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Development and testing of mycoinsecticides based on submerged spores and aerial conidia of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) for control of locusts, grasshoppers and storage pest

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CHAPTER 1

GENERAL INTRODUCTION

In many countries, especially in the tropics, locusts and grasshoppers are considered to be the major pests in agriculture causing significant damage in many field crops since the biblical times (Geddes, 1990; Steedman, 1990). Under favourable conditions, certain species exhibit gregarious and migratory behaviour, leading to the formation of spectacular swarms (Lomer *et al.*, 2001). The desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) is the most important locust species with an estimated invasion area of some 29 million km² affecting 57 countries, which is more than 20% the total land surface of the world (Steedman, 1990). During plagues the desert locust has the potential to damage the livelihood of a tenth of the world's population and in some countries they were reported as the determining factor between sufficient food for the people and starvation (Steedman, 1990). Similarly, the migratory locust, *Locusta migratoria* (R. & F.), the red locust, *Nomadcris septemfasciata* (Serville) and the brown locust, *Locustana pardalina* (Walker) (all belong to Orthoptera: Acrididae) found in most African countries south of the Sahara and during plagues, they cause damage comparable to that of the desert locust (COPR 1982; Steedman, 1990). On the other hand, grasshopper species such as *Hieroglyphus daganensis* (Krauss), *Zonocerus variegatus* (L.), *Cryptocatantops haemorrhoidalis* (Krauss), *Kraussaria angulifera* (Krauss) and *Cataloipus fuscocoerulipes* (Sjöstedt) are regular serious pests of subsistence and cash crops in most African countries (COPR, 1982). Control strategies for locusts and grasshoppers rely almost exclusively on the use of chemical insecticides (Prior and Streett, 1997). For example, the most recent outbreak of locusts and grasshoppers in Africa beginning in 1988 affected 23 nations and over US \$ 250 million were spent for control operation and relief for damage caused by locusts (Showler and Potter, 1991; Showler, 1995).

In good production years farmers in Africa harvest and store cereals and pulse crops for several months mostly in a simple storage structures at farm or village levels. However, as in field crops, all stored products are attacked by a wide range of insect pests (Hill, 1983, Subramanyam and Hagstrum, 1995). The maize weevil, *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae), and the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) are the two most important and destructive pests of stored grain in the tropics (Dobie *et al.*, 1984; Dick, 1988). The maize weevil was regarded as a cosmopolitan pest in tropical countries while the larger grain borer, native to Central America

and Mexico, was accidentally introduced into East and West Africa between the 1970s and 1980s and became a serious pest of stored maize and cassava (Dick, 1988). The favourable climatic conditions and poor storage systems in Africa often favour growth and development of these pests, resulting in considerable crop losses. For example, losses as high as 40 % were reported on stored maize due to *P. truncatus* and *S. zeamais* (Meikle *et al.*, 1998). In Africa, where subsistence grain production supports the population, such grain losses may be substantial (Golob & Tyler, 1994). In addition to grain weight loss, pests of stored grain also cause secondary fungal infection, resulting in a reduction in seed vigour, quality and commercial value. Synthetic chemical insecticides have been widely used for the control of pests of stored grain.

The widespread use of insecticides for the control of locusts and grasshoppers as well as stored-product insect pests is of global concern with respect to environmental hazards, insecticide resistance development, chemical residues in foodstuffs, side-effects on non-target organisms and the associated high costs (Morallo-Rejesus, 1987; Beeman & Wright, 1990; Krall *et al.*, 1997). To this effect, the increased public awareness and concern for environmental safety has directed research to the development of alternative control strategies such as the use of microbial control agents for locusts and grasshoppers (Inglis *et al.*, 1997; Lomer *et al.*, 2001) as well as for stored-product insect pests (Brower *et al.*, 1995). Entomopathogenic fungi have been shown to be effective biological control agents against several insect pests (Müller-Kögler, 1965; Burges, 1981, Inglis *et al.*, 2001). There are more than 100 genera of fungi that contain numerous species that are pathogenic to insects (Hall & Papierok, 1982; Zimmermann, 1986). However, the most important groups of insect pathogenic fungi presently used in biological control belong to the order Deuteromycotina (Butt and Goettel, 2000; Inglis *et al.*, 2001). This is partly because of their easiness to culture them *in vitro* for mass production and their wide host range (Payne, 1988; Charnley, 1991). Fungi are unique among the insect pathogens in that they infect their hosts primarily through the external insect cuticle although infection through the digestive tract occurs with some species (Butt and Goettel, 2000; Inglis *et al.*, 2001). Their spores attach to the cuticle, germinate, and penetrate the integument by means of a combination of physical pressure and enzymatic degradation of the cuticle. Once the fungus reaches the haemocoel, it grows as hyphal bodies (= blastospores). Host death usually occurs due to a combination of nutrient depletion, invasion of organs and the action of fungal toxins (Butt and Goettel, 2000; Inglis *et al.*, 2001).

Fungal biological control agents (BCAs) are also proved to offer alternative more rapid prospects for implementation (Prior and Greathead, 1989; Prior, 1997). Among these, entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana*, *B. brongniartii*, *Paecilomyces fumosoroseus* and *Verticillium lecanii* have shown considerable potential for the management of a variety of insect pests (Inglis *et al.*, 2001). Recently, considerable progress has been made in the development of fungal BCAs and to date there are more than 30 trade-named mycoinsecticide products registered or under-development worldwide (Wraight *et al.*, 2001). As regard to locusts and grasshoppers, two fungi have been developed as commercially available biopesticides. The first is Mycotrol®-GH OF, based on aerial conidia of *B. bassiana* strain GH produced by Mycotech (Wraight and Carruthers, 1999). The other fungal product is Green Muscle™ based on aerial conidia of *M. anisopliae* (*flavoviride*) var. *acridum* strain IMI 330189 and developed by the LUBILOSA research project (Bateman, 1997; Lomer *et al.*, 1999). Both mycoinsecticide products are developed as an oil formulation suitable for application by ULV. On the other hand “Boverosil” a powder commercial formulation based on aerial conidia of *B. bassiana* was registered for use in the former Czechoslovakia for the control of residual infestation of stored-product pests in grain stores and silos (Brower *et al.*, 1995).

More recently, submerged spores/conidia of *M. anisopliae* var. *acridum* were successfully produced in liquid culture (Kleespies and Zimmermann, 1992; Jenkins and Prior, 1993; Jenkins and Thomas, 1996; Stephan and Zimmermann, 1998) and dried using various drying techniques (Stephan and Zimmermann, 1998, 2001). Similarly, much progress has been made in recent years on the production and stabilisation of submerged spores/conidia of *B. bassiana* (Rombach *et al.*, 1988). However, the commercial-scale production/stabilisation systems developed so far are not competitive with existing technologies for conidia production (Wraight and Carruthers, 1999). Furthermore, submerged spores/conidia are hydrophilic in nature and it is very difficult to develop a pure oil formulation for ULV application. For the development of microbial control system, new isolates are continuously sought that require characterization and testing against specific target pests. Moreover, studies on the potential use of submerged spores/conidia of *B. bassiana* and *M. anisopliae* for control of storage pests are limited. Therefore, the central aim of this study was to fill the existing information-gap by addressing several pertinent questions with regard to the selection, production, stabilisation and formulation of *B. bassiana* and *M. anisopliae* for control of locusts, grasshoppers and key stored-product insect pests.