaf weeds had optimum efficacy when granules were 400 to 800 μm in diameter (Bailey et al. 2009). Broader coverage by the smaller granules and release of the biopesticide from these granules may have contributed to the improved efficacy by *P. macrostoma* over that of larger granules (Bailey et al. 2009).

Formulation a_w has a significant effect on survival of *P. fluorescens* BRG100, a grass weed bioherbicide. The population of *Pf* experienced minimal decrease in pesta at a_w 0.2, as compared to pesta at aw 0.5 and 0.8, respectively (Hynes and Boyetchko 2011). Drying pesta to 0.2 a_w maintained the population of *Pf* for 16 months at approximately 9 log10 cfu/g. Mugnier and Jung (1985) studied the survival of bacteria (*Rhizobium*, *Agrobacterium*, and *Arthrobacter* spp.), fungal spores (Penicillium sp.) and yeasts (*Saccharomyces* sp.) in relation to water activity (a_w). They reported that optimum survival of the microbial propagules was achieved using a_w ranging from 0.06 to 0.3.

Granular formulations of biopesticides applied to the soil or on to the target pest must disintegrate in a timely manner to inundate the pest with the biopesticidal agent. Starch

amendment to granular formulation recipes imparted variable rate disintegration properties to the granules (Hynes and Boyetchko 2011). For example, the order of fast to slow disintegration following addition of four different starches was pea>potato>corn> rice. Increasing pea, potato and corn starch content from 13 to 26% promoted faster disintegration of pesta, conversely, increasing rice starch content decreased disintegration (Hynes and Boyetchko 2011). This observation is due to differences in starch size, amylose and amylopectin content and their interaction with water (Li and Yeh 2001; Copland et al. 2009). This allows the formulator to customize the granular formulation to meet the needs of the active ingredient's mode of action.

Microencapsulation by complax coacervation of biopesticides

Microencapsulation by complex coacervation was developed as a way to deliver *Ct* and baculovirus to the target pests, scentless chamomile and cabbage looper. Coacervate formulations have been developed to maintain functionality of their key ingredients and shield them from harsh environmental conditions for the pharmaceutical (Suheyla and Oner 2000), cosmetic (Kalantar et al. 2007), food (Schmitt et al. 1998) and agriculture industries (Amiet-Charpentier et al. 2000). Plant growth promoting rhizobacteria were encapsulated in a complex coacervate and dried in a fluidized bed (Amiet-Charpentier et al. 2000). This protocol describes microencapsulating *Ct* suspended in vegetable oil (formulation core) with a protein and carbohydrate coat (shell). Formulation ingredients, including non-refined vegetable oil type, surfactant mixture, protein-carbohydrate shell components, and formulation processing parameters, mixing speed and time and ingredient ratios were examined for maximum retention of *Ct* conidia in the formulation. A significant breakthrough was the optimization of the parameters that allowed up to 95% retention of hydrophilic *Ct* conidia in the water-oil core of the complex coacervate (Hynes et al. 2010). The invert emulsion provides some protection from desiccation of the fungal mycelium on the foliage of the plant.

Conclusions

A biopesticide granule formulation protocol employing single screw extrusion-spheronization-fluidized bed drying resulted in a flowable granular product is described. Parameters such as granule size, water activity and adjuvants impact biopesticide efficacy. Low water activity, 0.2, promoted greater survival of the grass weed bioherbicide, *Pseudomonas fluorescens* BRG100, in pesta granules. The adjuvant, starch greatly affected the disintegration of the granules and dispersion of the biopesticide. The order of fast to slow disintegration following starch amendment was pea > potato > corn > rice. Corn starch-amended pesta promoted greater dispersion of *P. fluorescens* BRG100*gfp* in sand columns that non-amended pesta (Hynes and Boyetchko 2011).

Microencapsulation by complex coacervation of *Ct* conidia and baculovirus is a suitable formulation for delivery of biopesticides to the plant foliage. The platform formulation technology has the flexibility of being sprayed onto plant foliage or dried and applied as a dust to plant foliage or to the seed coat.

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