SESSION 4B

BIOLOGICAL CONTROL OF PESTS IN FIELD CROPS

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Platform Papers:	4B-1 to 4B-4
Poster Paper:	P4B-5

Biological control products in a changing landscape

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ABSTRACT

The history of applied biological control can be separated into three distinct eras, each characterised by different technologies, industrial efforts and socioeconomic conditions. The successes and failures of each era provide guidance for future efforts in both applied and basic biological control research.

INTRODUCTION

In the long history of biological control, which by some estimates dates back over 2,000 years, the development of commercial products, which began in earnest only in the 1950s, represents a mere snippet of time. However, it is in this short period that years of effort in biological control research have come to fruition in the form of biological control products (BCPs) used around the world – from the release of *Trichogramma* egg parasitoids on 32 million hectares in 30 countries (van Lenteren, 2000), to the planting of over 100 million hectares of crops engineered to produce *Bacillus thuringiensis* toxins (James, 2004). Onchocerciasis has been eliminated from seven West African countries using an integrated programme that includes *Bacillus thuringiensis* var. *israelensis* (Guillet *et al* 1990) and pesticide use has been reduced by 80 – 95% in European glasshouse vegetable production using beneficial macroorganisms (van Lenteren, 2000). There is no doubt that, despite the relatively brief period of time in which they have played a role in agriculture, biological control products—whether they are microbial biopesticides, beneficial macroorganisms, semiochemicals or botanical products—have had a significant and positive impact.

For every wonderful success story though, there are always the many blunders and mistakes upon which all progress rests. A rapidly changing landscape of scientific innovation, economic growth and public perception requires us to continually reassess and adjust priorities. It is in this spirit that I review the past 50 years of commercial biological control efforts in an attempt to understand what we have done that has worked best, what hasn't, and how we can apply this information to make future efforts — in both applied and basic biological control research — more productive. To do this, it is useful to divide the past 50 years into three eras defined by the technologies that were developed, the types of companies that developed them and the socioeconomic forces that shaped them.

1950 – 1980: VISIONARY ENTREPRENEURS

Although microbial biopesticides and beneficial macroorganisms were sold on a limited basis prior to World War II, their development was interrupted by the war and it wasn't until the 1950s that commercial BCPs became more readily available. Some of the pioneering products included Sporeine, Thuricide and Biotrol (*Bacillus thuringiensis* or *Bt*), Viron/H and

Elcar (*Heliothis* nuclear polyhedrosis virus), and Doom (*Bacillus popilliae*). Beneficial macroorganisms included *Trichogramma* and *Encarsia.* By the late 1970s, over ten species of beneficial macroorganisms were available worldwide (van Lenteren, 2000), microbial biopesticides based on bacteria, viruses, fungi and protozoans were commercially available and semiochemicals for pests such as the boll weevil, *Anthonomus grandis,* and the spruce bark beetle, *Ips typographus,* were being used.

Factors contributing to success

Inspired by the publication of Rachel Carson's *Silent Spring* in 1962 and by the success of many publicly funded classical biological control projects, early workers from universities, government agencies and the fledgling industry worked closely together. So closely, in fact, that the bulk of the research and development on field efficacy, production systems, formulation, safety testing and grower education for these early BCPs was carried out by publicly funded researchers in support of the new industry. Regulatory timelines and costs were relatively inexpensive for microbial biopesticides (considerably less than $1 million US), while beneficial macroorganisms were almost completely unregulated, further serving to increase the economic feasibility of BCPs.

Almost all of the BCPs manufactured during this era were produced outside of mainstream agribusiness channels by an assortment of visionary entrepreneurs that included small, family-owned companies, farmer cooperatives, pharmaceutical companies and government agencies that produced BCPs as a public service. *In vivo* production systems for beneficial macroorganisms and for obligate pathogens such as *Nosema locustae* and *Bacillus popilliae* were straightforward enough that several entrepreneurs began by producing them in their garages, backyards and home greenhouses. In the case of microbial biopesticides, many of which could be fermented *in vitro* in industrial fermenters, the need to fill idle tank space was a factor that motivated the involvement of companies such as Merck, International Minerals and Chemical Corporation, Sandoz and Abbott Laboratories.

It must have been an exciting time. Researchers in academia and industry with a deep sense of mission and a commitment to the principles of biological control were finally getting to see the fruits of their labours in the form of products in the field. Researchers such as Edward Steinhaus, John Briggs, Carlo Ignoffo, Lou Falcon, John Henry and Howard Dulmage (to name a few) personally championed various bacterial, protozoan and viral biopesticides to the marketplace in the US. In Brazil, Flavio Moscardi led a public/private collaboration to deliver the *Anticarsia gemmatalis* nuclear polyhedrosis virus to millions of hectares of soybeans, while Denis Burges and Constantin Vago paved the way in Europe. In the production of beneficial macroorganisms, scientist/entrepreneurs such as Everett Dietrick and Stanley Flanders lay the groundwork for this growing industry. Productive collaborations and strong personal friendships formed between the scientists in the industrial and research communities.

Factors inhibiting success

Farmer demand for BCPs during this period was low with sales during these early years never even approaching $20 million (US) per year. This was due primarily to the inherent limitations of biological control agents (host specificity, lack of environmental persistence, slow mode of action) and their inability to compete in most markets with cheaper and more

effective synthetic chemistry. Sales occurred mostly in niche markets where other pest control options were severely limited due to pesticide resistance, lack of other products or environmental safety concerns. In addition, the companies that produced BCPs were struggling with quality control, production costs and low profitability. It was the dedication and commitment of key visionaries and entrepreneurs, and not necessarily large profits, that kept these early companies afloat and that lay the groundwork for further progress.

1980 – 1995: VENTURE CAPITAL AND AGROCHEMICAL COMPANIES BECOME INVOLVED AND IRRATIONAL EXUBERANCE TAKES HOLD

Factors contributing to success

Conceivably, the most significant milestone during this era (for better or for worse) was the application of genetic engineering to biological control with the 1981 cloning of a *Bt* protein delta endotoxin in *Escherichia coli* (Schnepf & Whiteley, 1981). Coupled with advances in fermentation technology, the possibility that genetic engineering could be used to produce cheap and effective BCPs that could compete with conventional pesticides existed. In a related set of developments, changes in tax and intellectual property (IP) laws such as the Bayh-Dole Act of 1980 (Kennedy, 2005) made it possible for publicly funded research institutions to patent their biological discoveries and to provide exclusive licenses to companies that were interested in developing them. The modest market sizes and even smaller profit margins that had characterised biological control's earlier years seemed to be a thing of the past. Hopes were further inflated by a series of food safety scares (Carlson, 1989) and increased incidences of pesticide resistance (Georghiou, 1990) that had many projecting the rise of the biological control industry and the demise of conventional pesticides. Optimism was also fuelled by increased interest in organic agriculture (Figure 3) and the discovery of new isolates of *Bt* that had unexpected levels of activity against mosquitoes, black flies and beetles (Goldberg & Margalit, 1977; Krieg *et al*, 1983). With a wealth of pesticidal microbes waiting to be discovered and the potential to use them as raw materials in genetically improved biopesticides, the future looked bright.

As a result, biological control, and microbial biopesticides in particular, began to attract the attention of companies that had previously been uninvolved. Large agrochemical companies sought to diversify their portfolios and to cash in on the newly emerging market. New venture capital companies attracted millions of dollars from investors excited by the promise of the biotechnology revolution that was sweeping the agricultural, medical and pharmaceutical industries. Companies rapidly appointed high powered molecular biologists, acquired sophisticated, capital-intensive fermentation equipment and invested in the acquisition and/or generation of hefty patent estates. Genetically engineered bacteria, viruses, fungi, crop plants and even beneficial arthropods were the focus. The result was a proliferation of new microbial BCPs and rapid market growth with global sales that eventually reached over $200 million (Figures 1, 2). Some of the new products were manufactured by agrochemical and venture capital companies, and tended to rely on genetically modified and/or patent-protected microbes. However, an equal number of new BCPs were introduced by the original group of entrepreneurial companies. These products continued to be based on public domain research and on non-engineered microbes. The increased investment in biopesticides brought with it increased assessment of the value of the market. Prior to the 1980s, the industry had focused on small, niche markets that earned a

few hundred thousand dollars per year, at best. With the entry of larger and better financed companies, mainstream crops and pests were now targeted and market projections soared to $10 million per product per year or more.

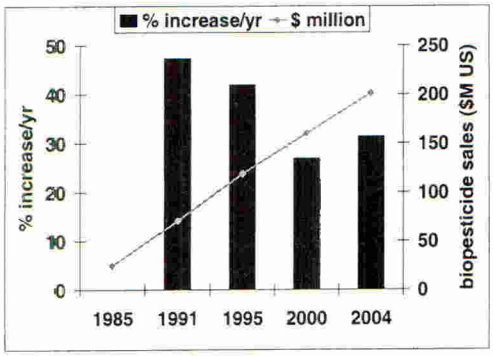

Figure 1. Sales of microbial biopesticides in North America and Europe. (Evans, 2005; B. Blum, International Biocontrol Manufacturers Association, personal communication).

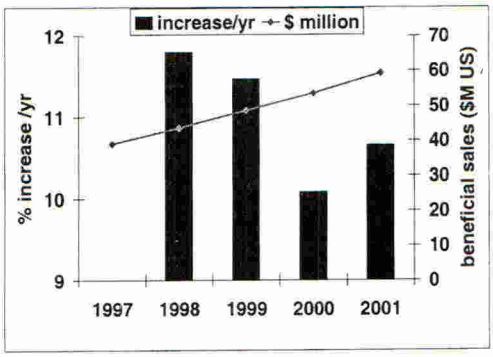

Figure 2. Sales of beneficial macroorganisms in Europe. (Anonymous, 2001; Evans, 2005; B. Blum, personal communication).

Factors inhibiting success

Were the large investments and increased market projections that dominated these years realistic? They certainly would have been, if the technology had been successful in creating BCPs that met the chemical paradigm; that is, if they performed as well as the best of the synthetic pesticides. In the case of genetically engineered crops that produced *Bt* toxins, that objective was eventually reached (see below) and adoption has exceeded all expectations, with annual global sales in excess of $1 billion (US) (James, 2004; Figure 4). However, in the case of other BCPs, only small improvements in performance were achieved during this era, and the average market size remained below $1 million per year per product. The features that made most BCPs so attractive from the standpoint of environmental and human safety also acted to limit the number of markets in which they were effective. Despite the fact that sales continued to grow at a healthy pace during this period (Figures 1, 2), a trend all the more interesting because conventional agrochemical sales during the same time period were essentially static (Anonymous 2005), the failure to meet irrationally high company forecasts created a strong, and, in retrospect, inappropriate degree of disappointment.

In addition to over-valuation of the biological control market, a further hindrance to progress was the belief, held especially within many of the venture capital companies, that all stages of BCP development, from discovery through to marketing, could be carried out with minimal assistance from the public sector. While this approach might have helped to carve out highly desired proprietary positions, the critical role that public subsidy had played over the past 30 years was ignored. As a result, the high costs of development for microbial biopesticides ($25 million or more) came as a surprise, especially when compared to the unexpectedly small markets of $1 million or less in which these products were finally sold. Perhaps no story illustrates this vicious cycle of mis-calculation better than that of *Bt* subspecies *morrisoni* strain *tenebrionis* (*Btt*). *Btt* was discovered by university researchers to have unique activity against the Colorado potato beetle (*Leptinotarsa decemlineata*) and other beetles in 1983 (Krieg *et al*), patented and commercialised within five years and ultimately made available as

seven different biopesticides produced by four different companies. However, by 2001, *Btt* products had all but vanished from the marketplace. Although several factors were responsible for its demise, the expense of development, which was borne almost wholly by the companies that introduced this product, was so out of proportion to the relatively meagre sales (they never reached $2 million among the four companies) that failure was inevitable (Gelernter, 2004).

The "go it alone" strategy espoused during this period was further reinforced by the value placed on the role of patents and exclusivity. Universities, government agencies and companies all fell into the trap of assuming that commercial interest in BCPs would be non-existent without the inducement of exclusive access to the technology. On this basis, patents were generated for microbes, insects, semiochemicals and botanicals; for *in vivo* production and *in vitro* fermentation; for formulation ingredients and even packaging design. In addition to adding to the cost of development, the prospect of costly licensing fees created the opposite of the intended effect by excluding the very companies that had the greatest likelihood of producing a commercial success—the experienced, but poorly financed entrepreneurial companies. The grandiose proprietary dreams of university and government technology officers and private company financial officers were a poor match for the small markets and profits generated by most BCPs. A quick scan of the list of BCPs available today bears this out—it is remarkable for the almost complete lack of patented products (most IP in the biological control industry these days is in the form of production technology and is handled instead as trade secrets). The undue emphasis placed on IP was at best a distraction, and at worst a financial drain, an impediment to development and a barrier to the exchange of scientific information. This is not to say that the patenting process was unprofitable for everyone involved. In addition to the patent attorneys (who were the first to profit), many of the early investors in venture capital biotechnology companies were rewarded handsomely when the patent estates of small companies such as Mycogen and Ecogen were acquired at great cost by Dow, Monsanto and others for use in the development of transgenic crops.

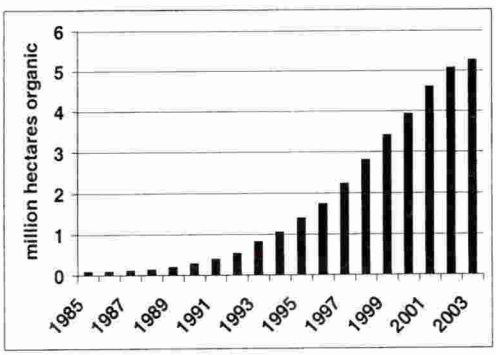

Figure 3. Organic farming trends in 15 European Union countries (Lampkin, 2004).

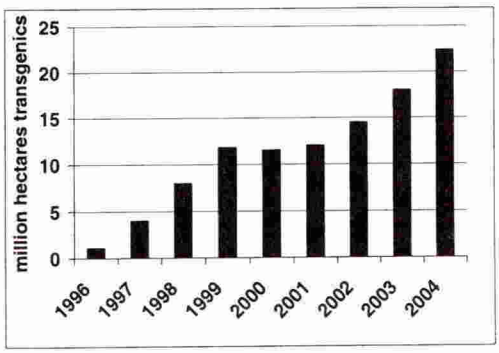

Figure 4. Global growth of *Bt* transgenic cotton and corn (James, 2004).

1995 TO THE PRESENT: BACK TO THE FUTURE?

This era is marked by the appearance of two related phenomena — the retreat of agrochemical and venture capital companies away from biological control and towards the development of transgenic crops (Figure 4), and a concomitant return of small companies as the dominant

force in the growing biological control industry. With their return has come a more rational assessment of the strengths and weaknesses of BCPs. Though sales have continued to exceed that of conventional agrochemicals for all five classes of BCPs (Figures 1, 2, Table 1), expectations for profitability, market size and product performance have been scaled down to more realistic forecasts.

Table 1. Estimated share of 2004 global product sales for biological control agents (Evans, 2005; B. Blum, personal communication)

Class of BCP	% market share (estimated)
Microbial biopesticides	65-70
Beneficial macroorganisms	15-16
Semiochemicals	10–19
Botanicals	4-8
Microbial soil and plant enhancers	1-2

The social and economic significance of biological control products

Though their role in the marketplace continues to be small (routinely estimated at 1 – 2 % of all pesticide sales), the influence of BCPs on agriculture, and society in general, has been profound. A few key examples include:

Serving as the raw materials for innovative pest control solutions: Whether you view *Bt* crops as BCPs or not, there is no dispute as to their origins in the efforts of researchers, companies and farmers who have worked with *Bt* over the years. And, although there is controversy regarding the benefits and risks of this technology, the 4.7% reduction in insecticide use that has directly resulted from their use, is a benefit worth considering (Benbrook, 2004). Furthermore, whether or not new microbial-based products such as spinosad, strobilurin fungicides and harpins should be classified as biopesticides, it is biopesticides that provided the incentive and the model for their development. Discovery of new and effective biological control agents is important not just for the short-term development of new BCPs, but also as a long-term strategy for identifying a diverse and environmentally compatible area of new active ingredients.

Providing tools for sustainable farming: As the stunning growth of organic agriculture continues around the globe (Figure 3), biological control methods of all types—conservation, classical and augmentive—have played a central role.

Filling the gaps left by conventional chemistry: Biological control methods also continue to play critical roles in situations where conventional pesticides are either ineffective due to resistance, are unavailable, or are prohibited. These situations include area-wide treatments for forestry and insect vector pests, pest control in the developing world where conventional pesticides are unavailable or inappropriate and greenhouse applications where worker safety is an issue.

Generating basic knowledge: The discovery, characterisation and development of BCPs has multiple spin-offs including invaluable insights into the complex interactions among crops, pests and the environment that will form the basis for the innovative pest management strategies of the future.

Maximising the potential of commercial biocontrol

Once we acknowledge the social and economic significance of BCPs, it is incumbent to ask how we can best ensure that their potential is fully utilised. This review of the past 50 years in commercial biological control suggests that the following points should be considered when new strategies and policies are developed:

Small markets: BCPs are characterised by limitations that restrict their usage in many situations. The most successful BCPs have been those that target markets where the benefits outweigh the limitations. In most cases, these are niche or speciality markets where annual sales range anywhere from $100,000 to $5 million (US). Experienced producers have learned to survive by streamlining their research, development and operation costs to take these realities into account. Expectations for financial and technical success in biological control need to reflect these realities.

Publicly funded biological control research is essential: One of the biggest mistakes made by the venture capital and large agrochemical companies during the middle era of commercial biological control was to underestimate the cost of product development and ignore the critical role of research, development and manufacturing expertise that had in the past been donated by public and even some private institutions. The profits generated from sales of most BCPs are not sufficient to pay for the entire expense of discovery and product development, and the role of public institutions in continuing to provide this support is critical.

Development of new regulatory policies requires input and technical support from experts outside the biological control industry: Until recently, the cost of conducting safety tests and registering a BCP totalled $1 – 2 million (US) and took 2 – 3 years to complete. Increased scrutiny of the regulatory system for BCPs raises many questions, some of which are valid and some of which probably are not, but all of which add substantially to the cost and time involved in developing a new product. Slim company profits may not be sufficient to support these costs and it may be necessary, if the product has value, for public institutions to become much more heavily involved in providing input, technical data and expertise.

A new approach to IP is needed: Most new biological control discoveries are made by scientists at universities and other public institutions. The trend to patent these discoveries, however, and the imposition of high cost, exclusive technology license fees are incompatible with the small scale nature of the biological control industry. As a result, university and government institutions have not obtained the royalties they anticipated and small businesses have not had the access to new technologies that they need. A re-evaluation of the goals of IP strategies is important and should include an assessment of who the patents are designed to protect and how they can ensure that the new technologies are utilised to their full potential for the benefit of society.

Values-driven entrepreneurs are an important resource: The world of commercial biological control is characterised as much by the passion of individuals committed to the principles of biological control as it is by business principles. This explains why the involvement of venture capital and agrochemical companies ended when the reality of small market sizes was realised. It also explains the re-colonisation of the industry by the smaller, entrepreneurial companies that have been consistently involved over the past 50 years. The modest sales and

even more modest profits that characterise biological control have not deterred these dedicated scientists and entrepreneurs from their mission. These individuals and companies need to be encouraged, supported and solicited for input; their expertise and insights are at the core of future successes in biological control.

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MASTER – Integrating biological control within IPM for winter oilseed rape across Europe

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ABSTRACT

MASTER is the acronym for MAnagement STrategies for European Rape pests, an EU-funded project (QLK5-CT-2001-01447). It seeks to develop economically-viable, environmentally-acceptable management strategies for six pests of winter oilseed rape in Europe by integrating biological control, using key parasitoids, predators and pathogens, within IPM. Key components of the strategies are reviewed.

The project MASTER

MASTER is the acronym for MAnagement STrategies for European Rape pests, the short title for the EU-funded project: 'Integrated pest management strategies incorporating biological control for European oilseed rape pests' (QLK5-CT-2001-01447). The project started in December 2001 and is of four and a half years' duration. The project consortium has partners from six EU countries, namely, Estonia, Finland, Germany, Poland, Sweden and the UK. The main objective of the project is to construct, develop, evaluate and promote an Integrated Pest Management (IPM) System for the European winter oilseed rape crop that integrates and maximises biological control of six target pests by their key natural enemies, while minimising pesticide use.

MASTER focuses on indigenous natural enemies, seeking to conserve and enhance the activity of parasitoids and predators in the crop as well as test inundative release of mass-reared pathogens. Literature on their identity, biology, taxonomy, status and potential has been reviewed and published as a book (Alford, 2003), funded by the EU-funded Concerted Action BORIS 'Minimizing pesticide use and environmental impact by the development and promotion of biological control strategies for oilseed rape pests' which preceded the MASTER project. Gaps in knowledge are being addressed through strategic research on natural enemy biology, phenology, distribution, feeding preferences and host location.

Target pests

MASTER is targeting six major pests of winter oilseed rape in Europe viz. *Psylliodes chrysocephala* (cabbage stem flea beetle), *Meligethes aeneus* (pollen beetle), *Ceutorhynchus assimilis* (cabbage seed weevil), *Ceutorhynchus napi* (rape stem weevil), *Ceutorhynchus pallidactylus* (cabbage stem weevil) and *Dasineura brassicae* (brassica pod midge). These attack the crop successively at various stages of its growth and damage different parts of the plant (Alford *et al.*, 2003). Of these six pests, *C. napi* is not found in the UK.

Key natural enemies

Parasitoids

Parasitoids can exert substantial natural control on rape pest populations. The six target pests are host to *ca.* 88 species of parasitoid, mostly hymenopterous wasps, that attack the egg/larval stages of the host (Alford, 2003). In the project MASTER we have focussed on 11 key species (Table 1), which we consider to be sufficiently widespread and abundant across Europe to be of potential economic importance for biological control.

Table 1. Key larval parasitoids of the MASTER target pests of winter oilseed rape in partner countries

Target pest	Key parasitoid	Family	Occurrence
P. chrysocephala	*Tersilochus microgaster* (Szépligeti)	Ichneumonidae	UK;SE;EE;P;DE
C. napi	*Tersilochus fulvipes* (Gravenhorst)	Ichneumonidae	P;DE
C. pallidactylus	*Tersilochus obscurator* Aubert	Ichneumonidae	UK;SE;EE;P;DE
M. aeneus	*Phradis interstitialis* (Thomson)	Ichneumonidae	UK;SE;EE;P;DE
	Phradis morionellus (Holmgren)	Ichneumonidae	UK;SE;EE;P;DE
	Tersilochus heterocerus Thomson	Ichneumonidae	UK;SE;EE;P;DE
C. assimilis	*Trichomalus perfectus* (Walker)	Pteromalidae	UK;SE;EE;P;DE
	Stenomalina gracilis (Walker)	Pteromalidae	UK;SE;EE;P;DE
	Mesopolobus morys (Walker)	Pteromalidae	UK;SE;EE;P;DE
D. brassicae	*Platygaster subuliformis* (Kieffer)	Platygastridae	UK;SE;EE;P;DE
	Omphale clypealis (Thomson)	Eulophidae	UK;SE;EE;DE

Predators

The polyphagous behaviour of most predators makes it difficult to determine which are key species for biological control within winter oilseed rape. Analysis of published literature showed that at least 160 taxa, mainly beetles (Coleoptera: Carabidae and Staphylinidae), spiders (Arachnida: Araneae), flies (Diptera: Hybotidae, Dolichopodidae, Muscidae), and bugs (Heteroptera: Nabidae and Anthocoridae) were abundant in fields of winter oilseed rape (Büchs, 2003). Their role as predators of the target pests is being determined by strategic research in Germany.

The role of carabids is better known and a focus of the project by all partners. Pitfall trapping has shown that, in winter rape, 14 carabid species achieve a dominance level of 5% or more in at least one partner country (Table 2).

Table 2. Carabid species dominant/subdominant in oilseed rape fields in MASTER partner countries in 2003 (all data from winter rape except Estonia where it is from spring rape) Dominance *** = >10%, ** = >5%, * = >1%

	UK	Germany	Sweden	Poland	Estonia
Amara similata	***	***	***		
Anchomenus dorsalis	*	*	***		*
Asaphidion flavipes	**				
Bembidion lampros	*		***	*	
Calathus melanocephalus					***
Harpalus affinis	*	*	***	*	*
Harpalus brevicollis				**	
Loricera pilicornis	***	***	*		
Nebria brevicollis	***	*			
Notiophilus biguttatus	***	*			
Poecilus cupreus	**	**	*	***	**
Pseudoophonus rufipes	*	*	***	*	***
Pterostichus madidus	***				
Pterostichus melanarius	***	*	**	**	***
Stomis pumicatus		**			

Each country has a rather unique assemblage of carabid species. Firstly, the rape fields of each country (except Sweden) are inhabited by carabids which are dominant exclusively to that country (e.g. *N. brevicollis, P. madidus* and *A. flavipes* in the UK; *S. pumicatus* in Germany; *H. brevicollis* in Poland). Secondly, for several species, there are extreme differences in the dominance level recorded (e.g. *A. similata* 47.2% in Germany, but only 0.2% in Poland; *P. cupreus* 73.2% in Poland, but 2.5% in Sweden; *N. brevicollis* 29.8% in UK, but 2.7% in Germany and 0.0% in Poland). Thirdly, the phenological patterns of certain carabid species differ to a greater extent between the partner countries than that of the pests

and their larvae. Generally, autumnal activity is greater in Eastern (Poland, Estonia) than in Western countries (UK, Germany).

For effective biological control, the carabid must coincide temporally and spatially with its prey and feed on it preferentially. The pests are most vulnerable to predation by carabids as eggs or young larvae in the soil or when, as mature larvae, they drop to the ground to pupate. UK and German partners are determining the temporal and spatial distribution patterns of carabids active on the soil surface of winter rape crops compared with those of pest larvae. Laboratory food preference tests in the UK indicate that, of those species that are both temporally and spatially associated with pest larvae, *Trechus quadristriatus* fed mostly on *P. chrysocephala* eggs, *Nebria brevicollis* on *M. aeneus* larvae and *Anchomenus dorsalis* on *C. assimilis* and *D. brassicae* larvae (Warner, 2001). In Germany, PCR analyses of gut contents of field-collected carabids are being used to determine their food preferences within the crop.

Pathogens

Pathogenic organisms known to exert some natural control of oilseed rape pests include entomopathogenic fungi (epf), nematodes (epn), bacteria and protozoa (Hokkanen *et al.*, 2003). To assess the natural incidence of soil entomopathogenic nematodes and fungi, soil samples were collected and analysed from 10 oilseed rape fields in each partner country in 2002/3. This survey showed that although epf and epn occurred commonly in most soils, incidence was too low in most countries to cause appreciable mortality of an extremely sensitive bait insect (*Tenebrio molitor)* and hence unlikely to be effective on the larvae of target pests.

MASTER partners are, therefore, investigating whether inundative introduction of pathogens is feasible; two organisms, the epn *Steinernema feltiae* and the epf *Metarhizium anisopliae* have been selected for this approach.

Steinernema feltiae

Finnish partners found that application of *S. feltiae* to the soil, at the rate of 1 million infective juveniles/m^2, shortly before the pupation of *M. aeneus* on spring oilseed rape, decreased emergence of new generation adults by 94%; application two months earlier, at the time of sowing of spring rape, did not significantly reduce pest numbers. An inoculation and conservation strategy using *S. feltiae* was tested in 2004 and in 2005 on winter oilseed rape in the different partner countries, with similar promising results. Current research is focussing on developing ways of applying the nematodes at a lower application rate and earlier in the season, using bagged and gel-formulated nematodes, and on crop rotation systems that encourage nematode survival by providing abundant alternative hosts.

Metarhizium anisopliae

In the UK, semi-field cage experiments showed that *M. anisopliae* was disseminated by honey bees to the flowering canopy of winter oilseed rape, where it infected adult *M. aeneus* feeding and ovipositing in the flowers and caused 61% mortality (Butt *et al.*, 1998). The isolate V245, from Finnish soil, was cultured and the conidia harvested, mixed with a diluent of 'Biobeads', and introduced to inoculum dispensers, which were then fitted to the entrances of honey bee hives. Further studies by UK partners have now shown that *M. anisopliae* is

also effective against *M. aeneus* larvae, which feed in the flowers. However, it appears not to cause mortality of *C. assimilis* adults, which feed in the flowers or of their larvae in the pods.

Integrating biological control within IPM

Three collaborative farm-scale field experiments are being conducted by MASTER partners, replicated across five countries (Estonia, Germany, Poland, Sweden and UK). These compare two pest management systems for winter rape within a cereal rotation: a Standard System (STN) and a Biological control System (BC), in which certain husbandry practices are modified to conserve parasitoids and predators.

Each experiment is of two year's duration with pest and natural enemy monitoring in the winter rape crop and in the following winter wheat crop. Data collection and evaluation of crop performance is by means of agreed indicators measuring yield and yield quality, energy and labour inputs, plant density, pod damage and plant architecture and incidence of stem diseases.

The crop husbandry practices modified to enhance biological control are soil tillage, plant density, cultivar choice and insecticide input (Table 3)

Table 3. Plot treatments in three collaborative European experiments comparing a standard system of growing winter oilseed rape (STN) with a system enhanced to conserve biological control agents (BC); ii, insecticide applied prophylactically; ie, insecticide applied only if economic threshold for control is exceeded; io, no insecticide applied. OSR, oilseed rape; TR, turnip rape

Year	Plot	Tillage	Row spacing (cm)	Seed/m^2	Seed mix OSR:TR %	Insecticide applications No.	Rate
2002/4	STNii	plough	12.5	50	100:0	3	Full
	BCio	non-inversion	12.5	50	98:2	0	-
2003/5	STNii	plough	12.5	60	100:0	3	Full
	STNie	plough	12.5	60	100:0	0-3	Full
	BCio	non-inversion	12.5	60	98:2	0	-
	BCie	non-inversion	12.5	60	98:2	0-3	Full
2004/6	STNii	plough	12.5	60	100:0	3	Full
	STNie	plough	12.5	60	100:0	0-3	Full
	BCio	non-inversion	25.0	40	98:2	0	-
	BCie	non-inversion	25.0	40	98:2	0-3	Half

Soil tillage

Many parasitoid and predator species overwinter in the soil of the rape field. Post-harvest soil

cultivations, particularly ploughing and rotary harrowing, can reduce their survival, whereas non-inversion tillage is less harmful. The effect of these soil cultivation practices on survival of the key natural enemies and crop performance is being evaluated.

Plant density

German partners are using different sowing rates and row spacings to investigate the effects of plant density, spatial distribution of plants and plant morphology on parasitism of the larvae of target stem-boring pests. Results suggest that larval parasitism is influenced by plant growth stage, the phenologies of both hosts and parasitoids, and the spatio-temporal within-plant distribution of host larvae. Plant infestation by stem-boring larvae increased as the number of plants/m^2 decreased (Nuss & Ulber, 2004), but no clear relationship was found between plant density and parasitism of *C. napi* and *C. pallidactylus* by *T. fulvipes* and *T. obscurator*, respectively. However, at wider row spacing (40 cm) the larval parasitism of *P. chrysocephala*, *C. napi* and *C. pallidactylus* was greater than that at narrow row spacing (13 cm). Hence in the collaborative experiments this year, a wider row spacing (25 cm) has been used in the BC system than in the STN system (12.5 cm).

Cultivar choice

Trap crops of the more attractive turnip rape (*Brassica rapa*) have been used for many years to lure pests away from the main oilseed rape (*Brassica napus*) crop. Within an IPM strategy, the turnip rape can be used as a trap crop, either surrounding oilseed rape or within it to reduce pest infestation of the main crop. Its potential and mechanism of action has been investigated by the UK partner (Cook *et al.* 2002). Polytunnel bioassays using whole plants, showed that plant growth stage influenced host plant choice by *M. aeneus*. When both oilseed rape and turnip rape were in flower, the beetles show no preference for species. When one species is in flower and the other in bud, the beetles preferred the one in flower. When both were in bud, the beetles preferred turnip rape to oilseed rape.

This approach has been incorporated into the BC system by the inclusion of 2% of the turnip rape cultivar Salut into the seed mixture sown. The preference for turnip rape over oilseed rape at the bud stage should help protect the latter from *M. aeneus* attack, at its most vulnerable green bud stage. Further semi-field experiments by the UK partner are investigating the responses of the key parasitoids to turnip rape.

Insecticides

Standard management of pests on winter oilseed rape throughout Europe still relies heavily on chemical pesticides, most often applied routinely and prophylactically without regard to pest incidence, and at best, according to threshold values of the pest population (Williams, 2004). Application often involves at least three treatments, firstly against *P. chrysocephala* in the autumn, secondly against *M. aeneus/C. pallidactylus* at green bud, and thirdly against *C. assimilis/D. brassicae* at the end of flowering of the main raceme. Over-use of chemical pesticides reduces the economic competitiveness of the crop, threatens biological diversity and risks for the development of insecticide resistance in target pests. Pesticides may also kill the natural agents of biological control. IPM strategies incorporating biological control therefore seek to minimise insecticide inputs without compromising profits.

Economic pest threshold levels above which insecticide application is recommended are an important component of IPM for the crop with potential for minimising insecticide input. They are available for most pests in most countries but vary with country and even within a country depending on severity of a particular pest species. In the UK, for example, the threshold for *P. chrysocephala* autumn and early winter treatment is an average of more than five larvae per plant. In the spring the threshold is 5-10 larvae per plant as the plants are more advanced and able to withstand larval damage. For *M. aeneus* the threshold is five beetles per plant on conventional cultivars, but one per five plants on composite hybrid cultivars. For *C. assimilis* it is usually two weevils per plant, but one per plant where there is also history of attack by *D. brassicae*. Most existing thresholds consider pest numbers only and not those of natural enemies; where the latter are effective, there should be potential to raise the thresholds as the crop should be able to tolerate more pests before sustaining economic damage.

Insecticide inputs to the MASTER collaborative experiments are comparing: a prophylactic three insecticide (pyrethroid) treatment regardless of pest incidence (ii), insecticides (0-3 applications) applied only when local economic thresholds are exceeded (ie) and then applied either at full or at half rate, and no insecticides (io) (Table 3). Insecticide application experiments by the Polish partner indicate that half rate application of insecticide is less harmful to parasitoids than full rate applications.

Broad-spectrum insecticides applied to the crop, particularly during or after flowering, kill many parasitoids and predators. MASTER is investigating ways of achieving better temporal and spatial targeting of insecticide applications to minimize mortality.

MASTER aims to construct a Phenological Model of key parasitoids, that relates their times of occurrence and activity on the crop to growth stage and climatic/weather conditions, for integration into the computer-based decision support system proPlant (Johnen & Meier, 2000). To do this, the phenologies of the occurrence, flight and activity of the key parasitoids to and on the crop are being monitored each year in five partner countries (UK, Sweden, Estonia, Germany and Poland) by means of yellow water traps placed at canopy height in the crop. This information will help to define spray windows compatible with natural enemy conservation. ProPlant already has pest Phenological Models for the six target pests of this project, based on eight years of field observations on the influence of the weather on their population dynamics in different regions of Germany. The program takes into account numbers of adult pests, weather-based forecasts of flight conditions, egg-laying periods and larval development. The models automatically collect regional meteorological data via internet or home-run meteorological stations to predict pest infestation and the need for control.

MASTER partners in UK, Germany and Poland are also investigating the within-field synchrony and co-incidence of the pests and their natural enemies to obtain a more detailed and informative picture of crop colonisation than hitherto achieved and to aid precision timing of treatments and any options for spatial targetting of pests for parasitoid conservation.

Information dissemination

An International Symposium 'Integrated Pest Management in Oilseed Rape', is being organised by the British Crop Production Council (BCPC) on behalf of the MASTER project, to disseminate project results. It will be held in Göttingen, Germany, from 3-5 April 2006. It

will include sessions on parasitoids, predators, pathogens, IPM, socio-economics and policy issues, and will include a review of the collated results from the collaborative field experiments. Details of the Symposium can be found on www.iacr.bbsrc.ac.uk/pie/master/master.htm. A full list of scientific and extension publications from the project can also be found on this website.

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A population based threshold model to demonstrate that physiological manipulation of endogenous reserves can increase the virulence of insect pathogenic fungi

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ABSTRACT

Physiological manipulation of the insect pathogenic fungus *Metarhizium anisopliae*, done by culturing it under water stress conditions, increased the concentration of the osmotically-active solute erythritol in conidia. Using a population based model to analyse the germination of conidia, it was found that physiological manipulation accelerated the *in vitro* germination of conidia over a range of water activities (a_w) by reducing the length of the lag phase of germination. In addition, conidia germinated more rapidly on the surface of the melon cotton aphid *Aphis gossypii,* and fungal virulence was increased under conditions of reduced water availability.

INTRODUCTION

Microbial biopesticides based on insect pathogenic fungi can be valuable components of Integrated Pest Management programmes. They infect their hosts using spores (conidia), which germinate on insect cuticle and penetrate the integument before growing through the body tissues. Because they have contact action, insect pathogenic fungi are particularly suited for use against sap feeding insects which do not acquire pathogens that infect *per os*.

The conidia of many species of insect pathogenic fungi can be mass produced easily and hence they tend to be used as inundative control agents, with little expectation that the fungus will reproduce and persist within the insect population. Their ease of use, combined with high levels of safety to non targets, means that they are potentially attractive options for pest management, particularly in situations where use of chemical pesticides is problematic because of resistance or environmental issues, or where there is pressure to reduce chemical inputs because of consumer concerns about residues. However, the infectivity and performance of inoculants tends to be inhibited in environments where water is lacking constantly or periodically at the insect surface. This is restricting the commercialisation of fungal biopesticides, and methods are needed to improve the efficacy of these products if they are to achieve their full potential.

Improvements in formulation, application, mass production and shelf life are known to give better and more reliable control with fungal biopesticides. However, less attention has been given to optimising inoculum quality. A key issue is the extent to which physiologically-useful compounds affecting the ability of fungal cells to obtain water can be accumulated in

fungal inoculants (Magan, 2001). Water balance in fungal cells is controlled by the synthesis of osmotically-active solutes in the cytoplasm (Jennings, 1995), and the concentrations of these compounds in inoculants can be manipulated by culturing fungi under controlled conditions of reduced water activity (a_w) (Hallsworth & Magan, 1994). The most important solutes for fungal osmoregulation are polyhydric sugar alcohols (polyols) and the sugar trehalose, which can be accumulated at high concentrations under water stress conditions without disrupting enzyme function.

We have quantified the effects of physiological manipulation on the germination over time of conidia of the insect pathogenic fungus *Metarhizium anisopliae* at a range of a_w levels, as well as its infectivity to the melon cotton aphid, *Aphis gossypii* (Homoptera: Aphididae).

MATERIALS AND METHODS

Physiological manipulation

Metarhizium anisopliae (Warwick HRI isolate code 416.96) was supplied by T. M. Butt, School of Biological Sciences, University of Wales Swansea, SA2 8PP UK. Cultures were grown on Sabouraud dextrose agar (SDA, water activity = 0.998 a_w), SDA + 2.27M glucose (Glu-SDA, 0.955 a_w), and SDA + 2.50 M glycerol (Gly-SDA, 0.955 a_w) at 20°C for 15 d. Media were buffered with 0.1 M MES and adjusted to pH 5.8 ± 0.1 with 5 M NaOH. Conidia were harvested in 0.05% Triton X-100 prepared in HPLC grade water, filtered, lyophilised, sonicated and boiled to extract osmolytes (Hallsworth & Magan, 1994). Osmolytes were separated using HPLC (Hallsworth & Magan, 1994) and quantified by comparison with the mean peak areas from standard solutions of known concentration. Osmolyte concentrations were transformed (ln + 0.1) to stabilise the residual variance and analysed by analysis of variance.

In vitro germination

The *in vitro* germination of conidia produced on SDA, Glu-SDA and Gly-SDA was measured over time at a range of water activities from 0.998 to 0.95 a_w on 30% nutrient SDA + 0.1 M MES buffer (pH 5.8) amended with different concentrations of polyethylene glycol (PEG) 200 and 600 to control water activity. Following serial dilution, aliquots of the suspensions were pipetted onto germination media in Petri dishes and incubated at 20°C for up to 30 h. Germination was terminated by applying lactophenol methylene blue, and numbers of germinated and ungerminated conidia were counted for approximately 300 conidia per dish. The germination of populations of conidia over time was described by a generalised linear model incorporating a logit transformation of the proportion germinated and a $\log_e$ transformation of time to normalise the population distributions. Estimates were made for the times for 5% of conidia to germinate, i.e. the lag phase of germination.

Germination on *A. gossypii*

The germination on *A. gossypii* of conidia of *M. anisopliae* 416.96 cultured on Gly-SDA was measured in a laboratory bioassay. Conidia suspensions were sprayed onto groups of 20 fixed age adult apterous *A. gossypii* which were then placed on a leaf of a 3 week old marrow plant (*Cucurbita pepo*, cv. Gold Rush). The leaf was enclosed in a Blackman box and plants

were transferred to a controlled environment room (20°C, 16 h photoperiod, 60% r.h.). Relative humidity was monitored in boxes modified to take a humidity probe and averaged 98 % rh. Groups of ten aphids were removed at regular intervals up to 50 h post-inoculation and preserved in 4% formaldehyde. For germination counts, aphids were stained in 0.5 ml 0.01% Calcofluor M2R and observed under a fluorescence microscope (Butt, 1987). The numbers of germinated and ungerminated conidia on the dorsal surface of the aphids were counted for each time interval.

Virulence bioassay

The virulence of *M. anisopliae* 416.96 was measured in a laboratory bioassay, in which *A. gossypii* were inoculated with conidia and maintained under saturation conditions for increasing periods before transfer to 60 % r.h. for the remainder of the bioassay. Conidia suspensions were prepared following culture on SDA and Gly-SDA and groups of approximately 20 fixed age *A. gossypii* adults were sprayed with suspensions of conidia as described previously. Aphids were then placed onto leaves of 3 week old marrow plants within Blackman boxes that contained gauze-covered ventilation panels at the front and rear. Water was misted into clear polythene bags which were secured over the boxes to provide a saturated atmosphere. The aphids were maintained for 32, 42, 50 h, or for the duration of the assay (9 d) in a controlled environment room (20°C, 16 h photoperiod, 60% r.h.). After the specified time interval, bags were removed to reduce the relative humidity of the bioassay cages to the ambient humidity of the room. The numbers of living and dead aphids were counted daily. Average survival times were calculated based on the total number of aphid cadavers supporting sporulating mycelium.

RESULTS AND DISCUSSION

Physiological manipulation

Conidia of *M. anisopliae* 416.96 produced on Glu-SDA exhibited a 57-fold increase in the concentration of erythritol compared to the control ($P < 0.05$), and a 45-fold increase when produced on Gly-SDA ($P < 0.05$) (Table 1). Production on Gly-SDA also significantly increased the concentration of glycerol in conidia ($P < 0.05$).

Table 1. Back-transformed concentration of osmolytes in conidia of *M. anisopliae* 416.96 produced under water stress conditions

Medium	Intracellular concentration (mg / g conidia)					
	trehalose	glucose	glycerol	erythritol	arabitol	mannitol
SDA	0.18	1.87	0.14	1.26	27.01	121.41
Glu-SDA	2.51	2.56	0.40	71.42	9.39	25.95
Gly-SDA	2.10	0.19	5.06	57.30	4.95	59.64

***In vitro* germination**

The *in vitro* germination of conidia was most rapid at 0.998 and 0.99 a_w, and was delayed markedly below 0.97 a_w (Figure 1). Analysis of deviance showed that there was no benefit in using single lines instead of parallel lines to describe the data set. This indicated that the

main effect of reducing the water activity of the germination medium was to delay germination without changing the distribution of germination times of the conidia population, i.e. to increase the length of the lag phase. In contrast, physiological manipulation by producing conidia on Gly-SDA or Glu-SDA accelerated germination (Figure 1, Table 2).

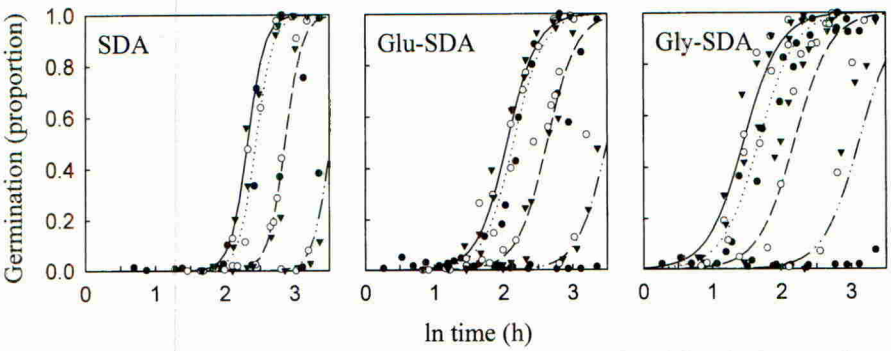

Figure 1. Germination *in vitro* at different water activities of conidia of *M. anisopliae* 416.96 produced on SDA, Glu-SDA and Gly-SDA. The experiment was repeated three times and all data points are displayed: replicate 1 (●), replicate 2 (○), replicate 3 (▼). The lines represent the mean response, back-transformed from GLMs. Water activity levels shown are: 0.998 a_w (—), 0.99 a_w (···), 0.97 a_w (– – –), 0.95 a_w (— · —)

Table 2. Germination *in vitro* at different water activities of conidia of *M. anisopliae* 416.96 produced on SDA, Glu-SDA and Gly-SDA: estimated times for 5% of conidia to germinate (= lag phase)

Culture medium	a_w	Lag phase, h (95% fiducial limits)	
SDA	0.998	6.89	(6.28 - 7.37)
	0.99	8.09	(7.45 - 8.59)
	0.97	12.00	(11.16 - 12.68)
	0.95	24.10	(21.58 - 25.40)
Glu-SDA	0.998	3.95	(3.33 - 4.45)
	0.99	4.99	(4.32 - 5.53)
	0.97	7.15	(6.05 - 8.03)
	0.95	14.49	(8.20 - 17.69)
Gly-SDA	0.998	2.09	(1.37 - 2.62)
	0.99	2.23	(1.39 - 2.88)
	0.97	4.35	(2.94 - 5.35)
	0.95	9.70	(4.89 - 12.61)

Germination on *A. gossypii*

Conidia produced on Gly-SDA germinated significantly faster on *A. gossypii* than conidia produced on SDA. Visual inspection of the time courses (data not shown) suggested that the acceleration of germination could be attributed to a reduction in the lag phase. Estimates

from the generalised linear model indicated that producing conidia on Gly-SDA reduced the lag phase of germination on *A. gossypii* by about half (Table 3).

Table 3. Estimated times for 5% of conidia to germinate (= lag phase) on *A. gossypii* following physiological manipulation

Culture medium	Lag phase, h (95% fiducial limits)
SDA	13.4 (9.28 – 16.88)
Gly-SDA	6.66 (4.15 – 8.86)

Virulence bioassay

Increasing the duration of the period of saturation humidity at the beginning of the virulence bioassay reduced the average survival time of the aphids (Table 4). Conidia produced on Gly-SDA caused shorter average survival times in *A. gossypii* for all humidity regimes ($P < 0.05$).

Table 4. Average survival time of *A. gossypii* infected with *M. anisopliae* 416.96 produced on SDA and Gly-SDA and maintained under saturation humidity conditions for increasing periods before transfer to 60 % r.h.

Initial saturation humidity period (h)	Average survival time (h)	
	SDA	Gly-SDA
32	140.8	132.1
42	137.0	124.3
50	135.1	118.5
constant	102.5	88.6

Physiological manipulation as a method of increasing biopesticide performance

It can be inferred from this study that physiological manipulation could have a significant impact on the efficacy and reliability of microbial biopesticides if it improves virulence in unfavourable environments, for example in glasshouses which have significant diurnal fluctuations in humidity. It is important that sufficient attention is paid to the quality of inocula produced for commercial biopesticides, alongside efforts to maximise yields of inocula in mass production. Further research is required to improve our understanding of the ecophysiological behaviour of inocula in the target environment, and to investigate how inoculum quality can be optimised in commercial production systems without adversely affecting yield. In this regard, it may be instructive to draw lessons from work in the plant seed industry designed to improve the uniformity and germination behaviour of seed batches with techniques such as seed priming, and to understand the effects of the environment on seedling emergence using population-based models (Finch-Savage, 2004).

While there is undoubtedly a requirement to improve the quality of inocula used in fungal biopesticides, it must be remembered that the active constituents are living organisms, and as such it is unrealistic to expect them to perform to the same levels of efficacy as conventional,

chemical pesticides. The development of fungal biopesticides tends to be done according to a chemical pesticide model, and while this approach has certain benefits, it has a serious negative consequence in that the fungal agent starts to be thought of as a chemical analogue, with false expectations of chemical-like performance. Under the chemical model, the unfavourable characteristics of fungal biopesticides compared to chemicals are highlighted, while attractive biological traits are overlooked, such as the ability to reproduce and persist within host populations (Waage, 1997). How then, can methods, such as the physiological manipulation of inocula, be used to improve biological control without reinforcing the treatment of fungal biopesticides as chemical clones? The answer must lie in combining technological improvements with knowledge of fungal ecology and the evolution of virulence. For example, physiological manipulation could be applied to the trade off that occurs in fungal isolates between speed of kill and the production of conidia on cadavers. The latter influences the ability of isolates to spread within insect populations, i.e. their epizootic potential. In biopesticide development programmes, fungal isolates with high epizootic potential are usually overlooked in favour of isolates that kill quickly. It may be possible to use physiological manipulation to improve the infectivity of inoculum produced from isolates with high epizootic potential but slow speed of kill, and thereby make them more attractive candidates for commercialisation. This approach might result in a more rapid initial kill with these isolates, but would not impair the ability of subsequent generations of the fungus to persist within the insect population by secondary cycling, resulting in extended pest control.

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Summary of the UK efficacy evaluation process and requirements for biological products

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ABSTRACT

The evaluation of efficacy forms part of the statutory regulation of pesticides. Approval depends on demonstrating a measurable benefit, through control of the target or reducing harmful effects. For most situations there is no absolute minimum level of control that must be achieved. The evidence presented in the application for approval should support the label claims, which must reflect both product performance and the conditions required to achieve this. The Pesticide Safety Directorate (PSD) operates a flexible system to address efficacy requirements, accepting relevant data from a range of sources including non-UK data, public domain information, and evidence from grower's trials. In addition, and particularly relevant for biological products, there is scope to use reasoned cases in certain areas. An alternative approach that may be available is mutual recognition of approvals or data packages from other Member States. PSD recognises the importance of biological products in developing a strategy for sustainable plant protection products. To lessen the regulatory burdens for biological and other 'alternative' products, there is currently a pilot scheme based around reduced data requirements and registration fees. Applicants are encouraged to meet with PSD at an early stage in product development, including discussion with efficacy specialists on requirements and appropriate trials design.

INTRODUCTION

Biological products can be a valuable component in integrated control programmes, one aim of which is to limit the use and impact of chemical plant protection products on non-target organisms and the environment. Their importance has increased in recent years, both with pressure to reduce conventional pesticide use, and as an additional tool in pesticide resistance management strategies. However there are currently relatively few biological products on the UK market. One of the main reasons given is the apparent regulatory burden, both in terms of data requirements and costs. To encourage more products onto the market, PSD set up a pilot scheme for alternative products. This is designed to assist in the compilation of reduced data requirement packages and is based on significantly reduced fees for pheromones, biological (microbial), and plant extract products. This experience will be used in the longer term to produce a reduced fee structure where appropriate. The first product to complete the registration process via this scheme was a pheromone mating disruption product approved in 2004. The pilot scheme also supports part of PSD's development of a 'National Strategy for the Sustainable Use of Plant Protection Products' (see www.pesticides.gov.uk). One aim is to examine ways to encourage the development and uptake of alternatives to chemical pest control. A perceived barrier in the registration of biological products is the efficacy requirements, both in terms of the standards applied and amount of data required. This paper gives an overview of the efficacy evaluation process and the approaches that can be used to address the data requirements,

including specific issues facing biological products.

REGULATORY BACKGROUND

One question asked is why efficacy is evaluated under the regulatory process instead of 'allowing the market to decide'. The statutory control of pesticides came into force as part of the Food and Environment Protection Act (FEPA) (1985). The mechanisms of the approval process were detailed in the Control of Pesticides Regulations (COPR) (1986). Efficacy consideration was defined by one of the aims of the act to 'secure safe, efficient and humane methods of controlling pests'. The harmonisation of regulation within the European Union was introduced in 1991 (Directive 91/414/EEC). Further directives detail the data requirements including efficacy (93/71/EEC), and guiding principles for Member States to ensure a consistent approach (97/57EC, 2005/25EC). Important efficacy concepts were introduced regarding good experimental practice (GEP), use of guidelines, dose justification, and consideration of resistance. In the UK the directives were implemented by the Plant Protection Products Regulations (PPPR) which applies to new active substances, and subsequently those existing active substances re-registered following their review. The approval of pesticides is a risk/benefit analysis, evaluating risk of exposure to consumers, operators and the environment. Consideration of efficacy determines the benefit of use, balancing effectiveness against negative impacts (e.g. crop safety). The independent assessment of product performance prevents unnecessary exposure to the environment, users or consumers, and unnecessary costs to the grower (including economic/resources). It is therefore not only a statutory requirement, but a key element in supporting the UK policy on the minimisation and sustainable use of pesticides.

ADDRESSING DATA REQUIREMENTS

The efficacy data requirements will not be discussed in detail, full information is available on the PSD website (www.pesticides.gov.uk) in 'Chapter 8' of the 'Data Requirements Handbook'. There are also a wide range of accompanying guidelines, addressing both specific crop/target situations and general issues e.g. numbers of trials and writing a biological dossier. The requirements examine both effectiveness and crop safety (including yield) and, where appropriate, impacts on succeeding and adjacent crops. Under PPPR there is further emphasis on various aspects of crop safety, and a need to submit preliminary data. Two significant new requirements, as mentioned above, relate to the principles of sustainable pesticide use. Dose justification is required for key label targets, whether economically important or difficult to control. Evidence must demonstrate inferior performance (lower or inadequate levels of control; shorter persistence of effect) at doses lower than those proposed (EPPO 1/225(1). This can be addressed by including lower doses (e.g. 0.6 – 0.8N) in the field trials. A resistance risk analysis is also required, based on resistance history of target and active, mode of action, and proposed use. In high risk situations modifying factors may be required to limit exposure (e.g. number of applications), and an appropriate resistance management strategy. For biological products their novel mode of action usually makes them positive contributors in resistance management programmes.

a) Use of preliminary data

Preliminary data includes laboratory based research, glasshouse screening data and small scale

trials. Biological products often involve novel techniques and background information is helpful to the evaluator in assessing the data and understanding how the product will be used. More importantly, such data can be used along with reasoned cases to address various areas of the data requirements. For products targeting pests and diseases this approach is relevant for various aspects of crop safety. Standard glasshouse pre- and post-emergence screens on a range of plant species can provide sound evidence of the lack of plant activity. This, alongside appropriate observations in effectiveness trials, could address crop phytotoxicity and impacts on succeeding/adjacent crops without the need for designated crop safety trials. (In contrast products with herbicidal activity will require specific crop safety trials at both 1N and 2N, with some taken to yield (EPPO guideline 1/226(1)). Preliminary data can also be used as evidence for dose justification when required, as well as supporting the effectiveness claims.

b) Location of trials

PSD has always accepted non-UK trials, indeed some product approvals are based entirely on such data, provided there is an appropriate case demonstrating comparability of relevant conditions (agronomic, edaphic, target, climate) Those conditions which are relevant will depend on the product's mode of action and use e.g. soil type is relevant to soil applied but not foliar applied products. Even where not comparable the data may still be acceptable provided the conditions are at least as challenging. For example, in warmer climates pest pressure may be greater because it allows for more generations per season and is, therefore, a more challenging situation to deliver effective control. Climate will need to be considered for all field applied products. The Crop Protection Association (CPA) prepared a climate comparability paper in liaison with PSD defining a zone across Northern Europe where climate is considered comparable to the UK. For trials conducted in this area applicants may simply refer to the zone without the need to submit any further specific meteorological data. The UK has encouraged this approach to be taken forward through EPPO, and a draft version of defined zones across Europe is awaiting final approval in the autumn.

b) Trial design and conduct

PSD provides guidance both on general principles of trials design and also specific crop/target situations. Under PPPR trials should be conducted in accordance with relevant European and Mediterranean Plant Protection Organisation (EPPO) guidelines. Guidelines set out minimum standards on key issues such as including untreated controls and standard treatments, plot size, number of replicates, and assessment methods. A common problem for biological products is that existing guidelines are either inappropriate or unavailable. PSD (and EPPO) recognise that deviations may be necessary (e.g. no available standard) or guidelines may not be relevant, particularly for products with novel modes of action. Therefore non-standard trials designs are acceptable provided there is a full explanation and appropriate justification of methods used (UK Efficacy Guideline 113, EPPO guideline PP1/223(1)).

Lepidopteran pheromone mating disruption products illustrate where alternative methodology is appropriate. Treated plots need to be large scale (around 5 ha) and separated from untreated areas to prevent continuous migration into treated plots. Monitoring flight activity to identify application timing cannot be done using standard pheromone traps because of potential target interference from the test products. Assessments focus on damaged fruit because the target itself is not controlled, and the site history of typical fruit damage is very important, particularly when there is significant distance between plots. The methodology is therefore radically different to a

standard randomised small plot trial for a conventional insecticide, but justified because of the mode of action and type of benefit being assessed (Efficacy Guideline 640).

Under 91/414 efficacy trials must be conducted according to GEP by testing organisations which are officially recognised. In the UK the scheme was introduced on 1st January 1998 (UK guideline 110) and the same principle applies in other Member States. Data generated after the relevant date by organisations not officially recognised cannot be considered as part of the core package of required trials. In some circumstances it may be permissible to accept as supporting data. Data from non-EU countries may be accepted where there is evidence that GEP was used. This requirement should be considered during the initial planning of developmental work. For research organisations involved in biological products it may be appropriate to consider applying for official recognition. This can be seen as a long term investment beneficial to all areas of development by ensuring maximum (regulatory) value for the data generated.

c) Data from other sources including other Member State approvals

Applicants may submit public domain evidence from e.g. published papers to support label claims provided their relevance is clearly explained. For microbial products this may include data on related microbial species. Factors to consider would include relevance of test conditions, dose, and formulation to the proposed use, and justification for any non-UK data. Evidence from grower's trials may also be accepted, provided they are actively supervised to ensure appropriate conduct and reporting of results (UK Guideline 112). An alternative approach may be to 'mutually recognise' an existing approval or previously evaluated data from another Member State. PSD consider these on a case by case basis to determine their relevance to UK conditions but there is no re-evaluation of any data. Details provided by the applicant on the conditions/location under which the supporting data were generated are very useful in determining their relevance. Where there are significant differences such data may still provide the basis for an approval with some limited confirmatory data to address particular concerns.

d) Amount of data required

Applicants need to address each area detailed in the relevant legislation. For biological products the use of preliminary and public domain data in some areas, particularly those relating to crop safety and other adverse effects, may be sufficient. Furthermore, for naturally occurring substances a comparison of dose/exposure levels with natural background levels can also be used as a reasoned argument instead of submission of data. This approach forms the basis of the OECD guidance document on pheromones and other semiochemicals. Arguments can be made in many areas of risk assessment (e.g. fate and ecotoxicology) and crop safety based on exposure levels being below those released naturally by organisms.

The main areas to be addressed will be effectiveness and supporting the product label. As a guide, for chemical pesticides the number of trials for a major target is normally ten spread over a two year period to demonstrate performance over a range of climatic and environmental conditions. An appropriate distribution of trial sites across main growing areas is important to ensure factors such as plant cultivar and target pressure are included. Minor targets/crops require only three trials. PSD guidance identifies specific major and minor targets for cereals, top fruit, oilseed rape and other brassicas, and will discuss individual crop/target situations with applicants. Some claims are for a target group rather than individual species e.g. 'caterpillars' which can be supported from a range of 2-3 trials on each of the key species. Various situations allow scope for a reduction in the number of trials necessary. In protected situations where

environmental conditions are more controlled fewer trials may be appropriate and can be conducted in one season. Other factors allowing a reduction include significant difficulties in trials conduct, a sporadic target, or, as with pheromones, the need for large areas when testing. It is important to stress that the number of trials required is flexible depending on the quality of data provided and supporting evidence available. For biological products the same approach can be taken of using evidence from a wide range of sources. Using all available information the applicant can then use a smaller number of appropriately conducted trials to confirm field performance and draft their label. The latter is important in providing specific guidance to users on appropriate conditions, for example any agronomic practices which help to maximise the effectiveness.

Extrapolation of existing data to support either new claims or formulation changes is a common approach. In some cases the extrapolation may require no further data e.g. closely related target/crop or minor formulation change. In others some confirmatory data over one season will be required. The extent of the existing database along with factors relating to the similarity in proposed new use will determine whether and what additional data may be required. For new actives, particularly when development resources are limited, it is worth considering supporting just one or two key uses/targets during registration. Once approved and marketed it then becomes more cost effective, and is a simpler registration process, to add additional uses.

LEVELS OF ACCEPTABLE EFFICACY

A key misconception is that approval is dependent on having high levels of control and being comparable to an existing standard. The approval of any product, regardless of mode of action, is dependent on evidence demonstrating a measurable benefit. The important point is that the label reflects the level of control (or benefit) achieved, which can be wide ranging, and any conditions under which lower or more variable levels of effectiveness may occur. The UK approach follows the EPPO principles of acceptable efficacy (guideline 1/214 (1)). Products should provide statistically significant benefits compared to the untreated control, reflecting the need to limit exposure of all products. Product performance should be of the same order as existing commercial standards (where available). However, lower effectiveness is acceptable when the product has other advantageous properties. These include a wider range or greater flexibility in uses, fewer limiting conditions, greater compatibility with cultural or other plant protection measures, lower resistance risk and fewer undesirable effects. Biological products meet many of these criteria and in addition their approval may also be justified by providing important alternatives in resistance management strategies for existing chemicals. Only in certain specific situations are high levels of control a requirement e.g. seed borne disease control must be at least 98% to comply with certified seed claims. The label claims for biological products can be tailored appropriately reflecting their mode of action. It may be more relevant, for instance, to refer to limiting or reducing levels of damage rather than control of populations, particularly where the target effects are on crop quality. As a guide, for fungicides and insecticides, claims for full control refer to control over 80%, 60-80% may be described as 'useful' or 'partial' control, and 40-60% as 'reduction'. Control levels less than 40% are still acceptable provided that there is a defined and proven benefit.

SUMMARY

Efficacy testing for biological products can present particular challenges but the regulatory requirements should not be seen as a barrier. Applicants should consider the requirements early in the development stages so that data are both relevant and generated in an appropriate fashion. Data requirements can be addressed by a combination of evidence from wide ranging sources, reasoned cases and, particularly for effectiveness, some field trials data. Alternatively, an approach based on approvals and data evaluated in other Member states may be possible. The need for non-standard trials design is accepted and applicants are advised to discuss their proposals with PSD initially. Trials should supplement existing evidence and be used to draft appropriate label claims with information, if relevant, on conditions where control may be more variable. Generally, a wide range of claims are acceptable provided a measurable benefit can be demonstrated. The more extensive the database, the greater is the potential to extrapolate to additional claims either directly or with some limited further data. It is recognised that biological products are an important tool in the sustainable use of pesticides and food production. PSD are looking to build on the experience of the pilot scheme and in the area of efficacy have used it to provide guidance on trials for pheromone products. More recently an efficacy working group has been set up with the International Biological Manufacturers Association (IBMA). The aims include developing more specific guidance with experts in relevant fields, as well as providing closer links with a sector of the crop protection industry that it is recognised is less familiar with the registration process. PSD is also involved in several European initiatives designed to reduce the amount of efficacy data required and to encourage the availability of more active substances, particularly for minor crops. This includes an EU contract to draft efficacy extrapolation guidance based on existing knowledge from all Member States.

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Auto-dissemination of a fungal pathogen for codling moth control in apple

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ABSTRACT

Here we describe work in progress to develop a highly specific approach to controlling codling moth by attracting male moths to a pheromone-baited inoculation device and infecting them with an entomopathogenic fungus. Laboratory and field trials demonstrated that pheromone dispensers made from plastic vials or rubber septa were equally effective, even though the extent of pheromone isomerisation was greater in rubber septa than plastic vials. A virulent isolate of the entomopathogenic fungus, *Beauveria bassiana*, was selected in laboratory bioassays. Inoculation devices were developed and shown to be effective at attracting male moths, contaminating them with fluorescent powder and releasing them again in laboratory and field trials. When a device containing the entomopathogenic fungus was used, up to 75% of recaptured males were infected in the field. Although this may be an overestimate due to the method of recapture used, it demonstrates the potential of this strategy.

INTRODUCTION

Codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), is a serious pest of apples in Europe. Due to the development of insecticide resistance in this pest, there is an urgent need for alternative, more sustainable management methods which minimise insecticide use. The authors are carrying out a collaborative research project to combine the use of a pheromone lure and an entomopathogenic fungus within an inoculation device for control of the codling moth. In response to the pheromone, male moths are attracted inside the inoculation device

where they become contaminated with, and infected by, spores of the fungus. After subsequent escape from the inoculation device, transfer of the fungus to females during mating and to the resultant eggs and larvae should provide multiple opportunities for breaking the life cycle. During the project the pheromone lure has been optimised, entomopathogenic fungi collected and evaluated against codling moth and non-target organisms and effective inoculation devices developed and evaluated in the field.

MATERIALS AND METHODS

Release rates and isomerisation of pheromone in different dispensers

Polyethylene vials (22 mm x 8 mm x 1 mm thick wall; Just Plastics, Norfolk, U.K.) and rubber septa (18 mm x 9 mm; Sigma-Aldrich; Dorset, U.K.) were evaluated as pheromone dispensers. Both types of dispensers (50 of each) were impregnated with (*E,E*)-8,10-dodecadienol (codlemone; 1 mg; International Pheromone Systems Ltd. (IPS Ltd), Wirral, U.K.) by adding a solution of the pheromone in petroleum spirit (b.p. 40-60°C) and allowing the solvent to evaporate. These dispensers were hung outside in white plastic delta traps (IPS Ltd.) during June 2004 in UK. Every 2-3 days for 16 days, three of each type of dispenser were removed and stored in a freezer (-20°C). To determine the amount of pheromone remaining in dispensers, the dispensers were extracted individually in hexane (5 ml) containing tetradecyl acetate (1 mg) overnight at room temperature. The resulting solution was analysed by capillary gas chromatography using a fused silica capillary column (30 mm x 0.32 mm id) coated with polar DBWax (Agilent Technologies; Cheshire), helium carrier gas (2 ml/min), splitless injection and oven temperature held at 60°C for 2 min then programmed at 6°C/min to 230°C. A flame ionisation detector was used and data were captured and processed by EZChrom Elite v3.0 software (Scientific Software Inc., California, USA). Results are the mean of the three samples analysed individually.

Field efficacy of different pheromone dispensers

White, plastic delta traps were baited with Natural Resources International (NRI) vials, NRI septa (as above) or commercially available rubber septa impregnated with codlemone (5 mg; PheroBank, The Netherlands). There were five replicates of each treatment. Traps were hung 20m apart in an orchard in Lleida, Spain and catches were recorded and discarded every 2-3 days. Three consecutive trials were carried out each lasting three weeks between July and September 2004. At the end of each trial, the dispensers were collected and sent to NRI for analysis as above. Results were analysed for two 10-11 day periods in each trial. Total catches in each replicate were transformed to log(x+1) and subjected to two-way analysis of variance. Differences between means were tested for significance at the 5% level by the Least Significant Difference (LSD) test.

Pathogen selection

The virulence of three isolates of the entomopathogenic fungus *Beauveria bassiana*, selected on growth and pathogenicity attributes (data not shown), against fifth instar codling moth larvae was compared. Isolates used were GHA (active ingredient of Botanigard®), EM1 from codling moth in UK and Ja1 from codling moth in Spain. All isolates were grown on Sabouraud dextrose agar, incubated at 23°C in darkness and the spores harvested after 14

days. Groups of ten, fifth instar larvae were dipped into 10ml suspensions of a range of concentrations from $1x10^2$ to $1x10^6$ conidia per ml. There was one replicate group of larvae for each concentration/ isolate combination and a control (just 0.03% Tween 80). The experiment was run on two occasions. Mortality was recorded at regular intervals after inoculation. Data from the two experiments were combined and probit analysis done to determine an LC50 value for each isolate at 7 DAT.

Field evaluation of castellation-type inoculation devices with entomopathogenic fungus

Experiments using fluorescent powder demonstrated that modifications of dish and castellation traps gave high levels of contamination and release of moths in both laboratory and field studies (Hartley *et al.*, 2005). In Spain, a castellation-type inoculation device containing spores of the entomopathogenic fungus *Beauveria bassiana* (1g; isolate GHA technical product from Emerald Biosciences, USA) was surrounded by four unmodified castellation traps (20 m apart). The inoculation device was left continuously in the field and the spores were replaced weekly. All inoculation devices and surrounding traps were baited with a rubber septum impregnated with codlemone (1 mg; IPS Ltd). The unmodified castellation traps caught live codling moth males and were deployed for four nights each week. Each day the adults captured were returned to the laboratory and kept individually under ambient environmental conditions until they died. Dead moths were evaluated for *B. bassiana* infection. Two replicates separated by 100 m were made between July and September 2004.

RESULTS

Release rates and isomerisation of codlemone

When polyethylene vial and rubber septa pheromone dispensers were exposed in delta traps in UK, analysis of the codlemone remaining at intervals showed the septa had a half-life of 7 days whereas the vials still contained 90% of the initial loading of pheromone after 16 days. Furthermore, the extent of isomerisation of the pheromone was much greater in the septa than the vials (Table 1). After 16 days the percentages of the active *EE* isomer were 98% in the vials and 63% in the septa. In fact there seems to have been appreciable isomerisation of the codlemone in the septa during the making up of the lures. The pheromone used contained 99.9% of the *EE* isomer, and this was unchanged in the vials. However, the codlemone in septa sampled immediately after completion of making up the lures contained only 95% of the *EE* isomer (Table 1).

Table 1. Isomeric ratio of pheromone remaining in lures used in NRI trap experiment (2-18 June 2004)

	VIALS (mean % each isomer, n=3)				SEPTA (mean % each isomer, n=3)			
	ZE	**EE**	EZ	ZZ	ZE	**EE**	EZ	ZZ
Day 0	0.13	**99.87**	0.00	0.00	0.78	**94.81**	1.78	2.62
Day 16	0.68	**98.01**	0.65	0.67	11.23	**63.44**	13.92	11.41

Field efficacy of different pheromone dispensers

There were no significant differences ($P > 0.05$) between catches of codling moth males with any of the three dispensers in any of the trials (Table 2). This was true for successive 10-11 day periods in each trial during which differing amounts of isomerisation would have been expected to have occurred. Analysis of the dispensers at the end of each trial confirmed much greater isomerisation of the codlemone in the septum dispensers than in the vials (Table 2). Subsequent experiments (data not shown) confirmed that the isomeric composition of the pheromone remaining in the dispenser was identical to that released and trapped on Porapak resin.

Table 2. Catches of male *C. pomonella* moths during trapping trials in Spain and percentage of active *EE* isomer of codlemone at end of each trial (July-September 2004; 5 replicates)

Dispenser[2]	Trial 1			Trial 2			Trial 3		
	Mean catch[1]		%EE	Mean catch[1]		%EE	Mean catch[1]		%EE
	8/7-19/7	19/7-29/7		29/7-9/8	9/8-19/8		23/8-2/9	2/9/-13/9	
NRI septum	2.1	5.3	76.4	2.0	2.1	70.7	0.9	0.3	80.7
NRI vial	2.5	4.0	90.1	2.1	2.7	90.5	0.6	0.5	96.0
PB septum	1.8	3.6	84.1	1.8	2.2	77.3	0.7	0.3	89.5

[1] Mean catch per trap per night

[2] NRI septum and NRI vial are lures prepared at NRI; PB septum from PheroBank

Pathogen selection

There was a significant difference between the LC_{50} values for the different isolates evaluated ($F_{2, 15}$=8.46, P = 0.003). Isolate GHA was the most virulent with the smallest LC_{50} value (Table 3) followed by isolate EM1 and then Ja1.

Table 3. Virulence of three isolates of *B. bassiana* (Ja1, EM1 and GHA) against fifth instar larvae of codling moth in experiments using a range of doses from $1x10^2$ to $1x10^6$ conidia/ml

Isolate	LC_{50} (CI)	Intercept (s.e.)	Slope (s.e.)
Ja1	$5.9x10^5$ ($5.7x10^4$-$1.1x10^7$)	-3.86 (1.06)	0.699 (0.186)
EM1	$1.1x10^5$ ($7.8x10^3$-$1.3x10^6$)	-3.38 (1.01)	"
GHA	$1.5 x10^3$ (10-$2.1 x10^4$)	-2.11 (0.902)	"

Field evaluation of inoculation devices containing entomopathogenic fungus:

When a castellation-type inoculation device containing the entomopathogenic fungus, *B. bassiana,* was surrounded by unmodified castellation traps to catch live moths, levels of infection of up to 75% in recaptured moths were observed (Figure 1). Overall mean level of infection was 51.1 % on seven sampling occasions. .

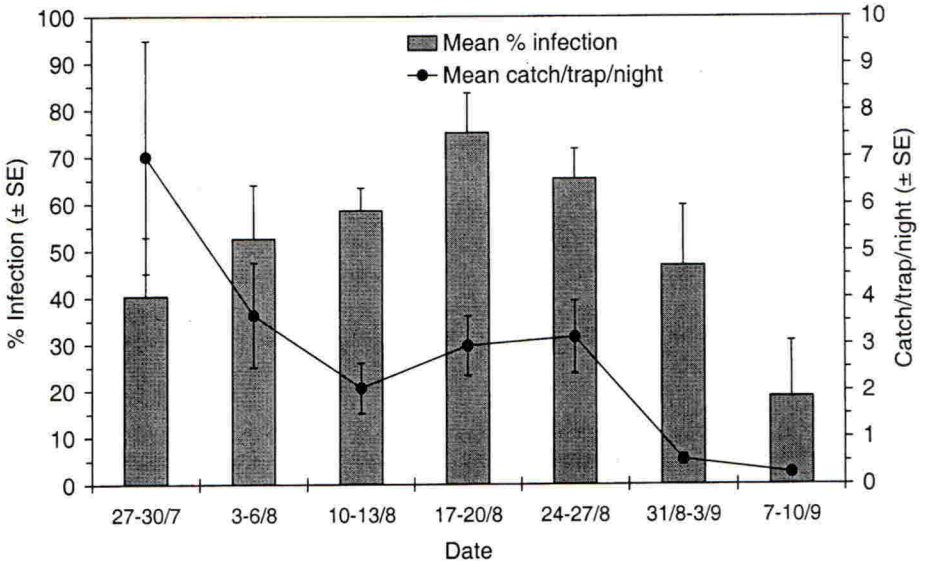

Figure 1. Percentage of male moths infected with *B. bassiana* after contamination in a castellation-type inoculation device and recaptured live in unmodified castellation traps (mean catches and % infection (± SE) over four nights on seven sampling occasions shown)

DISCUSSION

Although the extent of isomerisation of codlemone was much greater in rubber septa dispensers than in polyethylene vials, this did not affect the attractiveness of the lures to male codling moth in field trapping tests. Addition to the *EE* isomer of small amounts of the *ZE* isomer (El-Sayed *et al.*, 1998) or the *EZ* isomer (Witzgall *et al.*, 2001) have been reported to increase attractiveness in wind tunnel tests, although El-Sayed *et al.* (1998) found the thermodynamic equilibrium mixture of isomers (66% EE) was significantly less attractive than the pure *EE* isomer in field trapping tests. Furthermore, septa or vials impregnated with 1 mg of pheromone were as attractive as septa impregnated with 5 mg of pheromone, at least over the three-week period of the trials carried out here. A virulent isolate of *B. bassiana* was selected in bioassays against third instar larvae and in subsequent assays first instar larvae and adults were also found to be highly susceptible. When contamination by the castellation type inoculation device containing spores of *B. bassiana* was investigated, up to 75% of moths became infected. This is probably an overestimate as moths had to be recaptured live in unmodifed castellation traps so that cross-contamination between moths in the trap could have occurred. Nevertheless, it is further evidence that this trap can act as an effective attract-and-

release inoculation device with a simple formulation of fungal spores. Further field trials evaluating this approach to reduce damage by *C. pomonella* are being carried out in orchards in Spain and UK during 2005.

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