CHAPTER 9

PATHOGENS

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1.0 Background and history

There are many approaches to biological control of pests in stored products, including the use of predatory insects and mites, hymenopteran parasitoids and pathogens. To date, interest in pathogens has been constrained and most activity has been in quite basic research and rarely with pathogens as components of IPM strategies. Usually the aim appears to be a straight chemical pesticide replacement. The dangers of viewing pathogens as mere biological analogues of chemical pesticides have been outlined by Waage (1997), and it is increasingly clear that the major benefits of biological pesticides will result from exploiting their biological characteristics rather than trying to replace chemicals.

Chemical-based methods of pest control in stored products are under threat for many reasons, including costs, regulatory restractions, health fears and environmental dangers. However, end-users demand undamaged and uncontaminated goods, and alternative pest control technologies are required. Theoretically, pathogens offer many options including a degree of efficacy comparable with chemical pesticide and compatibility with most of the other components of IPM strategies.

Traditional biological control often entails a lag phase, which can be several months, between the increase in pest population and the increase in natural enemy. This may result in a rise pest populations to damaging levels before the control takes effect. The use of natural enemies in the manner of chemical pesticides has led to the concept of biopesticides. Although demonstrating some value, this approach has resulted in biopesticides being expected to mimic the chemical action, reducing appreciation of the superior characteristics of biopesticides (Waage 1997). Biocontrol of pests in grain requires the adding a biological control agent to the grain, and problems of perception exist. Perception issues should be fewer with a pathogen, invisible to the naked eye, than an arthropod predator or parasitoid. Opposition to the presence of beneficial microbes in food should be readily overcome. Consumers accept the consumption of live bacteria in yogurt and fungi when using yeast. Biocontrol should be of particular interest to certain sectors of the cereal industry, such as organic producers and end-users.

This chapter consists of a large dose of potentialities and little actual implementation. Most research into pathogens and stored-product pests is from laboratory studies, and the science is very much in its infancy. However remarkable advances have been made in insect pathology over the last decade, especially with the realization of the importance of understanding the ecology of pest/pathogen interactions. Within the relatively controlled storage situation, manipulation of pathogens may have a valuable role to play, and pathogen based-products could provide very effective, cheap, and safe measures. The largest challenge may be acceptance, and at present there are many uncertainties to consider.

The cereals industry of developed nations has implemented few microbial or other biological controls. Cost appears to be the major factor, and consequently equally effective but cheaper alternatives are more attractive. The withdrawal of chemical use or large increases in energy costs or consumer demand (from retailers or end-users) could alter these perspectives. However, end-users are also likely to be against the addition of biocontrol agents to grain, as this would be considered contamination (Cox and Wilkin 1996). At present, there is little research in the UK into the use of pathogens in stored-product protection.

End-user acceptance is always considered to be major obstacle and is often very difficult to predict. The "natural is good" view has many advocates and there are many examples of consumers exhibiting a scientifically unwarranted aversion to chemicals. The results of a major review (Cox and Wilkin 1996) showed that the Guild of Conservation Grade Producers of the UK endorsed biological control, but end-users of Conservation Grade cereals are more cautious, particularly because some of their products use cereal grains in a relatively unprocessed form. Any insect contamination, regardless of status, is unacceptable in such products. However, a recent disclosure that a significant proportion of UK breakfast cereals contain detectable levels of an array of arthropod remains (K. Wildey unpublished) did not cause mass panic and an immediate transfer to eating yogurt - with its live bacteria.

The present challenge therefore is to provide the consumer with food free from detectable pesticide residues without reducing standards of food hygiene. Pest management with beneficial insects or pathogens needs to be done without contaminating the product and as cheaply as possible. However, there is an increasing market for organic food in which the presence of cosmetic damage is often acceptable even at a price premium. At the moment, there is an enormous requirement for research studies in many aspects of pathogen use before viable control strategies are developed.

1.1 Advantages

Cox and Wilkin (1996) listed advantages and disadvantages of biological control in stored products (specifically grain). These are given below where relevant to microbial control:

(i) Uses naturally occurring organisms, many of which are already found in stored products.

(ii) No chemicals involved so that microbial control can be used on organic materials. However, some fungal pathogens have toxins, but not as the active components of the commercial biological pesticides.

(iii) Minimal risks to farmers, storekeepers, other end-users and consumers. Minimal does not imply absolute safety, but most safety issues, such as allergenic responses, can be avoided by use of the correct safety equipment.

(iv) Reduced risks to the environment compared to chemical control measures.

(v) Microbes subject to reduced regulatory requirements in many countries. Depending on venue, some approaches to microbial control fall outside regulatory constraints on the user or producer.

(vi) Fits readily into an integrated approach to pest control.

(vii) Generally inoffensive to the senses (lacking, for example, the offensive odor of conventional chemicals) and are inapparent after application.

(viii) Some highly infectious and prolific agents such as viruses can be applied at very low doses as they will persist and multiply in the pest population to produce long-term control.

1.2 Disadvantages

(i) Rarely eliminates pests. A realistic goal is pest suppression to below damage thresholds.

(ii) Target limitation due to host and stage specificity. Some, such as viruses, are highly selective, generally species-specific and attack only the larvae. This limits their markets. Since most infestations comprise multiple pest species, several different isolates or species of biological control agents may be needed.

(iii) Slow acting relative to conventional insecticides. Consequently, much damage may occur before control is effective.

(iv) Tends to be a management-intensive process, both in terms of application and subsequent monitoring. This is more likely to be true with pathogens used in IPM strategies than those used as biological pesticides.

(v) Involves contaminating the stored material with living organisms. However, physical removal of pathogens after storage might be feasible with suitable formulation, and processing often eliminates pathogens. Grain processing eliminates most *Bacillus thuringiensis* Berliner (*Bt*) spores (Subramanyam and Cutkomp, 1985). In a situation discussed later (rural stores in Kenya) the standard cooking processes of boiling, roasting and pounding cooked maize would remove residues.

(vi) May be more expensive than current chemical control measures in spite of lower development and registration costs. For example, in 1990 it was possible to register a new *Bt* product in the US in less than one year and for less than US $300,000 (Dales 1994). However, some modern production techniques are labor-intensive, which is considered a disincentive for commercial manufactures. Research is leading to more efficient techniques and quite successful biological pesticide production occurs in a number of developing countries.

(vii) Currently, little expertise or infrastructure to supply control agents or support the use of biocontrol. Biological pesticides may benefit from the infrastructure of the well-established chemical pesticide industry.

(viii) Not usually suitable for dealing with heavy, established infestations. Paradoxically, these may be exactly the right conditions for pathogen use, both with an initial primary contact on the pests and then with secondary increase of the pathogen.

(ix) Usually have limited shelf life and often must be obtained directly from the producer on an as-needed basis. Mycopesticides are proving to have good shelf-life potential (Moore et al. 1995), but the value of extended shelf life can be debated. One view is that the advantages of extended shelf life are exaggerated by pesticide producers; it enables them to receive payment early and to encourage the farmer to bear the cost of storage. An alternative view is that farmers are generally insistent on being able to store products, sometimes under adverse conditions.

(x) No patent rights to biological control agents, which may limit commercial interest. The specific uses, however, can be patented.

1.3 Microbial control agents

Bacteria

Many bacterial species are associated with insects. *Bacillus thuringiensis* and *B. cereus* Frankland and Frankland are probably the most significant in commercial pest control terms. (Some authorities would contend that *B. cereus* has no commercial significance whatever, except that *Bt* is considered by many to be a strain of *B. cereus*.) They usually invade the insect's hemocoel via the gut after ingestion, but transmission at oviposition by parasitoids has been reported (Toumanoff 1959).

Bacillus thuringiensis spores are associated with toxins that cause insect death. Although most well known strains affect Lepidoptera. Strains that kill Coleoptera, Diptera and other insects occur (Krieg et al. 1983). Different strains can produce different amounts of several types of toxins. Of primary interest are the various proteins comprising the δ-endotoxin of parasporal crystals (Schnepf et al. 1998). A second class of toxin, designated β-exotoxin or thuringiensin, is secreted from vegetative bacterial cells of some strains (Cantwell et al. 1964). Although β-exotoxin has broad insecticidal activity, most commercial preparations of *Bt* are composed of subspecies that do not produce the toxin because of its vertebrate toxicity and teratogenicity (Burges 1973; Perani et al. 1998).

The δ-endotoxin is a gut poison. Intoxicated insects become sluggish then moribund. Upon death, they become flaccid and turn dark brown, and they may be filled with bacteria cells. The midgut membranes are disrupted by the toxin, allowing ionic flow into the hemolymph. Depending on the type of susceptible insect, this disruption may kill quickly by loss of osmotic balance and general paralysis or more slowly by gut paralysis. In some insects, including the Mediterranean flour moth, *Anagasta kuehniella* (Zeller), death is caused by septicemia after spore germination in the midgut (Heimpel and Angus 1959). In the latter case, the bacteria cells multiply within the insect, and the potential exists for spores to be released as the cadaver decays, providing new inoculum in the environment.

The Mediterranean flour moth is among the few insects for which epizootics have been recorded (Krieg 1987). The rarity of epizootics is thought to be because the spores and crystals appear only in low numbers in the cadavers of affected hosts (Angus, 1968). Studies with five species of stored-product moths led Burges and Hurst (1977) to conclude that natural disease may curb insect infestations in debris, but it attacks too late to prevent excess damage to stored food. Those authors concluded that an admixture of $2x10^9$ spores/200 g of food is required for effective insect control.

Among the *Bt* strains with Coleoptera activity are some that have been shown to be pathogenic for stored-product pests such as *Tribolium castaneum* (Herbst) (Kumari and Neelgund 1985). Industry evaluation of Coleoptera strains has led to the conclusion that none of those currently in hand are sufficiently virulent to justify commercial development (R. J. Cibulsky, pers. comm.).

Bacillus thuringiensis is formulated and applied in the manner of conventional insecticides. Commercial preparations of *Bt* have been developed, including wettable powder (WP), liquid formulation or dust. Dusts and WPs of *Bt* have been tested in grain silos and bins in Midwestern USA (McGaughey 1985). Both formulations were effective at reducing populations of *Plodia interpunctella* (Hübner) by 50-60% in wheat and over 80% in maize. With inshell peanuts, McGaughey (1982) found a dust to give better coverage than a wettable powder and consequently better moth control and protection from damage. As with other microbial insecticides, formulations can be mixed with the grain as the last layer is augured into the storage bin, or by being raked into the material. Protecting the top 10 cm may be sufficient. Trials against *P. interpunctella* proved promising (McGaughey 1986a, b) and led to commercial development. The *Bt* product Dipel is registered in the US for the application to grain to control lepidopteran pests. It has yet to be registered by the Pesticides Safety Directorate for use in the UK.

Major advantages of *Bt* among microbial control agents are ease and economy of production, simple application and good storage stability. Deep tank liquid fermentation is used for the production of *Bt*. Although expensive in terms of initial capital investment and operational costs, this method gives consistent production of high quality material, and fermentation parameters can be adjusted to enhance toxicity.

Bacillus thuringiensis is rated as a safe microbial insecticide which is harmless to vertebrates including humans and to beneficial insects such as bees. In the US, commercial *Bt* is placed under the lowest toxicity category of the Environmental Protection Agency (EPA). It is exempt from residue tolerances on all raw agricultural commodities in the US (Dales 1994).

Despite an excellent safety record, there are isolated reports of problems with *Bt*. According to an unpublished report (Mardan and Harein, cited in Subrammyam and Cutkomp 1985), sheep fed on maize treated with 250 and 500 mg of formulation/kg developed hemorrhages and lesions in the heart, liver and lungs, and the bacterium was found in the infected organs. A component of the formulation was suspected to have been responsible for creating entry for the bacteria. In another instance, *Bt*-related corneal ulceration was reported (Samples and Buettner 1983) resulting in a recommendation for eye protection was as a safety procedure.

A general problem associated with *Bt* is the rapid development of resistance in many insect species. The first case of resistance to *Bt*, indeed the first documented case of resistance to any pathogen in an insect field population, occurred with *P. interpunctella* (McGaughey 1985). The resistance developed quickly and was stable when selection pressure was lifted. *Plodia interpunctella* and *E. cautella* developed resistance in one storage season in the US (McGaughey and Beeman 1988). *Bacillus thuringiensis* isolates vary greatly and judicious use of them may ameliorate the resistance problem (McGaughey 1986b).

A proposed novel approach to the use of bacteria in control of insects involves the application of *Pseudomonas syringae* Van Hall to cause ice formation in insects at higher than normal temperatures. Many insects have the capacity to withstand supercooling of body fluids, remaining unfrozen at -20°C (Lee et al. 1993, 1995). Since the isolation of the ice-nucleating *P. syringae* in the early 1970s, there has been interest in determining the value of such organisms as biological control agents (Fields 1991; Fields et al. 1995; Strong-Gunderson et al. 1992). Use of *P. syringae* against eight species of stored grain pest demonstrated increased levels of kill at subzero temperatures (Lee et al. 1992). Mignon et al. (1998) found dose, temperature and time responses in granary weevils, *Sitophilus granarius* (L.) and sawtoothed grain beetles, *Oryzaephilus surinamensis* (L.), treated with *P. syringae* at sub-zero temperature exposure.

Other organisms also possess ice-nucleating activity. The fungus *Fusarium avenaceum* (Corda *ex.* Fries) Saccardo was shown to raise the supercooling point of *Cryptolestes ferrugineus* (Stevens) from -17° to 6°C. This was not as effective as the *P. syringae* treatment (Fields et al. 1995). There may be undiscovered ice nucleators and combination use strategies, and the concept appears to be worthy of further exploration.

Fungi

When entomopathogenic fungi infect hosts, spores germinate and penetrate the cuticle using chemical and physical action to reach the hemocoel (Bidochka et al. 1997). Further proliferation results in death of the host. Under moist conditions the mycelium breaks out of the insect and sporulation occurs. Under drier conditions, sporulation may be internal, releasing spores as the host cadaver breaks down. Mycotoxins, some highly toxic, are present in significant quantities in some species, often with marked variation in level according to isolate. A pathogen that kills with mycotoxins is less likely to be commercialized because of safety and regulatory issues. In addition, if pathogenesis is toxin-related then post-application multiplication may be low and hence persistence may be limited. This differs from *Bt* wherein the δ-endotoxin in is not toxic for vertebrate and is the active agent for insects.

There are many species of entomopathogenic fungi recorded, but most of the limited work related to stored-product pests has been done with *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin. Early work by Searle and Doberski (1984) investigated *B. bassiana* against *O. surinamensis* and found humidity to be the critical factor. Very little infection was observed below 100% RH, and it was concluded that in grain stored at or below the recommended moisture content of 14% (70% RH), the fungus would be unlikely to control *O. surinamensis* populations. However, the work did demonstrate kill at low temperatures and a reduction of over 91% in numbers of progeny larvae and pupae when conidia were mixed with grain.

More recent work is more encouraging. Small-scale laboratory trials with *Beauveria brongniartii* (Saccardo) Petch and *M. anisopliae* against *S. zeamais* and

Acanthoscelides obtectus (Say) resulted in variable levels of kill (Rodrigues and Pratissoli 1990). *Beauveria brongniarti* proved superior under the particular bioassay conditions used. *Beauveria bassiana* was superior to *M. anisopliae*, *Nomuraea rileyi* (Farlow) Samson and *Verticillium lecanii* (Zimm) Viegas against *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *T. castaneum* in tests by Padin et al. (1995). Adane et al. (1996) examined a range of non-specific isolates of *B. bassiana* which are virulent against *Sitophilus zeamais* Motschulsky at very low doses. The least effective isolates were from Scotland, and this may have been a result 25°C bioassay temperature, which may have been too high for pathogens from temperate climate. Alternatively, the isolates may have been poor pathogens for the target insect. Moino et al. (1998) found only weak activity for *S. zeamais* among 61 *B. bassiana* isolates. These studies illustrates the need to evaluate performance differences among isolates and screen isolates with for desired characteristics such as virulence, persistence and suitability for mass production.

Formulation of fungi involves special considerations. In an attempt to avoid possible health problems, such as allergenic responses to dry conidia, Hidalgo et al. (1998) examined different formulation strategies, such as incorporation of conidia into fat pellets and suspending in oil. Results were variable, but high levels of mortality could be achieved. Hluchy and Samsinakova (1989) found Boverosil, a Czech commercial preparation of *B. bassiana*, to be ineffective against *S. granarius*. However, Boverosil, combined with the insecticide pirimiphos-methyl, was registered in the former Czechoslovakia for the treatment of empty stores and silos against residual infestations of stored-product pests. (Dales 1994).

In one of the few investigations on products other than grain, Jassim et al. (1988) applied *B. bassiana* to stored dates in Iraq. At a rate of 300,000 conidia/cm^2, mortality of 96% of *E. cautella* larvae was achieved.

Even until recently, many of outmoded views were limiting work done with fungi. The perceived major drawbacks to the use of fungi for insect control included poor stability in storage situations and climatic restraints (Kirschbaum, 1985). Both of these challenges are increasingly being met (e.g. Marois et al. 1988; Jin et al. 1993; Moore et al. 1995; Bateman 1997).

The conclusion of Dales (1994) that fungi are unlikely to be generally useful for the control of storage pests because the dry conditions that prevail in storage situations are unsuitable for fungal activity no longer stands. Paradoxically, in uncontrolled stores, the moisture may be too high for fungal persistency, and in cooled stores or those at high ambient temperature, the temperatures may also be outside effective ranges for fungi.

Baculoviruses

Baculoviruses include some of the most important naturally occurring insect pathogens. However, as host-specific obligate intracellular parasites, they have certain constraints to their use in applied pest control. The baculoviruses, a heterogeneous group of large viruses with double stranded DNA, are pathogenic to invertebrates, especially Lepidoptera (Granados and Federici 1986). They have also been recorded from Diptera, Hymenoptera, Neuroptera, Trichoptera and Crustacea (Cox and Wilkin 1996). Baculoviruses have rarely been reported from Coleoptera and not at all from stored-product beetles. They are the most common group in the insect viruses and are usually host specific or nearly so (Huber 1990). Most baculoviruses, both NPV (nuclear polyhedrosis virus) and GV (granulosis virus), produce crystalline occlusion bodies and are consequently protected until ingested by insects. The occluded form is preferred as a biological insecticide.

After ingestion, occlusion bodies are dissolved under the alkaline conditions of the midgut releasing the infectious baculovirus particles. The insect gut cells become infected followed by infection of the fat body tissues. The insect stops feeding within a few days of ingestion, and the body becomes flaccid as it fills with virus particles that are later released into the environment with gut expulsion and disintegration of the insect. The time to death is from 4 days to 3 weeks. Most, if not all, baculoviruses can be transmitted from an infected female to her progeny via the egg. This mode of transmission offers the potential to contribute to the success of the autodissemination approach to application (Vail et al. 1993). As is the case for many insect pathogens, there are sublethal or prelethal effects of baculoviruses that may reduce pest populations or economic impact in ways that are often unrecognised. For example, a sublethal infection of the granulosis virus of *P. interpunctella* is associated with reduced fertility (Sait et al. 1998).

Among the viruses of stored-product pests, the GV of *P. interpunctella* is the most studied and farthest along in development. A dose of 1.875 mg of formulated GV/kg of grain gave good control of *P. interpunctella* at 25°C (McGaughey 1975). Hunter et al. (1977) conducted studies with stored almonds and reported 134 days of control, substantial reduction in feeding damage, and as much as 88% reduction in rejects due to insect damaged nuts. Barrier treatments in the top 10 cm of the wheat or maize were almost as effective as treating the entire grain mass (Cox and Wilkin 1996), a finding of possible relevance to other pathogens. An economic assessment suggested costs would be comparable with fumigation and modified atmospheres (Vail and Tebbets 1991).

Commercial use of insect viruses to control stored-product pests is still in the future. However, the experimental success achieved with the *P. interpunctella* indicates that the method could have considerable impact if other pathogenic viruses can be isolated from the major insect pests of durable foodstuffs.

Protozoa

Protozoa are single celled organisms that usually enter the host by ingestion or passage from mother to progeny. The Gregarinida and Coccidia are parasites of the fat body, Malpighian tubes or gut of insects, and are characterized by resistant, spore-like or encysted stages (Cox and Wilkin 1996). The microsporidia are primarily found in the insect fat body and can be spread from insect to insect orally or via the egg stage. The widespread occurrence of Protozoa has been documented in surveys of stored-product insects in various regions including the former Yugoslavia (Purrini 1976), Bulgaria (Golemanski and Dukhlinska 1982), the U.S. (Hall *et al.* 1971), Tanzania (Purrini and Keil 1989) and the UK (Burges and Weiser 1973). There has been limited research on their effectiveness as microbial control agents (Dales 1994).

Adelina tribolii Bhatia (Coccidia), a fat-body parasite, infects *Tribolium destructor* Uyttenb., *Tribolium confusum* Jacquelin du Val (Listov 1979) and *T. castaneum* (Golemanski and Dulinska 1982). It is capable of causing epizootics in laboratory and natural populations of *T. confusum* (Brooks, 1988).

Eugregarines thought to be potentially pathogenic, (most are commensals) include *Ascogregarina* spp. (Brooks, 1988). *Ascogregarina bostrichidorum* Purrini and Keil has been isolated from *Prostephanus truncatus* (Horn) collected in

Tanzania (Purrini and Keil 1989), but only about 2% of the populations sampled were infected.

Neogregarines occur naturally in Lepidoptera, Coleoptera, and Orthoptera (Dale 1994). *Farinocystis tribolii* Weiser is a parasite of *Tribolium*, *Laemophloeus* and *Gracilia* spp. (Purrini 1976b). Infection results in a slow decline of *Tribolium* spp. in laboratory cultures. *Farinocystis* spp. have also been isolated from *P. truncatus* (Schulz and Laborius 1987).

A neogregarine of great potential is *Mattesia trogodermae* Canning. A cosmopolitan pathogen of *Trogoderma* spp, most notably *T. granarium*, it is capable of suppressing *T. glabrum* populations under simulated warehouse conditions using pheromone lures for spore dissemination (Shapas et al. 1977; Brooks 1988). Spore transfer by ingestion of dosed food or cannibalism of infected hosts proved successful. Use of pathogens in conjunction with pheromones will be further discussed in a later section.

Mattesia dispora Naville has been recorded from several Coleoptera and Lepidoptera species, including *Galleria mellonella* (L.) (Duhlinska 1986), *C. ferrugineus* (Finlayson 1950), *A. kuehniella* (Naville 1930) and *P. interpunctella* (Weiser 1954) among others. Its broad host range and spore stability make it a good candidate for development as a microbial control agent.

Mattesia spp. have also been recorded in *P. truncatus* (Henning-Helbig 1994; Leliveldt et al. 1988; Lipa and Wohlgemuth 1986). Natural infestation levels, occurring in about 30% of villages surveyed in Togo, averaged 2.1 % of insects. Adults, larvae and pupae can carry the infection, but greatest prevalence is in the adults. In heavy infections, parasites were found in the hemocoel, but generally the *Mattesia* were found in the fat bodies. Although natural infection levels were low, augmentation may result in appreciable levels.

The microsporidia, *Nosema* spp., have also been isolated from *P. truncatus* (Schulz and Laborius 1987), but most work with the group relates to *N. whitei* in *Tribolium* spp. (Dales 1994). Infection of *T. castaneum* with *N. whitei* had little effect on the sex ratio of subsequent offspring, but infection did reduce fecundity and survival (Armstrong 1982). A long time period may be necessary for significant population reduction to occur when using microsporidia (Lange and Wysiecki 1996). Characteristics such as vertical transmission (Lange 1997a) and the robustness of some protozoan spores (Lange 1997b) could allow the development of chronic infections with significant effects on the biology of pest populations. With *P. truncatus*, a relatively recent migrant into Africa, there could be benefits from increasing the levels of infection in the general population. Spores of *Nosema* spp. can be stored at 4°C for at least 15 months (Milner 1972). Preliminary work with synergy between chemical insecticides and the protozoa indicated some effects in increasing the larval period (Khan and Selman 1984), but this work does not appear to have been followed up.

There are undoubtedly many other pathogenic protozoa to be isolated from stored-product insects. Their limited effects on natural levels of insect infestation should not be taken as an indication that they are unsuitable as control agents. Their use in inoculative releases for long-term population impact merits serious study. Their safety characteristics and persistence are positive aspects, while slow action may reflect more of an education challenge than a fatal flaw as a control agent. Investigations into baits and pheromones may prove beneficial.

Nematodes

Nematodes are minute, worms that can actively seek out host insects. The infective stage of the nematode enters the mouth, anus or spiracles of their host and releases the entomopathogenic bacterium, *Xenorhabdus nematophilus* Poinar and Thomas, that multiplies in the insect hemocoel, killing the host and serving as food for the nematodes. Reproduction results in a massive increase in numbers of nematodes released from cadavers to infect new hosts.

There appears to be no work published on the use of nematodes against pests in storage (Cox and Wilkin 1996). There are a few records of *Steinernema feltiae* (Filipjev) being used in laboratory experiments against insects such as *S. granarius*, *Trogoderma granarium* Everts and *T. confusum* (Alikham et al. 1985; Kamionek and Sandner 1977). The accepted view, that nematode application to stored grain is extremely unlikely as they usually require a wet environment (Cox and Wilkin 1996) should not be accepted uncritically. There may be formulation possibilities, such as mixtures with oils, to protect against desiccation or encapsulation for release of nematodes in response to insect feeding damage.

2.0 Present usage

A fundamental difficulty with describing the control of pests of stored products is the sheer diversity of materials to be protected, of pests and of types of storage facility. The latter may range from sophisticated environmentally controlled silos to an earth floor in a hut. A major portion of research programs, both in terms of the numbers of workers and the level of funding, are aimed at specific problems in developing countries.

2.1 *Prostephanus truncatus* in Kenya.

Major efforts are being directed at developing a biological control strategy for use against the larger grain borer, *P. truncatus*. This pest is a recent introduction into East Africa and is causing extensive damage to a range of stored food products. It has proved difficult to halt its spread using conventional chemical control measures, so alternatives are being sought. Although the introduction of the predator *Teretriosoma nigrescens* (Lewis) has proved quite successful, there has been interest in pathogens such as protozoa (Henning-Helbig 1994; Lipa and Wohlgemuth 1986) and fungi such as *Beauveria* spp (Smith et al. 1999).

Storage facilities vary widely in East Africa. In Kenya, storage at its most simple may consist of maize cobs left on the floor or in bags. Cobs may be husked or not, with storage as cobs or as grain. Storage is often inside the house, sometimes in a loft area above the kitchen, benefiting from smoke. The maize is effectively subjected to ambient temperature and humidity conditions. Pests may be carried into the store with the grain or attracted later. Some farmers use chemical insecticides such as pirimiphos-methyl.

Fungus-based control of *P. truncatus* had to meet a number of requirements. Any treatment had to be cheap and persistent, effective under prevailing conditions, capable of controlling a range of insect pests, relatively harmless to the predator *T. nigrescens* and other natural enemies, and be safe to the consumer. Work in Kenya using field-collected *P. truncatus* and *S. zeamais* and non-specific isolates of *B. bassiana* demonstrated the greater susceptibility of the former beetle. This was followed by intensive surveys in the maize producing areas of Kenya to obtain isolates of fungi from insects in stores. This resulted in at least a dozen isolates of *B. bassiana* being found. *Beauveria bassiana* was obtained from *S. zeamais*, *Tribolium* spp (probably *castaneum)* and *Carpophilus* spp. (Oduor et al. 1999 in press). These isolates showed greater virulence against *S. zeamais* (S.M. Smith and G.I. Oduor pers comm).

A preliminary field trial, was carried out at Kiboko, southeast of Nairobi. Artificial infestations of *P. truncatus* and *S. zeamais* were established, and a range of treatments, including the addition of *B. bassiana* were applied. At the end of the five month experiment, *P. truncatus* had been eliminated, probably by competition from other insects, and *Tribolium* sp. had become the second most common insect after *S. zeamais*. Extensive Indianmeal moth immigration had occurred. The *B. bassiana* treatment achieved the following:

- Up to 84% of *P. truncatus* mortality could be attributed to *B. bassiana*.
- Three weeks after treatment, there was significant mortality of five species of storage pest.
- Although parasitoids were also infected, significant levels of parasitism were recorded.
- Cycling of the infection occurred in the store. The original inoculum lost viability within a few weeks, but high levels of infection were still present nine weeks after the experiment began.

The results suggested that increasing the persistence of the initial inoculum in the store would be important. Various options and research needs are apparent for this purpose. For example, isolates may be selected for persistence and improved capability to sporulate on cadavers, especially on the major species *S. zeamais*. Also important is the understanding of the relationships among temperature, moisture, and other factors with viability (Hong et al. 1997; 1998). Formulation and controlling storage environments may also contribute to success.

The work described above used dry conidia powder mixed in with the grain. Although mycopesticides are considered to be relatively safe, especially in comparison with chemical pesticides, the presence of dry conidia in foodstuffs is undesirable as they may present allergenic hazards. While these would be removed during the normal processes of washing and cooking during food preparation, effective formulations that remove this potential hazard need to be investigated.

Research in Kenya examined aspects such as storage properties of the formulated product, the effects of incorporating pheromones specific to *P. truncatus*, and the efficacy of dose transfer to the target insect (Smith et al. 1999). Hidalgo et al. (1998) worked with fat pellets made from hydrogenated rapeseed oil, incorporated conidia of *B. bassiana* and demonstrated kill of *S. zeamais* with an unknown (but extremely low) dose transfer to the insects. The material starts to melt at 32°C, clears by 38-40°C, begins to solidify at 29-32°C and is solid below 25°C. Materials such as pheromones or fungal conidia can be added while the material is liquid (40°C is not harmful to dried conidia). Dropping the liquid fat into 70% alcohol at 5-10°C causes rapid cooling and solidifying, usually in a hemispherical shape, occasionally spherical. Pellets containing conidia could be stored for over a year with minimal loss of viability, and dose transfer was, on occasions, very effective.

2.2 *Bacillus thuringiensis* in the United States

The sole example of operational use of microbial control agents in stored products in the US is that of *B. thuringiensis* var. *kurstaki* isolate HD-1. Although, *Beauveria bassiana* has a registration that would permit use in stored products and nematodes are exempt from registration requirements, they have not yet found their way into the stored product market. *Bacillus thuringiensis* has several attributes that would seem to give it a substantial advantage over the pathogens that require the relatively long incubation periods to achieve control and, more importantly, must be kept alive. Ease of use, low cost, and exemption from residue tolerances were primarily responsible for the interest in *Bt* of both the pesticide industry and the stored product handlers. Commercial use of *Bt* for stored-product pests began in the 1970s.

Isolate HD-1 is registered by the US Environmental Protection Agency (EPA) for use on stored grains, seeds, peanuts, soybeans, and tobacco. The target stored-product pests listed on the label are Indianmeal moth, almond moth, and tobacco moth. Apparently, the manufacturer, Abbott Laboratories, does not consider the numerous other *Bt*-susceptible moth species that infest stored products to be of commercial interest. This is somewhat surprising given that only a notification of EPA is needed to add a pest to a label.

Reliable data on the amount of the *Bt* used in stored products are not available. As of this writing, there is some US use on stored peanuts and corn. An informal phone survey failed to find any current use on small grains in the US wheat belt, and few of the grain elevator personnel contacted were familiar with *Bt*. Pesticide distributors, however, reported past sales. There are two major reasons for this dearth of use. First, the moths are not the major pests for which grain is treated. A variety of small beetles infest stored grain, and pest control personnel are not inclined to use a material that does not include the beetles in its spectrum of activity.

The second reason for the paucity of *Bt* use in small grains has unfortunate implications for the use of pathogens in general. There is a great deal of naturally occurring variation in the susceptibility of both Indianmeal moth and almond moth to *Bt* toxins (Kinsinger and McGaughey 1979). Further, there is variation within a given moth population to the toxins of various isolates (Kinsinger et al. 1980). The consequence of all this variation was rapid resistance development. McGaughey (1985) reported resistance to *Bt* in Indianmeal moth in 77 grain bins in a 4-state area. The resistance appeared from 1 to 5 months after *Bt* application to the bins. In laboratory work, Indianmeal moth larvae were exposed to a dose of 62.5 mg of *Bt* /kg of diet, a rate expected to produce 70 to 90% mortality. The survival rate of larvae given this treatment increased in each of four successive generations, going from 19% to 82% or approximately the survival rate of untreated larvae. In two generations of resistance selection, the LC_{50} increased 27 fold; in 15 generations it increased 97 fold. Nine generations with no *Bt* exposure did not restore the susceptibility. Not long after the discovery of *Bt* resistance in Indianmeal moth, McGaughey and Beeman (1988) reported resistance in almond moth.

There is scant reason for optimism regarding resistance management. The first discovered mechanism of the resistance developed by Indianmeal moth was a greatly reduced binding affinity for one of the five toxins in the HD-1 parasporal crystal, specifically CryIA(b) (Van Rie et al. 1990). This left open the possibility of rotating or combining strains and toxins to prevent resistance. There are many other toxins produced by other *Bt* strains. Unfortunately, the cross resistance to a broad range of toxins that was found to occur in *Heliothis virescens* (F.) (Gould et al. 1992) apparently has general applicability. McGaughey and Johnson (1994) studied the resistance spectrum of *P. interpunctella* selected for resistance to *Bt* varieties *aizawi*, *entomocidus* and *kurstaki*. The resistance tended to reflect the toxin composition of the variety used to select the insects, but there was evidence of cross resistance. The insects selected with variety *kurstaki* showed a low level of cross resistance to the endotoxins of the other two varieties. This is to be expected because varieties *aizawi* and *entomocidus* both contain low levels of CryIA(b). Selection for resistance to varieties *aizawi* and *entomocidus*, which have more diverse toxin composition than variety *kurstaki*, resulted in broader cross resistance. From their mathematical analysis of the data of McGaughey and Johnson (1994) and McGaughey and Beeman (1988), Tabashnik and McGaughey (1994) concluded that initial frequency of resistance alleles was greater than previously assumed and that mixtures of the above varieties offered no advantage over sequential use and would not prevent or greatly retard resistance.

The altered toxin receptor site mechanism is not the only one for *Bt* resistance in *P. interpunctella*. Oppert et al. (1997) reported that two *Bt*-resistant moth strains lack a major gut proteinase that activates the protoxin. The existence of multiple physiological means for adaptation to the *Bt* toxins can only confound efforts to prevent resistance.

McGaughey and Whalon (1992) reviewed the proposed strategies for *Bt* resistance management. They favor combinations of *Bt* with other mortality mechanisms and with refuges for susceptible insects among the available strategies, but they conclude that no available strategy offers clear advantages in all situations.

2.3 Practical utility

There are many options for the control of stored-product pests (Brower et al. 1995; Subramanyam and Hagstrum 1995). They include plant breeding and pre-harvest treatment of produce, physical and chemical (and possibly biological) cleansing of facilities, effective containment, controlled environments, the treatment of produce with chemicals, inert dusts or oils and many more. As a component of IPM strategies, pathogens offer many advantages. As a single control measure they are unlikely to be successful except in special cases. However, storage pests are not always effectively controlled by any one measure. The inclusion of pathogens in IPM programs requires efficient management but, if conducted well, should provide excellent protection.

Wilkin et al. (1990) proposed an IPM strategy for UK grain storage that requires monitoring of the physical condition of the grain and of pest numbers, a cooling strategy and restricted application of pesticides. The use of biological control agents to replace the chemical pesticides was thought doubtful on temperature grounds (Cox and Wilkin 1996).

The poor or inconsistent performances of experimental mycoinsecticides over the years can be put down to a misplaced emphasis on the active ingredient, usually the fungal spore, occasionally mycelium. Of equal importance were effective formulations and application techniques (Bateman et al. 1993; Jenkins and Thomas 1996). During the course of recent research, a fourth dimension has been added - the ecology of pathogen-host interactions within various ecosystems (e.g. Thomas et al. 1997a, b; Thomas et al. 1995; Thomas and Jenkins 1997; Blanford et al. 1998). Consequently, for maximum efficacy in any system, the specific requirements of the pest-pathogen interactions must be determined.

Natural occurrences of entomopathogenic fungi and bacteria have been reported on storage pests (Morris et al. 1998; Oduor et al. in press). It is most probable, judging from experience, that many virulent isolates could be found if adequate surveys were undertaken (Bateman et al. 1996; Prior et al. 1995).

Microbial insecticides usually have a little effect on non-target organisms, including beneficial species (Vinson 1990). Certainly their impact on nontarget organisms is less than that of chemical pesticides. Accordingly, a level of pest control by natural enemies can be maintained under a program of microbial control (Goettel et al. 1990). Consequently pathogens are more amenable to use within IPM systems.

The prevailing environmental conditions are unlikely to prevent effective use of the mycoinsecticide. Sunlight, a major concern with pathogens in many environments (Moore et al. 1996a), is absent from most storage systems, but formulations can be amended to ameliorate harmful effects in systems where they might occur (Moore et al. 1993). Temperatures are unlikely to be inhibitory to a great extent and would not usually be harmful to the stored mycoinsecticide (Hedgecock et al. 1995; Morley-Davies et al. 1996). Long-term storage is achievable with Hyphomycetes; this enables material to be produced, stored and used when required (Moore et al. 1995)

A mycoinsecticide is likely to have a greater persistence than legal chemicals. This is because the pathogen may recycle on the cadavers, re-introducing more inoculum into the system (Thomas et al. 1995; Wood and Thomas 1996; Thomas and Wood 1997; Thomas et al. 1997b). This release of infective material may extend over weeks or months and can result in significant secondary infection.

Pre-and non-lethal effects of infection greatly reduce the severity of pest attack. These effects include, among others, reduced feeding or the metabolic costs of defensive responses against infection (Moore et al. 1992; Thomas et al. 1997a).

Mass production of *B. bassiana*, one likely species to be the active ingredient in a mycoinsecticide, can be achieved cheaply in the quantities required, whether as one or many isolates.

The potential unlimited variability of isolates permits consideration of many options for use. They can be selected for suitability to various ecosystems enabling increased persistence of the initial inoculum and dramatically increasing the level and persistence of control. Likewise, they can be selected for adaptation to the characteristics of a particular pest complex (Doberski 1981; Thomas et al. 1996; Thomas and Jenkins 1997). The various options for use strategies reduce the already low risk that resistance to the mycoinsecticide would occur.

2.4 Safety and Regulatory Issues

Safety

Evidence of health hazards associated with the consumption of food treated with microbial insecticides is scant (Burges 1981; Siegel and Shadduck 1990; Saik et al. 1990). There are occasional cases of allergenic response usually associated with exposure to large quantities of conidia, as in mass production facilities. Immunologically compromised individuals have developed opportunistic infections of entomopathogens. For example, an invasive infection of *M. anisopliae* was reported in an immunosuppressed boy (Burgner 1998). There have also been very rare cases of entomopathogenic fungi being isolated from skin and corneal lesions in apparently healthy humans (De Garcia et al. 1997) although it is not clear if the fungi were causative. Siegel and Shadduck (1990) concluded that microbial control agents are not significantly toxic, virulent or pathogenic by conventional routes of exposure. The risks of adding *Bt* spores or conidia of *B. bassiana* to stored products may be less than those associated with insect infestation, such as particulate detritus that may be allergenic. In the less developed countries, spoiled grain itself may carry a massive dose of fungal spores, including those pathogenic to humans such as *Aspergillus* spp., which contain several types of aflatoxins (Stein and Gorham 1993).

Unfortunately, insect pathologists and industry may have done themselves a disservice by attempting to cover over relatively minor health risks. Biopesticides are not absolutely free or risk, but they are very safe. Most problems are associated with mass production and can be resolved by sensible use of basic safety equipment. Problems associated with exposure to fungal conidia include respiratory symptoms, dermal irritation and "fever-like" symptoms, likely an immune response. There are very occasional reports in the literature associating conidia with eye infections. However there may be real risks associated with mycotoxin content. Most problems can be addressed by selection of safe isolates, determined by regulated safety testing, and there is an urgent need to have cheap initial screening protocols developed.

Any risks associated with entomopathogens must be put into prospective and are generally far less than those presented by chemical pesticides where many adverse reactions have been noted. It is notable that none of the currently US-registered microbial pest control agents have required safety testing beyond Tier 1, the EPA minimum safety requirement (Betz et al. 1990).

Public Perception

A major obstacle to the use of entomopathogens on human or animal foodstuffs could be public perception. The term pathogen does not lend itself to reassurance. Of the groups involved, viruses probably carry the greatest stigma for a public that associates viruses with pestilence. Promoting the benefits of adding protozoa and nematodes to food may also be a challenge. Bacteria and fungi may prove more acceptable, being the basis for yogurts, fermented foods, etc. (Dirar 1993). The use of fungi in staples, such as bread and beer, and their direct consumption for their actual or supposed nutritional, medicinal and aphrodisiacal properties should also make fungi more acceptable to the public.

There is natural concern over the use of biological agents despite much research effort demonstrating that their careful use will be far safer than the use of chemical insecticides, in terms of both acute and chronic exposure. Despite a belief that safety studies are an industrial secret to be kept, safety results should be more freely available. Knowledge that the system is effective at weeding out dangerous isolates is reassuring. An informed public is key to acceptance of the use of microbial control agents. US Department of Agriculture-led, large-scale applications of *Heliothis* NPV on the Mississippi Delta met with no reported opposition as a result of a preemptive public information campaign that included brochure mailing, meetings with local physicians, presentations to local clubs and interest groups, and television interviews. Similar education efforts would surely ease the entry of microbial pest control into stored-product systems.

Regulation

Differences in regional attitudes toward safety and environmental issues have resulted in an international patchwork of regulations. The US, unlike most countries, does not require efficacy data for pesticide registration. The US EPA has developed a fast track system for registration of reduced risk pesticides, including microbial insecticides. As a result, microbial control agents have obtained full registration in as little as six months, and all of those registered for use on food items are exempt from residue tolerances. Canadian guidelines for registration are still in draft form at present. The stated aim is to harmonize guidelines with the US EPA, but progress has been slow. Consequently, *Bt* and viruses for forestry are the only microbes currently available for insect control in Canada.

Food safety regulations have been drawn up primarily to protect the consumer and to ensure that food for human consumption is not injurious to health. The UK 1990 Food Safety Act does not distinguish between beneficial and pest species in food products. The use of pesticides in the UK is controlled by a number of statutory measures. The Control of Pesticides Regulations sets out requirements for efficacy, and safety for the consumer, the environment, and non-target organisms that must be met before any pesticide can be offered for commercial use. There is a formal registration process in which data must be submitted by the manufacturer or distributor of the product, and this data package is then assessed by a series of experts. Unlike predatory or parasitic insects, mites and nematodes, the pathogens such as protozoa, fungi, bacteria and viruses require registration in the UK and most other countries.

Similar regulations exist in other European Community states, and there will be an increasing trend for uniformity within the community with data packages and registration from one member country applicable in all. To ensure greater accord of approach the "FAO Code of Conduct for the Import and Release of Exotic Biological Control Agents" may be applied to the European Community. There are several microbial candidates that could be developed, but the size and potential return from the market in the UK alone does not appear to justify the costs of registration (Cox and Wilkin 1996). Cheaper registration to cover a larger market could alter this situation.

Registration is expensive, but should be cheaper than the registration of comparable chemical pesticides. Regulations should not be modeled blindly on chemical systems. Any product must be effective against the species mentioned on the label, and a good biological pesticide should be as effective as standard products. Residues should be demonstrably safe for both the raw and processed product.

2.5 Compatibility of pathogens with other IPM techniques

Biological control agents tend to be flexible in that they can be used in conjunction with many techniques. Biologicals will not impede the efficacy of non-biological techniques, but they themselves may be vulnerable to such techniques. There are sometimes paradoxical cases of incompatibility in which very effective control techniques result in host populations that are too low to sustain a biological control agent. This is more likely to affect predators and parasitoids than pathogens, which can usually survive periods of low host populations in a resting phase. However, in those cases where greatest pathogen efficacy may require a high host population to renew pathogen inoculum via cadavers, a sustaining host population must be maintained. The mechanisms below are dealt with in more detail in other chapters of the book. These are just examples to demonstrate if compatibility is likely.

Chemical pesticides

Many pathogens have been found compatible with chemical insecticides, although there has been relatively little work on this aspect with stored product protection. Khan and Selman (1988) demonstrated some synergy when using chemicals and *Nosema whitei* Weiser. *Farinocystis tribolii* has also been shown to significantly increase the susceptibility of *T. castaneum* larvae to the insecticides malathion, chlorpyrifos-methyl, fenvalerate and cypermethrin (Rabindra et al., 1988).

The greatest effort in testing of pesticide-pathogen compatibility has been directed at the fungi (e.g. Anderson and Roberts 1983, Moorehouse et al. 1992, Todorova et al. 1998). Unfortunately, most studies have involved only growth on agar media, which may not be an accurate predictor of field performance. Luke (1999) investigated *M. anisopliae* conidia suspended in oil formulations of a range of chemicals plus neem oil and investigated their persistence in storage. Some were incompatible with the fungus, others had no adverse effects and neem appeared to enhance the persistence of the conidia.

In general, the active ingredients of chemical insecticides are compatible with entomopathogenic fungi, but some formulation ingredients, such as xylene (Anderson and Roberts 1983), are not. Rather surprisingly, many agricultural fungicides, including copper compounds, fosetyl-aluminum and thiophanate-methyl, can be tank mixed with *B. bassiana* without loss of germination or growth (Lord unpubl.).

Bacteria and viruses are likely to be even less sensitive to chemical toxicants than are fungi. Accordingly, integration of pathogens with traditional chemicals is certainly achievable.

Plant host resistance

Resistance mechanisms vary widely and have different effects on pest populations. Working with paddy rice, Clement et al. (1988) demonstrated that lemma tightness was important in restricting infestations by *S. oryzae* and *S. zeamais*. Cultivars showing low rates of lemma opening had lower pest populations. A test of maize cultivars demonstrated significant differences (50-fold) in population growths of *S. zeamais* with insect development time positively correlated with maize lipid content (Throne et al. 1995). There are many such examples, and their effects on pathogen efficacy are likely to be highly variable. In these examples, insects may be stressed and also exposed to pathogens for considerable periods of time, increasing probability of infection.

Botanicals

Botanicals have been the subject of great interest. Much research has focused on the use of oils or powders from plants. Those botanicals with fungicidal or anti-bacterial properties may be detrimental to some entomopathogens. Ground neem (*Azadirachta indica* A. Jussieu) seeds and leaves reduce adult fecundity and larval development of a range of pests including *S. oryzae, C. ferrugineus, R. dominica, Sitotroga cerealella* (Olivier) and *Cadra cautella* (Walker)(Pereira and Wohlgemuth 1982). Neem has weak fungicidal properties, and hence its use with some entomopathogenic fungi, could be problematic. However, its use with *Metarhizium* (Luke 1999) and *Beauveria* (Lord unpublished data) indicate that any problems can be overcome. It may be compatible with other pathogens. However, presence of *Aspergillus* on neem seeds under suitably warm and moist conditions bring's neem's fungicidal properties into doubt (Pereira and Wohlgemuth 1982).

Pheromones

The concept of using pheromones of stored-product pests with pathogens in an attracticide strategy has been tested with fungi (Smith et al. (1999), protozoa (Shapas et al. 1977) and viruses (Vail et al. 1993). The latter two works successfully demonstrated the concept in closed environments. The strategy is most logical for a pathogen that is highly transmissible and a pheromone that does not attract the entire population. If sufficiently high proportion of the population is attracted then killing them directly with a chemical would be logical, but if only a small proportion were attracted, dosing and releasing them into the storage environment where they initiated an epizootic may be a realistic option. Research into this is still at a preliminary stage, but in the work in Kenya, it was shown that a pheromone could be used in conjunction with the pathogens in pelleted formulations.

Dusts

Clays have often been used as protectants against stored-product pests. The kaolinite, montmorillonite and attapulgite clay types are also the predominant carriers or diluents in dry formulations of biopesticides. They improve shelf life by buffering pH and absorbing harmful metabolites (Ward 1984). Some clays appear to have a detrimental effect on the survival of stored conidia of *Metarhizium flavoviride* (Moore and Higgins 1997). However, many clays can be used in conjunction with fungi with at least an additive effect expected from the combined use.

Ash from the wild olive tree, *Olea europaea* L. *africana*, is used as grain protection in parts of Kenya. Its effect may be similar to that of other desiccant materials. It could be used with *B. bassiana* without detrimental effect on the effects of the fungus, but no synergy was obtained (G.O. Oduor personal communication).

Diatomaceous earth and amorphous sorptive silica dusts have low mammalian toxicity, but high insecticidal action due to their ability to absorb epicuticular lipids from insects contacting them, resulting in desiccation and death (Korunic 1998; Shawir et al. 1988). They are most effective in materials and stores of low humidity. They may act on lipophilic conidia in the same way as insects, leading to desiccation. When added to stores, fungal conidia would be very dry (as little as 5% moisture) and water loss may not be too great a problem. At germination, begun with uptake of water, the situation may be different. Alternately, insects weakened by low levels of injury may be more susceptible to disease. Diatomaceous earth and *B. bassiana* were synergistic against the lesser grain borer and sawtoothed grain beetles in laboratory tests. A concentration of 200 parts per million of diatomaceous earth on wheat killed only 2.5% of adult lesser grain borers independently, but it reduced the median lethal dose of *B. bassiana* four-fold (J. Lord unpublished). These materials are a logical combination because of their complementary humidity optima and potential for synergy.

Macrobials

When parasitoids and pathogens attack the same prey success is usually determined by the time of respective attack. Similarly, priority of host attack can decide the success of parasitoids and pathogens. Parasitoids may be able to detect and avoid attacking diseased insects and thereby could avoid wasting energy and eggs on unsuitable hosts. Predators appear less fastidious, and diseased insects often show reduced defensive capabilities, making them more susceptible to predation.

Predators and parasitoids are unlikely to be significantly adversely affected by the presence of bacteria, viruses or protozoa within the store environment, but parasitoid efficiency is likely to be reduced because of successful disease progression in hosts. On occasions, disease progression may result in the death of developing parasitoids. There are also many cases where parasitoid activity can aid disease dissemination with pathogens carried on the ovipositor. With contact agents such as fungi there are likely to be direct adverse effects or the parasitoids. Work in Kenya demonstrated that *B. bassiana* infected parasitoids of a complex of pest species (G.O. Oduor personal communication). The effects on pest control are not known because research is required to determine at what point parasitoids were infected and effects on their activity. If only old parasitoids, past or at the end of their reproductive stage were infected, the effect on pest control would not be very great, whereas infection of pre-reproductive females would be significant. In addition, the disease in insects can produce many unexpected results. It may be that infected parasitoids increase their egg-laying prior to death, an effect seen with other groups of insects (S. Blanford personal communication). Biological control of *P. truncatus* in Africa depends heavily on the predator *T. nigrescens*, and studies on the effects of *B. bassiana* on this species are required.

Oils

Both vegetable and mineral oils may have physical effects on insects and have been shown to be effective in reducing oviposition and adult longevity. Most oils are compatible with many pathogen groups, although little or no work appears to have been done with nematodes. Oils have been shown to significantly increase the efficacy of fungal pathogens (Prior et al. 1988). The reasons for improved performance are not clear. Among the possibilities are that oils may protect fungal conidia, or they may carry conidia to intersegmental membranes of the insects where penetration may be easier.

Controlled environments

Controlled environments, while unlikely to harm the pathogens may greatly reduce their efficacy, either directly or by reducing the activity of insect pests. Fungal isolates with good activity at low temperature are known, but reduced insect activity may make dose uptake less frequent. Controlling atmosphere composition is unlikely to directly effect longevity of the agents.

Stressing insects may make them more susceptible to disease. This may not necessarily be beneficial, as early death with fungi may result in poor sporulation on cadavers and hence limiting secondary cycling.

2.6 Economics

The *Bt* example demonstrates how economics change with market size and economies of scale. In addition to stored products, *Bt* is used on corn, cotton and many other crops. With pathogens other than *Bt*, the challenge is to demonstrate efficacy. Once that is achieved, the main cost challenge is in the area of mass production. Microsporidia, neogregarines and viruses are obligate parasites that do not grow outside a host, necessitating as yet cost-prohibitive cell culture or an insect production system. In vivo production has proved satisfactory for production of *Nosema locustae* Canning as a commercial control agent for locusts and grasshoppers (Jenkins and Goettel 1997) and for baculoviruses. Nematode mass production has proved feasible for use in high value crops, but their use in stored products is unlikely in the near future. Recent interest in fungi has resulted in many advances (Jenkins and Goettel 1997), some public knowledge and others not. It seems likely that at least some isolates of some species can be produced at well under $10 US per hectare of field crops (100g). Claims that *B. bassiana* may be produced for less than $10 per kilogram require substantiation. An order of magnitude increase in production at no extra cost would transform the economics of mycoinsecticides. This may require a radical re-think of methodology or many incremental improvements.

Bacteria production

It not surprising that the bacteria progressed most rapidly among the insect microbial control agents for all environments. In addition to their stability and simplicity, they benefit from extant large-scale production systems. Commercial scale production of *Bt* is carried out in deep tank industrial fermenters of up to 12,000 gal (Couch and Ross, 1980). Often, producers take advantage of the idle time between batches of drug-producing microbes. In this way, they are able to add to their realized revenues from existing equipment during periods when it would be otherwise unused.

Production costs are closely guarded proprietary information, but a crude estimate can be made from pricing. The only *Bt* products registered for use on stored products in the US are Dipel 2X and Dipel DF (Sumitomo, N. Chicago, IL). Dipel 2x contains 14.52 billion international units/lb. The cost to the consumer is about $16/lb. If production cost is estimated to be one third of the selling price, *Bt* would cost about $5/lb to produce. At the labeled use rate of 0.375 lb/100 bushels or 0.5 lb/500 sq ft (28g/ sq m), surface treatment of an 18 ft grain bin would cost ca. $4.00. Thus, the cost of the material should not be an impediment to its adoption.

Fungus production

Beauveria bassiana is the only entomopathogenic fungus with a registration that would allow application to stored food products in the US. *Metarhizium anisopliae* is the most promising alternative. Among the large companies with existing fermentation capacity, there is a preference for liquid culture over solid substrate for the fungi. The products of liquid culture, whether conidia, blastospores, or dried mycelium, are less stable than the conidia formed on solid substrates. A satisfactory compromise is the biphasic systems normally used for mass production of both fungus species. A liquid starter culture is transferred to a grain for final incubation to conidiation (Jenkins and Goettel, 1997). Handling and maintenance of aseptic, optimum growth conditions are the most demanding aspects of scale-up. Unlike bacteria production, the start up costs for commercial fungus production will limit the number of entrants into the market.

Production of viruses and Protozoa

All viral and most protozoan microbial control agents for insects require host cells for reproduction. For the foreseeable future, this means that the most economical means of production will be in vivo. Insect cell culture systems have been intensively researched for several decades with the hope of bringing costs down to acceptable levels. According to a 1993 estimate, 10- to 100- fold reduction in cost will be needed for in vitro production to be competitive (Copping 1993). Rhodes (1996) presented a model in which in vitro production would be cost effective. One of the underlying assumptions was 190 million dollars in sales, an unlikely prospect for stored products.

There has been marked improvement in in-vivo systems for virus production in recent years (Hughes 1994). In spite of the progress, production in vivo is labor-intensive, costly and results in a generally impure product. In some developing countries in vivo methods can be more economically feasible. In Egypt, *Spodoptera littoralis* (Boisduval) NPV production was comparable with standard control measures (Jones et al. 1994). At present, it is the only practical approach for most pathogens that have intracellular life stages.

Cost of application

In general, pathogens can be applied in the same manner as traditional contact insecticides. In the appropriate formulations, all classes of insect pathogens can be applied as dusts or aqueous sprays. Early trial work with *Bt* in stored grain was carried out with application methods that would limit its acceptance by grain producers. McGaughey (1986b) considered the need to mix the treatment into the grain, even by auguring into storage tanks, to be labor- intensive and unpleasant. Accordingly, he developed a method for application of *Bt* dust into corn by using the downward airflow of drying fans. This gave 25% penetration to the top several cm of corn (McGaughey 1986a). Given that the targeted moth larvae occupy the top few centimeters of stored grain, this should be adequate.

Some pathogens have the potential for inoculative release and dissemination via bait or pheromone autoinoculation devices. Autoinoculation should cut the costs of both materials and application. The autoinoculation approach has been the subject of several research reports and US patents (see Vega et al. 1995). Two infectious pathogens of stored-product insects have been applied experimentally by pheromone baiting on an arena scale. Shapas et al. (1977) prevented *T. glabrum* population build-up by pheromone attraction to *M. trogodermae*. Vail et al. (1993) achieved 60 and 50% mortality in F_1 and F_2 *P. interpunctella* by baiting adults. In order for such a device to be practical for use with pathogens, there must be a high level of contagion. Further, the autodissemination approach will require gregarious pest behavior, limited recruitment, a highly infectious pathogen, and time to take effect. If any of these factors is lacking, a more logical approach is to simply kill the target at the trap by a physical or chemical means. Additionally, there is the familiar market economy problem of minimum profit to justify investment. Inoculative materials are likely to be of little interest to major corporations, and success will depend on small specialty companies selling at very high profit margins.

2.9 Cost-benefit calculations

Cost-benefit calculations for microbial control of stored-product insects are impractical. In the less developed world, such calculations are often meaningless. How can one measure the value of protecting a village food supply while preventing pesticide poisoning? In developed countries, damage to stored products and the cost of inputs can be measured, but not the value of reduced risk to applicators and consumers. Even where cost-benefit analyses can be made with entomopathogens, the area is not large enough to draw the interest of economists to gather the data and carry out the work.

Perhaps the most underestimated cost of entry into the microbial pesticide business is the regulatory aspect. In spite of fast-track treatment given to microbials in the US, the initial cost of registration is a major impediment to entry into the small markets, including stored products. Furthermore, the expense does not end with the initial registration. Changes in formulation, packaging, targets, use rates, application methods, manufacturing methods, etc. all require federal, and often state, regulatory notification or approval. The microbial control agents with having the highest probability of use on stored products in the developed world are those that have applicability in other systems.

3.0 Research needs

In order to bring microbial control of stored product insects to fruition numerous research needs should be met. The following are some that we deem high priority.

Mass production and quality control.

Methods of mass production of aerial fungal conidia have been reviewed by Jenkins and Goettel (1997) and Jenkins et al. 1998. The major block to the use of pathogens in many situations is the perception that they are uneconomic. If pathogens are to be developed and used on a commercial scale, then there must be a reasonable profit margin for the producer. It is unlikely that pathogens will ever compete on cost with the less expensive chemicals. Other considerations drive their development. The economics may be greatly influenced by understanding ecological aspects of pathogen use (see below), but the active ingredients are often too expensive. Consequently, mass production research to obtain cheap active ingredients, such as fungal conidia, is essential. Reduction of production costs to 10% of present levels, is realistic. Industry confidentiality makes it very difficult to determine actual costs of production or even technologies used, but many of the necessary advances are probably already known. Sometimes the secrecy can result in duplicated research effort. For years companies were keeping secret from each other the necessary moisture levels to maintain good storage of conidia of *M. anisopliae* and *B. bassiana*. They had all come to the conclusion that single digit moisture content was optimum for spore longevity. Mass production research is a generic requirement for increased utility of pathogens and not specifically for stored product protection. Current commercial scale production of bacteria and fungi is remarkably efficient, but even incremental improvements could result in substantial improvements in economics.

Quality control is generally very good among large corporate microbial pesticide producers and quite uneven among small producers. In the developed countries, there are regulatory statutes that require extensive documentation of quality control methods and initial results in order to obtain a registration to sell a microbial pesticide. Once a registration is obtained, there is little if any, monitoring by regulatory agencies. Contaminated and low potency products can and do reach the marketplace. A single bad experience with such a product is likely to permanently turn an end user against the class of materials. An important role for public sector researchers is provision of the less sophisticated producers with methods to optimize their production methods for yield and purity.

Delivery systems.

Among the most demanding aspects of microbial insect control is delivery. None of the pathogens that have utility in most stored-product systems have search capacity and, consequently, the pathogens must be delivered so as to come into direct contact with the targets and/or be persistent enough to remain viable until the targets contact the pathogen. There is likely to be resistance to purchase of specialized equipment and modification of existing practices. Accordingly, methods that use conventional techniques for delivery of pathogens must be developed. This may be an argument for the autodissemination approach and the use of readily transmissible pathogens.

Ecology

Ecological research into pest-pathogen-environmental interactions in diverse agricultural systems has made dramatic advances in recent years. In the well-controlled, closed-environmental systems of storage facilities, such studies are more likely to produce predictable results and could bring major benefits. Ecological models of various components of the system have been developed. For example, population models have been developed of *P. truncatus* in rural maize stores in West Africa (Meikle et al. 1998) to better understand the potential of various control strategies. A model by Throne (1989) demonstrated that noncatastrophic factors can significantly impact the population dynamics of *Cryptolestes pusillus* (Schoenherr). Relatively small changes in duration of larval development and survival and adult fecundity (all characteristics which can be altered by pathogen infection) made significant differences to final populations and the theoretical need to intervene to control populations. Models of pathogen behavior have been created for other systems. For example, Hong et al. (1997; 1998) demonstrated that it is possible to model persistence of fungal conidia. Onstad and Maddox (1990) created a simulation model in which *N. whitei* could reduce a population confused flour beetles by 90%. Similar work with other pathogens of stored-product pests is needed.

Enhancing agent activity

There are many ways in which agent activity can be improved. An enormous reservoir of isolates exists in nature and new ones can be found relatively easily. A common mistake in initial research is to select isolates on their virulence to target insects when many other features need to be explored. Commercial concerns tend to select the isolates that are easiest, and hence cheapest, to mass-produce. In the future, selection parameters need to include useful biological characteristics such as those that lead to extended control periods. Searches and selection criteria may include combinations of control effects. In light of the ice nucleation by *F. avenaceum* (Fields et al. 1995), is it possible to obtain isolates of *B. bassiana* with ice-nucleating active properties, to work by pathogenesis at warm temperature while still being effective at low temperatures?

Gene manipulation, such as *B. thuringiensis* endotoxin gene insertion into recombinant baculoviruses to increase pathogenicity or the reduction of the susceptibility of fungi to chemical pesticides, can be done but may carry the danger of alienating the public. There has been little work done on formulations for use against storage pests, and quite simple formulations, such as in oils, can greatly enhance activity. Combining agents or IPM techniques may produce synergies.

The ideal is for a practical measure that controls all of the members of a pest complex, but many pathogens display some degree of specificity (as do many modern chemicals). Combining different types of agent, such as bacterial and fungal may also have value. This has been little researched, especially in the storage system. Initial work concentrating on storage and application of co-formulations of virus and fungi suggested no intrinsic problems (B. Luke unpublished data). Combining fungal isolates could give a product that is active against the various species of a pest complex and under varying conditions. The obvious impediments are the cost of producing a number of isolates and any necessity to register each isolate. The former problem is a matter of research and the latter one of common-sense regulations being in place.

Large scale field trials

Laboratory scale experiments under controlled conditions are necessary in order to generate reproducible data. While that is clearly a necessary step, nothing will be adopted by end users until it has been demonstrated on an operational scale. This must relate to pest ecology as well as technological requirements.

Integration with other tactics.

In consideration of the limitations of pathogens, combination strategies are appropriate. Synergies may enhance pathogens performance. For example, the widely used insecticide, imidacloprid synergizes some entomopathogenic fungi attacking root weevils (Quintela and McCoy, 1997). Diatomaceous earth (DE) and *B. bassiana* are two materials are also a logical combination because of their complementary humidity optima. A desiccant, DE is most active in dry conditions, while the fungi have a moisture requirement. As previously mentioned, the two materials were synergistic against the lesser grain borer in lab tests. No doubt there are other synergistic or simply complementary materials that would provide research opportunities for IPM.

Resistance management

With regulatory loss of many of the common chemical insecticides for stored products, there will be increasing resistance development pressure with the remaining materials. Pathogens offer unrelated modes of action that can be exploited in a manner equivalent to that of using alternating chemistries as is current practice in many cropping systems.

Methods of monitoring efficacy

Among the most difficult aspects of microbial control is quantification of benefit. Even qualitative information can be elusive, largely due to the pace of disease development. Taking prevalence rates as a measure of efficacy is a common practice among insect pathologists. The methods are fraught with difficulties and error sources, and the data are frequently unreliable. Combining sampled insects in containers can cause cross contamination. Inadequate surface disinfection gives false positives for fungi; too much can cause false negatives. Furthermore prevalence is not the type of information that the end user wants. The data that will bring microbial control to fruition are yield loss data taken in comparison with standard practices and related to the economics of use. This is the practice of industrial research groups, and it should be the practice of academic and government groups to the extent possible. With field crops this may extend beyond a single season because of secondary cycling of disease and sublethal effects. This would be rarely possible in temporary storage situations. Efforts should be made to bring other factors into consideration, such as non-target effects (again less applicable to most stored products systems) and persistence data. Persistence is a regulatory red flag for conventional insecticides, but it is still a desired trait among microbial agents. Persistence and even amplification are goals of microbial control.

Control of diseases

This chapter has concentrated on arthropod pests, but is there hope for controlling diseases damaging stored products? The control of harmful pathogens by antagonists is a minor industry at present. Microbial seed treatments are available (Maude 1996), designed to protect the resultant plant, but many of the scientific principles would be relevant to storage systems. Patents by McLaughlin *et al* (*1997*) and Wilson *et al* (1998) demonstrate the potential of such approaches against both pre-harvest and postharvest diseases.

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