Entomopathogens and biological control

Agricultural pests
Agricultural pests include plant pathogens (e.g. fungi, oomycetes, bacteria, viruses, nematodes), weeds, arthropods (primarily insects and mites), molluscs (slugs and snails) and a small number of vertebrates. They reduce the yield and quality of produce by feeding on crops, by transmitting diseases, or by competition with crop plants for space and other resources (weeds, for example).

There are estimated to be about 67,000 different pest species worldwide. They are a significant constraint on agricultural production, responsible for around 40% loss of potential global crop yields. These losses occur despite the considerable efforts made at pest control, and they suggest that improvements in pest management are significant way forward for improving yields and access to food.

Pest problems are an almost inevitable part of agriculture. They occur largely because agricultural systems (‘agro-ecosystems’) are simplified, less stable modifications of natural ecosystems. The creation and management of agricultural land disrupts the ecological forces that regulate potential pest species in natural ecosystems: these include physico-chemical conditions, food availability, predation, and competition. Thus, growing crops in monoculture provides a concentrated food resource that allows pest populations to achieve far higher densities than they would in natural environments. New food resources for pests are provided when a crop is introduced into a country. Cultivation can make the physico-chemical environment more favourable for pest activity, for example though irrigation or the warm conditions found in glasshouses. Finally, using broad spectrum pesticides will destroy natural predators that help keep pests under control.

Some of the most important problems occur when pests develop resistance to chemical pesticides. For example, over 500 arthropod species now show resistance to one or more types of chemical. Other serious problems are caused by alien (i.e. non-native) species that are accidentally introduced to a new country or continent and which escape their co-evolved natural predators More than 11,000 alien species have been documented . These cause highly significant damage to crops, biodiversity and landscape valued at billions of dollars per annum. There are also threats from emerging pests, such as new strains of plant pathogens that evolve to overcome varietal resistance in crops.

Farmers and growers are under immense pressure to reduce the use of chemical pesticides without sacrificing yields or crop quality, but at the same time the control of pests is becoming increasingly difficult due to pesticide resistance and the decreasing availability of products. Alternative control methods are needed urgently. These need to be used as part of Integrated Pest Management. There is a particular need to preserve the effectiveness of chemical pesticides, which will remain a key component of pest management for the foreseeable future.

Biological control
Biological control is the use by man of a living organism to help manage the population density of a pest organism. Natural enemies are organisms that kill or debilitate another organism. Biological control programmes operate throughout the world in agriculture and forestry. Biological control agents (BCAs) include the following: (1) predatory insects and mites, which eat their prey; (2) parasitoids, which are insects with a free living adult stage and a larval stage that is parasitic on another insect; (3) parasites and microbial pathogens, such as nematodes, fungi, bacteria, viruses and protozoa, which cause lethal infections. In Europe, typical examples of where these agents are being used include: (1) the application of parasitoids to control whiteflies in glasshouses; (2) parasitic nematodes against slugs; (3) mycoparasitic fungi to control plant

Biological control is the use of a living organism for pest management diseases of horticultural crops; (4) use of viruses to control codling moth in apple orchards; and (5) building habitats on farms to increase natural populations of predators and other beneficial organisms.

Natural enemies represent a large component of the world's biodiversity, for example parasitoids account for about 10% of the world's species, and each agricultural pest species is fed upon by up to hundreds of species of natural enemies. However, only a small proportion of the available species have been investigated for crop protection. Thus, while c. 750 species of insect pathogenic fungi are known, less than 20 have received serious attention as control agents of insect pests. The natural enemies inhabiting an agro-ecosystem play a key role in preventing pests reaching damaging levels.

The ways in which biological control agents are used vary according to the type of pest (plant, microorganism, vertebrate or invertebrate), the biological characteristics of the control agent, as well as the agricultural setting. There are three broad biocontrol strategies: Introduction (release of an alien control agent to control an alien pest), augmentation (application of natural enemies that already live in the area of use), and conservation (manipulating agricultural practices or the environment to enhance natural control). People who use biocontrol are bound by the FAO code of conduct on the import and release of biological control agents as well as national legislation on releases into the environment. The use of microbial agents is governed by Plant Protection Product regulations, in which authorisations proceed along similar lines to those for chemical pesticides although there are also some specific schemes.

BCAs have a range of attractive properties that include host specificity, lack of toxic residue, no phytotoxic effects, human safety, and the potential for pest management to be self sustaining. Many are able to actively locate their prey. BCAs can also be produced locally which can be important in terms of choosing and matching natural enemies to small scale needs. Successful use requires fundamental knowledge of the ecology of both the natural enemy and the pest. When this condition is satisfied, and the agent is used firmly within IPM, then biological control can sometimes be more cost effective than purely chemical control. However, under the present market system, many biological control products have not competed well with less expensive and more effective synthetic pesticides. The down sides of BCAs are that most are niche products, pest control is not immediate, there can be lack of environmental persistence, and efficacy can be low and unpredictable particularly in outdoor environments. The approvals process used for microbial agents results in significantly greater authorisation costs than for macro- agents.

Entomopathogens
Many farmers and growers are now familiar with the use of predators and parasitoids for biological control of arthropod (insect and mite) pests, but it is also possible to use specific micro-organisms that kill arthropods. These include entomopathogenic fungi, nematodes, bacteria and viruses. These are all widespread in the natural environment and cause infections in many pest species.

Entomopathogens contribute to the natural regulation of many populations of arthropods. Much of the research in this area concerns the causal agents of insect diseases and their exploitation for biological pest control. Many entomopathogens can be mass produced, formulated, and applied to pest populations in a manner analogous to chemical pesticides, i.e. as nonpersistent remedial treatments that are released inundatively. Entomopathogens have also been used as classical biological control agents of alien insect pests, and natural pest control by entomopathogens has been enhanced by habitat manipulation.

Entomopathogenic fungi.
There are thought to be about 750 species of fungi that cause infections in insects or mites. As a group, they attack a wide range of insect and mite species, but individual species and strains of fungus are very specific. The fungi produce spores which infect their host by germinating on its surface and then growing into its body. Death takes between 4 and 10 days, depending on the type of fungus and the number of infecting spores. After death, the fungus produces thousands of new spores on the dead body, which disperse and continue their life cycle on new hosts.

There are two main taxonomic orders of entomopathogenic fungi. The Entomophthorales occur in the phylum Zygomycota and include genera such as Pandora, Entomophthora and Conidiobolus. Many of the species are co-evolved, obligate pathogens that show specific ecomorphological adaptations to the life cycles of their hosts, such as the production of forcibly-ejected infective spores that are produced on insect cadavers during the night, when environmental conditions are most conducive to infection. These fungi often cause natural epizootics in insect and mite populations. However, some of them are very difficult to mass produce in culture, which is a challenge for people wanting to develop them as biopesticides.

Despite this, they can be used for pest control by being applied to the field (as mycelium or as infected insect cadavers). For example, isolates of Neozygites floridana from S. America are being investigated for classical biological control of the cassava green mite *Mononychellus tanajoa*, a major alien pest of cassava in Africa.

Elsewhere, natural epizootics of entomophthoralean fungi are being used as a natural form of pest control. For example, a prediction service developed by Don Steinkraus at the University of Arkansas is used to inform cotton growers in the southeast USA about outbreaks of Neozygites fresenii in cotton aphids, Aphis gossypii. These outbreaks can reduce aphid populations by about 80% in 4 days and can be predicted up to ten days in advance. Cotton farmers then have the choice to withhold expensive insecticide sprays.

The second major order of entomopathogenic fungi – the Hypocreales – occurs in the phylum Ascomycotina. There are many species in the Ascomycotina in which the sexual phase (teleomorph) is not known and which reproduce entirely asexually, and which have only be confirmed to be members of the Ascomycota by using molecular (DNA) data. These fungi are commonly known as anamorphic fungi.

Sexually reproducing hypocrealean fungi occur in the genera Cordyceps and Torrubiella. Cordyceps is thought to originate in Asia and Cordyceps species are important natural parasites of many insect and mite species in tropical forests. Cordyceps sinensis is a pathogen of ghost moth (Thitarodes) caterpillars that occur at high altitude in the Himalayas, Tibet and China. In China it is referred to as dong chong xia cao (winter animal summer grass). It is a highly valued herbal medicine and contains a range of pharmalogically active compounds.

Important anamorphic genera of entomopathogenic fungi include Beauveria, Isaria, Metarhizium and Lecanicillium. Species from all these genera are used as biopesticides of insect pests. Recently, molecular methods have revealed the telemorph connections of these fungi. Molecular studies are also shedding light on the phylogenetic relationships with other fungi. Much of this work is being pioneered in the US by mycologists such as Meredith Blackwell, Richard Humber, Steve Rehner and Joey Spatafora. They have shown that entomopathogenicity has evolved multiple times in the ascomycete fungi, characterised by host shifting from an intimate association with plants to insects and vice versa.

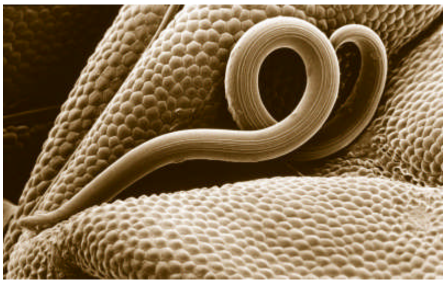
The spores of many species of the anamorphic entomopathogenic fungi can be mass produced on a variety of culture media, and so are suitable for development as biopesticides which are applied inundatively to pest populations. They have a range of desirable characteristics including safety to people, compatibility with other natural enemies, and a lack of toxic residues. They also offer the possibility of providing persistent control by multiplying in the pest population.

Because they have contact action, they are good for the control of sap feeding pests, like aphids and whiteflies, which cannot be infected by other types of biopesticide (such as bacteria and viruses) which are active only when ingested. The downside is that they are more expensive than chemicals and can be affected adversely by certain environmental conditions. There can also be a false expectation that biopesticides will have the same efficacy as chemical insecticides, whilst their desirable biological qualities are overlooked. In addition, the costs of registration are high in relation to the current market size, deterring companies from pursuing registration.

At present there are two commercial products are available to UK growers, namely Vertalec (for control of glasshouse aphids) and Mycotal (for glasshouse whitefly). Both are based on species of the fungus *Lecanicillium*: Vertalec is based on *Lecanicillium longisporum*, and Mycotal is based on *Lecanicillium muscarium* (until recently, these were considered to be one species, *Verticillium lecanii*, but the genus *Verticillium* has now been revised). They are produced by Koppert BV, and the products were originally developed at the Glasshouse Crops Research Institute, a forerunner of Warwick HRI. Products based on Beauveria bassiana and Metarhizium anisopliae are expected to be available shortly in the UK for the control of glasshouse pests and vine weevils.

Entomopathogenic nematodes

Entomopathogenic nematode worms are just visible to the naked eye, being about 0.5 mm in length. Two families – the steinernematids and the heterorhabditids - are obligate parasites of insects and used for microbial control. Juvenile nematodes parasitize their hosts by directly penetrating the cuticle or through natural openings. They then introduce symbiotic bacteria, which multiply rapidly and cause death by septicaemia, often within 48 hours. The bacteria break down the insect body, which provides food for the nematodes. After the insect has died, the juvenile nematodes develop to adults and reproduce. A new generation of infective juveniles emerges 8 – 14 days after infection.



Unlike other entomopathogens, nematodes are currently exempt from registration in the EU and so have been popular choices for commercialisation. Over 60 products are available in Europe and products from Becker Underwood (MicroBio), Koppert, Biobest, and Syngenta Bioline are sold in the UK. Nematodes require moist conditions to operate and have been marketed predominantly against soil pests, such as vine weevil and sciarid fly larvae. However they may also control foliar pests such as western flower thrips. Like other natural enemies, nematodes are affected by environmental conditions, but in the 1990s research at Warwick HRI by Roma Gwynn (now an independent biopesticide consultant) identified a nematode strain of Steinernema kraussi which is active at low temperatures and allows vine weevil to be controlled earlier in the season. This nematode is now available as a commercial product.

Baculoviruses

Over 1600 viruses have been recorded from more than 1100 species of insects and mites. Of these, three families (Baculoviridae, Polydnaviridae, Ascoviridae) are specific for insects and related arthropods. The baculoviruses are the most widely exploited virus group for biocontrol: they are very different from viruses that infect vertebrates and are considered very safe to use. The mode of pathogenesis and replication of entomopathogenic viruses varies according to the family, but infection nearly always occurs by ingestion. Virions then bind to receptors in the gut and penetrate epithelial cells. In the Baculoviruses, the infection often spreads to the haemocoel and then to essential organs and tissues, particularly fat bodies. Acute infections lead to host death in 5 – 14 days.

There are two genera of Baculoviruses: nucleopolyhedroviruses (NPV) & granuloviruses (GV). The host range of baculoviruses is restricted to the order, and usually the family, of the host of origin, and commercial baculovirus biopesticides are considered to present a minimum risk to people and wildlife. Mass production of Baculoviruses can only be done *in vivo*, but is economically viable for larger hosts such as Lepidoptera, and formulation and application are straightforward. At present, there are approximately 16 biopesticides based on baculoviruses available for use or under development. The majority of these products are targeted against Lepidoptera. For example, codling moth granulovirus, CpGV (*Cydia pomonella* Granulovirus) is an effective biopesticide of codling moth caterpillar pests of apples.

***Bacillus thuringiensis* (Bt)**
The majority of bacterial pathogens of insects and related taxa occur in the families Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceae. Most of these bacteria are weak pathogens that infect insects subject to environmental stress, but a minority are highly virulent. By far the most attention has been given to the Bacillaceae. *Bacillus popillae* causes milky disease in scarbaeids, while *Bacillus sphaericus* is a lethal pathogen of mosquitoes. *Bacillus thuringiensis* (Bt) is widespread in soil, is a lethal pathogen of a range of orders and is the most widely used entomopathogenic biological control agent. There are at present over 40 Bt products available for the control of caterpillars, beetles and small haematophagous fliees. Together, these products account for 1% of the world insecticide market.

Bt is a spore forming bacterium. Sporulation is usually associated with the synthesis of a proteinaceous protoxin crystal that has insecticidal activities, although some types of Bt produce crystals with no known activity. Ingested crystals dissolve within the gut and are cleaved by host proteases to form an active toxin, termed the δ-endotoxin. This binds to receptors in the midgut epithelium to cause the formation of ion pores, leading to gut paralysis. The ingested spores may contribute to bacterial septicaemia. Host death occurs quickly, often within a few days. About 70 Bt subspecies are known; they differ in host spectrum, but most show activities to Lepidoptera, Diptera and Coleoptera. Some strains also produce exotoxins, which have a wide spectrum of activity including vertebrates. Bt products used for pest control are normally based on exotoxin-negative strains.

Bt and GM crops
The genes that encode the Bt δ-endotoxin can be expressed in plants, which allows them to be protected against some species of insect pest. Genetically modified (GM) maize and cotton crops that express lepidopteran active endotoxins have been available for a number of years and have revolutionised farming in the countries in which they are grown. GM crops are now grown on about 5% of global cultivated land.

It is undoubtedly the case that the development of policies on GM crops in Europe has been affected by a lack 'upstream' engagement between governments, regulators, farmers, pressure groups, industry, the media, and other members of civil society. There has been a loss of confidence in scientific experts by the general public over issues of risk. While scientific expertise and evidence can help answer specific questions about GM crops, it cannot be the sole tool for developing policy. People entering the debate about GM have different points of view and hence a resolution may not be possible. Agriculture in Europe has its own distinctive social dimension and hence Europeans may well have different concerns about GM compared to citizens elsewhere. The ethical issues surrounding GM crops are many and complex, and include general welfare (i.e. the responsibility of governments to protect the interests of citizens), consumer choice and rights, principles of justice, and the boundary between what is considered natural / unnatural.
Those in favour of GM argue that transgenic crops can help increase yields (for example by reducing losses due to pests), can have improved nutritional content, require fewer inputs and have less post harvest spoilage and wastage. Arguments against GM crops include possible harm to human health, concerns that the technology consolidates the industrialisation of agriculture, that it is not natural, and that it may damage the environment (for example by having effects on natural enemies and other non target species). In Europe and elsewhere, detailed environmental risk analysis of potential effects of GM crops based on laboratory and field experiments is made before licences to release the technology are granted. Critics have argued that even farm scale trials cannot predict the effects of GM crops when grown at very large scales. Eight countries now grow > 1 million ha of GM crops (USA, Canada, China, India, S Africa, Paraguay, Argentina, Brazil) and hence, if negative effects do occur from GM crops, then it is reasonable to expect that they will become apparent soon if they have not done so already.

In the case of Bt crops, laboratory and field studies have shown either no impact or only a transient effect on natural enemies, which is mainly due to a reduction in the number of target pests as prey. Resistance management is a concern [106] as resistance has developed to Bt foliar sprays. A strategy has been devised based on the cultivation of areas of non Bt crops as refugia to maintain susceptible alleles within the pest populations . Surveys of farmers' pesticide use indicates that growing Bt crops can result in significantly reduced applications of conventional insecticides, up to 70% in some cases. However there are exceptions. Secondary pest problems caused by mirid bugs have occurred on Bt cotton grown in China. These bugs were controlled previously by broad spectrum pesticides but are not controlled by Bt cotton. Problems with mirids in China did not occur until a few years after the widespread uptake of Bt cotton (i.e. there was a time lag between adoption of Bt cotton and the onset of secondary pest problems). The unfortunate result is that some farmers growing Bt cotton in China are having to make more pesticide applications than before in order to control mirid outbreaks, with a net reduction in revenue compared to conventional cotton. Secondary pest outbreaks are a well-known phenomenon in agriculture, but without more evidence it is difficult to say whether this particular problem could have been foreseen. One lesson is clear; if GM crops, or any other new technologies, are to be used in ways that increase the sustainability of crop production, then they must be treated on a case by case basis and utilised according to basic IPM principles.

Further reading

If you are interested in this subject and want to know more, you will find the following references useful:

Bale, J.S., van Lenteren, J.C. & Bigler, F. (2008). Biological control and sustainable food production. *Philosophical Transactions of the Royal Society B*, **363**, 761 – 776.

Dent, D. (2000). Insect pest management. CABI publishing , Wallingford, UK.

Evans, J. (2008). Biopesticides: from cult to mainstream. *Agrow*, **October 2008**, 11 – 14. www.agrow.com

Flint, M.L. & van den Bosch, R. (1981). Introduction to integrated pest management. Plenum Press, New York, USA.

Freckleton R.P., Sutherland A.R., and Watkinson A.R. (2003). Deciding the Future of GM Crops in Europe. *Science* **302**: 994-996.

Goettel, M.S., Hajek, A.E., Siegel, J.p. & Evans, H.C. (2001). Safety of fungal biocontrol agents. In Fungi as biocontrol agents:progress, problems and potential. Butt, T.M, Jackson, C.W. & Magan, N. (Eds.). CABI Publishing, Wallingford, UK, pp.347-376.

Gurr, G. & Wratten, S. (Eds).(2000). Biological control: Measures of success. Kluwer Academic Publishers, The Netherlands.

Hajek, A. (2004). Natural enemies: an introduction to biological control. Cambridge University Press, Cambridge, UK.

Kogan, M. (1998). Integrated pest management: Historical perspectives and contemporary developments. *Annual Review of Entomology*, **43**, 243-70.

Li, G-P., Wu, K-M., Gould, F., Wang, J-K., Miao, J., Gao, X-W. & Guo, Y-Y. (2007). Increasing tolerance to Cry1Ac cotton from cotton bollworm, *Helicoverpa armigera*, was confirmed in Bt cotton farming area of China. *Ecological Entomology*, **32 (4)**, 366-375.

Lu, Y. H., Qiu, F., Feng, H. Q., Li, H. B., Yang, Z. C., Wyckhuys, K. A. G. & Wu, K. M. (2008). Species composition and seasonal abundance of pestiferous plant bugs (Hemiptera: Miridae) on Bt cotton in Chona. Crop Protection, 27, 465 – 472.

Nuffield Council on Bioethics (1999). Genetically modified crops: the ethical and social issues. http://www.nuffieldbioethics.org/go/ourwork /gmcrops/ publication_301.html

Oerke, E.C., Dehne, H.W., Schnbeck, F. & Weber, A. (1994). Crop production and crop protection: estimated losses in major food and cash crops. Elsevier, Amsterdam, The Netherlands.

Pimentel, D. (1997). Techniques for reducing pesticides: environmental and economic benefits. John Wiley and Sons, Chichester, UK.

Romeis, J., Meissle, M. & Bigler, F. (2006). Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology.*, **24**, 63-71.

Shelton, A.M., Zhao, J-Z. & Roush, R.T. (2002). Economic, ecological, food safety and social consequences of the deployment of Bt transgenic plants. *Annual Review of Entomology*, **47**, 845 – 881.

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J. & Kuris, A.M. (2003). Introduced species and their missing parasites. *Nature*, **421**, 628 – 630.

Van Driesche, R.G. & Bellows, T.S. (1995) Biological Control. Chapman & Hall, New York, USA.

Wang, S., Just, D.R. & Pinstrup-Andersen, P. (2006). Tarnishing silver bullets: Bt technology adoption, bounded rationality and the outbreak of secondary pest infestations in China. Selected paper prepared for presentation at the American Agricultural Economics Association Annual Meeting Long Beach, CA, July 22 – 26, 2006.