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Optimizing nutritional conditions for the liquid culture production of effective fungal biological control agents

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Spores of fungal pathogens of weeds and insects are unique in their ability to actively infect and kill their pest host. While these capabilities are advantageous in terms of their use as a contact biological control agent, or biopesticide, they also require special consideration during spore production. Directed approaches to medium optimization must consider not only spore yield but also spore qualities such as desiccation tolerance, stability as a dry preparation, and biocontrol efficacy. Nutritional conditions during culture growth and sporulation should direct the accumulation of appropriate endogenous reserves so that newly formed spores possess these advantageous qualities. Studies with the bioherbicidal fungus *Colletotrichum truncatum* and with the bioinsecticidal fungus *Paecilomyces fumosoroseus* have demonstrated the impact of nutrition on spore 'fitness' for use as a biological control agent. The optimization strategy used in these nutritional studies as well as a comparison of the results are presented.

Keywords: biological control; liquid culture production; weeds; insects; fungi

Inundative biological control

Chemical insecticides and herbicides have been extensively used over the past 50 years due to their effectiveness and ease-of-use. Undoubtedly, the widespread use of chemical pesticides will continue although public concerns about the safety of these chemicals has led to more rigorous registration requirements. Many of the most effective chemical pesticides have failed to pass reregistration. The removal of these chemical pesticides from the marketplace coupled with the emergence of herbicide- and insecticide-resistant pests has heightened interest in the development of biologically-based pest control strategies [8,40]. The use of aggressive, specific, indigenous fungal pathogens of weeds and insects is one such approach.

The 'inundative' or 'augmentative' method of biological control involves the massive application of a pest-specific, indigenous pathogen to weed- or insect-infested crops [9,40,41]. In many ways, the inundative application of fungal biopesticides resembles the use of chemical pesticides since the agent is applied as needed and must contact the pest [2]. The use of a stable, aggressive, pathogen which is capable of consistently killing the pest host under field conditions is a requirement. By using an indigenous isolate, biocontrol agent registration costs are reduced due to less stringent regulatory requirements. A limited number of commercial products using fungi in the 'inundative' approach to biological control are presently in use in the United States and around the world [9,30].

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Fungi as biopesticides

There are numerous advantages to the use of fungi as living microbial biocontrol agents. The ability of fungi to actively infect and kill the insect or weed host is certainly the most important. Fungal spores are capable of germinating and penetrating a healthy weed or insect and do not require a compromised host defense. Because of this advantage that fungi have over bacteria and the vast number of fungal pathogens of weeds, a recent list of biological control agents being evaluated for weed control listed 22 fungi and no bacteria [9]. It should be noted that two bacteria, *Xanthomonas campestris* and *Pseudomonas syringae*, are being evaluated for the control of the weeds, annual bluegrass and Canada thistle, respectively [1,16]. In both cases, effective control requires that the weeds' defenses be compromised physically or chemically.

Other advantages to the use of fungi include propagule stability. Fungal spores or sclerotial propagules are generally more stable compared to bacterial cells. Desiccation tolerance and stability as a dry preparation (shelf-life) are an absolute requirement for commercial use. The availability of numerous fungal pathogens for the control of weeds and insects is another advantage to the commercial use of these organisms as biocontrol agents. There are more than 8000 fungal species described which are pathogens of plants [7]. Fungal pathogens of insects are also widespread and readily available. Finally, fungi have the potential to develop an epidemic in the host population. This is particularly true in insect control strategies. Two commercial bioinsecticides, strains of *Metarhizium anisopliae*, for the control of termites and cockroaches, rely on the development of an epidemic in the insect colony [29].

While the ability or requirement for fungal spores to actively infect the insect or weed host is their greatest advantage, it also represents one of the major constraints to the commercial use of fungal biocontrol agents. Fungal

spores require free moisture during the germination and penetration process. This requirement for free moisture is a major environmental constraint to effective control under field conditions. Because of this constraint, a major focus of our research on optimizing nutritional conditions for the liquid culture production of fungal spores focuses on producing spores which will germinate rapidly.

The slow growth rate and relatively low propagule concentrations obtained with fungi as compared with bacteria is another disadvantage to their use as biocontrol agents. Longer fermentation times and lower yields translate into higher production costs. This is particularly true when solid substrate fermentation methods are employed [38,39,42]. Our research focuses on reducing fermentation times while increasing propagule yield. With some fungi, maximal spore concentration can be a fixed parameter based on nutritional requirements for sporulation in submerged culture. Because of this, the development of culture conditions which lead to more rapid sporulation and, therefore, reduced fermentation times can often be the most important factor in reducing production costs.

Constraints to the commercial use of fungal biopesticides

Plant and insect pathologists have identified hundreds of fungi which are candidates for development as commercial bioherbicides and bioinsecticides [8,9,40]. These fungi exhibit specificity toward their host pest and are usually highly aggressive in inciting disease and killing the insect or weed. Despite this success in discovering potential biocontrol agents, only four fungal bioherbicides and three fungal bioinsecticides have been registered for use in North America. The bioherbicides registered for commercial use are: *Colletotrichum gloeosporioides* (Collego®) for the control of northern jointvetch (*Aeschynomene virginica*) in Arkansas rice fields; *Phytophthora palmivora* (DeVine®) for the control of strangler vine (*Morrenia odorata*) in Florida citrus groves; *C. gloeosporioides* (BioMal®) for the control of round-leaved mallow (*Malva pusilla*) in various crops in Canada; and *Puccinia canaliculata* (Dr Biosedge®) for control of yellow nutshedge (*Cyperus esculentus* L) [9]. Of these products, Collego® and DeVine® reached the marketplace. Currently, only DeVine® is being sold commercially. The three commercially registered and marketed fungal bioinsecticides are *Beauveria bassiana* (Mycotrol®) for the control of whiteflies and other soft-bodied insects in vegetables and row crops and two strains of *Metarhizium anisopliae* (Bio-Blast® and Bio-Path®) for the control of termites and cockroaches, respectively. These products are recent introductions into the marketplace.

While regulatory issues and market demand have hindered the development of some potential bioherbicides, the overall lack of commercial success in using living microbial biocontrol agents stems from difficulties in producing and stabilizing these agents and from the lack of consistently effective weed control in field situations [28,42]. Production methods for fungal biopesticidal agents must be low-cost and yield high concentrations of viable, highly effective propagules. Also, these propagules must be amenable to long-term storage as dry preparations. The development of liquid culture fermentation processes which overcome these problems is essential for the commercialization of microbial biocontrol agents. The nutritional composition of the production medium has been shown to have a dramatic impact on propagule attributes such as biocontrol efficacy and desiccation tolerance [15,25–27].

Medium optimization schemes should be designed to address these factors. The goal of this paper is to outline strategies for optimizing liquid culture production techniques based on propagule yield and 'fitness' as a biopesticidal agent. Fitness is assessed in terms of biocontrol efficacy and stability as a formulated agent. Nutritional studies with the fungus *Colletotrichum truncatum*, a specific pathogen of the weed hemp sesbania (*Sesbania exaltata*), and *Paecilomyces fumosoroseus*, a pathogen of various soft-bodied insects including the silverleaf whitefly (*Bemisia argentifolii*), will be used to demonstrate the utility of this strategy.

Fungal biopesticide production methods

Analyses of the various production methods for fungal biopesticides have been the subject of several in-depth reviews [10,14,21,28]. In general, three methods exist for producing fungal propagules: the use of living host plants, solid substrate fermentation, and liquid culture fermentation. While the production of some potential fungal bioherbicidal agents (ie, rust fungi) can only be achieved using living plants, this method is generally not a commercial option due to high production costs, difficulties in propagule collection, and propagule quality control issues.

The production of fungal spores using solid substrate fermentation is often the first method evaluated because, in nature, most fungi form conidia on aerial hyphae. Since newly isolated fungal biopesticides are typically grown on nutrient agar, aerial conidia are usually the first propagules tested for host range and biocontrol efficacy. Solid substrate sporulation is advantageous since most fungi sporulate on solid-substrates, it is easily accomplished in the laboratory, and often the propagules produced in an aerial environment, conidia, tend to be more tolerant to desiccation and more stable as a dry preparation compared to spores produced in submerged culture [3,35]. Unfortunately, the commercial production of fungal spores using solid-substrate methods suffers from numerous technical and economic constraints. The scale-up of solid substrate production methods to a commercial level is difficult due to problems associated with substrate sterilization, gas exchange, temperature control, maintenance of pure culture, and product recovery from the substratum. Fermentation time for sporulation on solid substrates generally requires weeks rather than days, thereby increasing production costs. As an example, extensive studies on the commercial solid substrate production of conidia of the bioherbicide, *Alternaria cassiae*, showed that even when conditions were optimized, the production cost prohibited the commercial use of this organism for the control of sicklepod in soybeans [38,39]. In general, solid substrate sporulation methods are too costly for commercial use.

The successful use of solid substrate methods for producing fungal biopesticides relies on either a market for the

product which can tolerate a high input or a market where low-cost manpower is available for production, such as the case of production in third world countries. Mycotrol®, a product of Mycotech Corporation, consists of conidia of the fungus *Beauveria bassiana*. This commercial bioinsecticide is produced by solid substrate fermentation and the successful commercialization of this organism is based on the extremely high concentration of spores produced on solid substrate and on the high value crops (vegetables, cotton) on which this product is used [3].

At present, liquid culture fermentation is the most economical method for producing most microbial biocontrol agents. The production of antibiotics, amino acids, ethanol, and organic acids by submerged culture fermentation has provided an extensive knowledge base for optimizing processes and for designing fermentation vessels for the liquid culture production of biopesticides. Production methods for bakers' yeast, distillers' yeast, and bacterial starter cultures for the dairy industry have demonstrated that living biomass derived from liquid culture fermentations can be economically produced and can be stabilized as dry preparations. These commercial successes using liquid culture fermentation have strengthened industry's acceptance of this method. Three of the four fungal bioherbicides registered for commercial use in North America are produced using liquid culture fermentation.

By using submerged culture fermentation, a homogenous nutritional environment can be maintained and monitored. The homogeneity of a liquid medium simplifies production and processing methods and aids in the development of optimized nutritional conditions for production. In addition, environmental factors such as temperature, aeration, and pH are easily controlled compared to solid substrate fermentations. Controlled nutritional and environmental conditions, process scale-up capabilities, quality assurance issues, and ease of product recovery, in general, translate into lower production costs for fungal propagules using liquid culture production methods.

Medium optimization strategy for biopesticide production

Our strategy for optimizing nutritional conditions for the production of fungal biocontrol agents in submerged culture is based on developing a medium which maximizes not only propagule yield but also propagule fitness as a biocontrol agent. Propagule fitness for use as a biopesticide is equated with desiccation tolerance, stability as a dry preparation, and biocontrol efficacy.

The first step in our optimization strategy is the development of a defined or semi-defined medium which supports good culture growth and propagule formation by the fungal biocontrol agent. The propagule of interest will depend on the fungal biopesticide being evaluated and can, for instance, be spores, sclerotia, or mycelial fragments. A defined or semi-defined medium is essential so that nutritional components of the medium can be varied and the impact of these changes assessed in terms of propagule yield, biocontrol efficacy, and stability as a dry preparation. All of these factors must be considered during optimization since all are required if the biopesticide is to become a commercial reality.

Initial studies are directed at determining defined nutritional conditions which maximize growth. In general, significant biomass accumulation is necessary for optimal spore yield since spore production is usually dependent on the endogenous nutrients accumulated by the fungus during vegetative growth. Nutritional factors such as carbon sources, nitrogen sources, trace metals, vitamins, carbon loading, and carbon-to-nitrogen ratio can all have an influence on growth, propagule formation, and biocontrol efficacy. Once a defined medium has been developed which supports adequate growth, nutrients are varied in a directed way and their impact on spore yield and spore fitness can be assessed. The optimized defined medium serves as a nutritional framework from which a production medium can be formulated. In the production medium, the nutritional components of the defined medium are replaced with low-cost, complex substrates. Use of this directed optimization strategy not only aids in the development of production media for specific fungal biopesticides but also provides nutritional information which will be useful in developing production media for other microbial biocontrol agents. We have used this strategy to develop media for the production of numerous fungal biocontrol agents including *Colletotrichum truncatum* and *Paecilomyces fumosoroseus*.

Optimization of a production medium for *Colletotrichum truncatum*

The weed *Sesbania exaltata*, hemp sesbania, causes significant agronomic losses in the Southeastern United States in cotton, rice and soybeans [6]. Hemp sesbania is not easily controlled with conventional chemical pesticides and a strain of *Colletotrichum truncatum* has been isolated and patented which is a specific fungal pathogen of this weed [4,5]. Since, in preliminary studies, *C. truncatum* sporulated poorly using a standard liquid production medium (modified Richard's V-8), we began a medium optimization program for spore production at NCAUR in 1989.

Initial studies led to the development of semi-defined basal medium supplemented with trace metals, vitamins and organic carbon and nitrogen sources which satisfied the requirements for the growth and the conidiation of *C. truncatum* in submerged culture [24]. We routinely include trace metals and vitamins in our synthetic medium during initial experiments when various carbon and nitrogen sources are being evaluated. This is required to reduce the impact of the vitamins and trace metals which may or may not be present in the complex nitrogen and carbon sources being tested. During our studies with *C. truncatum*, numerous carbon and nitrogen sources were identified which supported submerged culture sporulation [24]. Glucose and vitamin-free Casamino acids (Difco, Detroit, MI, USA) were chosen as the standard carbon and nitrogen sources since their concentrations are easily measured in culture supernatants by high-performance liquid chromatography.

Two nutritional factors, carbon concentration and carbon-to-nitrogen (CN) ratio, were shown to have a dramatic impact on propagule formation by submerged cultures of *C. truncatum*. Carbon concentration was shown to

regulate conidiation and microsclerotia formation [24]. High concentrations of conidia were produced when *C. truncatum* cultures were grown in media with a carbon concentration of 4–16 g L^{-1}. Carbon concentrations greater than 25 g L^{-1} halted conidiation and promoted the formation of highly melanized hyphal aggregates which appear to be microsclerotia. These studies demonstrated that nutrition can dramatically impact sporulation and propagule formation. They also explain why early attempts to grow *C. truncatum* in a modified Richard's V-8 medium (~22 g carbon L^{-1}), a medium which supported heavy conidiation by the commercial bioherbicide *C. gloeosporioides*, yielded low conidia concentrations [4].

The CN ratio of the medium was also shown to dramatically effect spore yield. Using a carbon concentration of 4 or 8 g carbon L^{-1}, media with a CN ratio of 30:1 consistently produced more conidia than media with CN ratios of 10:1 or 80:1 [24]. The results prompted us to ask the question, 'Are spores produced in media with a CN ratio of 30:1 as effective in killing hemp sesbania as spores produced in media with CN ratios of 10:1 or 80:1?' Experiments were designed to evaluate the attributes of conidia produced under differing nutritional environments (CN ratios 10:1, 30:1, 80:1). These studies showed that conidial attributes were influenced by the nutritional environment during culture growth and sporulation [33].

Conidia produced in a medium with a CN ratio of 10:1 were longer and thinner than those produced in 30:1 or 80:1 media (Figure 1 [33]). The 10:1 conidia also germinated more rapidly, formed appressoria more frequently, and incited more disease in hemp sesbania seedlings. The association between rapid germination rate and a higher incidence of disease suggested that germination rate may be an important factor in biocontrol efficacy. Certainly, rapidly germinating spores would have a significant advantage in causing infection under field conditions where limited free-moisture represents a major constraint to biocontrol efficacy.

These CN ratio studies demonstrated that nutrition impacts not only spore production but also spore quality. Obviously, spore yield cannot be the sole criterion for medium optimization since the medium which yielded the highest spore concentrations (30:1) did not produce the most effective spores in terms of infecting and killing hemp sesbania seedlings. These results also demonstrated the importance of developing standardized inoculum production protocols to evaluate potential biopesticides. Comparing efficacy data on biocontrol agents produced in different media could be misleading since propagule efficacy may be altered by the nutritional environment.

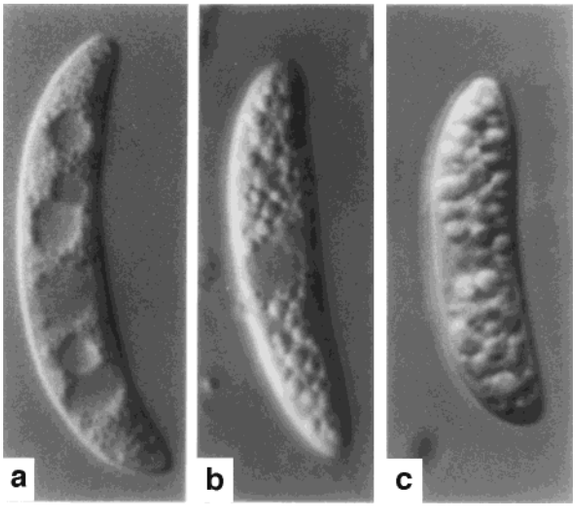

Figure 1 Morphological changes in conidia of *Colletotrichum truncatum* produced in submerged culture on media with carbon-to-nitrogen ratios of 10:1 (a), 30:1 (b) and 80:1 (c) [32].

An essential component of a medium optimization strategy involves understanding the physiological basis for changes in propagule attributes, ie, 'Why are the conidia produced in media with a CN ratio of 10:1 more effective than the conidia produced in 30:1 or 80:1 media?' Compositional analyses showed that 10:1 conidia contained more protein and less lipid than the 30:1 or 80:1 conidia [19]. Substrate utilization studies showed that the 10:1 medium was nutritionally balanced. That is, the carbon and nitrogen sources, glucose and amino acids, were both depleted after 2 days growth while the 30:1 and the 80:1 media contained excess glucose which was converted to lipid. These data also suggested an association between increased protein content and rapid conidial germination. This association was supported by optimization studies which showed that media with a CN ratio between 15:1 to 20:1 produced high concentrations of *C. truncatum* conidia ($1–3 \times 10^7$ conidia ml^{-1}) which were high in protein, germinated rapidly, formed appressoria frequently, and were efficacious in inciting disease in hemp sesbania seedlings [19]. Subsequent studies showed that media containing low concentrations of methionine, cysteine, and tryptophan reduced fermentation times and increased conidia yields in *C. truncatum* cultures [23]. Again, by using defined nutritional conditions we were able to not only optimize propagule yield and fitness as a biological control agent but also to identify the physiological changes which may be responsible for these differing attributes.

Additional studies were performed to optimize the production of effective *C. truncatum* spores. A complex medium for producing highly efficacious *C. truncatum* conidia was developed which yielded 5×10^7 conidia ml^{-1} in 4 days [34]. Oxygen delivery requirements for the germination, growth, and sporulation of *C. truncatum* in submerged culture were optimized using bench-top fermentors [36]. Unfortunately, attempts to stabilize conidia produced in liquid culture as dry or wet preparations were unsuccessful [35]. Recent formulation studies have shown promise in stabilizing these propagules as a dry, wettable powder (N Zidak, ARS, Montana State University, personal communication).

Problems in stabilizing conidial preparations of *C. truncatum* prompted us to consider microsclerotia for use as bioherbicidal propagules. Since many fungi produce desiccation-tolerant sclerotial propagules which allow the fungus to survive adverse environmental conditions, we decided to evaluate the usefulness of microsclerotia as bioherbicidal propagules. Consideration of microsclerotia as bioherbicidal propagules was warranted because we had previously identified nutritional conditions for the mass

production of these propagules in liquid culture. Subsequent submerged culture studies showed that media with a high carbon loading (80 g glucose L^{-1}) produced 6–10 $\times$ 10^6 sclerotial particles L^{-1} after 11 days growth (Figure 2 [20]). When stored at 4°C, dry preparation of *C. truncatum* microsclerotia (particle size range = 180–425 μm) retained over 90% viability after 36 months storage (unpublished data). Furthermore, soil incorporation of sclerotial propagules (150 microsclerotia per cc potting soil) killed over 95% of the emerging hemp sesbania seedlings in growth chamber studies [20]. Formulation studies with *C. truncatum* microsclerotia have shown that formulation with flour-based matrices improved biocontrol efficacy and microsclerotia stability when stored at elevated temperatures [11,18]. Microsclerotia have been shown to indirectly infect hemp sesbania seedlings at the soil line by producing spores which then germinate and infect the seedlings [32].

These studies suggest that microsclerotia may be useful as bioherbicidal propagules for controlling hemp sesbania. Our ability to produce high concentrations of *C. truncatum* microsclerotia in liquid culture is the key developmental step which has allowed us to consider microsclerotia as bioherbicidal propagules. Their biocontrol efficacy and shelf life are also qualities which suggest that these propagules have commercial potential.

By using a directed approach to the development of production media for the potential bioherbicide *C. truncatum*, we have gained a detailed understanding of how nutrition regulates propagule formation, conidial yield, and conidial efficacy. These studies have led to a method for producing *C. truncatum* conidia and microsclerotia in liquid culture. Field trials are currently underway in Mississippi to evaluate the commercial potential of this biocontrol agent.

Medium optimization studies with *Paecilomyces fumosoroseus*

Recent interest in developing *Paecilomyces fumosoroseus* as a bioinsecticide for the control of various soft-bodied insects, including the greenhouse whitefly and the silverleaf whitefly, stems in part from the ability of this fungus to develop epidemics in the host insect population [31]. Interest has also been spurred by the devastating impact of silverleaf whiteflies on agriculture. In the US alone, damage in cotton and vegetable crops in Texas, Arizona, California, and Florida is 100–500 million dollars annually [12]. Chemical control of the silverleaf whitefly is lacking in these cropping systems due to the rapid development of resistance to routinely used chemical insecticides [37].

Paecilomyces fumosoroseus, like many other entomopathogens, grows filamentously and conidiates on solid substrates but grows yeast-like in liquid culture. The yeast-like habit of *P. fumosoroseus* in liquid culture leads to the production of blastospores or hyphal bodies which are very similar to the organisms's mode of growth in the insect hemolymph [28]. The blastospores produced by various entomopathogens, including *Paecilomyces* spp, have been shown to be highly infective structures but have also been characterized as ephemeral and intolerant to drying [13,17]. Therefore, our goal in optimizing nutritional conditions for the production of *P. fumosoroseus* blastospores focused on blastospore yield and on tolerance to desiccation.

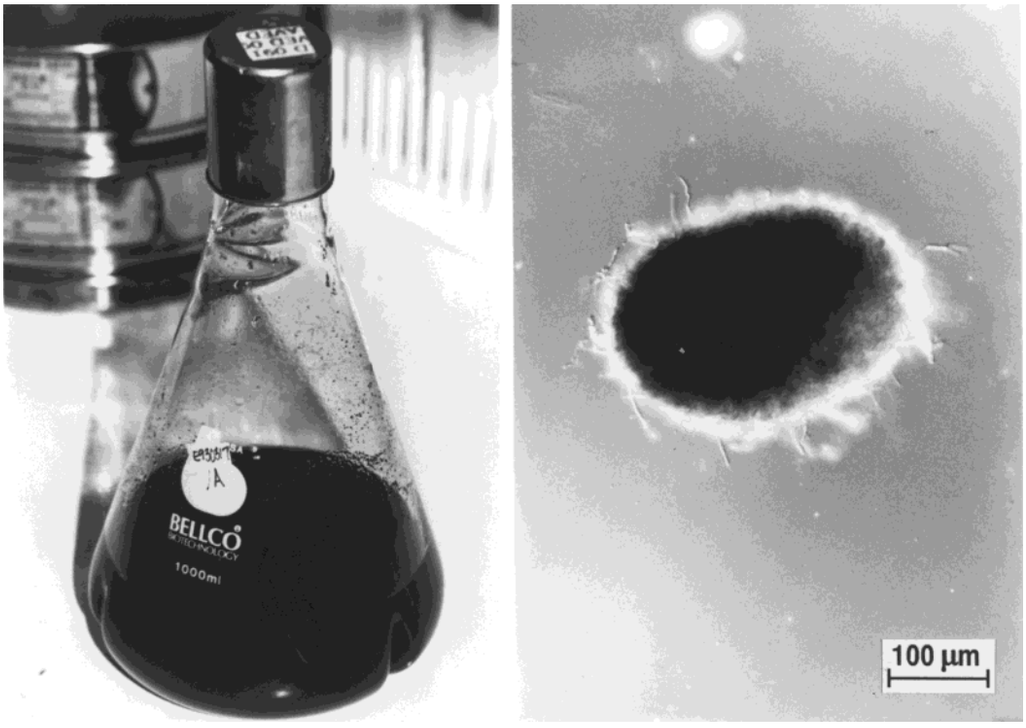

Figure 2 Production of microsclerotia of *Colletotrichum truncatum* in liquid culture (left). Photomicrograph of *C. truncatum* microsclerotium reveals that these particles are highly melanized compact hyphal aggregates which are approximately 200–400 μm in size (right).

Again, the strategy used to optimize nutritional conditions for the production of desiccation-tolerant blastospores of *P. fumosoroseus* involved developing a synthetic medium which supported good growth and sporulation. The basal medium used in studies with *C. truncatum* also supported growth of *P. fumosoroseus*. Like *C. truncatum*, an organic nitrogen source was required for adequate growth. Initial nutritional studies showed that various media (differing carbon-to-nitrogen ratios, carbon concentrations) supported good growth and sporulation by numerous strains of *P. fumosoroseus* [22]. Since blastospores are the product of yeast-like growth, media which contained high concentrations of nitrogen and carbon supported highest blastospore production. Blastospore formation, in the truest sense, is not sporulation but rather yeast-like growth. This contention is supported by the fact that blastospore formation was not dependent on nutrient depletion, as is the case with sporulation by many other fungi, but rather occurred during balanced growth [22]. Many entomopathogenic fungi exhibit dimorphic growth under appropriate nutritional conditions [28].

Highest concentrations of blastospores ($5–10 \times 10^8$) were produced under nutritional conditions where glucose was provided at a concentration of 20 g L^{-1} or higher [22]. While the production of blastospores occurred under various nutritional conditions, desiccation tolerance required appropriate nitrogen concentrations. Desiccation-tolerant blastospores (80% survival after air- or freeze-drying) of *P. fumosoroseus* were only produced in media containing between 13 and 40 g L^{-1} Casamino acids (1–3 g nitrogen L^{-1}). These results have demonstrated that desiccation-tolerant blastospores can be produced under appropriate nutritional conditions, thereby alleviating a critical constraint to their commercial use.

Subsequent studies have shown that numerous nitrogen and carbon sources supported the production of high concentrations of desiccation-tolerant blastospores provided that the nitrogen concentration was within the described limits. Scale-up experiments using 100-L fermentors (B Braun, Allentown, PA, USA) have shown that high concentrations of blastospores (1×10^9 ml^{-1}) can be produced in as little as 40 h fermentation time using appropriate nutritional conditions (unpublished data). Clearly, the yeast-like growth of this fungus reduced fermentation times compared to spore production by fungi which must grow vegetatively prior to sporulation. The short fermentation times for the production of blastospores of *P. fumosoroseus* are a major economic advantage.

Whitefly bioassays using air-dried blastospores showed that these spores were significantly more effective than conidia in infecting and killing silverleaf whiteflies [22]. This improvement in bioefficacy appears to be related to the ability of blastospores to germinate more rapidly than conidia. The germination rate of blastospores and conidia were compared. Blastospores germinated significantly faster than conidia, regardless of the medium used (Figure 3). As previously mentioned, the ability of spores to germinate and rapidly penetrate the host should enhance pathogen efficacy since free moisture is required during this infection process and is considered a critical constraint to consistent pest biocontrol, particularly under field conditions. As with

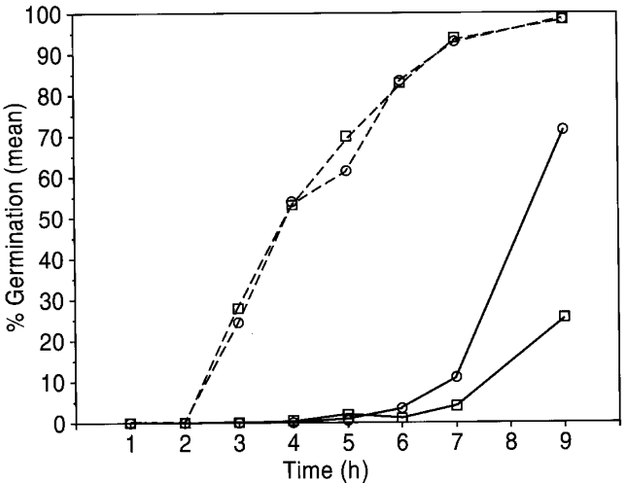

Figure 3 A comparison of germination rates for conidia (——) and blastospores (---) of *Paecilomyces fumosoroseus* on Noble water agar (□) and potato dextrose agar (○). While conidial germination rates were influenced by media components, blastospores were unaffected.

bioassays using *C. truncatum* to infect and kill hemp sesbania, spores of *P. fumosoroseus* which germinated more rapidly also incited more disease in the silverleaf whitefly.

Our current research with *P. fumosoroseus* is focused on evaluating the impact of the nutritional components of our production medium on blastospore germination rate and biocontrol efficacy. Preliminary results suggest that nutrition can impact the germination rate of the blastospores produced (data not shown). Bioassays are underway to compare the infectivity of blastospores which were produced in different nutritional environments and which have different germination rates.

Conclusions

A critical first step in evaluating the commercial potential of a fungal biocontrol agent should be determining if the organism of interest can be mass produced using a low-cost production method while maintaining a high level of effectiveness (see Schisler and Slininger, this issue). If the organism is difficult to produce, or cannot be produced in submerged culture, it would be prudent to select and develop other pathogens active against that particular pest. Given the number of pathogens which have been isolated by plant and insect pathologists for various insect and weed pests and the significant number of constraints to commercial development, this author feels that selecting organisms amenable to liquid culture production is imperative.

Medium optimization for the production of biological control agents must consider, not only propagule yield, but also propagule stability (desiccation tolerance, shelf-life) and propagule efficacy as a biocontrol agent. Our studies have demonstrated that nutrition can significantly influence propagule yield, stability, and biocontrol efficacy. The commercial use of fungal propagules as biocontrol agents requires that they possess all these attributes. Our medium development strategy, using a defined medium approach to optimization, allows the researcher an opportunity to understand how specific nutritional conditions affect propagule

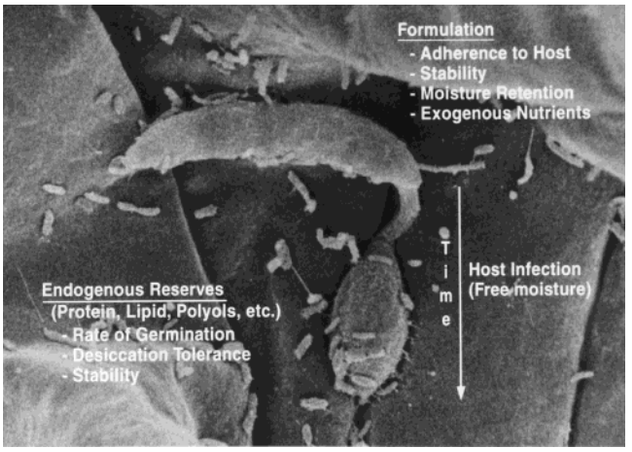

Figure 4 Electronmicrograph of *Colletotrichum truncatum* conidium which originated from a microsclerotium. The conidium has germinated and formed an appressorium on a hemp sesbania rootlet (photo provided by DA Schisler). By maximizing the appropriate endogenous reserves and by appropriate formulation, biocontrol efficacy can be improved by reducing the time required for host penetration and by retaining moisture and exogenous nutrients near the infective propagule.

yield, formation, stability and biocontrol efficacy. The principles which form the basis for this approach to medium optimization for submerged culture sporulation by bicontrol fungi should be applicable to the development of any microbial biocontrol agent, not just *P. fumosoroseus* or *C. truncatum*.

The major constraint to the use of fungal pathogens of insects and weeds as biological control agents is consistent performance under field conditions. Inconsistent performance is often attributed to a lack of free moisture. As previously described, free moisture is required by the fungal spore during the process of germination and penetration of the host. One of our primary goals in producing fungal spores for use as a biocontrol agent is to reduce the impact of the free moisture requirement by producing spores which germinate more rapidly and form an appressorium more frequently (Figure 4). Our studies with *C. truncatum* and *P. fumosoroseus* have demonstrated that the nutritional environment during culture growth can have an effect on spore attributes such as rate of germination and frequency of appressorium formation. We feel that by optimizing these spore attributes we can improve the biocontrol efficacy of these agents under field conditions.

If biological control agents are to become an important pest control tool, research efforts must be shifted from the discovery of potential biocontrol agents to solving the production, storage, and efficacy problems that plague all biopesticides. Commercial interest and user acceptance of fungal biocontrol agents as insect and weed management tools is dependent on the development of low-cost, stable products which give consistent control under field conditions. These biological constraints are general technical problems which impede the development of all microbial biocontrol agents. Solving these problems will require collaborative research between plant and insect pathologists, microbiologists, fermentation specialists, biochemists, and formulation scientists (Figure 5). Our medium optimization studies with *C. truncatum* and *P. fumosoroseus* have demonstrated that a multidisciplinary research approach is required if significant progress is to be made in overcoming the constraints which impede the commercialization of these agents.

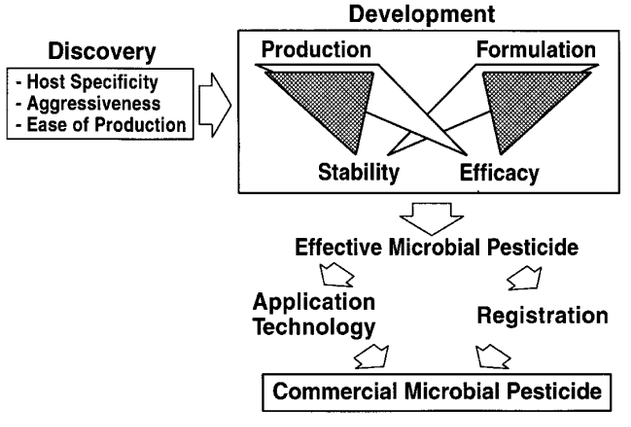

Figure 5 Developmental steps in the commercialization of microbial biocontrol agents.

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