SESSION 4C

DEVELOPMENT OF PATHOGENS FOR BIOCONTROL

SESSION ORGANISER DR N. E. CROOK

POSTERS 4C-1 to 4C-8

TECHNICAL IMPROVEMENTS TO BIOPESTICIDES

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ABSTRACT

Although the use of bacteria, fungi and viruses to control insects represents well under 1% of the crop protection market in terms of value, a number of recent developments have improved both the products and the prospects for biopesticides. In particular, the understanding of the mode of action of *Bacillus thuringiensis* (*Bt*), the active ingredient in most of the commercial biopesticides, has increased by the application of biotechnological methods. In addition, the recent steep increase in sales of *Bt* products (up around 80% in the past three years) has been due to improvements in formulation and production which have provided ever more cost-effective products, some of which can compete directly with chemicals. New inventive products are available in 1992 based on conjugation, genetic engineering, endophyte carriers and patented formulations that put well-known and understood pathogens into insect control products for the first time. Our recent survey of the biopesticide field suggests that these developments are likely to make these biological agents into more effective insecticides and disease control agents in the future.

BACTERIA

Bacillus thuringiensis (*Bt*), an aerobic gram-positive spore-forming bacterium, remains the focus of the majority of research on biopesticides. When it was discovered, and until 1978, *Bt* was thought to be active solely against a limited range of *Lepidoptera* and these strains form the basis of a number of well known products from companies such as Abbott, Sandoz and Novo - Dipel™, Thuricide™ and Biobit™ respectively. Cheaper, more active formulations of *Btk* such as Foray™ are increasingly being used to control insects over large areas of forests.

The recent discovery of strains effective against nematodes, animal ectoparasites like mites and endoparasitic protozoans, as well as the earlier discoveries of *Bt* active against *Coleoptera*, particularly the Colorado beetle (*Btt* and *Btsd*), and before that against *Diptera* including disease-carrying mosquitoes (*Bti* or *Bt* H-14), has created a range of major new commercial opportunities - Vectobac™, Teknar™ and Skeetal™ based on *Bti* and Diterra™, Trident™ and Novodor™ for use against Colorado beetles. In addition, many new products have been introduced over the past three years; more product launches are planned and it is possible to envisage a steady stream of new products based on the ever more diverse activities of new strains of *Bt*. Bactec, for example, launched the products Bernan I™, II™ and III™ based on novel patented *Bt* strains from the USDA. Field trials over several years suggested the trial product performed well; several of the products have recently received registration in the USA and additional registrations are pending eslewhere.

New products against *Diptera* include Acrobe™ launched by Cyanamid in 1991 and produced with Becker Microbial. It is claimed to have a unique aqueous formulation that can be applied with conventional ground and aerial release equipment.

The use of both genetic engineering and non-recombinant techniques are yielding novel *Bt* strains with increased insecticidal activity as well as novel formulations. Techniques such as mating (conjugation) and partial curing are used to generate strains with a novel combination of plasmids and the agricultural biotechnology company, Ecogen, has launched Condor™, Cutlass™ and Foil™, all based on non-recombinant novel strains. Although historically, non-recombinant methods have raised fewer regulatory issues than the use of recombinant DNA technology, this distinction will become of decreasing importance in the future. Another biotechnology company, Mycogen, has launched recombinant products MVP™ and M-Trak™ based on a Pseudomonad with *Bt* toxin genes incorporated. Killing the bacteria has eased fears about the release of genetically manipulated microorganisms. Recombinant technology has also been used to insert a *Bt* toxin gene into various different hosts, including *Escherichia coli*, *Bacillus subtilis*, a blue-green algae as well as into tobacco, tomato, maize and cotton plants to produce pest-resistant plants.

A novel approach has been taken in the development of InCide™ by Crop Genetics International which is scheduled to be launched in the US in 1993. The product is based on *Clavibacter xyli* var *cynodontis* CGI02, a maize endophyte incorporating a *Bt* endotoxin gene with activity against European corn borer. The endophyte rapidly colonizes the roots, leaves and stems of maize plants where it remains for the duration of the plant's life. It need only be applied in a small dosage (milligrams per hectare) as it multiplies inside the plants. The product functions only within target plants and will not survive outside it and is claimed to be environmentally safe. A range of endophytes are planned which are capable of colonizing maize, cotton, soybeans, wheat, rice and other major crops.

B sphaericus (*Bs*), once a subject for keen interest and then upstaged by *Bt* H-14 is again under active study due to its residual activity against mosquitos and blackfly. Although a number of new products were planned several years ago, none have yet appeared. However, the near future may see the introduction of Sphaerimos™, an as yet unregistered product developed by Duphar, now owned by Novo, for use against *Culex* species of mosquito which are not well controlled by *Bti*. Abbott is reported working on a species of *Bs* for control of mosquitoes in the Rhine.

Another bacterium reaching the market is the streptomycete, *Streptomyces griseoviridis* in Mycostop™, a biofungicide from Kemira. Tests suggest good control of a range of seed-borne and soil-borne pathogens including *Alternaria brassicola*, *Fusarium* on cereals, *Fusarium oxysporum* on carnation and *Botrytis cinerea* on lettuce. The bacteria releases antibiotic substances which inhibit the growth of *Alternaria* spp on cauliflower, *Rhizoctonia solani* on oilseed rape, *Pythium* spp on sugarbeet and cucumber, *Fusarium* spp on carnation, tomato and cereals, *Phomopsis sclerotioides* on cucumber and *Botrytis cinerea* on lettuce and carnation. Other bacteria currently under active study include *Bacillus pumilus* and *B. mycoides* which are being investigated for control of take-all; both worked in greenhouses and, to some extent, in outdoor trials. Strains of *Enterobacter cloacae* and *Erwinia herbicola* have been successful in reducing the incidence of *Pythium* seed rot and pre-emergence damping-off. A strain of the antagonistic bacterium, *Lactobacillus plantarum* has been found to inhibit the plant pathogens, *Xanthomonas campestris*, *Erwinia carotovora* and *Pseudomonas syringae in vitro* by researchers in South Africa. *Pasteuria penetrans* has been reported to give good control of nematodes although the organism is difficult to cultivate.

Genetic engineering may result in the improvement of other species to make them more effective against various targets. Protein engineering is being used for analyzing the molecular basis of toxin specificity to allow new types of toxin to be 'designed'.

FUNGI

Although there are several hundred entomopathogenic fungal species, only about 20 species have been studied as control agents and their commercial development has been slow due to problems of low pathogenicity, reduced viability of inoculum, differences in virulence within a pest species, production problems and constraints imposed by temperature/humidity requirements.

Verticillium lecanii was the first fungus to be commercialized in Europe for use in glasshouses but its temperature and humidity requirements made it unsuccessful for outdoor use and unreliable indoors. However, recent years have seen a new production method, new formulations and new entrants that may restore this fungus' commercial future. At present a number of commercial formulations have been registered. Mycotal™, from Koppert, controls glasshouse and cotton whitefly. The use rate is 3 kg/ha/treatment and 3 treatments per hectare are usually needed. Its companion product, Vertalec™, based on a closely related strain of *Verticillium* is used against aphids. MicroGermin™, from Christian Hansen, is similar to a combination of Mycotal™ and Vertalec™. Engerlingspilz™, from Andermatt-Biocontrol AG, is the first western commercial product based on *Beauveria brongniartii*, presently available only in Switzerland. Grown on barley grains, it controls larvae of the cockchafer, *Melolontha melolontha* at 1-2 applications of 30-50 kg/ha. Asper G™, a powder formulation of *Aspergillus* is being marketed by Shinsyu Creative G Co Ltd. Developed by University of Tokyo's Institute of Applied Microbiology, the fungus produces an insecticidal compound mellezine and is used to control the pine bark beetle *Bursaphelenchus lignicolus*. The product is buried around the base of the tree and is effective for two years.

The recent past has seen a dramatic reversal for *Metarhizium anisopliae* and *Beauveria bassiana*. Although many attempts for many years to make these fungi into viable commercial products had proved fruitless, new production and delivery methods and new formulations will see these fungi finally appearing on Western markets. It is worthwhile noting that neither research on strain selection nor improvement by direct genetic manipulation and protoplast fusion allowed these promising fungi to be commercialized. The clever trick was to find a better way to deliver the fungus to its target.

Bayer, as virtually the only major agrochemical company working on mycoinsecticides, have reported control equivalent to aldicarb with granulated fermenter 'pellets' of a wild-type strain of *Metarhizium anisopliae* against black vine weevil (*Otiorhynchus sulcatus)*. The experimental use rate of 0.2 to 1 g/l would require 20- 100 kg/ha to treat the top 1 cm of soil, suggesting that the product's main use for the present will be in high-value horticultural crops. The company began marketing BIO 1020™ on a small scale in Germany in 1991.

EcoScience, a recently funded US biotech company, has developed a novel delivery technology, the Bio-Path™ chamber system. Fungal spores are suspended on upside down petri dishes and the apparatus is designed so that insects, attracted into the device by various means, pick up doses of the lethal fungus. The fungus may also be carried into insect colonies, eliminating the whole population. The idea is to use microorganisms whose activity is well known, but whose formulation and delivery has not yet been effective in practice. EPA approval is being sought for a product for use against cockroaches based on *Metarhizium anisopliae* and the company plans a number of future products based on analogous technology.

Limitations

Experience with all these products, as well as considerable research has established the following key constraints to widespread use of many of the products. Apart from production problems associated with fermentation of active preparations in a consistent way, the main user problem relates to the slow effect. For most of the Deuteromycetes it may be between five and ten days from application before effects are seen. This is often unacceptable to the user who will, on the basis of previous experience using chemical pesticides, expect to find dead insects a short time after application. Even then, effects depend on the conditions, requiring warmth as well as a suitable pH for active growth. Products may be applied as a foliar spray, or as a soil application. With foliar application the effectiveness of the insecticide is influenced by both abiotic and biotic factors. Germination may be affected by the relative humidity or in open situations the spores may be washed off by heavy rain. The spores may also be inactivated by ultraviolet light or by residues of fungicides used previously. In general, our understanding of pathogen/host interaction and the level of customer know-how are not sufficient to obtain optimum performance from fungal insecticides nor are markets likely to grow rapidly.

For those fungi which are applied to the soil major problems may be encountered due to competition between the fungal product and soil flora. This may be a simple competition for available nutrients or actual antagonism by wild type soil microorganisms. The growth of the fungi may be limited by the physical nature of the soil, with such problems augmented again by residues of previously used fungicides and other pesticides.

Marketability and consumer acceptance of these products is also restricted by the present difficulty in defining the efficacy of a preparation or to compare products, or even formulations containing the same active ingredient due to the lack of approved or standardized bioassay procedures. Common techniques include spore counts, viable spore counts, viable percentages, percentage active ingredients vs inerts, etc. None of these show anything at all about efficacy. Other restrictions on the marketability of present products include costs that are high relative to the value obtained; short shelf-lives of almost all fungal products which means the products have to be refrigerated and replaced fairly often; and the highly specific nature of the host range means each product can be sold only for specific problems at specific times. This also makes market forecasting and production planning very difficult. Recently developed products have been aimed at either very specific pests where pesticide resistance is well established or at specific environments such as glasshouse crops or other small-scale trials where their beneficial qualities outweigh their cost and nuisance value. The sales of fungi to control whitefly are likely be between $50,000 and $100,000 for some years due to the reasons cited above plus high production costs, the relatively narrow range of environmental conditions under which it will work, the high level of grower skills required, and the need for substantial product support.

Fungi are widely used for crop protection in developing countries, Eastern Europe and the former Soviet Union. It is very difficult to determine the validity of efficacy data from these countries and until recently, the quality of crop protection and the economics of use may have been different from that required in the West. If production costs are low or unknown, uneconomically high use rates may be applied or, if labour costs are low, frequent re- applications may be feasible. Lenient requirements for registration may also permit the development of 'niche' products that would be uneconomic in the West.

Fungi's future

The main requirements for future research and development include better delivery systems, strain improvement for higher levels of infection, faster kill rates, improved and lower cost methods of production through improved fermentation and improved downstream processing and formulation in order to increase the shelf life and broaden the spectrum of effect. There appears to be no reason at present to think that most of these technical limitations are about to be overcome although progress is evident in the development of specifically formulated niche products like those discussed above.

New products for the near future include an Israeli strain of *Ampelomyces quisqualis* for control of powdery mildew on grapes and apples, presently being tested by E R Butts International. *Entomophaga maimaga* kills 85% of gypsy moth caterpillars attacking oak trees and is being researched by the Boyce Thompson Institute. *Neozygites* strains for the control of spider mites are being investigated by Ecogen. A *Paecilomyces fumosoroseus* which gives 99% control against sweet potato whitefly in all stages of development has been patented by the University of Florida and W R Grace hopes to commercialize the fungus. A strain of *V. lecanii* has been field tested by the USDA to assess its efficacy for the control of soybean cyst nematodes. An Australian sterile red fungus (a Basidiomycete) has been studied in field trials to control take-all. Biotech International are developing a delivery system for the fungus which must be re-inoculated with every new sowing. The same fungus can be used for control of *Fusarium* in carnations, *Pythium* in wild flowers and *Pleiochaeta* in lupins. *Zoophthora radicans* can easily be cultured in liquid medium and attacks *Plutella xylostella* and the leafhopper which it can kill in 3-4 days.

Disease biocontrol

The number of potential biofungicides is increasing, with a number of large companies actively participating in the field. Few products are yet available commercially and the ones that have been on the market have not made much impact thus far. Although some of the claimed benefits of existing and experimental products are doubtful, the products are being sold in niche markets such as fruit trees or soilless glasshouse cropping. Two examples are Binab T™ from Binab which has been on sale in various markets for more than 10 years and F-Stop™ developed by Kodak and Cornell University and registered for sale in the US. However, Kodak is currently seeking to dispose of the product and project to another company. In general, disease biocontrol is more difficult and less well advanced than insect biocontrol.

VIRUSES

Baculoviruses have been the focus for much of the work on viral insect control. No member of the baculovirus family afflicts man or animals so they are believed to be safer than other virus families; extensive testing has revealed no adverse effects on man, animals or plants. The high specificity of baculoviruses also means that they are more environmentally friendly, attacking only one or two species of insect and having no effect on non-target organisms. Effective control of insects relies on a number of factors: the speed with which the virus kills its host and releases new viruses to infect others; the quantity of new virus released; the initial density of the pest insects and their social behaviour; and environmental conditions.

The earliest viral product is Elcar™, now made by Sandoz. New research suggests that

using it on weed species early in the season reduces pests by 88-95%. The United States Forest Service has developed, registered and distributed several products for control of forest pests including Gypchek™ against gypsy moth (*Lymantria dispar*) and Neochek™ against pine sawfly (*Neodiprion sertifer*). In the UK, the Natural Environment Research Council also developed an NPV product against pine sawfly which was registered and commercialized as Virox™. The product is effective and can be used at relatively low rates against the gregarious pine sawfly. However, it is used mainly against pests on young trees so the market is small and limited. MicroGenSys (US) introduced Decyde™ for the control of codling moth (*Cydia pomonella*) on apples. Andermatt-BIOCONTROL (CH) produces three virus products for sale only in Switzerland thus far: Capex 2™ based on a granulosis virus used against summer fruit tortrix moth (*Adoxophyes orana*); Madex 2™ (for amateurs) and Madex 3™ (for professionals) based on a granulosis virus of codling moth. Hoechst plans to sell the same GV in Germany as Granupom™. In 1992 Calliope SA of France will be selling in France, Germany, Belgium, Switzerland and the UK a product based on the same virus, Carpovirusine™, and Mamestrin™, based on the cabbage moth (*M. brassicae*) NPV but also claimed effective against cotton boll worm and diamondback moth (*Plutella xylostella).*

The future

The prospects for successful commercialization of viral insecticides have improved significantly within the past few years as technical innovations begin to overcome limitations. At present, large quantities of virus are needed for efficacy. Recent work on combining viruses with conventional insecticides or insect growth regulators and the discovery of 'viral enhancing factor' should reduce the quantities needed. Production of viruses has always been expensive, using live insects either in an insectary or collected from the wild. Recent work suggests that the *in vivo* production costs may now be coming down through the use of alternative host insects with higher productivity. The InStar Division (formerly Espro) of Crop Genetics International, claims to have developed two large-scale commercial processes for the production and purification of consistent quality baculoviruses (*Autographa californica*) which are reported to be successful in field trials against gypsy moth, beet armyworm and codling moth.

Although production *in vitro* using insect cell cultures was even more expensive, these costs may also be coming down through improved fermentation methods that allow cells to be grown continuously and infected with viruses in 'fattening' tanks, and through the use of cheaper nutrients like egg yolk in place of serum. Additional improvements would lower production costs and possibly make viral products cost-competitive with many chemicals.

Use of viruses has been limited by their slow kill, allowing insects to do further damage. Now, viruses have been genetically engineered to produce enzymes or hormones to block moulting, or extra toxins such as *Bt* δ-endotoxin or scorpion toxin to add to their speed and virulence without reducing specificity. The collaboration between NPS Pharmaceuticals (Utah, USA) (formerly Natural Product Sciences) and FMC Corporation (Pennsylvania, USA) has introduced a spider toxin gene. Viruses are also particularly sensitive to UV radiation in sunlight; new formulations that encapsulate them in starch may prolong their effective field life. There is considerable scope for refinement of viral insecticides and improvements in production technology and formulation could generate significant rewards. New, faster acting, higher potency, cheap-to-produce products should be well within the capabilities of a number of companies.

TECHNIQUES FOR QUANTIFYING THE ECOLOGICAL AND PATHOLOGICAL CHARACTERISTICS OF ENTOMOPATHOGENIC FUNGAL STRAINS

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ABSTRACT

In order to classify strains of entomopathogenic Hyphomycete fungi (Beauveria and Metarhizium spp.) according to their ecological characteristics and behaviour during the infection process, a number of techniques have been developed and evaluated. These include the use of in vitro assays to determine the ability of strains to grow at low water potential, fluorescent staining to track the fate of propagules during the infection process, and selective recovery of conidia from soil. When used in conjunction with conventional bioassay approaches, such techniques allow a strain profile to be assembled, based on traits which are required of a successful biopesticide.

INTRODUCTION

Fungal strains which are to be used as biopesticides must satisfy a number of criteria. The first, and most obvious, of these is virulence towards the target pest. Strains have frequently been selected for field evaluation on the basis of virulence alone, as determined in simple laboratory assays. However, a number of other traits may be equally important in determining the performance of a given strain in the field. Recently, the upsurge in interest in biological control has led to significant progress in expanding the range of characteristics of entomopathogenic fungal strains which can be quantified in laboratory bioassays. The purpose of this paper is to discuss the use of such techniques as part of a strain selection and characterisation programme.

Although single-dose assays may provide some measure of the intrinsic virulence of a fungal strain, more sophisticated bioassay procedures are likely to yield valuable information on dose/response relationships and speed of kill, both of which may be expected to influence the performance of the strain in the field. A tiered cascade of bioassays was described by Milner (1992) in which strains were assayed firstly for intrinsic pathogenicity, secondly for virulence as assessed by LC50, and finally for efficacy under simulated field conditions.

Ecological parameters may also be introduced into such a screening cascade. Fungal propagules in the aerial environment may be affected by humidity, light, temperature and the chemical environment. Relative humidity appears to be of particular importance in limiting efficacy of entomopathogenic fungi (Walstad et al., 1970), although there is some evidence that fungal strains may differ in their ability to infect at low humidity, and that this may be affected by the microclimate surrounding the host insect

(Marcandier & Khachatourians, 1987). Soil physics and ecology exert a profound influence on the behaviour of fungi in the soil (Studdert et al., 1990), while the persistence of fungal propagules in the soil may differ between strains (Fargues & Robert, 1995). Attempts have therefore been made to include some measure of infectivity of propagules in soil into screening cascades (Milner, 1992).

CULTURE COLLECTION AND STORAGE

Fungal strains obtained from a variety of sources, including mycosed insects, soils and culture collections, are routinely purified on Sabouraud's Dextrose Agar, which may also be used for isolation from mycosed cadavers. The selective medium of Doberski and Tribe (1980) proved preferable for isolation of Beauveria bassiana from other sources, since it allows colonies of the fungus to be distinguished from other species. Cultures were stored at -20C, using the PROTECT bacterial preservation system. Although designed primarily for storage of bacterial cultures, this non-destructive system appears to be suitable for preservation of conidia of Beauveria and Metarhizium species, at least over the short term.

EFFECT OF WATER POTENTIAL

Despite the available evidence suggesting that moisture is a limiting factor in both infection of insects and the establishment of fungal epizootics, there have been few studies which compare the ability of individual strains to grow across a range of moisture potentials. The objective of this preliminary investigation was to assess whether techniques developed to compare the moisture requirements of spoilage and plant pathogenic fungi could be adapted in order to characterise strains of entomopathogens (Magan & Lacey, 1984).

Relative humidity is related to water potential (expressed in bars) according to the following equation (Papendick and Campbell, 1981):

$$\Psi = RT/V \ (\ln RH)$$

where: Ψ = water potential (bar)
R = ideal gas constant (8.31×10^{-5} m^3 bar $mole^{-1}$ K^{-1}
T = absolute temperature (K)
V = partial molal volume of water (1.8×10^{-5} m^3/mole at 4°C)

Water potential in vitro may be adjusted by addition of solutes to the medium, taking account of the effect of the major medium components. This in turn may be related to relative humidity according to the above equation. Water potential was adjusted in this study using glycerol, although preliminary experiments suggested that the threshold value for conidial germination of Metarhizium anisopliae was identical whether water potential was adjusted with glycerol, polyethylene glycol or sodium chloride. Replica plating of sporulating colonies on to a series of glycerol-amended plates of Sabouraud's Dextrose Agar, followed by measurement of colony diameter after 9 days, proved to be more effective and reproducible than the use of solute gradients or liquid media in microtitre plates.

By adjusting water potential to values equivalent to relative humidities between 94.5% and 99.5%, growth of Metarhizium anisopliae and Beauveria

bassiana was found to be inhibited by decreasing water potential (Figure 1). Regression analysis indicated that a water potential equivalent to between 90% and 94% r.h. was necessary for growth of the strains tested in this study (Figure 2). Use of this technique allows rapid determination of the ability of entomopathogenic fungal strains to grow across a range of relative humidity values.

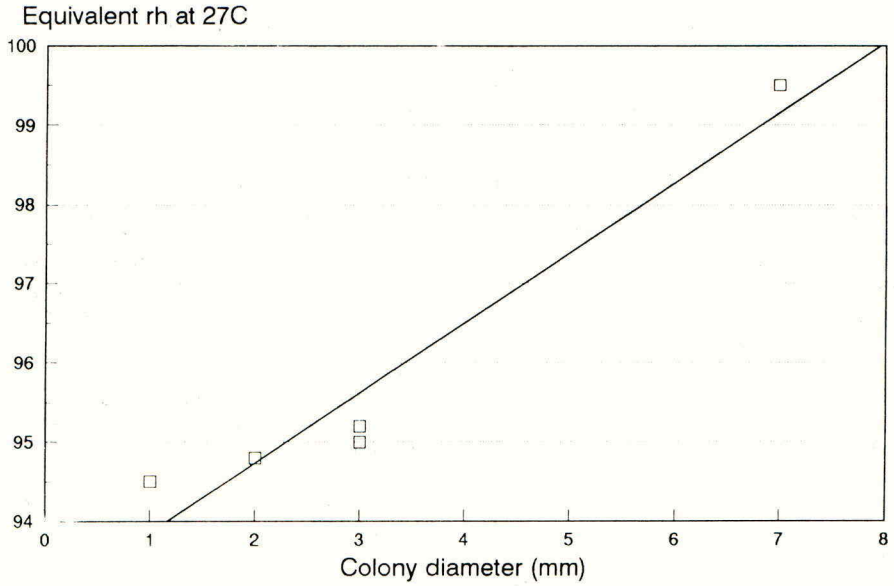

Fig. 1. Effect of water potential on the growth of Beauveria bassiana

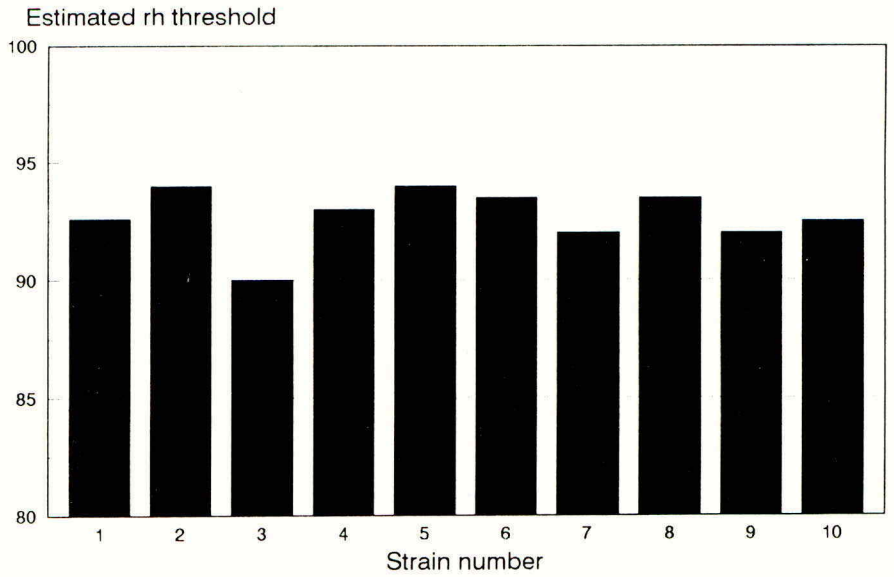

Fig. 2. Threshold water potential for growth of 10 entomopathogenic fungal strains

OBSERVATION DURING PATHOGENESIS

Some measure of the virulence of fungal strains can be obtained by determining LC50 values for a given host in the laboratory, and these undoubtedly vary between strains (Milner, 1992). However, such studies provide no quantitative information on the fate of the inoculum, or the point at which invasion by individual propagules is blocked. In order to address this problem, a technique for direct observation of the infection process was evaluated. The method was adapted for quantititive purposes from that described by Drummond & Heale (1985), in which a fluorescent brightener, Uvitex BOPT, was used to visualise the fungus Verticillium lecanii on the insect surface.

The fluorescent brightener Uvitex BHT was added to spore suspensions of Beauveria bassiana immediately before application to insects through a Potter Precision Spraying Tower. This appeared to have no adverse effect on the infectivity of the spores towards any of the species tested. Treated insects were then incubated for up to five days after treatment, after which they were squash-mounted and examined using fluorescence microscopy. The spores, germ tubes and appressoria retained sufficient brightener to distinguish fungal structures throughout the period of observation, although a further application of Uvitex BHT was required in order to visualise blastospores. Three randomly-selected fields of view were photographed per insect, and the photographs used to quantify the number of propagules per field.

Using this technique, it was possible to quantify the time of appearance of all initial stages of the infection process, and the number of propagules involved at each stage (Figure 3). Strains could therefore be characterised and compared according to the timing and efficiency of the infection process.

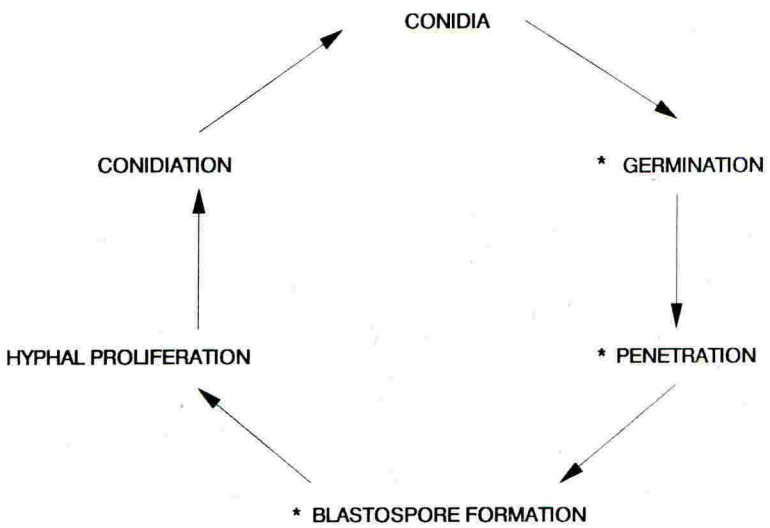

Fig. 3. Infection cycle of Beauveria bassiana. Stages which could be quantified using fluorescence microscopy are marked with an asterisk.

SOIL ECOLOGY

Interactions between fungi and soil are complex. The establishment of entomopathogenic fungal populations in the soil is determined by the chemical and physical properties of individual soils, as well as the properties of the fungal strain (Fargues & Robert, 1985). Characterisation of the behaviour of entomopathogenic fungi in soil is complicated by the fact that total colony counts on selective media fail to distinguish between the infective propagules (conidia) and other forms of fungal biomass. Apparent increases in biomass may therefore be due to saprophytic or parasitic growth, or simply to sporulation of hyphae.

Heat treatment eliminates hyphal biomass while retaining the viability of conidia in the soil (Harrison R.D. et al, unpublished). This allows conidiation, and the persistence of conidia in the soil, to be monitored directly. The proportion of the total propagule count which consists of conidia may vary considerably between soil samples. In field soil artificially infested with hyphal fragments of Beauveria bassiana, the proportion of total recoverable propagules which consisted of conidia was found to vary between 16% and 37% (Figure 4).

Use of this technique allows the behaviour of fungi in soil to be characterised and quantified in terms of rate of conidiation, persistence, soil specificity, and susceptibility to variations in the soil environment.

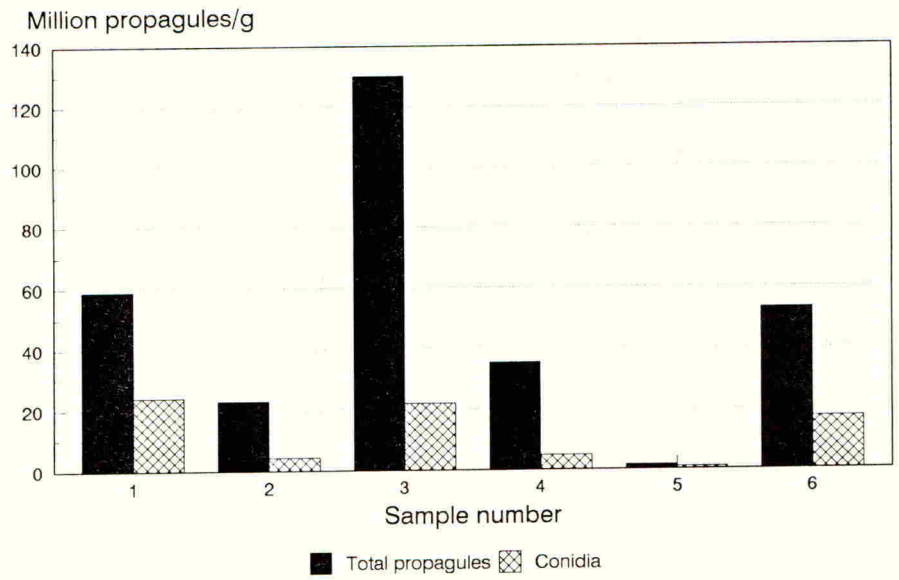

Fig. 4. Total propagule counts and conidial numbers in field soil infested with hyphal fragments of Beauveria bassiana.

CONCLUSIONS

As the biopesticide industry grows and develops, the screening cascades used to characterise and select candidate microbial strains will undoubtedly become more sophisticated. Significant advances are being made in this field, in terms both of quantifying virulence of entomopathogenic fungi, and of measuring ecological and other properties which contribute to the efficacy of a given strain as a biopesticide. In addition to the techniques discussed here, new approaches such as genetic characterisation and multiple enzyme assays may further contribute to the efficiency of the screening process in the future.

REFERENCES

Doberski, J.W.; Tribe, H.T. (1980) Isolation of entomogenous fung from elm bark and soil with reference to the ecology of Beauveria bassiana and Metarhizium anisopliae. Transactions of the British Mycological Society, **74**, 95-100.

Drummond, J.; Heale, J.B. (1985) Vital staining of the entomopathogen Verticillium lecanii on a live insect host. Transactions of the British Mycological Society, **85**, 171-173.

Fargues, J.; Robert, P-H. (1985) Persistence des conidiospores des hyphomycetes entomopathogenes Beauveria bassiana (Bals.) Vuill., Metarhizium anisopliae (Metsch.) Sor., Nomuraea rileyi (F.) Samson et Paecilomyces fumoso-roseus Wize dans le sol, en conditions controlees.

Magan, N.; Lacey, J. (1984) Effect of temperature and pH on water relations of field and storage fungi. Transactions of the British Mycological Society, **82**, 71-81.

Marcandier, S.; Khachatourians, G.G. (1987) Susceptibility of the migratory grasshopper, Melanoplus sanguinipes (Fab.) (Orthoptera:Acrididae), to Beauveria bassiana (Bals.) Vuillemin (Hyphomycete): influence of relative humidity. Canadian Entomologist, **119**, 901-907.

Milner, R.J. (1992) Selection and characterization of strains of Metarhizium anisopliae for control of soil insects in Australia. In: Biological Control of Locusts and Grasshoppers, C.J. Lomer and C. Prior (Eds), Wallingford: CAB International, pp. 200-207.

Papendick, R.I.; Campbell, G.S. (1981) Theory and measurement of water potential. In: Water Potential Relations in Soil Microbiology, J.F. Parr, W.R. Gardner and L.F. Elliott (Eds), Madison: Soil Science Society of America, pp. 1-22.

Studdert, J.P.; Kaya, H.K.; Duniway, J.M. (1990) Effect of water potential, temperature, and clay-coating on survival of Beauveria bassiana in a loam and peat soil. Journal of Invertebrate Pathology, **55**, 417-427.

Walstad, J.D.; Anderson, R.F.; Stambaugh, W.J. (1970) Effects of environmental conditions on two species of muscardine fungi (Beauveria bassiana and Metarhizium anisopliae). Journal of Invertebrate Pathology, **16**, 221-226.

CONTROL OF THE MIGRATORY LOCUST, *LOCUSTA MIGRATORIA CAPITO*, IN MADAGASCAR: THE POTENTIAL FOR THE USE OF A MYCO-PESTICIDE.

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ABSTRACT

The potential of a mycopesticide, based on *Metarhizium flavoviride* conidia formulated in oils, to control *L. migratoria capito*, was investigated. Although environmental factors such as ultra-violet radiation reduce conidial viability, there appear to be no major difficulties which could not be overcome. Field trials are recommended for 1993.

INTRODUCTION

The island of Madagascar, lying east of the mainland of Africa, has regular outbreaks of the migratory locust, *Locusta migratoria capito* (L. m. c.) This locust has rearing grounds largely in non agricultural land in the south west of the country but if the hopper bands are not controlled the locusts may aggregate and swarm, fly north and attack the rice producing areas. Major swarms have been reported in 1992; these are being tackled with chemical pesticides.

The Service de la Protection des Végétaux (SPV) of Madagascar, with logistical support from the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), have a strategy for treating hopper bands in the transitional phase (as the locusts begin to gregarise) so that major swarms do not develop. The process depends on accurate scouting with a rapid response to enable ground treatment by ULV sprays of fenitrothion or dusting with the methylcarbamate Propoxua (3% D.P.). If ground treatments are inadequate aerial spraying of fenitrothion takes place. In years where only ground treatment takes place the maximum area sprayed is about 20,000 ha; this area may be at least doubled when aerial applications are used.

The International Institute of Biological Control, in conjunction with the International Institute of Tropical Agriculture (IITA) Cotonou, Benin, and the Département de Formation en Protection des Végétaux (DFPV) Niamey, Niger are collaborating on the biological control of locusts and grasshoppers. The strategy envisaged is to formulate spores of entomopathogenic fungi in vegetable or mineral oils, increasing their efficacy (Prior et al, 1988) and decreasing reliance on conditions of high humidity, and apply as a biological

pesticide with conventional ultra-low volume (ULV) equipment. Preliminary work resulted in an isolate of *Metarhizium flavoviride* (IMI 330189, isolated from *Ornithacris cavroisi* (Finot) (Orthoptera: Acrididae) in 1988 in Niamey, Niger) being selected as a suitable agent for development. The isolate has a degree of specificity, being more virulent to Acrididae than Pyrgomorphidae and apparently having little virulence to non target organisms at a normal field dose.

In March 1992 a preliminary visit was made to Madagascar to explore the possibility of carrying out a major field trial in 1993. Objectives of the visit included assessing the logistics of application under the prevailing climatic and ecological conditions. This involved spray trials using blank formulation (oil without *M. flavoviride* conidia) and yellow and red tracers on transitional phase locusts.

EXPOSURE TO UV AND TEMPERATURE

Both ultra violet radiation and high temperatures are potentially limiting factors to the use of myco-insecticides (Zimmermann, 1982). In Madagascar temperatures of 55°C in direct sunlight were recorded over the mid-day period and the temperature was over 40°C for at least eight hours each day. The sunlight intensity was assessed by the use of polysulphone films (Davis and Gardiner 1982), the level of UV irradiation being expressed as an equivalent dose of 305nm monochromatic radiation in Wh m^{-2}.

The effects of both environmental factors were studied in the laboratory at IIBC. The effects of UV were assessed using an Oriel sunlight simulator and a comparison made between the level of field irradiation and the loss of conidial viability in formulations exposed to the simulator.. Formulations of oil and *M. flavoviride* were also exposed to temperatures ranging between 50-80°C for various periods of time and conidial viability assessed by germinating conidia on gelatine plates incubated for 24 or 48 hours at 25°C.

RESULTS

Chemical control of L.m.c.

Chemical control has proved successful in achieving good kill of locust bands and this is believed to have prevented the development of swarms during the previous years. The 1992 season had high locust populations and, by the end of March, 50, 000ha were treated with about 25, 000 l of pesticide. However negative environmental and social effects have occurred from past reliance on chemical pesticides. Post application assessments have shown many arthropod predators killed as well as predatory birds. In addition an important social custom amongst people of Southern Madagascar is the wrapping of the dead in silk shrouds for burial ceremonies. The Mahafaly people obtained the silk from the cocoons of two wild species inhabiting the woodlands. Spraying of locusts in previous campaigns is considered to be responsible for the decline in the indigenous silk worm numbers and the virtual elimination of the silk industry in Madagascar (Schomerus-Gernböck, 1981).

Environmental factors.

Work carried out at IIBC showed that conidia of *M. flavoviride* can tolerate short periods of very high temperatures. Five hours of exposure to 60°C failed to cause a

significant decline in conidial viability and many conidia survived exposure of 70° and 80°C (Figure 1).

In contrast laboratory studies indicated that direct exposure to sunlight would be very disadvantageous for the conidia. One hour of exposure to simulated solar radiation can dramatically reduce conidial viability (assessed after 24 hours incubation). This reduction is variable according to many features such as age of the fungal culture and whether the fungus is formulated in oils or water (Figure 2). With the configuration used the exposure corresponds to an energy input of 2.358 Wh m^{-2}; this input can be exceeded in a single hour of direct exposure between 10.00-16.00 hours in Madagascar and a single day of direct sunlight would effectively reduce viability to an unacceptably low level (Figure 3).

The results indicated the marked adverse effects of both temperature and U.V. but also indicated the ameliorating effects of a suitable time of application and the nature of the prevailing, largely graminaceous, vegetation. By 15.00 hours the temperature has dropped to only mid 30's and the U.V. dose has decreased from the midday maximum greatly reducing the danger of conidial inactivation by UV.

Application Strategy

An area with locally severe maize crop damage was selected to carry out a preliminary assessment of direct droplet impingement on insects using a hand-held 'Micro Ulva' sprayer. The site was near Analatelo village (22°20' S, 43°45' W), and infested with populations of *L. m. c.* with over 12,000 adults/hectare - mostly in the transiens phase.

The volume application rate was approximately 1 l/ha (flow rate 60 ml/min, swath width 10 m.), and the droplet size 60-70μm VMD (5 batteries producing 10,000 RPM). Spraying took place at 09.40 (windspeed 4-5 km/hr; temperature 32°C, r.h. 38.7%.) A one hectare zone of open grassland (30-50 cm tall) was divided into two 70 x 70m plots and treated with two different ultra-violet tracers (lumogen and flame orange).

This whole area was treated with pesticide approximately 80 minutes later. Fenitrothion was applied with a Piper aircraft fitted with two AU3000 atomisers. Five hours later, moribund and dead locusts were recovered from the central 30 x 30m of each plot for droplet counting. A severe rain storm had occurred during the interval, however being oil-based, the droplets were still discernible with an ultra violet lamp on the following night. Results for locusts recovered in one of the plots are presented in Figure 4.

More than half of the insects examined had four or fewer droplets on them representing at best 0.8 nl of formulation, and nearly 15% of the locusts had no visible traces at all. The wings account for a substantial proportion of the droplet capture, even when the insects are stationary, and infectivity is almost certainly lower for spores deposited on these parts. In order to achieve high (>95%) mortality, secondary uptake would need to be an important means of dose transfer for at least 20% of the insects.

Secondary uptake can be of great importance in the control of locusts with chemical pesticides (Nguyen, 1980). Although the relative importance of direct contact of the spray drops and of secondary uptake from vegetation is not known with our formulation it is likely that the pathogen must contact a mobile host within 24-48 hours to be effective.

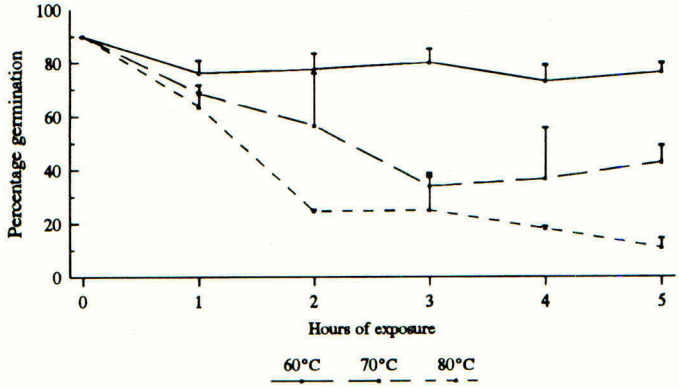

Figure 1. Germination of *Metarhizium flavoviride* conidia (±SE) after exposure to high temperatures

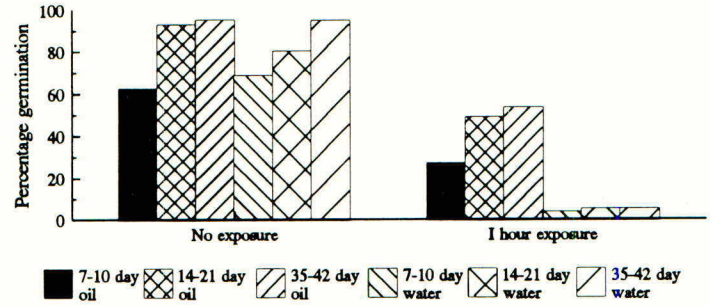

Figure 2. Effect of UV radiation on conidial viability and interactions with formulation and conidial age

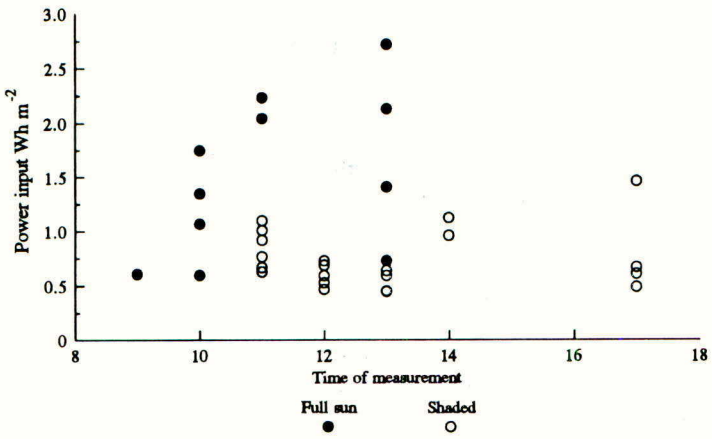

Figure 3. Sunlight intensity Madagascar March 1992

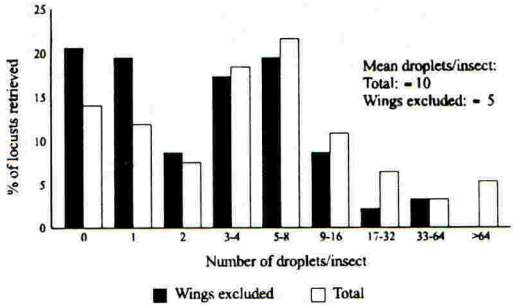

Figure 4. Droplet recovery on *Locusta migratoria capito*

Mid-afternoon spraying ensures that at least 18-20 hours pass before damaging heat and U.V. levels occur on exposed areas and even after that time period dense vegetation will provide further periods of protection. Adverse temperature effects would be unlikely as conidia on vegetation or locusts would not reach the extremes of over 50°C experienced in the open sun.

In reality the period of most danger would be during large scale applications with drums of formulation left in the sun for perhaps a week or two. These could quite rapidly reach temperatures of 50°C or more each day, leading to a reduction in conidial viability (G. V. McClatchie unpublished data). However simple measures would reduce this hazard; shading the drums from direct sunlight, allowing ventilation, using white reflective drums and using evaporative cooling should keep the temperatures down below 40°C. The conidia in the formulation would survive these temperatures for the time covering specific spraying episodes. An alternative strategy would be to store the conidia as a concentrate added to the formulation just prior to application.

Time to kill

One possible disadvantage of a mycopesticide is that kill may take 5-8 days, although certain chemical pesticides may also be slow acting (Bateman 1992). It should be noted that the disease causes a reduction in feeding and flying and hence a kill achieved in 8 days really amounts to the locusts ceasing to be active pests after 5-6 days (Moore et al 1992; E. Seyoum and D. Moore unpublished). In the present situation the problem of slow kill is greatly reduced as the control strategy is to kill locust bands in non-agricultural areas, weeks before the locusts reach the rice producing areas of the north.

Specificity

Although further testing is required it is generally true that *Metarhizium* spp. have varying degrees of specificity. With IMI 330189 the specificity appears to be significant to the family level with the Acrididae being most susceptible, other orthopteran families less so and different orders being infected only rarely and then usually under extreme conditions. *Metarhizium* spp. are notable for the lack of records of severe infections of non-target organisms. The exceptional isolates do occur, but screening of each isolate against a few

important species, such as bees and silkworms should be sufficient safeguard to remove those that are non specific. These screens should use realistic field doses and should be carried out in conjunction with comparable chemical insecticides. This would demonstrate the relative safety of the myco-pesticide. In addition the use of a mycopesticide in arid conditions means that it acts like a contact pesticide, without external sporulation on the cadavers (which would occur only in humid conditions) and an epizootic would not be set up, further reducing risk to non target organisms.

CONCLUSION

The strategy of *L. m. c.* control in Madagascar is carried out very effectively but this strategy brings with it environmental problems. Replacement of the chemical pesticide with the mycopesticide would remove these problems. The results of this preliminary visit suggested no reason why the mycopesticide should not be successful and a full scale field trial is planned for 1993.

ACKNOWLEDGEMENTS

The project on biocontrol of locusts and grasshoppers is funded by the Canadian International Development Agency, the Netherlands Directorate General for International Cooperation, the UK Overseas Development Administration and the US Agency for International Development. Thanks are also due to Dr. E. Rabehevitra Rakatobe, Chef du Service de la Protection des Végétaux, Madagascar for cooperation with the initial visit.

REFERENCES

Bateman, R. P. (1992) Controlled droplet application of mycopesticides. In: *Biological Control of Locusts and Grasshoppers*, C.J. Lomer and C. Prior, (Eds.), CAB International Wallingford, pp 249-254.

Davis, A.; Gardiner, D. (1982) An ultra-violet radiation monitor for artificial weathering devices. *Polymer Degradation and Stability*, 4, 145-157.

Moore, D.; Reed, M.; Le Patourel, G.; Abraham, Y. J. and Prior, C. (1992 in press) Reduction of feeding by *Schistocerca gregaria* Försk. after infection by *Metarhizium flavoviride* (Gams & Rozypal). *Journal of Invertebrate Pathology*.

Nguyen, N.T. (1980) Insecticide acquisition by drift sprayed hoppers. *Australian Plague Locust Commission Annual Report Research Supplement, 1970-80*, pp78-85.

Prior, C.; Lomer, C.J.; Herren, H.; Paraïso, A.; Kooyman, C. and Smit, J.J. (1992) The IIBC/IITA/DFPV collaborative research programme on the biological control of locusts and grasshoppers. In: *Biological control of Locusts and Grasshoppers*, C.J. Lomer and C. Prior (Eds.), CAB International Wallingford, pp 8-18

Schomerus-Gernböck, L. (1981) *Die Mahafaly: eine ethnische gruppe im Süd-Western Madagascars*. Reimer, Berlin, 232 pages (See page 115).

BIO 1020: GRANULAR METARHIZIUM - A NEW PRODUCT FOR BIOCONTROL OF SOIL PESTS

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ABSTRACT

A mycelial granular formulation of a wildtype strain of Metarhizium anisopliae (common name: BIO 1020/Metarhizium anisopliae) has been developed for use in horticulture. The mycelial granules are mixed into the compost at planting, or potting, at an application rate of 1.0 g BIO 1020/litre soil. After application infectious spores form on the granules. BIO 1020 can be used for control of different soil pests but is especially effective against the black vine weevil, Otiorhynchus sulcatus. This pest represents an as yet unsolved problem particularly in horticulture. Trials in greenhouses on ornamentals and in nurseries on nursery stock showed the high efficacy of BIO 1020 against the black vine weevil. Incorporation of the product into the complete compost gave better control than a broadcast application to the soil surface. BIO 1020 showed activity against all stages of O. sulcatus. Plant compatibility was excellent.

INTRODUCTION

Biological control agents are an important means of pest control and can play a part in integrated pest management systems. They require specific environmental conditions and cultural practices to optimise their activity and should, therefore, be targeted first to crops where conditions are favourable. These requirements can be fulfilled, especially in horticulture with its intensive cultivation of plants.

The black vine weevil (Otiorhynchus sulcatus) is of increasing importance in horticulture in many countries, mainly in Central-Europe and North America (Schread 1972, Boehringer 1983, Parella and Keil 1984). Its preferred host plants, for example ornamentals and hardy ornamental nursery stock, are found in professional horticulture as well as house and roof gardens. Yews, 'peat bed' plants such as rhododendrons and azaleas, also grape vine and strawberries are heavily infested.

Feeding by the adult weevils produces the typical indented leaf margins, reducing the commercial value of plants. The main damage to plants is caused by the feeding of larvae on the roots and, particularly, on the stem base which results in impaired plant growth, wilting and finally the death of the plants. Curative treatments applied at the time of symptom development are often unable to prevent damage. Therefore the demand from growers for new strategies is urgent.

BIO 1020, a granular formulation of Metarhizium anisopliae, is under development for use as a biological soil insecticide (Andersch et al. 1990, Reinecke et al. 1990). This paper presents data on the characteristics and biological effectiveness of this product.

MATERIAL AND METHODS

The organism used is a strain (DSM 3884) of the entomopathogenic fungus M. anisopliae. The product (common name: BIO 1020/Metarhizium anisopliae) consists of dust free and insoluble mycelial granules of the fungus which are produced according to a patented procedure (EP 0268177A2). The granules were stored under vacuum at 4°C until testing.

For biological testing the mycelial granules (GR) were mixed in commercial compost as a soil treatment at different application rates. To ensure high sporulation rate of the fungus under unfavourable conditions, e.g. where temperatures were low at potting time, a premixture (PM) was prepared by mixing granules into the compost and incubating it at temperatures between 15°C or 25°C for 7 or 4 days respectively, until sporulation on the granules was completed. The premixture was used for potting directly after this incubation period.

All trials on biological efficacy of BIO 1020 presented in this paper were carried out with the black vine weevil, Otiorhynchus sulcatus. Dependent upon the experiment different stages of O. sulcatus were added at different times before or after treatment. The test plants were planted and either incubated in the greenhouse or placed directly outside. Plants were irrigated and supplied with fertilizer as necessary. No other plant protection agents were applied during the experiments. Efficacy was evaluated according to Abbott's formula.

Sporulation of granules was measured by extracting the spores from soil samples using a 1% (v/v) aqueous solution of Tween 80. Soil extracts were then diluted and inoculated onto a selective medium. Population density of M. anisopliae was expressed as spores (colony forming units) per gram of dried soil. The recovery rate was evaluated for each analysis.

RESULTS

BIO 1020 develops its biological activity in two steps. The first step is the formation of infectious spores on the granules after rehydration and the second is the infection of the pests after contact. Both processes are dependent on environmental conditions, mainly temperature. Soil humidity above the permanent wilting point of ornamentals is in general sufficient for the sporulation and for the infection process. In soil saturated with water sporulation is reduced.

Temperature dependency

When soil temperatures reached 15°C for several hours, more than 10^6 spores/g dried soil were produced within 4-7 days after an application rate of 1 g BIO 1020 granules/litre soil. Lower temperatures, e.g. 10°C, led to an increased time requirement for the production of spore titer sufficient to control black vine weevil. Once sporulation was complete, lower temperatures or even frost during cultivation of the plants, e.g. outside in nurseries in spring, did not influence the viability of the fungal spores. At temperatures higher than 35°C the fungus did not grow.

For the second step, the infection process, temperature was the most important factor. It took one week (at temperatures higher than 14°C) to maximal two weeks (at temperatures of 10°C or 5°C at night/15°C in the day) incubation in a premixture until first reduction of feeding activity of black vine weevil larvae was observed (Table 1). The larvae died some days later. After three to four weeks efficacy was 100% at temperatures of 10°C or higher. At 4°C the effectiveness of BIO 1020 was minimal, since at this temperature the black vine weevil larvae did not feed in treated nor in untreated compost during the incubation period of 32 days.

TABLE 1. Influence of temperature on effectiveness of BIO 1020 measured by feeding activity and mortality of O. sulcatus larvae (L2-3). Larvae were incubated in a BIO 1020-premixture (1.0 g/litre) and feeding activity and mortality were evaluated at different time intervals. Feeding activity of larvae in untreated compost = 100%.

	% feeding activity of larvae during incubation in BIO 1020-premixture at time interval (days)						% mortality of larvae at day	
	0- 5	6- 8	9-12	13-15	16-22	23-32	22	32
4°C	-[a]	-	-	-	-	-	0	25
10°C	106.6	30.2	20.0	22.9	0	0	17	100
14°C	37.8	21.2	25.5	0	0	0	88	100
18°C	50.3	13.1	1.4	0	0	0	100	100
23°C	18.1	17.2	0	0	0	0	100	100
5°C/15°C[b]	104.3	121.6	25.9	7.6	25.4	0	86	100
8°C/18°C[b]	101.0	12.4	0	0	34.2	0	100	100

[a] no feeding by larvae

[b] Temperature with day/night change (12h/12h)

Mobility in soil

Table 2 demonstrates the limited mobility of spores of BIO 1020 in soil. After several weeks incubation under field conditions, 95.2 to 99.2 % of the spores were recovered from the treated upper soil layer. Biological testing in separated soil layers demonstrated the lack of efficacy in deeper parts of the soil. Efficacy in the upper layers was excellent.

TABLE 2. Mobility of BIO 1020 after surface application and incubation for 4 weeks (in greenhouse pots, containers) or 7 weeks (outdoors in turf) and efficacy in different soil layers against O. sulcatus. Pots and containers were planted with azalea. Treatment and irrigation as follows:

	Treatment with BIO 1020		
	Irrigation		
11 cm pot:	0.5 g/pot	30 ml/day	
7.5 l container:	7.5 g/container	450 ml/day	
turf:	50.0 g/m²		37 mm rainfall in 7 weeks

	layer (cm)	% spores in soil layer	% efficacy in soil layer
11 cm pot	0 - 3	97.9	80
greenhouse	4 - 7	2.1	40
7.5 l container	0 - 6	99.2	82
greenhouse	7 -13	0.4	0
	14 -20	0.4	0
turf	0 - 3	95.2	100
outdoors	4 - 7	4.8	40

Because of the low mobility the efficacy of BIO 1020 was dependent upon the application method (Tab. 3). Protective treatment either by mixing the granules into the compost or using a premixture, gave a mean efficacy of approximately 85% (Abbott) against black vine weevil larvae. Application of the granules to the soil surface was less effective. The lower efficacy of surface applied granules in comparison to the surface applied premixture in these trials was caused by insufficient sporulation of the granules. These were placed unprotected on the surface of the potting soil and were exposed to high temperature and very low humidity.

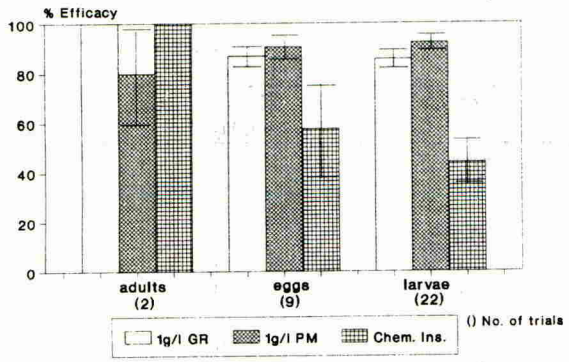
TABLE 3. Influence of application method on the efficacy of BIO 1020 against O. sulcatus on different plants. BIO 1020 was applied at 1.0 g/l compost as a soil mix or as a top dressing treatment at an amount appropriate to the soil mix.

Plant	Site	Premix/ Granules	% Efficacy with application method: mix	top dressing
fuchsia	greenhouse	GR	95	57
azalea	greenhouse + outdoors	GR	86	0
grapevine propagation	plastic tunnel	PM	76	51
balcony pots	outdoors	GR	80	26
		PM	83	52

Stage-dependent activity

Often biological measures are effective only against certain developmental stages of an insect. BIO 1020 showed good efficacy against all stages of O. sulcatus (Fig. 1), and even adult beetles were controlled sufficiently. Efficacy against eggs and newly hatched larvae was evaluated by connecting the number of larvae following 8 weeks incubation of a known number of eggs per pot. Infection of eggs by M. anisopliae was proven by microscopic examination in laboratory trials.

Fig. 1: Efficacy of BIO 1020 against developmental stages of O. sulcatus.



Longterm control

Spores of M. anisopliae survived for a long time in the soil after application. In these trials an activity period of up to 8 months, approximately one vegetation period, was achieved.

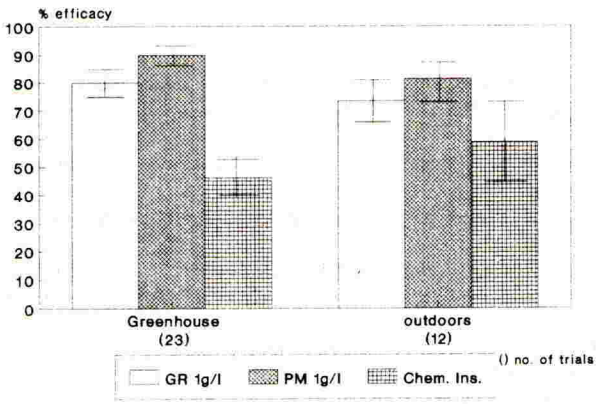
Efficacy

For the trials in greenhouses and outdoors all treatments were applied either as the premixture (PM) or as granules (GR) incorporated in the soil. Temperatures during growth of the plants were between 10°C and 30°C in greenhouses and between 5°C and 25°C outdoors. The containers were infested by natural populations or, if necessary, by artificial infestations with eggs, larvae or adults. With natural infestation the efficacy was assessed 6-12 months after treatment. Artificial infestations was carried out immediately or up to 8 months after treatment. Efficacy was evaluated one to three months later. Fig. 2 summarizes 35 trials with BIO 1020 against the black vine weevil. Plant compatibility was excellent on all plants tested so far using the recommended method and rate of application.

In the greenhouse under practical conditions BIO 1020 showed high efficacy. On azalea, fuchsia, chrysanthemum, begonia, impatiens, kalanchoe, parthenocissus veitchii, euonymus and taxus seedlings the number of larvae was reduced by an average of 80% (GR) or 90% (PM) independent of the plant species. The reduction in larvae resulted in reduced plant damage and, therefore, reduced plant loss. The level of control was more variable in cyclamen but an average of 60-70% activity was achieved.

BIO 1020 was also effective in trials under outdoor nursery conditions carried out in the summers of 1989, 1990 and 1991. On rhododendron, taxus, euonymus and in balcony pots on begonia, chrysanthemum and fuchsia 74% (GR) or 81% (PM) control of the black vine weevil was obtained.

Fig. 2: Comparison of the efficacy of BIO 1020 against O. sulcatus on ornamentals in the greenhouse and on nursery stock outdoors.



CONCLUSION

BIO 1020 is a new biocontrol product which is easy to handle by the user and, under the conditions of the experiments, showed good and reproducible effectiveness. This product offers a new opportunity to control black vine weevil in horticulture. As expected, the efficacy was better in the greenhouse than outdoors. This is due to the more favourable conditions and confirms previous experience that better results are obtained with biological agents when the environmental conditions are controlled. The premixture was 8-10% more effective than the granule treatment both indoors and outdoors because the conditions for sporulation were more favourable in the premixture.

If the plants are incubated after treatment at soil temperatures below 15°C, the product should be used only as a premix to ensure a protection. Also, for good biological activity in terms of insect infection levels, soil temperatures should not be lower than 15°C. Slow action is a characteristic of all entomopathogenic fungi; an acute, rapid onset cannot be expected. BIO 1020 needs 1-2 weeks at least to develop its full effectiveness and is therefore unsuitable for curative treatment if prompt control of a pest is required.

Efficacy after broadcast application is clearly dependent on feeding behaviour of the pest because of the limited mobility of BIO 1020 in soil. The pest must be present in the treated area e.g. when larvae feed on the stem base, or when adult beetles hide during the day in the upper soil layer. Although some control was obtained when applying the premix to the soil surface of the container, a treatment after planting can only be recommended if the root system of the plants grows near the soil surface (e.g. on roof gardens). The advantage of the immobility of M. anisopliae spores is however the long term control provided.

Like M. anisopliae in general (Zimmermann 1984), BIO 1020 was also less effective on cyclamen. The reasons are, firstly, fungistatic metabolites of cyclamen, the saponins, which are produced in the tubers (Schönbeck and Schlösser 1976) and which impair the development of the fungus (Stenzel, unpubl. data). Secondly, the larvae can migrate into the tuber, thus being prevented from contact with the fungus. Also chemical insecticides often show reduced activity in cyclamen. To ensure a sufficient fungal population at the tuber bases treatment of cyclamen with BIO 1020 should start as early as possible at the seedling stage.

For commercial reasons, particularly in ornamental plants, the absence of phytotoxis of a protection agent is an important criterion. Used correctly with the recommended application rate (1 g/litre), BIO 1020 showed no negative effects on plant growth. The rooting of cuttings was not impaired. Since some trials actually led to the promotion of plant growth, the use of BIO 1020 can be expected to produce positive rather than negative effects.

REFERENCES

Andersch, W.; Hartwig, J.; Reinecke P.; Stenzel, K. (1990). Production of mycelial granules of the entomopathogenic fungus Metarhizium anisopliae for biological control of soil pests. *Proceedings of the Vth International Colloquium on Invertebrate Pathology*, 2-5.

Boehringer, M. (1983) Increasing incidence of Otiorrhnychus sulcatus. *Deutscher Gartenbau*, 37, 698-700.

Parella, M.P.; Keil, C.B. (1984) Black vine weevil: an increasing problem for California nurseries. *California Agriculture*, 38, 12-14.

Reinecke, P.; Andersch, W.; Stenzel, K.; Hartwig, J. (1990) BIO 1020, a new microbial insecticide for use in horticultural crops. *Proceedings of the Brighton Crop Protection Conference - Pests and Diseases 1990*, 1, 49-54.

Schönbeck, F.; Schlösser, E. (1976) Preformed substances as potential protectants. *Physiological Plant Pathology*, R. Heitefuss and P.H. Williams (Eds), Berlin: Springer, pp. 653-678.

Schread, J. C. (1972) The black vine weevil. *Circular Connecticut Agricultural Experiment Station*, 211, 1-8.

Zimmermann, G. (1984) Weitere Versuche mit Metarhizium anisopliae (Fungi imperfecti, Moniliales) zur Bekämpfung des Gefurchten Dickmaulrüßlers, Otiorhynchus sulcatus F., an Topfpflanzen im Gewächshaus. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes*, 36, 55-59.

SYNERGISM BETWEEN ENTOMOPATHOGENIC FUNGI, *METARHIZIUM* SPP., AND THE BENZOYLPHENYL UREA INSECTICIDE, TEFLUBENZURON, AGAINST THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*

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ABSTRACT

The entomopathogenic fungus *Metarhizium anisopliae* (applied in an aqueous solution of Tween 80) and the benzoylphenyl urea insecticide teflubenzuron (applied in acetone) can act synergistically to kill 3rd instar desert locusts, *Schistocerca gregaria*. This was apparent from analyses of single dose bioassays but not from experiments involving several doses of fungus and insecticide. However, multidose bioassays with a more specific Acridoid pathogen, *Metarhizium flavoviride* isolate IMI 330184, resulted in significant synergy, when the agents were applied as a combined formulation in mineral oil.

INTRODUCTION

Persistent synthetic chemical pesticides have been used effectively in the past to tackle locust outbreaks. However, the banning of dieldrin has left a significant gap in locust control. Alternative less persistent insecticides have increased costs and reduced efficiency of control (Brader, 1988; Symmons, 1992). In addition scientific and political opinion is against the large-scale spraying of pesticides for locust or grasshopper control. Entomopathogenic fungi, particularly *Metarhizium* spp. show promise as an alternative to chemicals (Lomer and Prior, 1992). However, a mycopesticide would probably be deployed best as part of an integrated programme of measures. The particular strategy addressed here is the combined application of low doses of chemical pesticides with fungi. If the two agents act synergistically, lower doses of both may be used producing more effective and perhaps cheaper control. Benzoylphenyl urea insecticides commend themselves to this approach because; 1. they interfere with chitin synthesis in insects (but not fungi) and thus could facilitate entry of the fungus through weakened host cuticle, 2. insect growth regulators such as chitin synthesis inhibitors are specific and therefore have less environmental impact than many other insecticides, 3. We have shown previously that the benzoylphenyl urea diflubenzuron can act synergistically with *Metarhizium anisopliae* against the tobacco hornworm, *Manduca sexta* (Hassan and Charnley, 1983).

MATERIALS AND METHODS

Locusts were reared on wheat seedlings and bran supplemented with yeast as described previously (Dillon and Charnley, 1986). The fungi *Metarhizium anisopliae* isolate ME1 and *Metarhizium flavoviride* isolate IMI 330189 were cultured on quarter strength Sabouraud's dextrose agar (SDA). For bioassays involving *M. anisopliae,* conidia were harvested in 0.04% Tween 80 in distilled water from 2 week old cultures (Dillon and Charnley, 1986). Newly ecdysed 3rd instar locusts (12h ± 12h old) were immersed for 3s in suspensions of conidia in 0.04% tween. Teflubenzuron (99.5% AI) was dissolved in acetone and applied in a 1µl aliquot topically to metathoracic sternites. Controls were dipped in 0.04% Tween 80 or dosed with 1 µl of acetone. Experimental and control insects were maintained at 30° C and 100% RH for 24h after inoculation then at 30°C and 30%RH for the duration of the experiment (5d). Fresh wheat seedling was supplied as food.

For bioassays involving *M. flavoviride*, conidia were harvested in the mineral oil ondina EL from 2 week old cultures. Teflubenzuron was dissolved also in mineral oil. A combined formulation was produced by mixing the two preparations. 1µl aliquots of single or combined formulations of the agents in mineral oil were applied to the metathoracic sternites of newly ecdysed 3rd instar locusts (12h ± 12h). Controls were treated with 1µl of mineral oil. Experimental insects were maintained at 30° C and 30%RH throughout the experiment (5d). Fresh wheat seedling was supplied as food.

RESULTS

Initial bioassays were carried out to establish the efficacy of single treatments. The LC50 for *M. anisopliae* was 7.9×10^5 conidia ml^{-1} (95% confidence limits - 1.3×10^5, 41.5×10^5) and the LC50 for teflubenzuron was 0.26 µg/ul (95% confidence limits - 0.11, 0.81).

Combined treatment - agents applied separately

Preliminary experiments showed that teflubenzuron did not significantly affect either germination or growth of *M. anisopliae* at the doses used in the bioassays. The combination experiment was carried out by applying the two agents separately, the insecticide first in acetone, the fungus next in Tween 80. There was no evidence that three replicate experiments behaved differently and thus the data were combined (see table 1).

An independent action model (as defined at the bottom of table 1) was applied to the results. The predicted mortalities are shown in parentheses (table 1). The data differ significantly from the model therefore it may be concluded that fungus and insecticide are acting synergistically.

In a second experiment the insecticide and fungus were again applied separately but at several doses. This enabled a more detailed investigation of the presence of synergy. Once again data were combined from two replicates as there was no evidence of significant residual deviance between the replicates (table 2).

TABLE 1. Effects of combined applications of teflubenzuron and *Metarhizium anisopliae* against 3rd instar locusts

	Metarhizium anisopliae	
	0	$3x10^5$ conidia ml^{-1}
Teflubenzuron		
0	0 (0)	17 (20.4)
0.003 µg $µl^{-1}$	1 (1.2)	24 (20.60)

Combined data from 3 replicates. Data are number dead out of 25.
Figures in parentheses indicate mortality predicted by the independent action model:
proportion killed when insecticide and fungus applied together
$= (1 - (1 - p_M) (1 - p_N))$
where p_M is the proportioned killed by *Metarhizium* alone and p_N is the proportion killed by teflubenzuron alone

Four models which might reveal synergy were fitted to this data (viz. parallel dose response curves *vs* fungus, parallel dose response curves *vs* insecticide, equivalent doses, independent action). Unfortunately in all cases the data do not deviate significantly from the model so there is no evidence to disprove the hypothesis that the two compounds behave independently.

Bioassays using a combined formulation in mineral oil

Evidence from the two previous experiments was conflicting; in the first experiment synergy was apparent in the second it was not. These bioassays were performed with isolate ME1 of *M. anisopliae*. Although this is a virulent pathogen of locusts it is also pathogenic for other insects including *Calliphora vomitoria* (Diptera) and *M. sexta* (Lepidoptera) (unpubl.; St. Leger *et. al.*, 1988). Therefore we decided to switch our attentions to isolate IMI 330189 of *M. flavoviride*. This isolate appears to have a much more restricted host range than *M. anisopliae* ME1 (unpubl) and it has proven virulence for locusts and a number of other Acridoids.

Bateman *et. al.* (1992) have shown that oil-based ultra low volume sprays containing conidia of *Metarhizium flavoviride* kill locusts at low humidity. This provides the basis for future control programmes of locusts with a mycopesticide. If combined application of fungus and insecticide is to be used practically against locusts, the two agents would be best applied together in a combined formulation (a "tank mix") in mineral oil. Before bioassays were carried out with a combined formulation, the viability of conidia in a mineral oil solution of teflubenzuron was determined. 80% viability was retained for at least 2 months at room temperature and at 6° C (data not shown).

Results of bioassays of the combined formulation of teflubenzuron and *M. flavoviride* in oil against 3rd instar locust hoppers are shown in figure 1. Since an

analysis of deviance showed no evidence of differences between the three experiments the data

TABLE 2. Multidose bioassay of teflubenzuron and *Metarhizium anisopliae versus* 3rd instar locusts

	Dose of *M. anisopliae* conidia ml^{-1}			
Dose of Teflubenzuron $\mu g\ \mu l^{-1}$	0	$3x10^3$	$3x10^4$	$3x10^5$
0	0	-	-	-
0.003	-	2	4	10
0.03	-	0	4	10
0.3	-	6	7	10

Combined data from 2 replicates. Data are number dead out of 10 for each treatment.

have been amalgamated. In dual treatments, increasing dose of insecticide brought about a large increase in mortality over that observed in either single treatment. The approach used to test for synergism in this case was to fit separate dose response lines versus dose with and without teflubenzuron. This analysis showed that the LC50s of insecticide only and fungus-insecticide treatments were significantly different, indicating synergism. If an "independent action" model was the correct model to describe the results of the experiment (ie there was no synergy) the LC50 (the dose which kills 50% of the insects not killed by the fungus, or which die naturally) would be unaffected by the presence of the fungus; it is affected clearly so there is evidence of synergism.

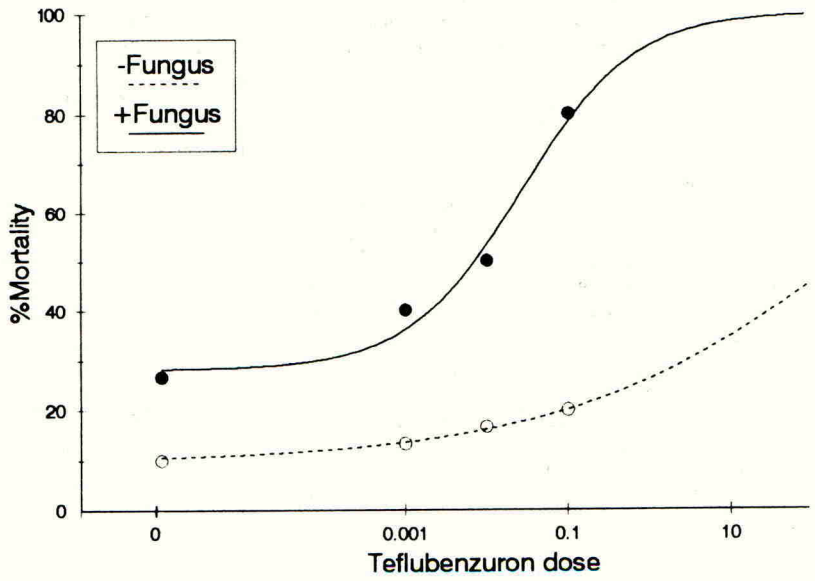
DISCUSSION

The present work establishes that teflubenzuron and *Metarhizium* spp. can act synergistically to kill 3rd instar hoppers of the desert locust. The effect was more marked with the *M. flavoviride* isolate IMI 330189 than with *M. anisopliae* ME1. The effectiveness of a combined formulation in oil suggests that this approach may have practical significance. Locusts are not a perennial problem, though many grasshoppers, particularly in West Africa, are (Fishpool and Popov, 1984). Teflubenzuron proved effective against grasshoppers in field trials in Mali (Krokene, 1991), and *Metarhizium flavoviride* performed well against the grasshopper *Zonocerus variegatus* in Benin (Lomer, pers comm). From which it may be concluded that combined applications of fungus and benzoylphenyl urea insecticide may be useful for grasshopper as well as locust control.

The literature is replete with reports on the interaction between chemical pesticides and microbial pathogens against insects. However, only in comparatively

few cases has synergy been shown using established statistical protocols. In most combination experiments the insecticide is purely seen as a general stressor making the insect more prone to disease. Thus it may not be too suprising that the effects of combined treatments are often at best only additive rather than synergistic.

Figure 1. effects of an oil-based formulation of teflubenzuron and *Metarhizium flavoviride* on 3rd instar locusts



Closed circles = mortality with fungus present ($3x10^5$ conidia ml^{-1}) as well as insecticide
Open circles = mortality with teflubenzuron alone
Teflubenzuron concentration is in $\mu g\ ul^{-1}$
N = 30 for each treatment

However, in the present case there was good reason to believe that the insecticide may specifically promote invasion of the fungus by weakening the cuticle. Hassan and Charnley (1989) showed in an ultrastructural study that this was the case for *M. sexta* larvae treated with diflubenzuron and infected with *M. anisopliae*. This illustrates the value of a rational rather than an empirical approach to the choice of chemical synergist for a microbial pesticide.

ACKNOWLEDGEMENTS

We would like to thank Chris Vennard for excellent technical asistance and Dr Ray Cannon (Shell Research) for the gift of the teflubenzuron. The work was funded in part by an EEC grant.

REFERENCES

Bateman, R.P.; Godonou, I.; Kpindu, D.; Lomer, C.J.; Paraiso, A. (1992) Development of a novel field bioassay technique for assessing mycoinsecticide ULV formulations. In: *Biological Control of Locusts and Grasshoppers*, C.J. Lomer and C. Prior (eds), Oxford: C.A.B. International, pp. 255-262.

Brader, L. (1988) Control of grasshoppers and migratory locusts. *Brighton Crop Protection Conference - Pests and Diseases 1988*, **2**, 283-288.

Dillon, R.J.; Charnley, A.K. (1986) Inhibition of *Metarhizium anisopliae* by the gut bacterial flora of the desert locust, *Schistocerca gregaria*: evidence for an antifungal toxin. *Journal of Invertebrate Pathology*, **47**, 350-360.

Fishpool, L.D.C.; Popov. G.B. (1984) The grasshopper faunas of the savannas of Mali, Niger, Benin and Togo. *Bulletin de l'Institut Francaise d'Afrique Noire*, **43**, 275-410.

Hassan, A.E.M.; Charnley, A.K. (1983) Combined effects of diflubenzuron and the entomopathogenic fungus *Metarhizium anisopliae* on the tobacco hornworm, *Manduca sexta*. *Proceedings of the 10th International Congress of Plant Protection*, **3**, 790.

Hassan, A.E.M.; Charnley A.K. (1989) Ultrastructural study of the penetration by *Metarhizium anisopliae* through dimilin-affected cuticle of *Manduca sexta*. *Journal of Invertebrate Pathology*, **54**, 117-124.

Krokene, P. (1991) Reduced rates of teflubenzuron and their effect on grasshoppers (Acridoidea) and non-target arthropods in Mali. Cand. Scient thesis in ecology, University of Oslo.

Lomer, C.J.; Prior, C. (Eds.) (1992) *Biological Control of Locusts and Grasshoppers*, Oxford: C.A.B. International.

St. Leger, R.J.; Durrands, P.K.; Charnley, A.K.; Cooper, R.M. (1988) The role of extacellular chymoelastase in the virulence of *Metarhizium anisopliae*. *Journal of Invertebrate Pathology*, **52**, 285-294.

Symmons, P. (1992) Strategies to combat the desert locust. *Crop Protection*, **11**, 206-212.

OPPORTUNITIES FOR A NEW *BACILLUS THURINGIENSIS* BIOINSECTICIDE IN GRAPES

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ABSTRACT

A new *Bacillus thuringiensis* (BT) bioinsecticide is under development worldwide for control of lepidopterous pests in grapes, tomatoes and cabbage. This product is based upon strain GC-91, serovar. *aizawai*. It was selected for development by CIBA-GEIGY because of its novel spectrum of activity when compared to existing *B. thuringiensis* strains.

In grapes it is used against the second and third generations of the grape moth, *Lobesia botrana* and the vine moth, *Eupoecilia ambiguella*. At the recommended dose of 100 g formulated product/hectolitre, the efficacy is comparable to chemical standards.

INTRODUCTION

In Europe, *Eupoecilia ambiguella* and *Lobesia botrana* are the major pests in grapes. *E. ambiguella* predominates in the cooler wine growing areas of Switzerland, Austria and Germany, whereas *L. botrana* is the major pest in the warmer, Mediterranean climates like Western Switzerland, Italy, France and Spain. Insecticide treatments against larvae of the second and third generation are needed to protect the grapes from losses caused by feeding damage to the berries as well as infection of the damaged berries with the fungus *Botrytis cinerea*. In the past, *Bacillus thuringiensis* insecticides were only infrequently used in grapes because of poor and unreliable efficacy, difficult determination of the proper application time and of the need to add sugar (Charmillot et al.,1991).

MATERIALS AND METHODS

The *B. thuringiensis* strain GC-91

Strain GC-91 was constructed by P. Jarrett and D. Burges (1986) using conjugation between two wild type strains, which produce delta-endotoxins with different insecticidal spectra. It produces delta-endotoxins of both parent strains and is useful for control of a range of lepidopterous pests, broader than the range of either parent. It was patented by Agricultural Genetics Company in Cambridge/U.K. from which CIBA-GEIGY obtained a sole licence. The product based upon GC-91, serovar. aizawai is now developed under the code no. CGA 237'218 and will be commercialized under the trademarks TUREX ™ and AGREE ™.

Apart from grapes, it has also been shown to be efficacious in cabbage (*Plutella xylostella, Mamestra brassicae, Pieris rapae, Trichoplusia ni*), tomatoes (*Heliothis*

armigera, Plusia spp.), leek (*Acrolepia assectella*) and soya beans (*Anticarsia gemmatalis*) (Flückiger, 1992).

Production of test material

The product based upon GC-91 is produced by cultivating strain GC-91 by deep liquid fermentation. At the end of sporulation, the solids comprising of viable spores, parasporal delta-endotoxin crystals and inert media residues are harvested by centrifugation and spray dried. The powder obtained typically contains 1.2 % w/w delta-endotoxin (Bernhard, 1992) and is referred to as technical product. It is formulated by mixing with an equal amount of inert ingredients. The formulated product is thus referred to as WP 50.

Field trials in grapes

The product was applied as high volume spray at 1000 l/ha. Dosages were 50 - 200 g formulated product/hl. The first application was made at the beginning of egg hatching. In trials with two applications, the second application followed 8 - 12 days after the first. If not stated otherwise, 1 % (w/v) sugar was added to the spray broth as recommended by Charmillot et al. (1992). Evaluation of efficacy occurred by counting larvae on the bunches.

RESULTS

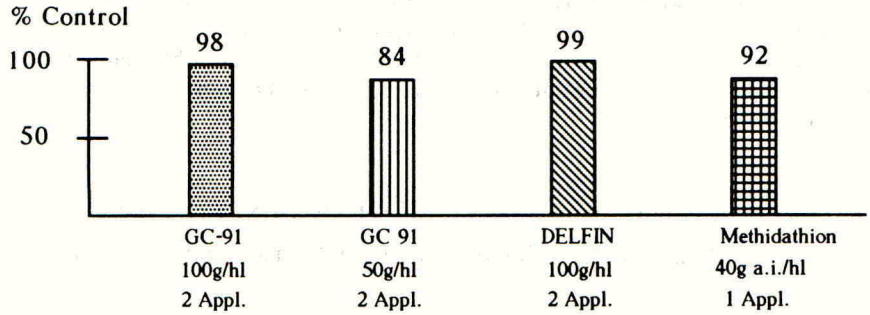
This report summarizes results of 38 tests with strain GC-91 over three years in most of the important European wine growing countries against larvae of all generations of *L. botrana* and *E. ambiguella*. Experimental data of only a selected number of typical trials are shown in this report.

In trials against first generation larvae of *L. botrana* and *E. ambiguella* only 40 - 60% control were achieved. Similar results were obtained with other *B. thuringiensis* preparations and some synthetic insecticides (Data not shown).

Efficacy trials against *E. ambiguella* and *L. botrana*

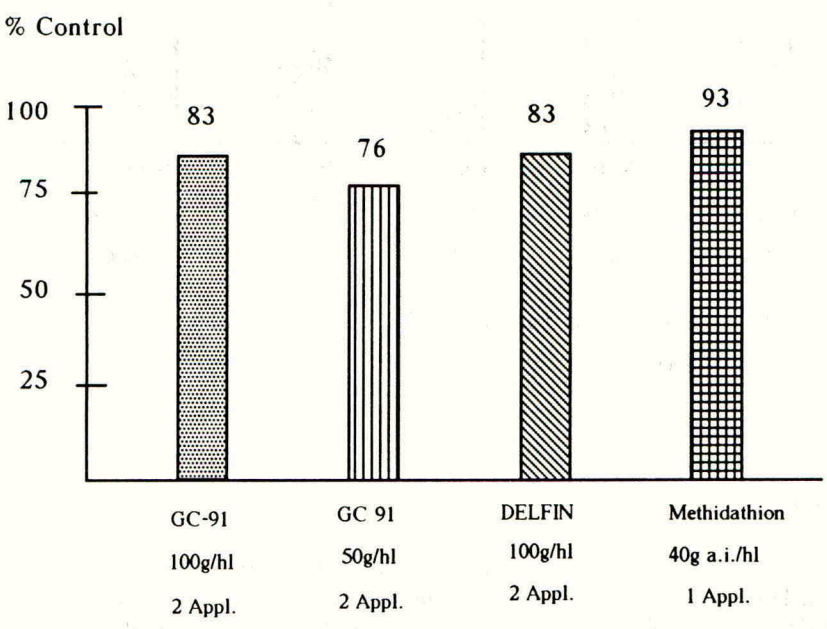
As shown in figure 1 excellent control of *L. botrana* was achieved against the second generation at 100 g product/hl. Two applications are superior to just one. Good efficacy with two applications was also obtained against second generation larvae of *E. ambiguella* as shown in figure 2. The product was also efficacious against third generation larvae of *L. botrana* in France and Spain (Data not shown).

Figure 1: Efficacy of GC-91 against the second generation of *Lobesia botrana* in Solana de los Barros, Spain 1991



Application dates: 13.6. and 20.6. for GC-91 and DELFIN, 20.6. for methidathion
Untreated check: 53 larvae per 10 bunches

Figure 2. Efficacy of GC-91 against the second generation of *Eupoecilia ambiguella* in Eisental, Germany 1991

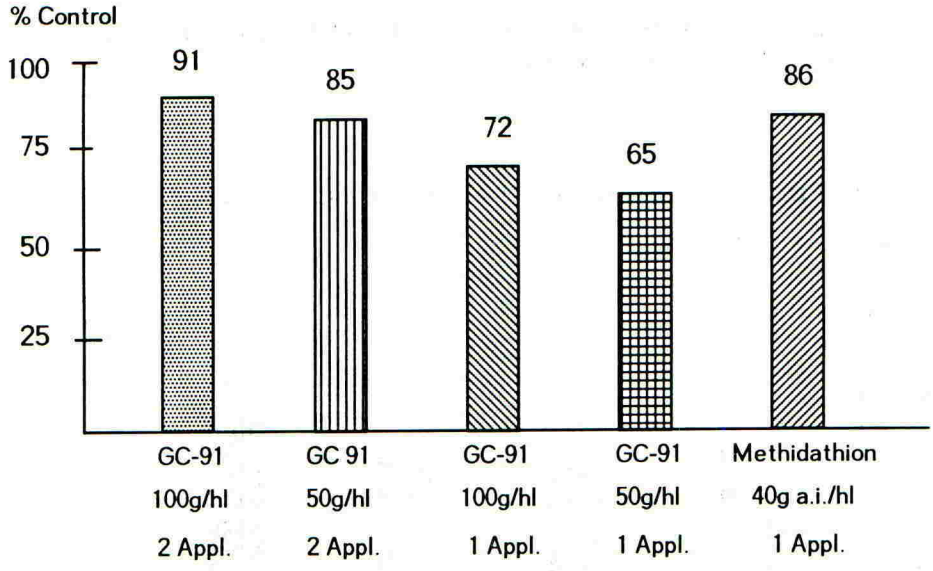


Application dates: 25.7. and 1.8 for GC-91 and DELFIN; 1.8. for methidathion
Untreated check: 20.5% attacked bunches

Dosage rate finding and number of applications

Strain GC-91 was applied once or twice at different dosage rates against second generation larvae of *L. botrana*. Best results, equal to the chemical standards can be achieved with two applications of GC-91 at 100 g product /hl and addition of 1% (w/v) sugar to the spray broth as shown in figure 3. Although best results were obtained with two applications, one application may be sufficient if the second generation flight is short and thus well synchronized.

Figure 3. Efficacy of different dosage rates of GC-91 applied once or twice against second generation of *Lobesia botrana* in Saillon (Valais), Switzerland 1991



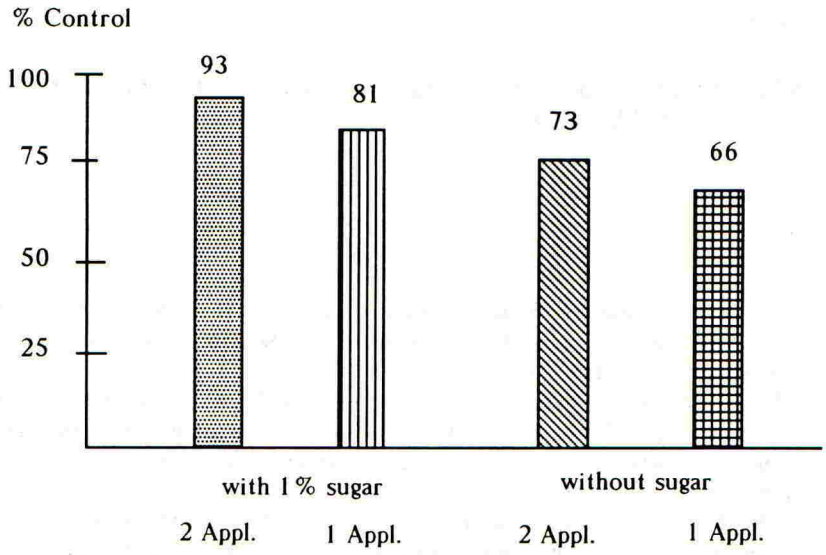
Application dates: 22.7. and 5.8. for GC-91; 31.7. for methidathion

Untreated check: 142 larvae per 100 bunches

Activity enhancement with sugar

In order to test the effect of adding 1% sugar to the spray broth, a number of tests were performed in several countries against second generation larvae of *L. botrana*. The results summarized in figure 4 demonstrate that sugar enhances the activity of GC-91. It has to be noted however that addition of sugar did not cause a significant effect upon efficacy in all trials.

Figure 4. Efficacy of GC-91 applied at 100 g/hl, alone or with 1 % sugar, in 5 trials carried out in France, Italy and Switzerland 1990 against the second generation of *Lobesia botrana*



DISCUSSION

Excellent control, equal to the chemical standards against second generation larvae of *L. botrana* and *E. ambiguella* can be achieved with two applications of GC-91 at 100 g product/hl and addition of 1 % (w/v) sugar to the spray broth.

In the past *B. thuringiensis* bioinsecticides were not widely used in grapes. The reason was farmer's mistrust based upon poor and unreliable control achieved with older products. Difficulties in determining the proper time of application and the recommendation to add sugar to the spray broth were also deterrents.

With GC-91, control of the first larval generation of *E. ambiguella* and *L. botrana* is 40 - 60 %. Similar results were obtained with other *B. thuringiensis* products and some synthetic insecticides. The relatively weak control is most likely due to long flights of the moths and rapid plant growth at the same time, causing rapid dilution of applied insecticides. Since damage caused by first generation larvae is generally insignificant, no attempts to control them are made in most wine growing areas.

The mode by which sugar enhances activity of *B. thuringiensis* is not well understood. Since enhancement seems to be dependent upon local factors, we recommend to follow advice by the local extension services on whether or not to add sugar to the spray broth.

In some countries farmers are required to cut the green ground cover prior to insecticide application to protect bees. This has a detrimental effect upon predator populations, and is unnecessary if GC-91 is used, which is safe to honey bees. The use of highly efficacious bioinsecticides like GC-91 is therefore also labour saving and helps implementing IPM in grapes.

ACKNOWLEDGEMENTS

The authors wish to thank all their colleagues in the different countries who have coordinated and conducted field trials.

REFERENCES

Bernhard, K. (1992) Quantitative dDetermination of delta-endotoxin contents in spray dried preparations of *Bacillus thuringiensis* strain GC-91. *World Journal of Microbiology and Biotechnology* 8, 24-29.

Charmillot, P.J. ; Pasquier, D. ; Antonin, Ph. (1991) Efficacité et rémanance de quelques préparations à base de *Bacillus thuringiensis* (BT) dans la lutte contre les vers de la grappe eudémis et cochylis. *Revue suisse de Viticulture, Arboriculture et Horticulture* 23 (3), 187-194.

Charmillot, P.J. ; Pasquier, D. ; Antonin, Ph. ; Mittaz C. (1992) Lutte contre les vers de la grappe eudémis et cochylis au moyen de *Bacillus thuringiensis* (BT) : résultats de 1991. *Revue suisse de Viticulture, Arboriculture et Horticulture* 24 (2), 109-116.

Flückiger, C.R. (1992) Resultate der Feldversuche mit TUREX (*Bacillus thuringiensis*) zur Bekämpfung von Schädlingen im Rebbau, auf Tomaten und auf Kohl. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 65 (1-2), 197.

Jarrett, P. & Burges, H. D. (1986) *Bacillus thuringiensis* : Tailoring the strain to fit the pest complex on the crop. *BCPC Monograph* No.34 Biotechnology and Crop Improvement and Protection, Cambridge: British Crop Protection Council, pp 259-264.

NovoBtt - A NOVEL *BACILLUS THURINGIENSIS* SSP *TENEBRIONIS* FOR SUPERIOR CONTROL OF COLORADO POTATO BEETLE, AND OTHER LEAF-FEEDING CHRYSOMELIDAE

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ABSTRACT

NovoBtt, a novel *Btt* strain has been developed for the control of chrysomelid pests. NovoBtt shows a consistent benefit over competitor strains, both in terms of percent control, percent defoliation and window of application when applied against Colorado potato beetle (*Leptinotarsa decemlineata*). Examples are given on the use of *Btt* against other chrysomelids and in modern pest control systems.

INTRODUCTION

In 1982, a novel strain of *Bacillus thuringiensis* was discovered which exhibited activity against larvae of certain chrysomelid species. It was designated *Bacillus thuringiensis* ssp *tenebrionis* (Btt) (Krieg *et al*., 1983).

The original patent on *Btt* was, in November 1991, purchased by Novo Nordisk A/S, and the company now assumes all rights and license agreements associated with the *Btt*-products.

NOVODOR is a commercial product based on *Btt* strain NovoBtt, produced by Novo Nordisk A/S, Plant Protection Division.

THE STRAIN

All the commercialised *Btt* strains produce rhomboidal crystals containing one major identical protein (delta endotoxin), CryIIIA (Krieg *et al*., 1987).

TABLE 1. Mean size of protein crystals and delta-endotoxin production yields (shakeflask trials) produced by original *Btt* strain and strain NovoBtt.

	Mean length of crystals µm	Delta endotoxin yield PIA (BTTU/g)
Org. Btt - strain	0,7	1293
NovoBtt strain	2,3	4169

NovoBtt is a mutant obtained by classical techniques. The mutation has resulted in a low sporulating strain, producing crystals which are significantly bigger and have a higher protein content then the crystals produced by the original Btt strain (Table 1).

Activity spectrum

The activity spectrum of Btt is relatively specific within the leaf beetles, chrysomelidae (Krieg et al., 1983; Huger et al., 1986).

The most important species is the Colorado Potato Beetle (CPB), Leptinotarsa decemlineata, but other susceptible species include Alder leaf beetle (Agelastica alni), Cereal leaf beetle (Oulema melanopa), Eucalyptus leaf beetle (Chrysophtharta bimaculata), Elm leaf beetle (Xanthogaleruca luteola) and Large red poplar leaf beetle (Chrysomela populi).

Formulation

NovoBtt is presently available as a FC formulation made from fermenter solids and formulating ingredients. The lead formulation contains 3% active proteins, but in some countries a 2% formulation is available.

Mode of action

Like other Bt strains NovoBtt is a stomach poison. Upon ingestion by the larvae, the crystal proteins are dissolved by the action of the gut juices. They are proteolytically converted into the toxic core fragment. The toxins subsequently then bind to specific receptor sites on the midgut epithelial cells, and it is believed that the toxins now induce the formation of small pores in the cell membrane. As a result the cells swell and lyse (Höfte & Whiteley, 1989). The larvae normally stop feeding within a few hours after ingestion and die within 2-5 days. The early larval stages (L1-L2) being more susceptible then larger instars (L3-L4).

Safety

Standard toxicological testing has shown NovoBtt to be very safe for workers, mixers and applicators. NovoBtt contains no ß-exotoxin and does not show any pathogenic or infective properties.

Ecotox studies have shown that NovoBtt is not considered to be toxic or pathogenic to mammals, avian wildlife or freshwater fish, and NovoBtt is not considered to pose any significant risk to nontarget wildlife like aquatic invertebrates and nontarget insects.

FIELD TRIAL RESULTS

Colorado Potato Beetle (CPB)

A series of trials was carried out in the northeastern states of US during 1991 to compare different dose rates / application timings of NovoBtt (3% FC formulation) to other Bt formulations for the control of CPB. In this region resistant populations of CPB are a severe problem. Control measures include treating the field several times at high dosages at intervals of approx. 7-10 days.

Averaged across all the locations in which treatments were evaluated in at least 3 of the locations, NovoBtt exhibited a clear rate response based on percent control (Figure 1).

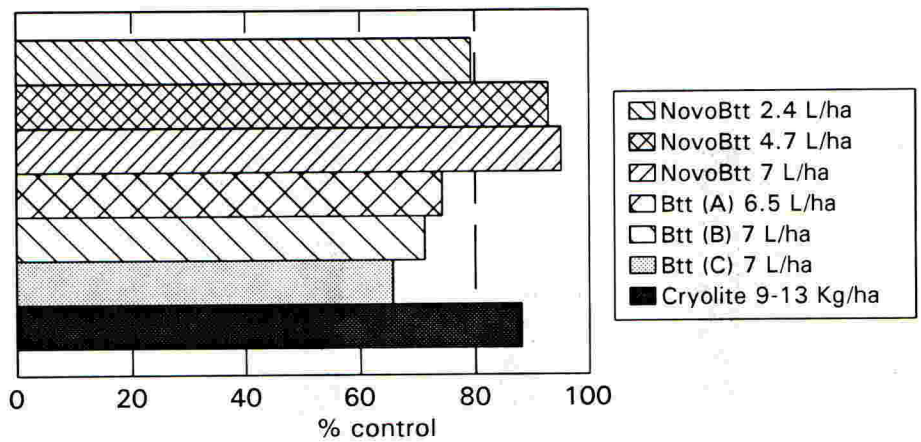

FIGURE 1. Percent control of CPB based on numbers of surviving L3 and L4 larvae. Mean of trials, US 1991.

The average yield improvement based on the trial sites where the information was given shows the best treatments to be cryolite and NovoBtt at 7 l/ha, with an average yield increase of 4,5% and 4,4% respectively.

In one trial the potatoes were planted later than normally, and subsequently experienced a very erratic and uneven distribution of first generation larvae. The larvae population was cleaned up, and a number of CPB adults were collected and released into the plots. This resulted in a very even distribution of second generation eggs throughout the plots. The various treatment were then applied at 10%, 40% and 60% egg hatch, to simulate an early, optimum and a late application scheme.

The optimum timing for Bt's was confirmed to be around 40%, but even at a late application timing, NovoBtt resulted in outstanding control of CPB compared to other Bt products (Figure 2). Defoliation data from the trial shows the same trend (Figure 3).

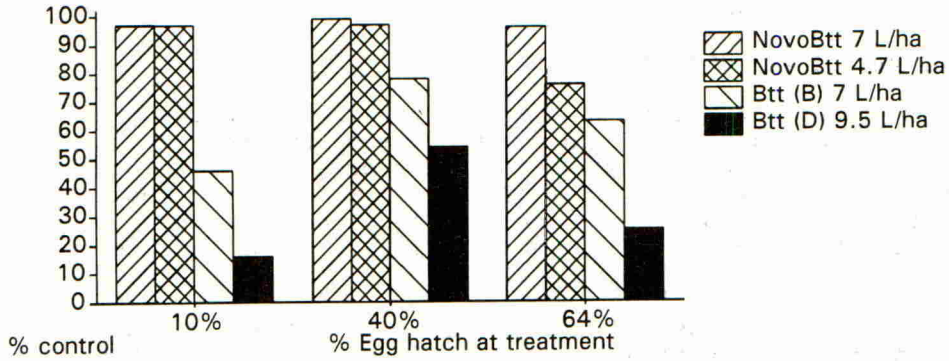

FIGURE 2. Timing Study US 1991. Control of Colorado Potato Beetle based on numbers of surviving L3 and L4 larvae.

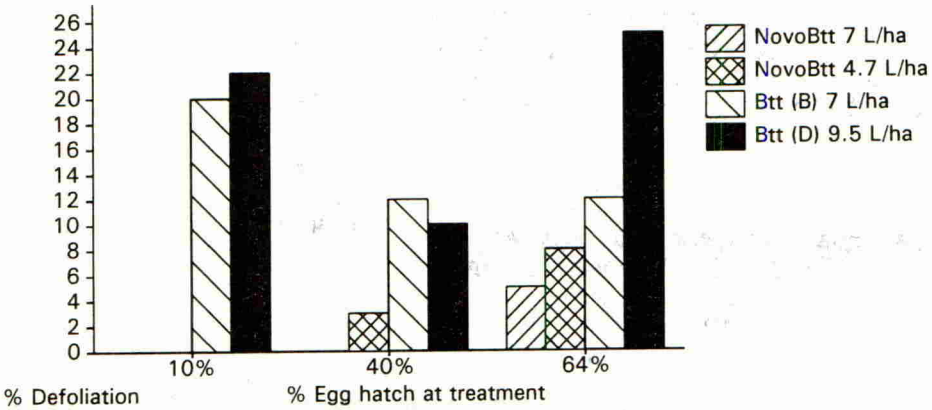

FIGURE 3. Timing study US 1991. Defoliation of potatoes infested by CPB.

All the results presented in figures 1-3 indicate, that applied at comparative dose rates, NovoBtt shows a consistent benefit over competitor formulations, both in terms of percent control, percent defoliation and window of application.

Other chrysomelids

Trials have been undertaken in Hungary during 1990 and 1991 against the Cereal leaf beetle (Oulema melanopa). The outcome of these trials have been, that NovoBtt is now under registration with a proven recommendation for the control of cereal leaf beetles in the dose rates 2 - 4 l/ha.

The eucalyptus leaf beetle (Chrysophtharta bimaculata) is capable of causing extensive damage to the eucalyptus grown

commercially in Tasmania. The standard approved chemical treatment is by using pyrethroids. The knockdown effect of pyrethoids are much faster, but in spite of the lower effectiveness in % control recorded in the trials for NovoBtt, it has been shown that NovoBtt is equally effective then cypermethrin in the protection of leaf area. As the aim of the chrysomelid control is to protect the trees from leaf area loss (and subsequent improve tree growth), the results of the leaf area loss assessments are more important then the population reduction data, in determining the success of each treatment.

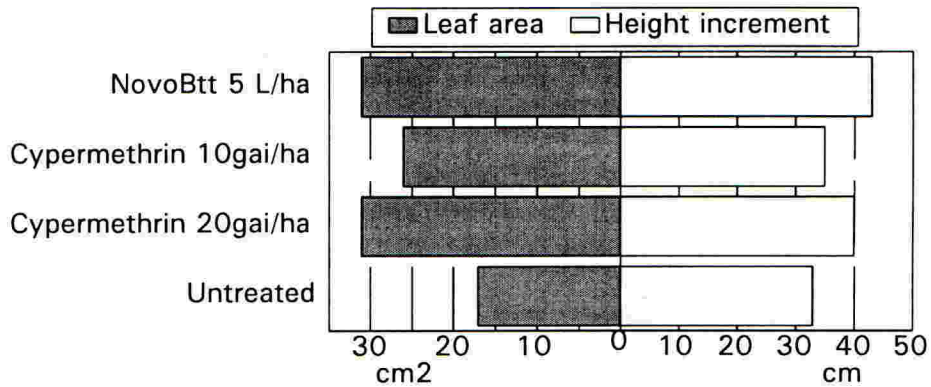

FIGURE 4. Protection of Eucalyptus from leaf beetles. Average leaf area in cm^2 and recorded height increment in cm. Tasmania 1989.

USE AREAS

The different mode of action of Btt's from that of chemical insecticides, makes them a good tool in resistance pest management. In the northeastern of USA, Colorado potato beetle has evolved resistance to important insecticide groups like organochlorides, carbamates and pyrethoids (Forgash, 1985).

Bt's containing cryIIIA-proteins are now used as an important tool in the resistance management strategy. There have been no evidence of field resistance to Btt in CPB, and no indications of cross resistance to any insecticides. On the contrary, a recent resistance monitoring study in Maryland concluded, that populations exhibiting a high level of pyrethroid resistance were the most sensitive to Btt. Since there is no evidence of a related biochemical mechanism involved, it was presumed that some fitness cost associated with pyrethroid resistance may be responsible for the increase in Btt sensitivity (Everich et al., 1992)

Following the Chernobyl Nuclear Accident of 1986, considerable nuclear fallout affected the surrounding agricultural land of three C.I.S. countries - Ukraine, Byelorussia and Russia. In view of the resulting impact on the land, it was decided by the relevant governments not to add further environmental pressure, and so prohibited the use of chemical insecticides.

To protect the vital potato crop from being decimated by the Colorado Potato Beetle, NovoBtt (2% FC) were supplied by Novo Nordisk and equally distributed to the worst affected regions of the three countries. Used at rates of 3-5 litres/ha, NovoBtt gave good control in all the involved areas. For example, from one site in Byelorussia, 61% of plants were infested with 1st & 2nd instars, about 2000 larvae/100 plants (L1L2). Ten days after 5 litres of NovoBtt, there were 142 larvae/100 plants (L3L4). At another site, a 40% infestation with 764 larvae/100 plants had been reduced to 31 larvae/100 plants, ten days later.

It seems very likely that NovoBtt will have a considerable commercial impact on potato growing in the C.I.S.

REGISTRATION

NovoBtt is currently registered and sold in a number of countries:

Bulgaria	Switzerland	Czechoslovakia	Poland
Romania	C.I.S.	Yugoslavia	U.S.A.
Denmark	Spain		

Furthermore NovoBtt has been submitted for registration in the following countries:

Australia	Turkey	Austria	France
Germany	Greece	Hungary	Italy

REFERENCES

Everich, R.C.; Dively, G.P.; Linduska, L.L. (1992) Baseline monitoring of Colorado Potato Beetle Sensitivity to Bacillus thuringiensis and Associations with Pyrethroid Resistance. Resistant Pest Management, **4**, 1, pp. 14-15.

Forgash, A.J. (1985) Insecticide resistance in the Colorado potato beetle. pp33-53. In: D.N. Ferro and R.H. Voss (Eds) Proceedings of the symposium on the Colorado potato beetle. XVIIth International Congress of Entomology. Res. Bull #704, Mass. Agric. Expt. Sta., Amherst.

Huger, A.M.; Krieg, A.; Langenbruch G.A., Schnetter, W. (1986) Discovery of a new strain of Bacillus thuringiensis var. tenebrionis, effective against Coleoptera. Mitt Biol. Bundesanstalt. Ld. und Forstw., 83-96.

Höfte, H.; Whiteley, H.R. (1989) Insecticidal Crystal Proteins of Bacillus thuringiensis, Microbiological Reviews, **53**,242-255.

Krieg, A.; Huger A.M.; Langenbruch G.A.; Schnetter W. (1983) Bacillus thuringiensis var. tenebrionis: ein neuer, gegenüber Larven von Coleopteren wirksamer Pathotyp., Zschr. angew. Entom., **96**, 500-508.

Krieg, A.; Huger A.M.; Schnetter W. (1987) Bacillus thuringiensis var. san diego Stamm M-7 ist identisch mit dem zuvor in Deutschland isolierten käferwirksamen Bacillus thuringiensis var. tenebrionis Stamm BI 256-82. Zschr. angew. Entom., **104**, 417-424.

IMPROVEMENT OF A BACULOVIRUS PESTICIDE BY DELETION OF THE EGT GENE

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ABSTRACT

The *egt* gene of the insect baculovirus *Autographa californica* nuclear polyhedrosis virus (AcMNPV) encodes the enzyme ecdysteroid UDP-glucosyltransferase. Expression of *egt* enables the virus to inhibit moulting of its infected host. In this study, the effect of *egt* on the growth and development of infected insects has been investigated. These studies revealed that, since insects normally stop feeding during ecdysis, expression of *egt* prevents the insect from experiencing this feeding arrest. Thus, *egt* functions to prolong the length of time the insect feeds after infection, with a resultant increase in the weight gain of the insect. Deletion of the *egt* gene significantly improves the pesticide characteristics of AcMNPV. Larvae infected with an *egt* deletion mutant display considerably reduced feeding and earlier mortality than wild-type AcMNPV-infected larvae.

INTRODUCTION

Insect baculoviruses have been used for the control of several insect pests (Entwistle & Evans, 1985), and are considered to have great potential as environmentally benign biological control agents. In general, baculoviruses infect only arthropods, and individual virus strains infect only one or a limited number of species (Gröner, 1986). They are not known to infect mammals, other vertebrates, or any plant species. Despite these attractive features, the more widespread commercial application of baculoviruses has been limited for a number of reasons. The length of time taken to kill the infected insect is particularly problematic; depending on the strain of virus and the pest species, it may take from several days to weeks before the infected insect at least ceases feeding. During this period the insect pest can cause serious damage to the crop. There is currently intense interest in the possibility of reducing this lag before feeding arrest by genetic engineering means. A variety of strategies have been proposed, all based on the concept of engineering the virus to express new genes which are deleterious to the insect. Genes which have been tested so far include genes encoding insect-specific toxins, such as *Butheus eupeus* insect toxin-1 (Carbonell *et al.*, 1988), *Bacillus thuringiensis* endotoxin (Martens *et al.*, 1990; Merryweather *et al.*, 1990) and the insect-specific neurotoxin of the straw itch mite (Tomalski & Miller, 1991). In addition, several groups are investigating the potential of disrupting hormonal regulation by expressing genes such as diuretic hormone (Maeda, 1989), eclosion hormone (Eldridge *et al.*, 1991), and juvenile hormone esterase (Eldridge *et al.*, 1992; Hammock *et al.*, 1990).

The *egt* gene of the baculovirus AcMNPV encodes an enzyme known as ecdysteroid UDP-glucosyltransferase (EGT) (O'Reilly & Miller, 1989). This

enzyme, which is secreted into the haemolymph of infected insects, catalyses the conjugation of ecdysteroids (insect moulting hormones) with glucose or galactose (O'Reilly *et al.*, 1992). The sugar group is attached to the hydroxyl group at position C22 of the ecdysteroid molecule (O'Reilly *et al.*, 1991). The release of ecdysteroids into the haemolymph is the principal trigger for ecdysis. During infection by AcMNPV, EGT circulating in the haemolymph prevents the accumulation of high levels of ecdysteroids, and moulting or metamorphosis of the infected host is prevented (O'Reilly *et al.*, 1992; O'Reilly & Miller, 1989).

In this study, the effects of *egt* expression on the growth and development of infected insects have been characterized. These data reveal that, through the expression of *egt*, AcMNPV ensures that the host insect continues to feed after infection. Thus, the lag time before feeding cessation is actively prolonged by the virus, presumably to facilitate its own propagation. Consequently, the pesticidal properties of AcMNPV can be improved simply by deleting the *egt* gene and impairing the ability of the virus to lengthen feeding of the infected insect. This approach contrasts with other strategies for the improvement of baculovirus pesticides in that it does not involve the insertion of foreign DNA into the virus. These data have been discussed in more detail in O'Reilly & Miller (1991).

INSECT GROWTH AND DEVELOPMENT FOLLOWING INFECTION

To investigate the effects of *egt* expression on the growth and development of infected insects, *Spodoptera frugiperda* larvae were infected by injection with wild-type (wt) AcMNPV or with a recombinant AcMNPV in which the *egt* gene had been destroyed by genetic engineering (O'Reilly & Miller, 1990). Groups of 30 insects were infected approximately 24 hours after ecdysis into the 6th (final) larval instar, and their growth and development were monitored daily. The infected insects were compared to control insects which had been injected with tissue culture fluid containing no virus. The mean weights of the insects are presented in Fig. 1. As expected, the control insects fed and gained weight extensively for the first 2-3 days, but then displayed a dramatic weight loss during the wandering phase preceding pupation. All these insects pupated from 5 to 6 days post-infection (p.i.). The insects infected by the *egt*$^-$ virus also stopped feeding and displayed significant weight loss after 2 days p.i. These insects developed to a pharate pupal stage but they all died before completing the pupal moult. In contrast, because of the expression of *egt*, none of the wt-infected insects initiated wandering behaviour or stopped feeding. Instead they gained weight until 4 days p.i. and continued to feed until shortly before death. Some of these wt-infected insects attained weights significantly larger than those normally observed in uninfected insects. Similar observations were made with insects infected at the beginning of the penultimate instar. In addition, in both instars, the insects infected with the *egt*$^-$ virus displayed earlier mortality. Thus, infection by a virus lacking *egt* in either 5th or 6th instar results in reduced feeding and more rapid death of the infected insects.

BIOASSAY IN NEONATE LARVAE

The data presented in Fig. 1 were obtained following injection of 6th instar insects with non-occluded virus prepared in cell culture. We have also compared the virulence and infectivity of wt and *egt*$^-$ AcMNPV following

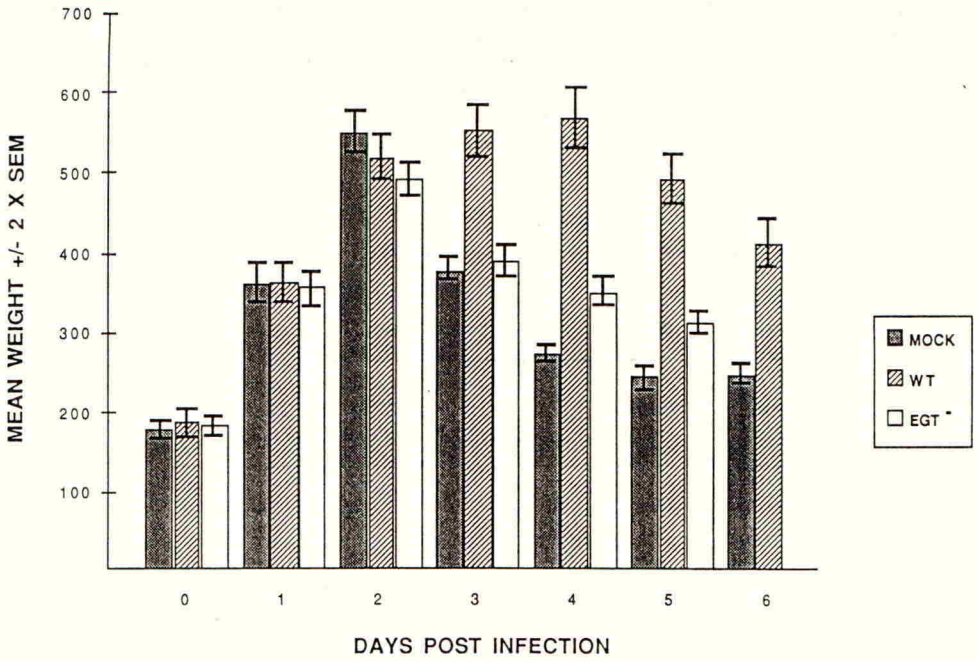

Figure 1. Daily mean weights +/- 2X SEM of control insects (mock) compared to insects infected with wt or egt⁻ AcMNPV.

per os infection of neonate insects with viral occlusion bodies (OBs). For the dose-mortality studies, groups of 60 insects were fed artificial diet containing different concentrations of viral OBs. After 24 hours, the insects were transferred to virus-free diet, and the mortality

TABLE 1. Dose-mortality response

Virus	LC_{50}[a]	95% fiducal limits upper	lower	slope
wt AcMNPV	1.42	2.67	0.79	1.59
egt⁻ AcMNPV	2.39	3.75	1.63	0.84

[a] OBs x 10^{-6}/ml of infected diet

at each dose was recorded 9 days later. The data obtained are presented in Table 1. It can be seen from these data that the LC_{50}s of the two viruses do not differ significantly.

The time-mortality responses following infection with either wt or *egt*⁻ AcMNPV were assessed by feeding groups of 90 neonate larvae an LC_{95} dose of OBs, and recording mortality at 24 hour intervals. As shown in Table 2, *egt*⁻ AcMNPV is significantly more virulent, with an ST_{50} 27.5 hours shorter than that of wild-type virus. These data show that deletion of the *egt* gene increases the virulence but does not change the infectivity of the virus.

TABLE 2. Time-mortality response

Virus	ST_{50}(h)	95% fiducal limits upper	lower	slope
wt AcMNPV	127.2	132.0	122.6	11.5
egt⁻ AcMNPV	99.7	104.1	95.6	9.6

DIET CONSUMPTION BY INFECTED INSECTS

The data presented above demonstrate that *egt*⁻ virus-infected insects feed for shorter periods of time after infection than wt virus-infected insects. To determine the actual reduction in food consumption, the amount of diet consumed by day 1 final instar insects from the time of infection until death was measured. The mean dry weight of diet consumed by insects infected by wt AcMNPV was 402 ± 133 mg. In comparison, *egt*⁻ AcMNPV-infected insects consumed 287 ± 88 mg. This difference was highly significant ($p = 0.01$) by the Mann-Whitney Wilcoxon test. As expected from the weight gain studies (Fig. 1) there was no significant difference between the amount of food consumed by *egt*⁻ AcMNPV-infected and mock-infected insects (286 ± 79 mg) during this time. These data demonstrate that wt-infected insects consume approximately 40% more diet than insects infected with an *egt*⁻ virus.

CONCLUSIONS

This study demonstrates that *egt* expression by the baculovirus AcMNPV, through the inhibition of ecdysis, maintains the insect in an actively feeding state during infection. From a virological point of view, a critical question is why AcMNPV expresses an *egt* gene. We have found that the prolonged feeding due to *egt* results in an increase of approximately 30% in the yield of progeny OBs per infected insect (O'Reilly & Miller, 1991). Such an increase could certainly confer a selective advantage on the virus. However, these data were obtained in controlled laboratory settings with developmentally synchronous insects which were infected with large virus doses. It remains to be seen whether *egt* expression increases virus yield to the same extent in a field situation, where all these parameters will be highly variable. Other effects of *egt* expression may also be important to the virus. For example, insects which normally leave the host plant to pupate will not do so following infection with an *egt*⁺ baculovirus. Such a behavioural change could be of considerable importance in facilitating the spread of the virus in the field.

Our data show that *egt* deletion accelerates the virus-induced mortality and reduces the insect feeding period (up to 3 to 5 days shorter in 6th instar; Fig. 1). Measurements of the amount of food consumed by the infected insects indicates that, in 6th instar, the prolongation of feeding by wt AcMNPV causes a 40% increase in the amount of diet consumed. This is in good agreement with previous data on food consumption by baculovirus-infected insects (Subrahmanyam & Ramakrishnan, 1981) which also noted that feeding continued until death, and that infected insects consumed more than uninfected controls.

The results presented in this study demonstrate that the pesticidal properties of a baculovirus can be improved simply by deletion of a gene. This represents a novel approach to the engineering of improved baculovirus pesticides, and contrasts with other strategies, all of which have involved the expression of some foreign gene. One of the safety concerns with the generation of recombinant viral pesticides is that introduction of a foreign gene will alter their properties in some undesirable way. We expect therefore, that *egt*$^-$ baculoviruses will be more easily registered by pesticide regulatory agencies as genetically improved viral pesticides.

ACKNOWLEDGEMENTS

S. frugiperda eggs were kindly provided by D. Perkins (USDA; Tifton, Georgia). This work was supported in part by Public Health Service grant AI 23719 from the National Institute of Allergy and Infectious Disease to Lois K. Miller (University of Georgia, Athens, Georgia).

REFERENCES

Carbonell, L.F.; Hodge, M.R.; Tomalski, M.D.; Miller, L.K. (1988) Synthesis of a gene coding for an insect-specific scorpion neurotoxin and attempts to express it using baculovirus vectors. *Gene*, **73**, 409-418.

Eldridge, R.; Horodyski, F.M.; Morton, D.B.; O'Reilly, D.R.; Truman, J.W.; Riddiford, L.M.; Miller, L.K. (1991) Expression of an eclosion hormone gene in insect cells using baculovirus vectors. *Insect Biochemistry*, **21**, 341-351.

Eldridge, R.; O'Reilly, D.R.; Hammock, B.D.; Miller, L.K. (1992) Insecticidal properties of genetically engineered baculoviruses expressing an insect juvenile hormone esterase gene. *Applied and Environmental Microbiology*, **58**, 1583-1591.

Entwistle, P.F.; Evans, H.F. (1985) Viral Control. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 12*, G.A. Kerkut and L.I. Gilbert (Eds), Oxford: Pergamon Press, pp. 347-412.

Gröner, A. (1986) Specificity and safety of baculoviruses. In: *The Biology of Baculoviruses. Vol. I*, R.R. Granados and B.A. Federici (Eds), Boca Raton: CRC Press, pp. 177-202.

Hammock, B.D.; Bonning, B.C.; Possee, R.D.; Hanzlik, T.N.; Maeda, S. (1990) Expression and effects of the juvenile hormone esterase in a baculovirus vector. **344**, 458-461.

Maeda, S. (1989) Increased insecticidal effect by a recombinant baculovirus carrying a synthetic diuretic hormone gene. *Biochemisty and Biophysics Research Communications*, **165**, 1177-1183.

Martens, J.W.M.; Honee, G.; Zuidema, D.; Van Lent, J.W.M.; Visser, B.; Vlak, J.M. (1990) Insecticidal activity of a bacterial crystal protein expressed by a recombinant baculovirus in insect cells. *Applied and Environmental Microbiology*, **56**, 2764-2770.

Merryweather, A.T.; Weyer, U.; Harris, M.P.G.; Hirst, M.; Booth, T.; Possee, R.D. (1990) Construction of genetically engineered baculovirus insecticides containing the *Bacillus thuringiensis* subsp. *kurstaki* HD-73 delta endotoxin. *Journal of General Virology*, **71**, 1535-1544.

O'Reilly, D.R.; Brown, M.R.; Miller, L.K. (1992) Alteration of ecdysteroid metabolism due to baculovirus infection of the fall armyworm *Spodoptera frugiperda*: host ecdysteroids are conjugated with galactose. *Insect Biochemistry and Molecular Biology*, **22**, 313-320.

O'Reilly, D.R.; Howarth, O.W.; Rees, H.H.; Miller, L.K. (1991) Structure of the ecdysone glucoside formed by a baculovirus ecdysteroid UDP-glucosyltransferase. *Insect Biochemistry*, **21**, 795-801.

O'Reilly, D.R.; Miller, L.K. (1989) A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase. *Science*, **245**, 1110-1112.

O'Reilly, D.R.; Miller, L.K. (1991) Improvement of a baculovirus pesticide by deletion of the *egt* gene. *Bio/Technology*, **9**, 1086-1089.

O'Reilly, D.R.; Miller, L.K. (1990) Regulation of expression of a baculovirus ecdysteroid UDP-glucosyltransferase gene. *Journal of Virology*, **64**, 1321-1328.

Subrahmanyam, B.; Ramakrishnan, N. (1981) Influence of a baculovirus infection on molting and food consumption by *Spodoptera litura*. *Journal of Invertebrate Pathology*, **38**, 161-168.

Tomalski, M.D.; Miller, L.K. (1991) Insect paralysis by baculovirus-mediated expression of a mite neurotoxin gene. *Nature*, **352**, 82-85.

SESSION 5

NEW COMPOUNDS, FORMULATIONS AND USES – FUNGICIDES

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PYRIMETHANIL: A NEW FUNGICIDE

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ABSTRACT

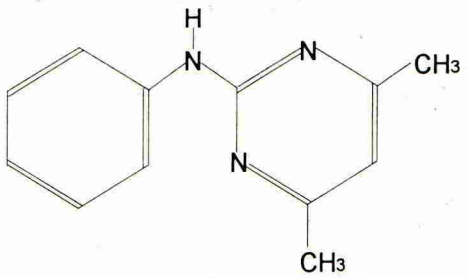
Pyrimethanil is a new anilino-pyrimidine fungicide. It is being developed by Schering AG as a foliar fungicide to control grey mould (*Botrytis cinerea*) on vine, fruits and vegetables. Pyrimethanil is highly effective against all strains of *Botrytis* and has not shown cross-resistance to commercially available botryticides.

INTRODUCTION

Pyrimethanil is the draft ISO common name for N-(4,6-dimethylpyrimidin-2-yl)aniline, a new anilino-pyrimidine fungicide, property of Schering AG, Berlin, Germany. It is being developed by Schering AG for the control of grey mould. This paper describes the properties of pyrimethanil and its performance on several economically important crops.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical class: Anilino-pyrimidine
Chemical name: N-(4,6-dimethylpyrimidin-2-yl)aniline
Common name: Pyrimethanil (draft ISO)
Structural formula:



Molecular formula: $C_{12}H_{13}N_3$

Molecular weight: 199.26 g/mol
Appearance: white crystalline solid
Melting point: 96.3°C
Solubility: 0.121 g/l in water (25°C), soluble in most organic solvents
Partition coefficient: log P = 2.48 (n-octanol/water)
Stability: essentially stable within the relevant pH range
Vapour pressure: 2.2 x 10-3 Pa at 25°C

TOXICOLOGY

Pyrimethanil is of very low acute toxicity to a variety of species as follows:

Acute toxicity:	mouse oral LD50	4061-5358 mg/kg
	rat oral LD50	4150-5971 mg/kg
	rat dermal LD50	>5000 mg/kg
Irritation and sensitisation:	rabbit skin irritation	negative
	rabbit eye irritation	negative
	guinea pig skin sensitisation	negative
Mutagenicity:	negative in Ames test, *in vitro* cytogenetics assay, *in vivo* micronucleus test and *in vivo* UDS assay.	
Teratogenicity:	not teratogenic in rats or rabbits.	
Wildlife toxicity:	mallard duck and bobwhite quail	LD50 >2000 mg/kg
	mallard duck and bobwhite quail (5 day)	LC50 >5200 ppm
	mirror carp 96 hr LC50	35.36 mg/l
	rainbow trout 96 hr LC50	10.56 mg/l
	earthworm 14 day LC50	625 mg/kg
	honey bee (oral and dermal) LD50	>100 µg/bee

FORMULATIONS

Pyrimethanil is currently available as a flowable formulation (SC 200 and 400 g/l), as well as a water dispersible granule formulation (WG 80 %) and will be marketed under various trademarks including 'Scala'.

BIOLOGICAL ACTIVITY

Mode of action

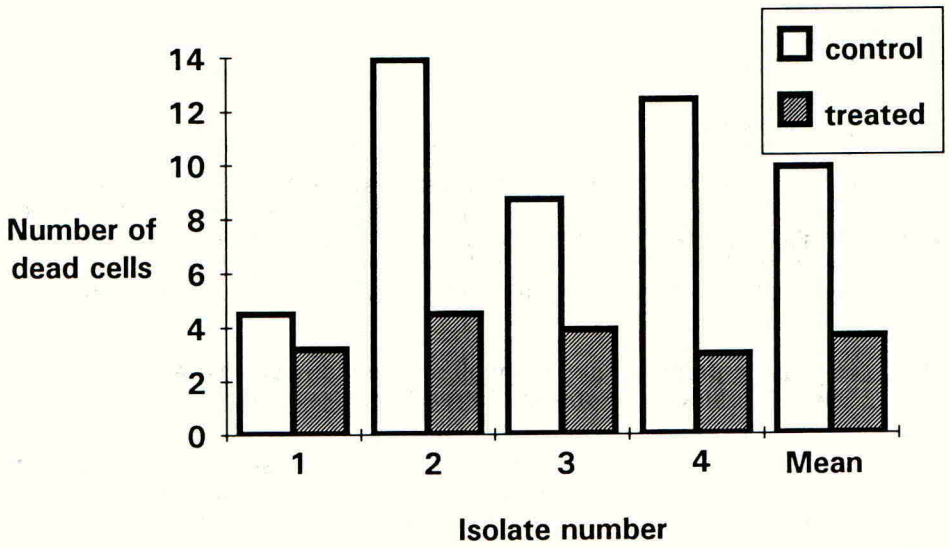
The effect of pyrimethanil on the infection process of *Botrytis* spp. has been investigated. It does not inhibit spore germination or the number of cells per germ tube, but germ tube extension is inhibited. A decrease in the extent of penetration into the host epidermal cells has also been observed, and the number of dead epidermal cells resulting from an individual penetration site is considerably reduced (see Fig. 1). Pyrimethanil appears to be fungistatic in its action.

A number of studies has been conducted to determine the biochemical mode of action of pyrimethanil in controlling *Botrytis* spp. It has been shown neither to inhibit respiration nor to act as an uncoupler of oxidative phosphorylation. It does not cause lipid peroxidation or affect the integrity and osmotic stability of the cells. Preliminary tests showed that ergosterol biosynthesis is not inhibited. There is also no effect on the biosynthesis of protein, RNA, DNA or chitin.

Further studies are ongoing to clarify the mode of action. Inhibition of protein secretion by the pathogen has been demonstrated, including reduced levels of some hydrolytic enzymes which are thought to play a role in penetration into and necrosis of the host tissue. These findings are consistent with the observed effects of pyrimethanil on the infection process *in vivo*.

FIGURE 1. Effect of a 48 h pre-inoculation treatment with 200 mg/l of pyrimethanil on the number of epidermal cells killed per penetration site, in four different isolates of *Botrytis fabae*.

(Dr A Daniels, University of Nottingham, 1991)



BIOLOGICAL PROPERTIES

Systemicity Data

Pyrimethanil has been shown to exhibit protectant control but no significant systemic activity by leaf to leaf transfer (Table 1).

TABLE 1. Control of *Botrytis cinerea* on tomatoes following a 24 hour protectant spray of pyrimethanil.

Compound	Rate mg/l	% disease control* systemic	protectant
pyrimethanil	250	20	91
	100	13	93
iprodione	250	1	95
	100	0	90

* Systemic control was assessed on the 3 leaves above the treated leaf. Protectant control was assessed on the treated leaf.

Although the systemic activity from leaf to leaf is negligible, the translaminar activity is significant (Table 2).

TABLE 2. Translaminar activity of pyrimethanil.

Treatment (pyrimethanil 200 mg/l)	% protection of untreated areas
Lower surface treated + upper surface inoculated	34
Upper surface treated + lower surface inoculated	73
Left half leaf treated + right half leaf inoculated	63

Pyrimethanil has shown a very high level of systemicity when applied as a root dip for 30 minutes (Table 3). After dipping, the roots were rinsed in distilled water and the plants potted in a John Innes base compost. Inoculation with *B. cinerea* was carried out 48 hours later. Care was taken to exclude any vapour effects. Disease level on untreated plants was 60 % at assessment time.

TABLE 3. Systemic profile of pyrimethanil on tomatoes against an MBC sensitive strain of *Botrytis cinerea* (root dip application).

Rate (mg/l)	disease control (%) with pyrimethanil	disease control (%) with benomyl
50	92	70
25	82	50
5	75	47
1	0	38

Vapour Activity

Vapour activity of the compound has also been observed. Unsprayed tomato plants were placed next to sprayed plants and then enclosed under a plastic cover (Table 4).

TABLE 4. Control of *Botrytis cinerea* on tomato plants by pyrimethanil applied as a foliar spray.

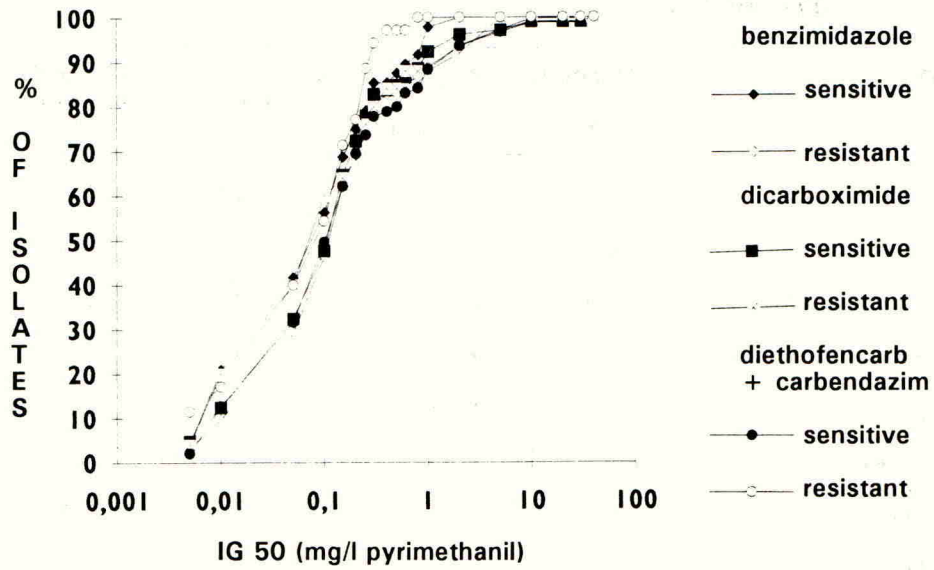
Rate (mg/l)	% control in treated plants	% control in adjacent untreated plants
100	86	61
50	82	30

Control of Resistant strains

Pyrimethanil is able to control strains of *Botrytis cinerea* resistant to dicarboximides, MBC (benzimidazoles), dicarboximides and MBC, diethofencarb, and diethofencarb and MBC.

In Figure 2, 130 isolates collected from French vineyards have been classified into 6 phenotypes according to their sensitivity to MBC, dicarboximides and diethofencarb + carbendazim. Pyrimethanil was equally active against all strains.

FIGURE 2. Cumulative IG 50 (50 % control of mycelium growth) distribution values of different phenotypes of *Botrytis cinerea* from vines.



FIELD TRIALS DATA

Grapes

Pyrimethanil gave outstanding control of *B. cinerea* which was significantly better than the commercial standards (Table 5).

TABLE 5. Average % control of *Botrytis cinerea* in 64 European trials conducted over 7 years.

Year	85	86	87	88	89	90	91
No. of trials [1]	2	2	4	6	17	13	20
pyrimethanil [2]	85	91	90	81	70	74	81
best standard [3]	29	52	30	64	44	51	66
untreated (% infestation)	50	25	33	37	45	40	26

1): trials conducted in France, Germany, Italy, Greece and Spain
2): 2 to 4 applications at 50 g AI/hl or 800 g AI/ha
3): same number of applications of vinclozolin 37.5 g AI/hl or diethofencarb + carbendazim 50 + 50 g AI/hl.

Comparison of trials where pyrimethanil alone was used 4 times per season or with sequential spray programmes including other fungicides indicated that the efficacy of sequential spray programmes can be as effective as the use of pyrimethanil alone. Therefore, pyrimethanil can be recommended for use in an alternating spray strategy for resistance management (Table 6).

TABLE 6. Assessment of *Botrytis cinerea* on Sauvignon grapes, 19 September 1991, INRA Bordeaux.

Treatment	Dose per ha	Application stage				Damage per bunch	Number of diseased bunches
	g AI	T1	T2	T3	T4	%	%
Untreated						76.0	100.0
Vinclozolin	750	x	x	x	x	62.0	97.8
Diethofencarb + carbendazim	500 + 500	x	x	x	x	21.7	72.7
Vinclozolin + thiram	500 + 3200	x	x	x	x	21.7	69.9
Pyrimethanil	800	x	x	x	x	14.7	59.8
Diethofencarb + carbendazim	500 + 500	x					
Pyrimethanil	800		x				
Vinclozolin	750			x	x	16.0	57.7
Pyrimethanil	800	x					
Diethofencarb + carbendazim	500 + 500		x				
Vinclozolin	750			x	x	22.9	74.8
Diethofencarb + carbendazim	500 + 500	x					
Vinclozolin	750		x		x	29.7	82.3
Pyrimethanil	800			x			
Newman & Keuls (5 %)						F = 54.99 C.V. = 10.2%	F = 34.56 C.V. = 7.5 %

Pyrimethanil had no adverse effects on wine fermentation and did not affect the organoleptic quality of the wine in official tests in France, Italy and Germany.

Strawberries

Many trials over 3 years in normal and everbearing varieties have demonstrated an outstanding performance of pyrimethanil for the control of *B. cinerea*. Two examples from official trials conducted in Belgium (Research Station of Gorsem), are shown in Tables 7 and 8.

TABLE 7. Control of *Botrytis cinerea* on everbearing strawberries (cv. Selva) on 21 October 1991 after 12 treatments.

Treatment	g AI/ha	% control
Pyrimethanil	800	92
Diethofencarb + carbendazim	250 + 250	94
Procymidone	500	16
Vinclozolin	500	42
Tolylfluanid	1250	75
Thiram	1600	45

In spray programmes, best results, equivalent to pyrimethanil alone, were achieved in alternation with the best compounds of the above table (Table 8). The sequence with procymidone was the least effective due to presence of dicarboximide-resistant strains.

TABLE 8. Control of *Botrytis cinerea* on everbearing strawberries (cv. Selva) after different triple sets of treatments in the spray programme (evaluation on 21 October 1991).

Treatment (rates as Table 7)	% control
3 x P/3 x DC/3 x P/3 x DC	95
3 x P/3 x T/3 x P/3 x T	91
3 x P/3 x Pc/3 x P/3 x Pc	69
P/Pc/DC (continous sequence)	87
12 x P	91

Key: P = pyrimethanil, DC = diethofencarb + carbendazim, T = tolylfluanid, Pc = procymidone

Tomatoes

Pyrimethanil shows excellent activity at 50 g AI/hl or 750-800 g ai/ha in tomatoes. In Table 9, the results of a typical trial are given. The trial was conducted under glass in Spain and, due to the vapour activity of pyrimethanil, 2 untreated plots were included: one within the experimental area (4 randomised blocks), and one separate but close to the area of the trial.

TABLE 9. Control of *Botrytis cinerea* on glasshouse tomatoes after 6 sprays at 14 d intervals.

Treatment	g AI/ha	% control on dates: (T_6+8 d)	(T_6+29 d)	(T_6+39 d)
Pyrimethanil	750	100	100	100
Vinclozolin	750	62	83	50
Diethofencarb + carbendazim	468	81	96	89
% of infestation in				
Untreated within area of treated		11	12	14
Untreated outside area of treated		22	33	45

The disease levels in untreated plots within the experimental area were lower than outside the area. This suggests vapour activity.

Onions

Excellent activity has been demonstrated against *Botrytis squamosa* in onions. Table 10 shows the average results of 5 trials conducted in 1991 in the Netherlands using 8 applications.

TABLE 10. Control of *Botrytis squamosa* in onions

Treatment	g AI/ha	% control	% yield
Pyrimethanil	800	69	105
Diethofencarb + carbendazim	250 + 250	50	103
Untreated (% infestation)		31	100

Flower bulbs

An outstanding effect on *B. cinerea* in the Netherlands in artificially infected, forced tulips can be seen in Table 11 (mean of 3 trials). Pyrimethanil treated plants gave the largest yield per pot and the highest number of first class tulips at harvest (max. score 10).

TABLE 11. Control of *Botrytis cinerea* in forced tulips at harvest.

Treatment	g AI/hl	crop yield (g/pot)	1st class tulips (out of 10 per pot)
Pyrimethanil	400	294	9.4
Pyrimethanil	200	303	9.1
Fluazinam	125	275	7.5
Captan	136.5	245	6.7
Procymidone	75	162	4.0
Infected/untreated		180	3.7
Uninfected/untreated		293	9.2

Other crops

Pyrimethanil is highly effective and crop safe at appropriate rates in a number of additional field crops including fodder peas, peas, beans, cucumbers, aubergines and in ornamentals (*Erica, Rhododendron, Begonia, Cyclamen*).

CONCLUSION

Pyrimethanil is a new protective, translaminar and root systemic botryticide for various uses in many crops. It fits very well into seasonal spray programmes. Pyrimethanil has not shown cross-resistance to currently available botryticides and represents a true alternative or partner in a modern *Botrytis* management programme.

ACKNOWLEDGEMENT

The authors wish to thank all their colleagues from many countries, and all the cooperators who have contributed to the data presented in this paper.

BAS 490 F - A BROAD-SPECTRUM FUNGICIDE WITH A NEW MODE OF ACTION

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ABSTRACT

BAS 490 F (methyl-(E)-methoximino[α-(o-tolyloxy)-o-tolyl]acetate) is a new synthetic fungicide derived from the fungal secondary metabolite strobilurine. Its broad fungicidal spectrum has demonstrated the following profile in several years of field trials: excellent control of scab in apples and pears, of powdery mildew in apples, grape vine, cucurbits and sugar beet and good control of mildew, scald, net and glume blotch in cereals, of blast and sheath blight in rice, and of downy mildew on grape vine and vegetables. Control of many diseases in several other crops has also been observed. Generally, BAS 490 F has been used safely on mono- and dicotyledoneous crops. In grapes, selectivity of BAS 490 F was good; crop tolerance was slightly reduced only at higher rates.
The fungicidal activity is due to the inhibition of the fungal respiration. The product is safe both to users and to the environment. To date, no adverse toxic effects have been observed.

INTRODUCTION

In 1983, BASF began a programme to evaluate natural products as potential leads for new synthetic pesticides. In the course of this programme, we obtained strobilurine A - a secondary metabolite from the fungus *Strobilurus tenacellus* - from Prof. Anke of the University of Kaiserslautern, FRG in July 1983. Its remarkable in vitro activity (Anke *et al.*, 1977), its new mode of action (Becker *et al.*, 1981), its weak but still measurable fungicidal activity in glasshouse tests and the simplicity of its structure convinced us to focus on this compound as a new fungicidal lead.
At the same time, Prof. Steglich and his group at the University of Bonn, FRG had already succeeded with the synthesis of the first analogues of the β-methoxy-α-phenyl-acrylate type (Schramm 1980, Anke *et al.*, 1989), which also showed some fungicidal activity. Cooperation with the Steglich group led to further structural variations which resulted in several new types of strobilurine analogues (see Appendix) with remarkably enhanced fungicidal properties (Schirmer *et al.*, 1985). Independently, a similar approach to this field of chemistry has been made by others (Beautemont *et al.*, 1991).
Our research in this field has resulted in the fungicide BAS 490 F, which is currently under development (Wenderoth *et al.*, 1986). Its broad-spectrum fungicidal properties, which have been determined in laboratory, glasshouse and field trials, are described below.

CHEMICAL AND PHYSICAL PROPERTIES

Code number	:	BAS 490 F
Chemical name (IUPAC)	:	methyl-(E)-methoximino[α-(o-tolyloxy)--o-tolyl]acetate

Structural formula :

Molecular formula	:	$C_{18}H_{19}NO_4$
Molecular weight	:	313.36
Appearance at 20 ^{0}C	:	colourless, odourless crystals
Melting point	:	97.2 - 101.7 ^{0}C
Vapour pressure at 20 ^{0}C	:	2.3 x10^{-8} mbar
Solubility	:	2 mg/l water at 20 ^{0}C
Partition coefficient at 25 ^{0}C	:	log P = 3.4 (n-octanol/water at pH7)
Stability	:	no hydrolysis at pH7 at 20 ^{0}C within 24 h

TOXICOLOGY

Acute toxicity of the technical active ingredient:

Acute oral LD50 rat	:	>5 000 mg/kg body weight
Acute dermal LD50 rat	:	>2 000 mg/kg body weight
Skin irritation rabbit	:	non-irritating
Eye irritation rabbit	:	non-irritating
Ames test	:	negative
Teratogenicity	:	not teratogenic according to the data currently available

FORMULATION

Three formulations have been used: 50 DF, 50 WP with 500 g AI/kg and 500 SC with 500 g AI/l.

BIOLOGICAL ACTIVITY

Material and methods

LC50-values were determined using BAS 490 F-amended agar plates, at a range of concentrations, which had been inoculated with discs of fungal mycelium and assessed by comparison with untreated plates.
In glasshouse experiments, pot grown plants were used which had been cultivated under standard conditions. Inoculations were made by either using aqueous spore suspensions or dusting the plant with spores (*Erysiphe, Puccinia*) with subsequent cultivation under conditions favourable for disease development. Visual assessment of the disease development was made in percent leaf area affected.
The field trials were laid out in randomized blocks with 4 replications. The size of the blocks varied from 10 to 20 m^2. All trials were sprayed at the beginning of attack either using special small plot tractor-spray equipment or a hand-held precision plot sprayer. Treatments were applied in 200 - 800 l water/ha. A visual assessment of the infected leaves or ears was made in percent for the plot as a whole. Growth stages (GS) are described for cereals according to Zadoks and for other crops according to Weber *et al.*, (1990).

Results

Mode of action

From the literature, the natural lead strobilurine was known to be an inhibitor of mitochondrial respiration by blocking the electron transfer at the cytochrome bc_1 complex (Becker *et al.*, 1981). Detailed studies with the first synthetic analogues by Brandt *et al.* (1988 and 1991) revealed the ubihydroquinone:cytochrome-c oxidoreductase to be the binding site. According to our experiments (Röhl, F., BASF, personal communication), BAS 490 F binds to the same site and thus inhibits respiration. Using a yeast electron transport particle preparation, the rate of cytochrome c reductase was inhibited to 50 % by $2.9 \pm 0.6 \times 10^{-8}$ mol/l BAS 490 F in comparison with an untreated control.

In vitro activity

In in vitro tests, BAS 490 F showed the following activities against a representative group of fungi.

TABLE 1. LC50-values in mg AI/l of a variety of fungi towards BAS 490 F in amended agar plates.

Alternaria solani	< 1	*Mucor circinelloides*	> 500
Aspergillus niger	> 500	*Mycosphaerella fijiensis*	< 1
Botryotinia fuckeliana	> 500	*Nectria galligena*	> 500
Cercospora kikuchii	< 1	*Penicillium digitatum*	< 1
Chaetomium globosum	< 50	*Penicillium expansum*	< 1
Choanephora cucurbitarum	< 500	*Phaeosphaeria nodorum*	< 1
Cladosporium herbarum	< 10	*Phomopsis longicola*	> 500
Cochliobolus sativus	< 50	*Phytophthora cactorum*	> 500
Colletotrichum coffeanum	< 50	*Phytophthora infestans*	< 1
Coniophora puteana	< 1	*Pyrenophora avenae*	< 10
Corticium rolfsii	< 1	*Pyrenophora teres*	< 10
Corticium salmonicolor	< 50	*Pythium ultimum*	> 500
Cylindrocladium scoparium	> 500	*Rhizopus stolonifer*	< 10
Fusarium culmorum	< 50	*Sclerotinia fructigena*	> 500
Fusarium oxysporum	< 50	*Sclerotinia sclerotiorum*	< 1
Glomerella cingulata	< 10	*Sclerotium cepivorum*	< 1
Guignardia citricarpa	< 1	*Serpula himantoides*	< 1
Leptosphaeria maculans	< 10	*Tapesia yallundae*	> 500
Leptosphaeria salvinii	< 1	*Thanatephorus cucumeris*	< 10
Macrophomina phaseolina	> 500	*Trichoderma viride*	< 10
Magnaporthe grisea	< 100	*Venturia inaequalis*	< 1
Monographella nivalis	< 1	*Verticillium dahliae*	< 10

Glasshouse results

The good and broad activity of BAS 490 F was established in glasshouse tests against plant pathogenic fungi belonging to the Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes. Special tests revealed a strong curative activity of BAS 490 F in the control of *Venturia inaequalis* on apple. A single foliar spray-treatment with an aqueous suspension containing 31 mg/l BAS 490 F completely controlled this disease, even 72 h after inoculation. The trial was assessed 14 days after inoculation. Drenching hydroponic rice with BAS 490 F gave no indications for systemic activity against *Pyricularia oryzae* (teleomorph *Magnaporthe grisea*).

Field results in pome fruits

A very good control of scab (*Venturia inequalis*) and powdery mildew (*Podosphaera leucotricha*) in apples (Table 2) has been established. The strong curative properties of BAS 490 F are advantageous under field conditions. No problems of phytotoxicity have been observed in a number of cultivars tested. Equally good results were obtained in Spain in the control of scab on pears (*V. pirina*) using 50 or 100 g AI/ha.

TABLE 2. Control of *Venturia inaequalis* and *Podosphaera leucotricha* in the Federal Republic of Germany, 1991 (cvs. Golden Delicious, Idared, Gravensteiner.).

Treatment[1]	Dose (g AI/ha)	Mean % leaf area affected	
		V. inaequalis	*P. leucotricha*
BAS 490 F	50	4	7
BAS 490 F	100	1	5
Flusilazole + metiram	30 + 1200	9	9
Untreated		79	36
Number of trials		3	2

[1] 9 - 10 Treatments (2-4 days after favourable conditions for scab infection).

Grape vine

BAS 490 F has the potential to effectively control powdery (*Uncinula necator*) and downy (*Plasmopara viticola*) mildew on grape vine. Under strong disease pressure, both diseases on the leaves and berries can be significantly reduced.

TABLE 3. Control of *Uncinula necator* in grape vine in Spain 1991.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area or berries affected	
		leaves	berries
BAS 490 F	50	8	9
BAS 490 F	100	3	3
BAS 490 F	150	2	1
Myclobutanil	30	4	4
Untreated		50	70

[1] 4-6 Treatments in 10 - 14 days' interval (Mean of 2 sites).

TABLE 4. Control of *Plasmopara viticola* in grape vine in France and Brazil 1990-1991.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area or berries affected			
		France		Brazil	
		leaves	berries	leaves	berries
BAS 490 F	375	12	11	6	5
Metiram	2800	8	15	11	1
Metalaxyl + folpet/mancozeb	200-320 + 800-1280	5	14	1	1
Untreated		49	84	51	83

[1] 9 Treatments in France and 5 in Brazil (Mean of 2 sites).

Slight phytotoxicity was sometimes observed on young expanding leaves of grape vine at rates > 200 g AI/ha. This was dependent upon the variety grown and on the prevailing climatic conditions. No phytotoxicity symptoms have been observed on fully developed leaves.

Cereals

BAS 490 F offers good opportunities to control the major diseases in cereals due to its broad spectrum of activity.

TABLE 5. Control of *Erysiphe graminis* (eradicative), *Phaeosphaeria nodorum* (anamorph *Septoria nodorum*) and *Puccinia recondita* (prophylactic) on winter wheat in the Federal Republic of Germany 1990.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area affected			Relative yield
		E.graminis	*P.nodorum*	*P.recondita*	
BAS 490 F	250	3	1	6	150
BAS 480 F + fenpropimorph	125 + 375	6	1	0	154
Untreated		28	12	45	100

[1] Applied at GS 32 and GS 51 (Mean of 3 trials).

Field tests revealed the good potential of BAS 490 F to control *Phaeosphaeria nodorum* and *Mycosphaerella graminicola* (anamorph *Septoria tritici*) on leaves and ears in winter wheat. In prophylactic situations, BAS 490 F has a good fungicidal activity against rust diseases in cereals, such as *Puccinia recondita* on winter wheat or rye and *P. striiformis* on winter wheat.
In barley, net blotch (*Pyrenophora teres*) and scald (*Rynchosporium secalis*) are well controlled by BAS 490 F.

TABLE 6. Control of *Pyrenophora teres* and *Rynchosporium secalis* on winter barley in the Federal Republic of Germany 1992.

Treatment	Dose (g AI/ha)	Mean % leaf area affected	
		P. teres [1] 30 DAT	*R. secalis*[2] 30 DAT
BAS 490 F	100	3	10
BAS 490 F	200	2	9
Flusilazole + tridemorph	160 + 350	1	4
Untreated		25	19
Number of trials		2	4

[1] One treatment applied at GS 49, [2] one treatment applied at GS 32 - 51.

Rice

In rice, BAS 490 F has a good potential to control blast (*Magnaporthe grisea*, anamorph *Pyricularia oryzae*) and sheath blight (*Corticium sasakii*) by foliar application.

TABLE 7. Control of *Magnaporthe grisea* and *Corticium sasakii* on rice in Taiwan 1991.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area affected			
		M. grisea		*C. sasakii*	
Days after last treatment		8	14	8	14
BAS 490 F	200	6	7	8	20
BAS 490 F	300	3	5	7	12
Kasugamycin + fthalide	10 + 200	9	14	31	53
Pencycuron	125	13	18	6	19
Untreated		19	25	53	74
Number of trials		3		4	

[1] 2-4 Treatments.

Sugar beet

A good control of powdery mildew (*Erysiphe betae*) and leaf spot (*Cercospora beticola*) has been observed with BAS 490 F in sugar beet.

TABLE 8. Control of *Erysiphe betae* on sugarbeet in Spain in 1992.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area affected	
Days after last treatment		0	10
BAS 490 F	100	5	4
BAS 490 F	200	4	1
Difenoconazole	300	4	1
Untreated		52	72

[1] 2 - 3 Treatments in 2-weeks' interval (Mean of 2 sites).

TABLE 9. Control of *Cercospora beticola* on sugarbeet in the Federal Republic of Germany 1991.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area affected		
Days after second treatment		20	30	Relative yield
BAS 490 F	300	13	26	108
BAS 490 F	400	11	23	110
Fentin-acetate + maneb	324 + 96	7	28	108
Untreated		28	30	100

[1] Treatment at the beginning of infection by *C. beticola* at GS 48 and at re-attack at GS 49 (Mean of 3 sites).

Potato

Early and late blight (*Alternaria solani* and *Phytophthora infestans*) in potatoes can be significantly reduced by BAS 490 F.

TABLE 10. Control of *Phytophthora infestans* on potatoes in the Federal Republic of Germany 1991.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area affected		
		GS 81	GS 85	Relative yield
BAS 490 F	400	8	45	111
Metiram	1400	7	40	108
Untreated		31	84	100

[1] 3-5 Treatments (Mean of 3 sites).

TABLE 11. Control of *Alternaria solani* on potatoes in Brazil 1991,1992.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area affected	
		7 DAT	14 DAT
BAS 490 F	200	22	37
	400	15	37
Metiram	1600	49	71
Untreated		59	84

[1] 2 - 4 Treatments (Mean of 3 sites).

Similarly good control of *P. infestans* and *A. solani* in tomatoes has been observed. Good control of other *Alternaria* spp., such as black spot on oil seed rape (*A. brassicae*) and leaf blight on carrots (*A. dauci*), has also been observed; 375 g AI/ha performed clearly better than 500 g AI/ha iprodione.

Cucurbits

Powdery mildews (*Sphaerotheca fuliginea*) on cucurbits are another feature of the fungicidal profile of BAS 490 F.

TABLE 12. Control of *Sphaerotheca fuliginea* on cucurbits in Spain in 1990.

Treatment[1]	Dose (g AI/ha)	Mean % leaf area affected		
		melon 10 DAT	zucchini 10 DAT	cucumbers under glass 7 DAT
BAS 490 F	50	8	30	3
BAS 490 F	100	5	18	1
BAS 490 F	200	3	13	0
Ethirimol	420	3	26	0
Hexaconazole	50	9	41	14
Untreated		43	69	65

[1] 2 - 3 Treatments.

CONCLUSION

BAS 490 F is a new, very active, broad-spectrum fungicide with strong protective, curative, eradicative and long residual disease control. BAS 490 F has excellent potential to control diseases caused by Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes in many crops. A pronounced feature is its strong efficacy against *Venturia*, *Uncinula*, *Erysiphe* and *Alternaria* species by foliar treatments. Minor gaps in its fungicidal spectrum can be filled by combinations with other fungicides.
Disease control achieved by the use of BAS 490 F resulted in significant yield increases.

ACKNOWLEDGEMENTS

We would like to express our thanks to those many colleagues who have contributed to the international development of BAS 490 F.

REFERENCES

Anke, T.; Oberwinkler,F.; Steglich, W.; Schramm, G.(1977) The strobilurins - new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus*. The Journal of Antibiotics. **30**, 806 - 810.

Anke, T.; Steglich, W. (1989) "β -*Methoxyacrylate Antibiotics: From Biological Activity to Synthetic Analogues*" in Schlunegger, U.P. (Ed.) "Biologically Active Molecules", Springer Berlin.

Beautement, K.; Clough, J.M.; de Fraine, P.J.; Godfrey C.R.A. (1991) Fungicidal β-methoxyacrylates: from natural products to novel synthetic agricultural fungicides. Pesticide Science **31**, 499-541.

Becker, W.F.; von Jagow, G.; Anke, T.; Steglich, W. (1981) Oudemansin, strobilurin A, strobilurin B and myxothiazole: new inhibitors of the bc_1 segment of the respiratory chain with an E-β-methoxyacrylate system as common structural element. FEBS Letters **132**, 329 - 333.

Brandt, U.; Schägger, H.; von Jagow, G. (1988) Characterisation of binding of the methoxyacrylate inhibitors to mitochondrial cytochrome c reductase. European Journal of Biochemistry **173**, 499 - 506.

Brandt, U.; von Jagow, G. (1991) Analysis of inhibitor binding to the mitochondrial cytochrome c reductase by fluorescence quench titration. European Journal of Biochemistry **195**, 163 - 170.

Schirmer, U.; Karbach, S.; Pommer, E.H.; Ammermann, E.; Steglich, W.; Schwalge, B.; Anke, T. European Patent 203 606 (30.05.1985); European Patent 203 608 (30.05.1985); European Patent 226 917 (20.12.1985) and European Patent 229 974 (20.12.1985).

Schramm, G. (1980) Neue Antibiotika aus höheren Pilzen (Basidiomyceten). PhD Thesis, University of Bonn, FRG.

Weber, E.; Bleiholder, H. (1990) Explanations of the BBCH decimal codes for the growth stages of maize, rape, field beans and peas. Gesunde Pflanzen **42**, 308 - 321.

Wenderoth, B.; Rentzea, C.; Ammermann, E.; Pommer, E.H.; Steglich, W.; Anke, T. European Patent 253 213 (16.07.1986).

APPENDIX

We prefer the term "strobilurine analogues" instead of "β-methoxyacrylates" for two reasons: 1. strobilurine A is structurally the simplest natural lead molecule of this class and 2. the β-methoxyacrylate moiety, in its narrow chemical definition, is not essential for fungicidal activity. Several variations beyond it, e.g. BAS 490 F, have the same mode of action with similar fungicidal activity.

FLUQUINCONAZOLE, A NOVEL BROAD-SPECTRUM FUNGICIDE FOR FOLIAR APPLICATION

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ABSTRACT

Fluquinconazole, code number SN 597265, is a new quinazolinone based triazole fungicide discovered as a result of a chemical design and synthesis programme aimed at producing novel inhibitors of ergosterol biosynthesis. It possesses a broad spectrum of activity when used as a foliar spray against Ascomycetes, Deuteromycetes and Basidiomycetes, which cause economically important diseases of broad-leaf and cereal crops.
SN 597265 is particularly active against diseases of apple, giving excellent control of *Venturia inaequalis* and *Podosphaera leucotricha*. Other pathogens controlled include powdery mildews, *Monilinia* spp., *Cercospora* spp., *Phoma* spp., *Septoria spp.*, *Pyrenopeziza brassicae*, *Puccinia* spp., *Hemileia* spp., and *Sclerotinia* spp. SN 597265 possesses protectant, eradicant and systemic properties combined with excellent crop safety. It is being developed alone and with various mixture partners in a range of formulations. Expected rates of use are 2.5-15 g AI/hl, and 100-500 g AI/ha, depending on the crop.

INTRODUCTION

SN 597265 is a novel quinazolinone based compound discovered and patented by Schering Agrochemicals Ltd. It arose as a result of a chemical design and synthesis programme aimed at producing novel inhibitors of ergosterol biosynthesis. Early stage research showed SN 597265 to possess excellent fungicidal properties frequently superior to currently available ergosterol biosynthesis inhibitors. This paper presents preliminary data on the chemical and biological properties of SN 597265, with particular emphasis on fungal pathogens of top fruit.

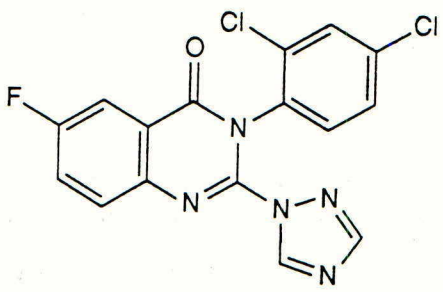
CHEMICAL AND PHYSICAL PROPERTIES

Chemical name (IUPAC)	:	3-(2,4-dichlorophenyl)-6-fluoro-2-(1*H*-1,2,4-triazol-1-yl) quinazolin-4(3H)-one
Draft ISO common name	:	Fluquinconazole
Molecular formula and weight	:	C_{16} H_8 Cl_2 F N_5O; 376
Appearance and melting point	:	Off-white particulate, 191.5-193°C
Vapour pressure	:	6.4×10^{-9} Pa at 20°C

Solubility (g/l at 20°C) : water 0.001, acetone 44, xylene 10, ethanol 3, DMSO 150

Partition coefficient : 3.2 (n-octanol/water)

Structural formula :



TOXICOLOGY

The acute toxicological profile for SN 597265 is presented in Table 1.

TABLE 1. Acute toxicity summary data: SN 597265

Route		Species	Sex	LD50 mg/kg
Oral		Rat	M	112
			F	112
		Mouse	M	325
			F	180
Dermal		Rat	M	2679
			F	625
Skin irritancy		Rabbit		-ve
Eye irritancy		Rabbit		-ve
Skin sensitisation		Guinea Pig		-ve
Mutagenicity:	Ames test			-ve
	In vitro chromosome aberration test			-ve
	Mouse micronucleus test			-ve
	Unscheduled DNA synthesis test			-ve

SN 597265 shows a wide margin of safety when used as recommended.

BIOLOGICAL ACTIVITY

The biological activity of SN 597265 was first recognised in a series of tests conducted in controlled environment rooms and the glasshouse. Excellent results were achieved in the control of a wide range of Ascomycete, Deuteromycete and Basidiomycete fungi. Control of Phycomycetes was negligible. Of particular note were excellent results obtained in control of diseases of apple. All results presented in this paper relate to the use of a 25% WP formulation.

Activity on apples

The control of diseases of apples poses many problems for the grower. Of these, the requirement to apply fungicide sprays at the correct time is a major factor. In order to help the grower achieve this, various prediction and warning schemes have been devised and will no doubt be refined in the future. In themselves these schemes are excellent but as conditions suitable for disease development and spread are not always perfect for spray application and it is not always possible to respond immediately to a disease warning, the success of the schemes will depend on fungicides with particular biological properties. One of these is the ability to protect uninfected foliage and to eradicate early infections in the period following a disease warning, thus providing the grower with the flexibility in application timing he requires in order to profit from the warning scheme. SN 597265 provides this flexibility, as shown in Table 2.

TABLE 2. Protectant and eradicant activity of SN 597265 against *Venturia inaequalis* (glasshouse trial).

Treatment	Rate mg/l	% disease control 28 days after inoculation
24h protectant	12.5	100
24h eradicant	200	100
48h eradicant	200	100
96h eradicant	200	100

As can be seen, SN 597265 gave total disease control of *V.inaequalis* when applied up to 4 days after inoculation.

Although *Venturia* is the principal disease of apple, powdery mildew caused by *Podosphaera leucotricha* is a major secondary disease which frequently occurs in a complex with *Venturia*. SN 597265 combines excellent *Venturia* control with similarly excellent protectant and eradicant control of *Podosphaera*, as shown in Table 3.

TABLE 3. Protectant and eradicant activity of SN 597265 against *Podosphaera leucotricha* (glasshouse trial).

Treatment	Rate mg/l	% disease control 15 days after treatment
24h protectant	5	100
24h eradicant	100	98 (42)*
5 day eradicant	100	96 (65)
10 day eradicant	100	77 (81)

* Disease levels on untreated at assessment time.

In order to achieve high activity against these diseases, systemicity is a valuable asset. SN 597265 possesses excellent systemic properties on apple as shown by data in Table 4.

TABLE 4. Systemic control of *P.leucotricha* by SN 597265.

Treatment systemic effect evaluated	Rate mg/l	% Disease Control SN 597265	
		Protectant	Systemic
Soil drench, root uptake	2000	-	58
	500	-	57
Leaf to leaf, phloem mobility	200	100	0
	50	100	0
Leaf base to leaf tip xylem transfer	200	100	100
	50	100	100
Leaf tip to leaf base phloem mobility and some xylem diffusion	200	100	22
	50	100	0
Lateral ie leaf side to leaf side	200	100	78
	50	100	63
Translaminar, leaf under surface to leaf upper surface	200	-	97
	50	-	95

These data show that xylem transfer of SN 597265 is extremely good, resulting in highly efficient disease control in areas remote from the site of application. Lateral movement can be explained by leakage from leaf blade vessels to the midrib, followed by xylem vessel transfer to the opposite leaf side. There is no evidence for phloem transfer. The translaminar effect illustrates well that SN 597265 penetrates leaf tissue, is not automatically translocated to remote areas by the xylem vessels but is transported across

the leaf tissues to control disease on the opposite surface. This is an important property, ensuring efficient disease control.

Data for field activity are shown in Tables 5 and 6. All sprays were made at 10-14 day intervals with 5-9 applications depending on location. Spray volume was adjusted according to tree size. Initial studies examined dose rates up to 15 g AI/hl. This was soon realised to be too high, with disease control being consistently 100% irrespective of pathogen so lower rates were evaluated.

TABLE 5. Field activity of SN 597265 against *V. inaequalis*.

Treatment	Rate g AI/hl	% Disease control	
		Foliar	Fruit
SN 597265	3.25	82	-
	5.0	90	90
	7.5	94	94
	10.0	95	94
DMI standards (mean)		89	76
Untreated disease level (range)		42 (28-94)	44 (11-90)

(Data from Italy, France, Germany, UK, Spain, Belgium, 1990).

SN 597265 offered excellent control of *Venturia* at dose rates of 5g AI/hl and above. This was particularly noticeable in control of fruit scab, where SN 597265 was consistently the superior product.

TABLE 6. Field activity of SN 597265 against *P. leucotricha*.

Treatment	Rate g AI/hl	% Disease control
SN 597265	5.0	82.6
	7.5	86.1
	10.0	87.3
DMI standards (mean)		83.0
Untreated disease level (range)		43 (12-95)

(Data from France, Germany, UK, Spain, Belgium, 1990).

The activity of SN 597265 applied at 5.0 g AI/hl was at least comparable to that given by the majority of DMI fruit sprays.

The performance of SN 597265 in a *Venturia* spray programme based on scab warnings was evaluated in Belgium in 1991. SN 597265 was used as a mixture with mancozeb. Data are presented in Table 7.

TABLE 7. The activity of SN 597265 plus mancozeb when used in a *Venturia* scab warning spray programme.

Treatment	Rate g AI/hl	% Disease control Time from scab warning		
		4 day	5 days	7 days
SN 597265 + mancozeb	5+ 80	95	93	68
Pyrifenox + mancozeb	4+ 80	92	75	64

SN 597265 gave excellent disease control at the normally accepted spray interval of 4 days, and retained the ability to extend this to 5 days with negligible decrease in efficacy.

During the course of the investigations into control of apple diseases, considerable attention has been paid to crop safety. In all investigations and at dose rates far in excess of those reported here, SN 597265 has shown no problems of crop safety to foliage, pollination processes or final fruit.

Activity on cereals

Data for control of diseases of wheat are shown in Table 8.

TABLE 8. Field control of diseases of wheat by SN 597265.

Treatment	Rate g AI/ha	% Disease control		
		Erysiphe graminis	*Puccinia spp.*	*Septoria spp.*
SN 597265	125	58	84	49
	250	59	84	60
	375	74	94	70
Propiconazole	125	46	78	40
Fenpropimorph	750	68	74	-
Prochloraz	450	-	-	55

(Data from UK, France, Germany, applications made to, and disease control assessed on, the flag leaf).

Control of *E. graminis* on barley was not as good as that on wheat. Control of *Rhyncosporium secalis* and *Pyrenophora teres* was moderate, but inferior to that obtained with prochloraz. SN 597265 gave poor field control of *Pseudocercosporella herpotrichoides* (teleomorph *Tapesia yallundae*) where a mixed W and R type population was present. In common with some other triazoles, SN 597265 showed good laboratory activity against W types but negligible activity against R types.

Activity on vines

SN 597265 has been extensively evaluated for control of vine diseases. Typical trials data are shown in Table 9.

TABLE 9. Control of *Uncinula necator* by SN 597265.

Treatment	Rate g AI/hl	% Disease control
SN 597265	2.5	95
	3.75	97
	5.0	98
Penconazole	1.5	98
Triadimenol	3.75	95

(Data from USA, Germany, France. 14 d spray intervals).

Control of *Uncinula* by SN 597265 was very comparable to that given by the best DMI standards. Although not yet fully evaluated, control of *Uncinula* was frequently associated with control of "rot brenner" (*Pseudopeziza tracheiphila*) and with suppression of *Botrytis cinerea*.

Activity on other crops

The activity of SN 597265 extends to many other crops. On oilseed rape, extensive trials in Germany, France and UK have shown that 250 g AI/ha gives exceptionally good control of *Sclerotinia*, *Pyrenopeziza*, *Phoma* and *Erysiphe* with good control of *Botrytis*. Control of *Alternaria* was only moderate. On sugar beet, control of *Cercospora*, *Ramularia* and *Erysiphe* is excellent at rates of 125-187.5 g AI/ha, while control of *Cercospora* on peanuts in the USA at 125-250 g AI/ha was far superior to that achieved by chlorothalonil.

Other crops where excellent disease control has been achieved include:

		g AI/ha
Coffee :	*Hemileia vastatrix*	200-500
Turf :	*Thanatephorus cucumeris*	320
Legumes :	powdery mildew	125
Rice :	*Thanatephorus cucumeris*	125-500
	Cercospora spp.	125-500
	Helminthosporium spp.	125-500
		g AI/hl
Stone fruit :	*Monilinia* spp.	5-10
	powdery mildews	5-10
	Cladosporium carygenum	5-10

FUTURE DEVELOPMENT

SN 597265 is being developed in the markets indicated and evaluated in many more. Where appropriate for reasons of spectrum or activity enhancement, or for fungicide resistance management strategy, it is being developed in a range of mixtures and formulation types with other compounds. The mixture partner and formulation type is being decided according to the crop and pathogen involved.

METCONAZOLE, AN ADVANCE IN DISEASE CONTROL IN CEREALS AND OTHER CROPS

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ABSTRACT

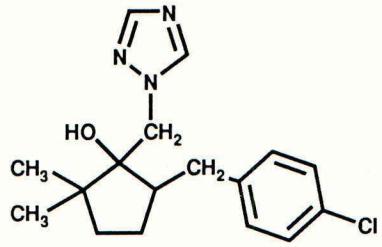
Metconazole, (1RS,5RS;1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol, is a new broad spectrum triazole. With its systemic properties, it gives excellent control of foliar infections of Septoria spp., Puccinia spp., Rhynchosporium secalis and Pyrenophora teres, and seed-borne infections of Tilletia caries, Ustilago spp. and Pyrenophora spp. Useful control of other diseases on cereals is also obtained. A major characteristic of the compound is its outstanding performance against Septoria and rust diseases on cereals. The high prophylactic and strong therapeutic activity have proved to be particularly useful against Septoria spp. Uses on a wide range of other crops are under evaluation.

INTRODUCTION

Fungicidally active substituted azole derivatives have been studied for more than twenty years. The large number of possible substitutions on the molecules have encouraged many chemical companies to synthesise related molecules, looking for variations in the levels of biological activity or in the spectrum of activity. Metconazole, the subject of this paper, is one such material, first synthesised and patented by the Kureha Chemical Industry Co.Ltd. and under development with the Shell Group of companies.

CHEMICAL AND PHYSICAL PROPERTIES

Structural formula:



Chemical name (IUPAC):	(1RS,5RS;1RS,5SR)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol
Common name (BSI/ISO):	metconazole
Shell code:	WL136184 is the cis (1RS,5SR) isomer of metconazole, with the hydroxy and benzyl groups on the same side of the cyclopentyl ring, and for which all data applies unless otherwise stated
Empirical formula:	$C_{17}H_{22}ClN_3O$
Molecular weight:	319.8
Physical state:	crystalline solid
Melting point:	110-113ƎC
Colour:	white
Odour:	odourless
Solubility in water:	15 mg/kg
Stability:	good thermal and hydrolytic stability

TOXICOLOGY

Acute toxicity for technical material

Acute oral LD50 to rat:	1459 mg/kg
Acute dermal LD50 to rat:	>2000 mg/kg
Skin irritancy to rabbit:	not irritant
Eye irritancy to rabbit:	slight irritant
Skin sensitisation:	negative
Ames test:	negative

BIOLOGICAL ACTIVITY

Mode of action

Metconazole is, in common with most other azole fungicides, an ergosterol biosynthesis inhibitor. However, the spectrum of disease control and inherent fungicidal activity varies considerably between the different azole molecules.

Laboratory testing

Initial in-vitro tests with metconazole identified it as an extremely active molecule against a very large range of species. At the same time, cis and trans isomers were identified and produced separately. Both were found to be fungicidally active. Against some diseases the isomers had similar activity, but, overall, the cis isomer was shown to be substantially more active. Data were produced using cultures on freshly prepared potato, sucrose and agar (PSA) media, all at pH 6.0. Temperatures and incubation periods varied from fungus to fungus but were mainly 28°C and three days. Examples of the activities determined in these tests are given for the separate isomers in Table 1. Data are expressed as the mycelial growth inhibition concentration (MIC) in concentrations of mg/l.

TABLE 1. In-vitro activities of metconazole isomers.

Pathogen	MIC in mg/l cis	trans
Alternaria alternata	25	100
Botryotinia fuckeliana	1.6	12.5
Cercospora beticola	25	>100
Cochliobolus miyabeanus	25	25
Fusarium oxysporum f.sp. cucumerinum	1.6	12.5
Fusarium oxysporum f.sp. niveum	3.1	25
Fusarium oxysporum f.sp. raphani	1.6	6.3
Glomerella cingulata	1.6	12.5
Nakataea sigmoidea	25	100
Leptosphaeria nodorum	<0.8	<0.8
Pyricularia oryzae	6.3	12.5
Thanatephorus cucumeris	100	>100
Monilinia laxa	<0.8	1.6
Sclerotinia sclerotiorum	1.6	25
Valsa ceratosperma	<0.8	1.6

In-vivo activity

Some similar differences were observed in pot tests in the glass-house. For example, disease control obtained with the isomers in same-day applications against grey mould on kidney beans and brown rust on wheat, (see Table 2 below), also shows the cis isomer to be substantially more active than the trans isomer against these diseases.

TABLE 2. Preventive activity of metconazole isomers.

Treatment	mg AI/l	% disease control	
		Botrytis cinerea	Puccinia recondita
cis isomer	7.8	50	100
	31.3	79	100
	125	100	100
trans isomer	7.8	5	90
	31.3	44	98
	125	60	100

Field testing

Because of the differences demonstrated above, the cis isomer, code number WL136184, was selected for further evaluation in field programmes. It is still referred to as metconazole in the remainder of this report. Table 3 gives a broad view of the activity spectrum determined in field tests to date. Trials reported were conducted in the U.K., France and Germany.

TABLE 3. Metconazole fungal activity spectrum.

Crop	Pathogen	Disease control rating
Cereals (seed treatment)	Fusarium spp.	**
	Puccinia striiformis	***
	Pyrenophora gramineum	***
	Leptosphaeria nodorum	***
	Tilletia spp.	***
	Ustilago spp.	***
Cereals (foliar spray)	Erysiphe spp.	**
	Fusarium spp.	**
	Pseudocercosporella spp.	*
	Puccinia spp.	***
	Pyrenophora teres	***
	Rhynchosporium secalis	***
	Septoria spp.	***
Sugar beet	Cercospora beticola	***
	Erysiphe betae	***
	Ramularia betae	***
Apple	Podosphaera leucotricha	**
	Venturia inaequalis	***

* low activity ** moderate activity *** high activity

Cereal seed treatments

As a seed treatment on wheat and barley, metconazole provided good control of the major diseases. It was highly active against Tilletia caries and Ustilago spp., normally giving complete control with doses of 2.5 g AI/100 kg of seed, and often with much less. Good levels of control of Pyrenophora graminea and Puccinia striiformis have also been obtained in the field, and in pot tests in France good activity was shown against Leptosphaeria nodorum and Fusarium roseum, and moderate activity against Fusarium nivale. Examples of the interesting activity against leaf stripe and yellow rust are given in Tables 4 and 5. Most of the data were produced from 'wet' treatments using a 100 g/litre EC.

TABLE 4. The effect of metconazole seed treatment on leaf stripe (Pyrenophora graminea) on barley.

Treatment	g AI/100 kg seed	% effect 1991	% effect pre 1991
Metconazole	2.5	71	71
Metconazole	5.0	91	86
Metconazole	7.5	92	91
Triadimenol	30	7	5
Triadimenol + triazoxide + anthraquinone	30+2+50	98	-
Oxine-copper	20	60	46
Untreated (% infected plants)		16	15
Number of trials (all in France)		3	4

TABLE 5. The effect of metconazole seed treatment on yellow rust (Puccinia striiformis) on wheat, cv. Slejpner.

Treatment	g AI/100 kg seed	% effect
Metconazole	0.5	73
Metconazole	1.0	79
Metconazole	2.5	79
Metconazole	5.0	83
Triadimenol	30	71
Untreated (% infection)		33

Mean of 2 U.K. trials assessed at the early booting stage.

Barley foliar treatments

Data collected from winter barley trials showed good control of the four main diseases which infect this crop. Brown rust, Puccinia recondita f.sp. hordei, was sometimes present as a late infection. This was controlled well, virtually 100% effect being achieved by all doses for five weeks after spraying. Control of the other diseases, Rhynchosporium secalis, Pyrenphora teres and Erysiphe graminis f.sp.hordei, are shown in Table 6. These results are all based on assessments made four to six weeks after spraying, mostly from a single spray using a 60 g/l SL. All of the trials summarised in Table 6 were located in England.

TABLE 6. Disease control with metconazole treatments on winter barley.

Treatment	g AI/ha	% control on - R.secalis	P.teres	E.graminis
Metconazole	30	72	-	-
Metconazole	48	84	72	89
Metconazole	60	-	75	93
Metconazole	72	88	76	94
Metconazole	90	89	-	-
Tebuconazole	250	74	81	95
Cyproconazole	100	-	60	93
Untreated (% infection)		76	25	8
Number of trials		2	6	5

Wheat foliar treatments

With the exception of powdery mildew, foliar diseases of wheat are also well controlled. Infections of Erysiphe graminis f.sp.tritici may be moderately well controlled by prophylactic treatments, but on varieties sensitive to mildew, therapeutic treatments, in common with most other azoles, need a specific mildewicide partner.

Brown rust, (Puccinia recondita f.sp.tritici), is an excellent indicator species for the persistance of azole treatments in wheat. In most trials all metconazole treatments resulted in complete rust control for a long period. Yellow rust (Puccinia striiformis) was also very well controlled.

In addition to rusts, the strength of metconazole is it's activity against the septorias. Data presented in Table 7 are from high natural infections of Septoria tritici and Leptosphaeria nodorum. Data from two U.K. trials which were inoculated with L.nodorum are given in Table 8.

TABLE 7. Activity of metconazole against the Septoria spp.

Treatment	Dose g AI/ha	% control on leaves 1 and 2 S. tritici	L. nodorum	
Metconazole	48	81	-	-
Metconazole	60	83	-	-
Metconazole	72	87	84	53
Metconazole	90	-	87	62
Tebuconazole	250	82	86	65
Cyproconazole	100	82	-	-
Flusilazole+ carbendazim	200+100	-	84	-
Untreated (% infection)		60	39	28
Trials/country/years		8 U.K. 91/92	9 FR 92	5 GE 91/92

TABLE 8. Activity of metconazole against inoculated Leptosphaeria nodorum.

Treatment	Dose g AI/ha	L. nodorum % control prophylactic Ear	Leaf	therapeutic Ear	Leaf
Metconazole	48	53	86	46	87
Metconazole	60	63	90	54	88
Metconazole	72	65	89	58	92
Tebuconazole	250	60	85	13	8
Cyproconazole	100	46	62	36	57
Untreated (% infection)		15	60	32	93
Treatment timing		10 days pre-inoculation		4 days post-inoculation	

Cereal yield responses

Cereal yield responses were usually very good, reflecting the differences in infection levels between control plots and the treatments. In seed-treatment trials with high infection levels, yields from treated plots were often several times that of untreated.

In the U.K. trials from which the above foliar disease data were taken, average yield increases of 25% were achieved in winter barley (mean control yield 6.5 t/ha) and 13% in winter wheat (mean control yield 8.9 t/ha) giving 1.6 t/ha and 1.2 t/ha extra grain respectively. Similar yield responses to disease control were seen in France and Germany.

DISCUSSION & CONCLUSIONS

The above data demonstrate that metconazole has a high level of activity and a wide spectrum of activity for this class of compound. Doses as low as 10 g per tonne of seed can completely control some seed-borne diseases. It is active against all diseases in cereals, although, in common with most other azoles, control of established mildew in sensitive wheat cultivars is not adequate. Mixtures of metconazole with mildew-specific products overcome this weakness, although data have not been presented in this paper. In barley, all diseases are well controlled, and activity against Rhynchosporium secalis is particularly high. In wheat, strong prophylactic and therapeutic activity combine to give high and persistent activity, particularly against the rusts and septorias. Persistence of disease control for more than six weeks may allow the use of a single treatment for effective crop protection in many wheat growing areas.

Metconazole is not just another azole, but a useful addition to the armoury against cereal diseases.

Trials to define the activity of metconazole against diseases in broad-leaved crops are continuing.

MON 24000: A NOVEL FUNGICIDE WITH BROAD-SPECTRUM DISEASE CONTROL

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ABSTRACT

MON 24000 is a new thiazolecarboxanilide fungicide. It is being developed by the Agricultural Group of Monsanto Company as a foliar fungicide for rice, cereals, field crops and turf, and as a seed treatment for both cereal and non-cereal crops.

An extensive field testing programme carried out during 1989-1992 has revealed the excellent activity of MON 24000 against a wide range of diseases, especially the Basidiomycete fungi. Efficacy as a foliar application has been observed particularly against plant pathogenic fungi in the genera *Rhizoctonia*, *Puccinia* and *Corticium* and as a seed treatment at low rates against species of *Ustilago*, *Tilletia* and *Pyrenophora*. The mode of action of this compound is by inhibition of the enzyme succinate dehydrogenase in the tricarboxylic acid cycle of fungi.

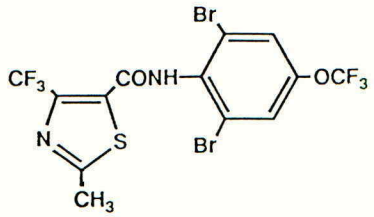
INTRODUCTION

MON 24000 is a new thiazolecarboxanilide fungicide which was discovered and patented by the Agricultural Group of Monsanto Company. It is being developed as a fungicide for foliar, seed and soil treatment. The biological properties of MON 24000 have been evaluated since 1989 on a wide range of crops including rice, cereals, potatoes, peanuts and turf. This paper is a review of its performance against diseases of major importance in these crops.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical Class:	Thiazolecarboxanilide
Chemical Name:	2',6'-dibromo-2-methyl-4'-trifluoromethoxy-4'-trifluoromethyl-1,3-thiazole-5-carboxanilide

Structural Formula:



Molecular Formula:	$C_{13}H_6Br_2F_6N_2O_2S$
Molecular Weight:	528.1
Appearance at 20°C:	White to light brown powder
Melting Point:	177.9 - 178.6 °C (pure material)
Solubility in water at 20°C:	1.6 mg/l
Partition Coefficient:	log P = 4.1 (n-octanol/water)
Hydrolysis:	Stable at pH 5.0 - 9.0

TOXICOLOGY AND ENVIRONMENTAL STUDIES

Mammalian toxicity

Acute oral LD_{50} rat:	> 5000 mg/kg
Acute dermal LD_{50} rabbit:	> 5000 mg/kg
Eye irritation rabbit:	moderately irritating
Skin irritation rabbit:	slightly irritating

Mutagenicity

Ames test:	negative
Micronucleus (mouse):	negative

Toxicity to wildlife

Avian	Bobwhite quail LC_{50} > 5620 ppm, practically non-toxic
	Mallard duck LC_{50} > 5620 ppm, practically non-toxic
Aquatic	Blue sunfish LC_{50} (96 h), 1.2 mg/l, moderately toxic
	Rainbow trout LC_{50} (96 h), 1.3 mg/l, moderately toxic
	Daphnia magna LC_{50} (48 h), 1.6 mg/l, moderately toxic
	Carp LC_{50} (96 h), 2.9 mg/l, moderately toxic

MODE OF ACTION

MON 24000 inhibits the enzyme succinate dehydrogenase in the tricarboxylic acid cycle of fungi. Efficacy within the Basidomycete sub-division of fungi is not restricted to particular strains of any genus as evidenced by its activity against all anastomosis groups of *Rhizoctonia*.

FORMULATIONS

Several formulations of MON 24000 will be available including a wettable powder formulation (WP 25) as well as water based flowable formulations (SC 20, SC 50), granules (G02, WG50) a dust (DL 0.85) and a flowable concentrate for seed treatment use (FS15).

BIOLOGICAL ACTIVITY

Materials and methods

Rice

The trials were conducted using a randomized block design with 4 replicates, on plots 5-10 m^2. One or two applications were made from panicle differentiation to late heading using spray volumes of 200-400 l/ha. Disease assessments were made at grain maturation (20-30 days after heading)

Cereals

All foliar trials reported had plot sizes of 10-20 m^2 with 4-5 replications using randomized complete block design. Treatments were applied at GS 30-32 (stem base) or GS 37-59 (foliar). Spray volume was 200-300 l/ha. Disease severity was assessed on stems, leaves or ears as appropriate. Seed treatment applications were made using a fluidised bed or mini rotostat machine.

Potatoes

Trials were conducted using a randomized block design with 4 replicates. Plot sizes were 10-40 m^2 and treatments were applied prior to planting as an ultra low volume mist at a rate of 2 l/tonne. The severity of both stem canker and black scurf was assessed.

Peanuts/Turf

Trials had plot sizes of 2-20 m^2 with 4 replications using randomized complete block design. Treatments were applied as foliar sprays in volumes of 600-1500 l/ha. Peanut white mould trials were evaluated at lifting. Disease severity was measured as the number of disease loci per plot. Turf brown patch trials were assessed as percent disease severity at intervals appropriate to application timing .

Coffee

Trials were conducted using randomized complete block design with 4 replicates. Plot sizes were 5-10 m^2. Applications were made using a spray volume of 1000 l/ha. Disease assessments were performed six to seven weeks after the last application.

Results and discussion of field trials

Rice

MON 24000 gave outstanding control of rice sheath blight (*Thanatephorus cucumeris)* as a foliar application in both Asia and USA. (Table I). The compound was also effective as a granule applied to paddy water at 50 to 20 days before heading. In the 75 official trials performed by the Japan Plant Protection Association since 1989, MON 24000 obtained a class "A" or "B" category in 73 of these tests, confirming its strong potential for practical use.

TABLE I. Control of sheath blight on rice in USA and Asia 1990-1991.

Treatment	Dose g AI/ha	% sheath blight USA 1990	USA 1991	ASIA 1990-1991
Untreated		83	56	36
MON 24000	100	-	-	3
MON 24000	140/130*	17	29	3
MON 24000	280	15	16	-
Benomyl	1120	56	43	6
Pencycuron	560/330*	32	22	4
Number of trials		12	7	4

* Dose in USA and Asia respectively

Cereals

MON 24000 has consistently shown good activity against the common stem base disease sharp eyespot (*Rhizoctonia cerealis*). In high disease situations, foliar applications at GS 30-31 gave optimum control and yield benefits of 5% (Table 2).

TABLE 2. Control of sharp eyespot on winter wheat 1990-1991.

Treatment	Dose g AI/ha	1990 %stem infection	Yield (t/ha)	1991 %stem infection
Untreated		34	6.3	25
MON 24000	125	12	6.6	-
MON 24000	150	-	-	8
MON 24000	200	-	-	6
MON 24000	250	8	6.6	-
Prochloraz	450	28	6.7	20
Flusilazole	200	27	6.7	20
Number of trials		8*	8*	28

Average of trials in France, UK, Denmark, Belgium and Ireland
* Yields and efficacy originated in same trials

Stem disease surveys conducted by Monsanto of 100 commercial winter wheat crops randomly sampled at GS 75 in France and UK in 1990 and 1991 revealed a high incidence of sharp eyespot (Table 3). A low incidence of this disease was observed in Germany and Denmark in 1991. In France and UK, 20-30% of crops sampled recorded significant levels of the disease. Wheat, oilseed rape and legumes as previous crops were found to be equally conducive to sharp eyespot development.

TABLE 3. Incidence of sharp eyespot in 100 winter wheat crops in France, UK, Germany and Denmark 1990-1991.

Country	% crops with sharp eyespot 1990	1991
France	92	60
UK	92	70
Germany	-	43
Denmark	-	26

MON 24000 also provides control of cereal rusts especially *Puccinia recondita* (Table 4). Good control of *Ustilago* spp and *Tilletia caries* has been observed when used as a seed treatment at 7.5-30 g AI/100 kg seed (Table 5). Suppression of barley leaf stripe (*Pyrenophora graminea*) also was noted.

TABLE 4. Control of *Puccinia recondita* and *P.striiformis* on winter wheat 1990-1992.

Treatment	Dose g AI/ha	% leaf infection* *P.recondita*	*P.striiformis*
Untreated		24.7	16.3
MON 24000	125	2.0	-
MON 24000	150	-	4.6
MON 24000	200	0.5	3.9
MON 24000	250	0.8	-
Cyproconazole	60	-	1.4
Fenpropimorph	562	3.3	1.7
Number of trials		6	4

Average of trials in France, UK and USA
* Assessed 20-40 days after last treatment

TABLE 5. Control of *Ustilago nuda* and *Pyrenophora graminea* on winter barley and *Tilletia caries* on winter wheat 1990-1991.

Treatment	Dose g AI/100 kg seed	% infection		
		U.nuda	*P.graminea*	*T.caries*
Untreated		13.10	18.0	15.9
MON 24000	7.5	-	-	0
MON 24000	15	-	6.8	0
MON 24000	30	0.03	7.4	0
MON 24000	60	0	9.5	0
Carboxin	60	0.11	-	0
Imazalil	5	-	2.2	-
Triadimenol + fuberidazole	37.5 + 4.5	0	0	0
Number of trials		5	2	4

Average of trials in France, UK, Belgium and Ireland

Potatoes

MON 24000 at 50 g AI/tonne seed gave control of stem canker (*Thanatephorus cucumeris*) superior to reference compounds pencycuron and tolclofos-methyl (Table 6). Good activity also was observed on the black scurf phase of the disease.

TABLE 6. Control of stem canker and black scurf on potatoes 1989-1990.

Treatment	Dose g AI/tonne	% infection	
		Stem canker	Black scurf
Untreated		16.0	34.1
MON 24000	50	0.9	1.4
MON 24000	100	0.6	1.4
MON 24000	150	0.3	2.0
Tolclofos-methyl	125	6.4	0.7
Pencycuron	150	4.2	0.3
Number of trials		3	4

Average of trials performed in UK, Belgium and Ireland

Peanuts

Activity against white mould (*Corticium rolfsii*) has been observed. Foliar broadcast applications at pegging at rates of 280-560 g AI/ha demonstrated control of this devastating disease superior to the reference compound quintozene (Table 7). Control of limb rot (*T.cucumeris)* and rust (*Puccinia arachidis*) also has been observed.

TABLE 7. Control of white mould and limb rot on peanuts USA 1989-1991.

Treatment	Dose g AI/ha	% Disease *C.rolfsii**	*T.cucumeris*
Untreated		30	27
MON 24000	280	20	15
MON 24000	560	17	12
Quintozene	5600	26	-
Tebuconazole	500	-	17
Number of trials		5	3

*Mean disease loci per 10-30 linear m of row

Turfgrass

MON 24000 has been shown to provide long duration control of brown patch (*T.cucumeris*) on turf. Rates of 1500-3000 g AI/ha gave 21 to 28 day control of the disease at least equal to commercial standards (Table 8). MON 24000 is also effective in controlling red thread (*Laetisaria fuciformis*).

TABLE 8. Control of brown patch on turfgrass at various times after treatment USA 1989-1990.

Treatment	Dose g AI/ha	% Disease 14 DAT	21 DAT	28 DAT
Untreated		-	19.7	17.9
MON 24000	1500	-	2.8	3.9
MON 24000	3000	-	2.9	2.3
Iprodione	3000	-	2.8	11.1
Chlorothalonil	9500	3.9	-	5.4
Number of trials		3	3	6

Coffee

MON 24000 at 250 g AI/ha gave control of leaf rust (*Hemileia vastatrix*) equal to the reference compound triadimefon and superior to copper (Table 9). Activity was observed at least 40 days after the last treatment application.

TABLE 9. Control of leaf rust on coffee in Brazil 1991-1992.

Treatment	g AI/ha	% leaf infection* 1991	1992
Untreated		48	46
MON 24000	250	24	25
Triadimefon	250	26	22
Copper	3528	-	38
Number of trials		2	1

* Assessed 44-47 days after last treatment

Other Diseases

MON 24000 applied as a foliar, seed or soil treatment has shown activity against the following other disease targets : wheat take-all, Rhizoctonia damping-off of cotton and canola, and aerial blight of soyabeans. Excellent efficacy against Rhizoctonia rot of poinsettias also has been observed, representing strong potential for use of MON 24000 against Basidiomycetes in a wide range of high value vegetable and ornamental crops.

CONCLUSIONS

MON 24000 has shown excellent activity in the last three years in a number of crops especially against the Basidiomycete fungi. Its mode of action makes it particularly attractive for use alone or in mixtures with existing products.

ACKNOWLEDGMENTS

The authors are indebted to many Monsanto colleagues who contributed to the international development of MON 24000, especially A.Amano, G.Barnes, M.Halsey, J.N.Mutz, M.O'Keeffe, H.Rasmussen, J.Rejda-Heath, R.Schumacher, B.Shortt, and C.Stride. We also thank cooperators who have contributed data presented in this paper.

ICIA5504: A NOVEL, BROAD SPECTRUM, SYSTEMIC β-METHOXYACRYLATE FUNGICIDE

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ABSTRACT

ICIA5504 (methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate) is a highly active fungicide providing a broad spectrum of disease control. LC_{95} values below 1 mg AI/l have been demonstrated against major ascomycete, basidiomycete, deuteromycete and oomycete plant pathogens in glasshouse in vivo studies. ICIA5504 has a novel mode of action and controls fungal pathogen strains resistant to the 14-demethylase inhibitors, phenylamides, dicarboximides or benzimidazoles.

ICIA5504 has eradicant, protectant, translaminar and systemic properties, offering the potential for use as a foliar, seed, soil or paddy water treatment. The breadth of spectrum of ICIA5504 has been demonstrated in field trials against a wide range of economically important crop pathogens.

INTRODUCTION

Becker *et al.* (1981) first reported that the fungicidal activity of the natural products strobilurin A, strobilurin B, oudemansin A and myxothiazol, all derivatives of β-methoxyacrylic acid, stemmed from their ability to inhibit mitochondrial respiration by blocking electron transfer between cytochrome b and cytochrome c_1. Indeed, subsequent work has established that these natural products bind at a specific site on cytochrome b (Mansfield & Wiggins, 1990). No compounds currently sold as agricultural fungicides have the same specific mode of action, a feature which should preclude cross-resistance between the β-methoxyacrylates and other classes of fungicide. Therefore, ICI was particularly interested in the β-methoxyacrylates as an area for fungicide synthesis.

Samples of oudemansin A and myxothiazol (kindly provided by Prof. T.Anke and Dr.H.Reichenbach respectively) were tested by ICI in 1982 and shown to have fungicidal activity *in vivo*. Although no sample of strobilurin A was available, we felt it important to determine whether this was similarly fungicidally active since it is structurally the simplest of the natural β-methoxyacrylates and, consequently, the most attractive starting point for the synthesis of analogues. In preparing a sample of strobilurin A for testing *in vivo*, we established that the wrong configuration had previously been assigned to the strobilurins. Subsequently, we were able to assign the correct configuration (Beautement & Clough, 1987). Disappointingly, strobilurin A showed no in vivo fungicidal activity in glasshouse tests. Nevertheless, it was active against fungi growing on agar in the dark and strongly inhibited mitochondrial respiration *in vitro*. Tests showed that the lack of in vivo fungicidal activity of strobilurin A stemmed from its photochemical instability and relatively high volatility.

We have designed and synthesised analogues of the strobilurins in which

the structural features responsible for fungicidal activity have been retained, while those responsible for photochemical instability and volatility have been modified (Beautement *et al.*, 1991). During the course of an extensive chemical synthesis programme, we have established the relationships between structure and fungicidal activity for the β-methoxyacrylates and this has led to the preparation of ICIA5504, the subject of this paper.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical name (IUPAC) : Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate.

Code number : ICIA5504

Structural formula :

Molecular formula and weight:	$C_{22}H_{17}N_3O_5$; 403.4
Physical state:	White crystalline solid
Melting point:	118-9°C
Density:	1.33 g/cm^3
Water solubility:	10 mg/l at 25°C
n-Octanol-water partition coefficient:	440 (logP = 2.64)
Vapour pressure:	<< 10^{-5} Pa at 20°C

TOXICOLOGY

Rat acute oral LD50:	>5000 mg/kg (males and females)
Rat acute dermal LD50:	>2000 mg/kg (males and females)
Rabbit skin irritation:	slight
Rabbit eye irritation:	slight
Guinea pig skin sensitisation:	negative
Mutagenicity:	Ames negative

BIOLOGICAL PROPERTIES

Test methods

Laboratory, glasshouse and field study methods closely follow those

described in previous publications by ICI Agrochemicals (Heaney *et al.*, 1988; Waller *et al.*, 1990).

Spectrum and features of activity

In vivo glasshouse tests highlight the breadth of spectrum and level of activity of ICIA5504, with excellent fungicidal activity displayed against ascomycetes, basidiomycetes, deuteromycetes and oomycetes (Table 1). ICIA5504 shows protectant, eradicant, translaminar and systemic properties.

TABLE 1. Glasshouse efficacy *in vivo*.

Pathogen	Plant host	Type of application	LC95 value (mg AI/l) ICIA5504	Commercial standard	
Ascomycetes					
Erysiphe graminis f.sp. *tritici*	Wheat	Erad,01	4	7	Tebuconazole
Mycosphaerella graminicola	Wheat	Prot,01	0.3	2	Tebuconazole
		Erad,05	0.3	2	Tebuconazole
Pyrenophora teres	Barley	Prot,01	0.3	6	Flusilazole
		Erad,01	20	10	Flusilazole
Venturia inaequalis	Apple	Prot,01	2	5	Hexaconazole
		Erad,03	5	0.6	Hexaconazole
		Xlam,01	11	2	Hexaconazole
Basidiomycetes					
Puccinia recondita	Wheat	Prot,01	0.2	0.7	Tebuconazole
		Erad,05	3	7	Tebuconazole
		Syst,02	4	34	Tebuconazole
Thanatephorus cucumeris	Rice	Prot,01	1	3	Pencycuron
		Syst,02	35	33	Flutolanil
Deuteromycetes					
Pyricularia oryzae	Rice	Prot,01	0.03	0.9	Tricyclazole
		Erad,02	1	0.6	Kasugamycin
		Syst,02	0.9	4	Pyroquilon
Alternaria solani	Tomato	Prot,01	0.1	3	Hexaconazole
Oomycetes					
Plasmopara viticola	Vine	Prot,07	1	8	Mancozeb
		Erad,01	13	1	Cymoxanil
		Syst,02	8	2	Cymoxanil
Phytophthora infestans	Potato	Prot,01	0.4	109	Mancozeb

Erad = Foliar eradicant
Prot = Foliar protectant
Syst = Root drench (systemic) protectant
Xlam = Translaminar protectant
01 = 1 day between inoculation and chemical application (02=2 days etc.)

ICIA5504 is a particularly potent inhibitor of spore germination and, in addition to its ability to inhibit mycelial growth, also shows marked anti-sporulant activity.

Uptake into leaves

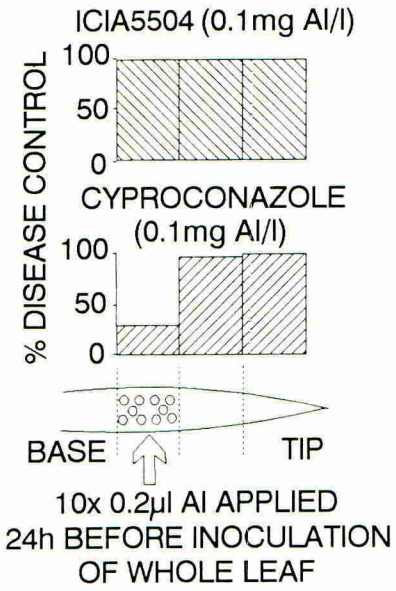
Foliar uptake of ICIA5504 into wheat, barley and vines is low with typically ≤ 10% AI penetrating the leaf by 24 hours after application.

Photostability

In a simulated sunlight test, ICIA5504 did not suffer the photoinstability of strobilurin A (time for 50% loss of ICIA5504 = 24 hours cf. strobilurin A = 12 seconds).

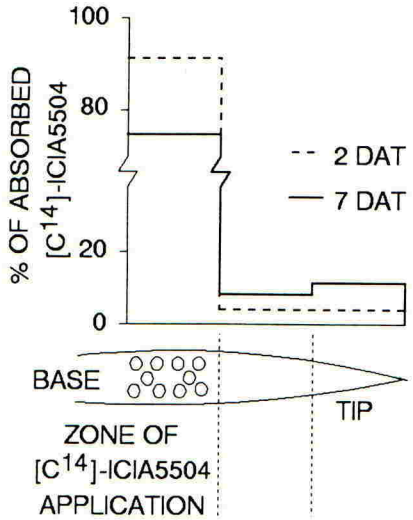
Translocation in wheat

FIGURE 1. Distribution along wheat leaf (*Puccinia recondita* bioassay)



Bioassays to examine movement of AI along the leaf in wheat highlight the systemic properties of ICIA5504; disease control at the leaf tip can be achieved within 24 hours of application of ICIA5504 to a zone at the leaf base (Figure 1). In addition, and in contrast to highly systemic fungicides such as cyproconazole, disease control is retained at the zone of application with ICIA5504 in this test. The uniformity of distribution of ICIA5504 within the cereal leaf ensures an excellent persistence of biological effect without rapid AI accumulation at the leaf tip.

FIGURE 2. Distribution along wheat leaf (radiolabel)



Studies with [C^{14}]-ICIA5504 have confirmed that translocation of the AI takes place acropetally only and occurs slowly, resulting in uniform distribution of ICIA5504 throughout the leaf (Figure 2).

Translocation in vines

Plasmopara viticola bioassays and radiolabelled studies showed ICIA5504 to have penetrant and local redistribution properties in vine leaves.

Field performance

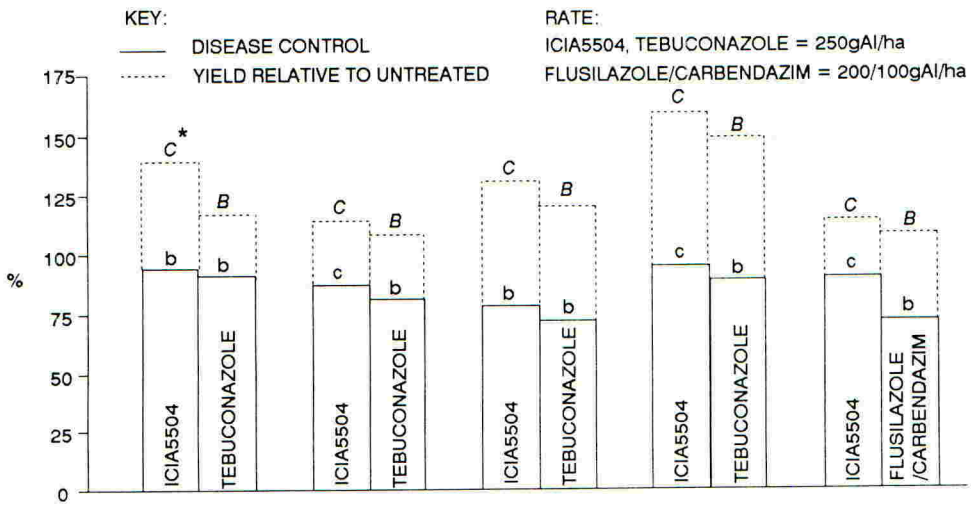
Cereal foliar spray

Representative data from European cereal field trials confirm the very good efficacy of ICIA5504 against *Puccinia* spp., *Mycosphaerella graminicola* (*Septoria tritici*), *Leptosphaeria nodorum* (*Septoria nodorum*) and *Pyrenophora teres* (Figure 3). The persistence of fungicidal effect with ICIA5504 was impressive, maintaining the green leaf area of the upper foliage until late in the season. A similarly high level of protection was afforded to the ear by sprays applied at GS 55-59. Consequently, particularly large yield benefits have been achieved (Figure 3).

Despite only moderate control of wheat and barley powdery mildews (*Erysiphe graminis*) on the foliage, ICIA5504 applied at GS 55-59 has regularly given good control of wheat powdery mildew on the ears. ICIA5504 showed negligible activity against true eyespot (*Tapesia yallundae*) but was highly effective against sharp eyespot, caused by *Rhizoctonia cerealis*.

ICIA5504, applied over a wide range of application timings and environmental conditions, has consistently been very crop safe on wheat and barley.

FIGURE 3. Disease control and associated yield increases on wheat and barley in five representative trials



DISEASE:	*Puccinia recondita*	*Septoria tritici*	*Septoria nodorum*	*Puccinia hordei*	*Pyrenophora teres*
ASSESSMENT:	(L1,30DAT2)	(L1,42DAT2)	(Ear,45DAT1)	(L2,15DAT2)	(L1,42DAT2)
ACTUAL DISEASE IN UNTREATED:	29%	35%	31%	80%	76%
ACTUAL YIELD IN UNTREATED: (t/ha)	3.0	8.3	6.7	6.1	8.6

*DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES AT 5% LEVEL BETWEEN TREATMENTS WITHIN INDIVIDUAL TRIALS (UNTREATED EQUIVALENT TO *A* OR *a*)
L = LEAF NUMBER (NUMBERING DOWN FROM FLAG LEAF =L1)
30 DAT2 = 30 DAYS AFTER SECOND TREATMENT etc

Rice

ICIA5504 is unique in showing control of both rice blast (*Pyricularia oryzae* on the leaves and panicles) and sheath blight (*Thanatephorus cucumeris*). ICIA5504 has been effective in Japanese field trials when applied either as granules directly to the paddy water (Table 2) or as a spray to the foliage.

Vines

Under high disease pressure in France, ICIA5504 at 25 g AI/hl applied on a prophylactic schedule has typically given control of vine downy mildew (*Plasmopara viticola*) on the leaves and bunches superior to the commercial standards (Table 3). In a representative Italian trial, ICIA5504 applied at a rate of 12.5 g AI/hl has given good control of a heavy attack of vine powdery mildew (*Uncinula necator*) on the leaves and bunches (Table 3). Its performance was superior to sulphur, but slightly inferior to hexaconazole.

ICIA5504 may cause transient chlorotic symptoms on younger foliage. Fruit quality and/or quantity has not been adversely affected and no phytotoxic symptoms have been seen on the fruit at any stage of development.

TABLE 2 Control of rice diseases by granule application to paddy water.

Treatment	Rate (g AI/ha)	% disease control: Leaf blast (37 DAT)	Panicle blast (36 DAT2)	Sheath blight (23 DAT)
Untreated (% disease)		0 A (23)	0 A (45)	0 A (38)*
ICIA5504	1600	-	-	66 B
	1800	91 B	79 B	-
Probenazole	2400	96 B	78 B	-
Flutolanil	2000	-	-	50 B

* Mean lesion height (cm)
Treatment means within data columns followed by different letters indicate significant differences at the 5% level.
36 DAT2 = 36 days after second treatment etc.
\- = treatment not in specified trial.

TABLE 3 Control of vine diseases.

Treatment	Rate (g AI/hl)	% disease control: *Plasmopara viticola* Leaves (15 DAT9)	*Plasmopara viticola* Bunches (15 DAT9)	*Uncinula necator* Leaves (13 DAT7)	*Uncinula necator* Bunches (13 DAT7)
Untreated (% disease)		0 A (46)	0 A (69)	0 A (30)	0 A (96)
ICIA5504	12.5	-	-	90 C	92 C
	25	98 B	99 B	-	-
Mancozeb	280	87 B	91 B	-	-
Cymoxanil + mancozeb	12+ 140	80 B	87 B	-	-
Sulphur	280	-	-	77 B	61 B
Hexaconazole	2	-	-	100 D	100 C

Treatment means within data columns followed by different letters indicate significant differences at the 5% level.
15 DAT9 = 15 days after ninth treatment etc.
\- = treatment not in specified trial.

Potatoes

A prophylactic schedule of ICIA5504 at 200 g AI/ha has typically given control of potato late blight (*Phytophthora infestans*) equivalent to the

commercial standard, mancozeb. ICIA5504 applied at rates up to 500 g AI/ha on a range of potato varieties has shown no phytotoxic symptoms.

Apples

ICIA5504 applied at 120 mg AI/l on a prophylactic schedule has provided equivalent foliar and superior fruit scab (*Venturia inaequalis*) control to captan or flusilazole. At the higher rate of 200 mg AI/l, ICIA5504 has given control of *Alternaria mali* equivalent to a bitertanol/thiram/ziram mixture.

ICIA5504 has caused no phytotoxic effects on apples, with the exception of a limited number of varieties on which necrosis of young leaves and buds has been observed.

Cereal Seed Treatment

ICIA5504 applied as a seed treatment to winter barley at 100 mg AI/kg seed has shown good crop safety and given powdery mildew control equivalent to the commercial standard, ethirimol + flutriafol + thiabendazole ('Ferrax'), in UK field trials. Clarification of the potential for ICIA5504 as a cereal seed treatment continues.

CONCLUSIONS

ICIA5504 is a broad spectrum fungicide which has displayed good efficacy in field trials against a wide range of economically important crop diseases, often matching or outperforming the best current commercial standards. Crop safety, except on a limited number of apple varieties, has been good to excellent. Other exciting features of ICIA5504 are its systemic properties, novel mode of action and persistence of fungicidal effect.

ACKNOWLEDGEMENTS

The authors would like to thank their many colleagues in ICI Agrochemicals who have participated in the ICIA5504 project.

REFERENCES

Beautement, K.; Clough, J.M. (1987) Stereocontrolled syntheses of strobilurin A and its (9E)-isomer. *Tetrahedron Letters*, **28**, 475-478.

Beautement, K.; Clough, J.M.; de Fraine, P.J.; Godfrey, C.R.A. (1991) Fungicidal ß-methoxyacrylates : from natural products to novel synthetic agricultural fungicides. *Pesticide Science*, **31**, 499-519.

Becker, W.F.; von Jagow, G.; Anke, T.; Steglich, W. (1981) Oudemansin, strobilurin A, strobilurin B and myxothiazol: new inhibitors of the bc_1 segment of the respiratory chain with an E-ß-methoxyacrylate system as common structural element. *FEBS Letters*, **132**, 329-333.

Heaney, S.P.; Shephard, M.C.; Crowley, P.J.; Shearing, S.J. (1988) ICIA0001: a novel benzamide fungicide. *Proceedings 1988 Brighton Crop Protection Conference - Pest and Diseases*, **2**, 551-558.

Mansfield, R.W.; Wiggins, T.E. (1990) Photoaffinity labelling of the ß-methoxyacrylate binding site in bovine heart mitochondrial cytochrome bc_1 complex. *Biochimica et Biophysica Acta*, **1015**, 109-115.

Waller, C.D.; Eschenbrenner. P.; Godwin, J.R. (1990) Hexaconazole - a new flexible cereal fungicide. *Proceedings 1990 Brighton Crop Protection Conference - Pests and Diseases*, **2**, 447-454.

XRD-563 - A NOVEL FOLIAR APPLIED FUNGICIDE FOR THE CONTROL OF POWDERY MILDEW IN CEREALS

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ABSTRACT

XRD-563 is a novel benzamide fungicide discovered by DowElanco. It is effective against wheat and barley powdery mildew (Erysiphe graminis f.spp. tritici and hordei respectively).

The fungicide can be applied as a foliar spray and penetrates into plant tissue very rapidly where it is translocated acropetally. XRD-563 shows curative, eradicant and protectant activity against mildew.

XRD-563 shows similar activity to morpholine fungicides under field conditions when applied at equivalent rates. No phytotoxicity has been observed on any of the cereal cultivars tested. In laboratory and field trials XRD-563 was effective against powdery mildew showing reduced sensitivity to EBI (DMI) fungicides.

The compound has been mixed with azole fungicides to give products for broad-spectrum disease control in cereals. The tank-mixtures also afford valuable resistance management tools to reduce the resistance risk to both components of the mixture. The product, alone or in mixture, will provide a valuable new chemistry to combat cereal powdery mildews.

INTRODUCTION

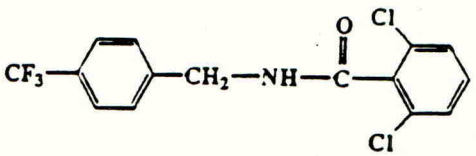
XRD-563 is a new systemic fungicide discovered by DowElanco. This paper describes the properties and

performance of XRD-563, under both glasshouse and field conditions, against powdery mildew of wheat and barley.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical name(IUPAC): 2,6-dichloro-N-(4-trifluoromethyl-benzyl)benzamide

Structural formula:



Molecular formula: $C_{15}H_{10}Cl_2F_3NO$

Molecular weight: 348.154

Solubility : water 3.5 mg/l
: readily soluble in organic solvents eg methanol, acetone

TOXICOLOGY PROPERTIES

Technical material

Mammals
Acute oral - rat : LD50 > 5000 mg/kg
Acute oral - mice: LD50 > 500 mg/kg
Acute dermal - rabbit: LD50 > 2000 mg/kg (no mortality at this level)

Reproduction studies
Rat teratology study: 1000 mg/kg daily - no evidence of teratogenicity

Irritation
Rabbits
Primary eye: slight conjunctives cleared in 72 hr
Primary skin: slight erythema cleared in 72 hr.

Mutagenicity
Gradient Plate Assay (Modified Ames) test with/without metabolic activation:negative
DNA repair assay in primary rat hepatocytes:negative

Avian
Bobwhite (adult): Acute Oral - LD50 > 2000 mg/kg (no mortality at this level).
Dietary - 8 day exposure LC 50 > ca. 4990 ppm in the diet

Aquatic
Bluegill: LD50 (96 h) > 100 mg/l)
Rainbow trout: LD50 (96 h) between 50 and 100 mg/l
Daphnia: 24 h static test - LD50 > 100 mg/l

BIOLOGICAL PROPERTIES

Glasshouse studies

Wheat or barley plants, grown in a glasshouse at 18-20°C under a 16 h light: 8 h dark regime were inoculated with Erysiphe graminis f.sp. tritici or f.sp. hordei five days before treatment (eradicative), two days before treatment (curative) or two days after treatment (protective). Treatments of XRD-563 or reference fungicides were applied with a deVilbiss spray gun. XRD-563 was tested as either technical material or as a 120 g/l EC. Standard fungicides were fenpropimorph 750 g AI/l EC, fenpropidin 750 g AI/l EC, tridemorph 750 g AI/l EC, propiconazole 250 g AI/l EC, triadimenol 250 g AI/l EC and nuarimol (technical material). Unless otherwise stated in the Tables, formulated material was used in laboratory and field studies.

XRD-563 showed eradicative, curative and protectant activity against Erysiphe graminis f.sp. tritici on wheat rates comparable to reference fungicides (Table 1). A similar range of activity could be demonstrated against Erysiphe graminis f.sp. hordei on barley (Table 2).

TABLE 1. The eradicative, curative and protectant activity of XRD-563 and reference fungicides against Erysiphe graminis f.sp. tritici of wheat (cv. Rapier) under glasshouse conditions.

Treatment	Dose mg AI/l	% Disease control 14 d after inoculation		
		eradicative	curative	protectant
XRD-563	6.25	94	99	42
	25.0	94	99	77
Fenpropidin	6.25	94	99	54
	25.0	98	99	88
Propiconazole	6.25	98	95	86
	25.0	94	99	94
Tridemorph	6.25	31	50	31
	25.0	66	96	42
Disease in controls		36%	63%	54%

TABLE 2. The eradicative, curative and protectant activity of XRD-563 and reference fungicides against Erysiphe graminis f.sp. hordei of barley (cv. Golden Promise) under glasshouse conditions.

Treatment	Dose mg AI/l	% Disease control 14 d after inoculation		
		eradicative	curative	protectant
XRD-563	6.25	82	79	59
	25.0	89	100	79
Fenpropidin	6.25	85	97	83
	2.50	91	99	95
Propiconazole	6.25	91	95	79
	25.0	93	99	95
Tridemorph	6.25	56	48	18
	25.0	68	99	73
Disease in controls		59%	60%	46%

The efficacy of the compound is not affected by rainfall when applied as either formulated or technical material (Table 3). Plants were treated with doses ranging from 25 to 100 mg/l and allowed to dry before subjecting to 12.5 mm of artificial rainfall. The efficacy of the compound to control wheat powdery mildew was excellent under the rainfall conditions.

TABLE 3. The efficacy of XRD-563 on the control of wheat powdery mildew (Erysiphe graminis f.sp. tritici) after plants had received rainfall.

Treatment	Rate mg AI/l	Disease Control Rating*	
		No rain	12.5 mm rain
XRD-563 EC	25	8	7
	50	8	8
	100	9	8
XRD-563 Tech	25	8	7
	50	8	8
	100	8	8
Control	0	1	1

*Rating scale=1-9; where 1=no control, 9=100% control

It has been demonstrated that the compound is readily translocated in leaves. In laboratory experiments, the application of technical XRD-563 in a known concentration on a small portion of the upper leaf surface resulted in the control of mildew on untreated acropetal portions of the upper and lower surface of the leaf blade.

XRD-563 demonstrated excellent control of wheat powdery mildew conidia as shown in Table 4. These data suggest the material is fungicidal rather than fungistatic.

TABLE 4. The efficacy of XRD-563 on wheat powdery mildew (Erysiphe graminis f.sp. tritici) conidia after exposure for 15 min*.

Treatment	Rate mg/l	Disease Control Rating♦
XRD-563 Tech.	10	1
	100	9
Nuarimol Tech.	10	2
	100	9
Control blank	0	1

*Wash conidia from wheat; add compound; let stand 15 minutes; spin down; wash conidia with distilled water; inoculate wheat plants.
♦Rating scale=1-9; where 1=no control, 9=100% control

FIELD ACTIVITY

XRD-563 was evaluated in field trials in France, United Kingdom and U.S.A. using 15-25 m^2 plots and 3-4 replicates. Applications were made with Azo sprayers fitted with medium flat fan nozzles. In the trials reported in this paper, two applications of XRD-563 and reference materials were made on a 21-28 day schedule.

XRD-563 at rates in excess of 225 g AI/ha gave control of Erysiphe graminis f.sp. tritici comparable to the reference powdery mildewicides, propiconazole, triadimenol and tridemorph (Table 5). Data for each trial reported under each experiment show a similar rate range of control in both the U.K. and France.

TABLE 5. The efficacy of XRD-563 and reference fungicides against Erysiphe graminis f.sp. tritici of wheat in field trials in France and U.K.

	Dose	% Disease Control 35 Days after Treatment 1					
Treatment	g AI/ha	206*	212*	123*	822♦	823♦	411♦
XRD-563	112	43	75	73	73	54	75
	224	84	91	80	93	70	91
	338	91	88	83	97	73	96
	450	93	94	84	97	81	96
Tridemorph	750	84	86	80	62	58	73
Triadimenol	125	91	90	84	94	80	-
Propiconazole	125	81	79	75	84	48	91
Disease in Controls		34%	51%	52%	87%	65%	60%

* Trial code number, UK. ♦ Trial code number, France.

Good control of Erysiphe graminis f.sp. hordei was provided by XRD-563. In trials where triazoles (propiconazole, triadimenol) were not providing good mildew control (207R-208R, Table 6), indicating the presence of DMI insensitive mildew, XRD-563 continued to provide good control.

TABLE 6. The efficacy of XRD-563 and reference fungicides in controlling Erysiphe graminis f.sp. hordei of barley in field trials in France and U.K.

	Dose	% Disease Control 35 Days after Treatment 1					
Treatment	g AI/ha	204*	018*	824♦	824♦	207R*	208R*
XRD-563	112	43	85	71	57	20	18
	224	84	87	78	87	51	59
	338	85	91	82	87	71	79
	450	93	92	85	97	95	93
Tridemorph	750	96	99	85	97	95	93
Triadimenol	125	83	88	85	70	20	10
Propiconazole	125	73	97	87	73	51	38
Disease in Controls		55%	14%	20%	7%	91%	90%

* Trial code number, UK. ♦ Trial code number, France.

MIXTURES WITH AZOLE FUNGICIDES

Cereals are attacked by a complex of diseases in addition to powdery mildew. These include rusts (eg Puccinia recondita, P.striiformis) and Septoria spp. in wheat and Rhynchosporium secalis and rusts in barley. Broad-spectrum disease control can be achieved with XRD-563 by either tank-mixing or co-formulating it with broad-spectrum azole fungicides.

XDE-563 has been successfully tank-mixed (Table 7) at low rates with the recommended rate of propiconazole to provide broad-spectrum disease control. As well as providing broad-spectrum disease control these combinations will also provide a 'Resistance Management Strategy' analogous with that provided by mixtures of azoles with morpholines (Heany et al., 1988).

TABLE 7. Field activity of XRD-563, alone or in combination with propiconazole, against a range of cereal diseases.

Treatment	Dose g AI/ha	Mean % disease control: Puccinia recondita wheat(5)*	Erysiphe graminis wheat(3)	Rhyncho-sporium secalis barley(7)	Erysiphe graminis barley(4)
XRD-563	225	8	84	8	64
XRD-563+ propiconazole♦	225+ 125	82	82	80	70
Fenpropimorph+ propiconazole♦	375+ 125	83	81	88	77
Propiconazole	79	79	70	79	65

()* Number of trials ♦ Tank-mix

CROP SAFETY

XDE-563 has been evaluated at rates up to and including 1000 g AI/ha in over 100 trials in wheat and barley in Europe and the U.S.A. No injury has been reported on any cultivar tested.

CONCLUSIONS

XRD-563 will provide a safe new chemistry, which is highly selective to the crop, to combat powdery mildew of cereals. This is particularly important in view of the widespread reduced sensitivity to DMI compounds (eg Locke, 1986) and reports (Brown et al., 1990; de Waard et al., 1992) of population shifts to reduced sensitivity of Erysiphe graminis to morpholine fungicides.

REFERENCES

Brown, J.K.M.; Slater, S.E.; Howe, P.M.; See, K.A. (1990). Mildew of Barley. **U.K. Cereal Pathogen Virulence Survey, 1989 Annual Report, Cambridge: U.K. Cereal Pathogen Virulence Survey Committee**, 24-31.

De Waard, M.A.; Banga, M.; Ellis, S.W. (1992). Charaterisation of the sensitivity of Erysiphe graminis f.sp. tritici to Morpholines. **Pesticide Science**, 34, 374-376.

Heany, S.P.; Martin, T.J.; Smith, J.M. (1988). Practical approaches to managing anti-resistance strategies with DMI fungicides. **Proceedings of the 1988 Brighton Crop Protection Conference - Pests and Diseases** 3, 1097-1106.

Locke, T. (1986). Current incidence in the United Kingdom of fungicide resistance to pathogens of cereals. **Proceedings of the 1986 British Crop Protection Conference - Pests and Disease** 1, 781-786.

A NEW CONCEPT IN CROP PROTECTION : AN ACTIVE ADJUVANT FOR FUNGICIDES - THE CASE OF COPPER TALLATE

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ABSTRACT

Copper tallate is a combination of copper hydroxide with the acid distillate (fatty and resin acids) from pine wood. Low in toxicity, it has been extensively tested in France since 1985, in the first instance as a fungicide. However, its main interest lies in its excellent properties as a surfactant and its synergy with a number of foliar fungicides including benzimidazoles, chlorothalonil, cymoxanil, folpet, fosetyl-aluminium and prochloraz. Copper tallate's mode of action has not been fully explained, but as little as 50g Cu metal/ha gives statistically significant improvements in efficacy. Registered in France as an *active adjuvant* to fungicides used against Botrytis and downy mildew of grapes, it is expected to be of interest in fungicide programmes in top-fruit, horticulture, cereals and many tropical crops.

INTRODUCTION

This research programme on copper tallate started in 1985 and has demonstrated that its fungicidal activity is less interesting than its secondary characteristics such as surfactant activity. The improvement in efficacy of several fungicide molecules by the addition of copper tallate is such that a true synergy is demonstrable in many cases. This led to the new concept of active surfactant or *active adjuvant*, a category of surfactant with biological efficacy and synergistic effect with potential for the reduction of rates of some fungicide partners. This potential is of interest for various reasons, particularly where fungicide resistance problems occur. Up until now, available wetting agents, surfactants and other additives have been intrinsically inactive in applications in which they are used.

CHEMICAL AND PHYSICAL PROPERTIES

Derivation and Formulation

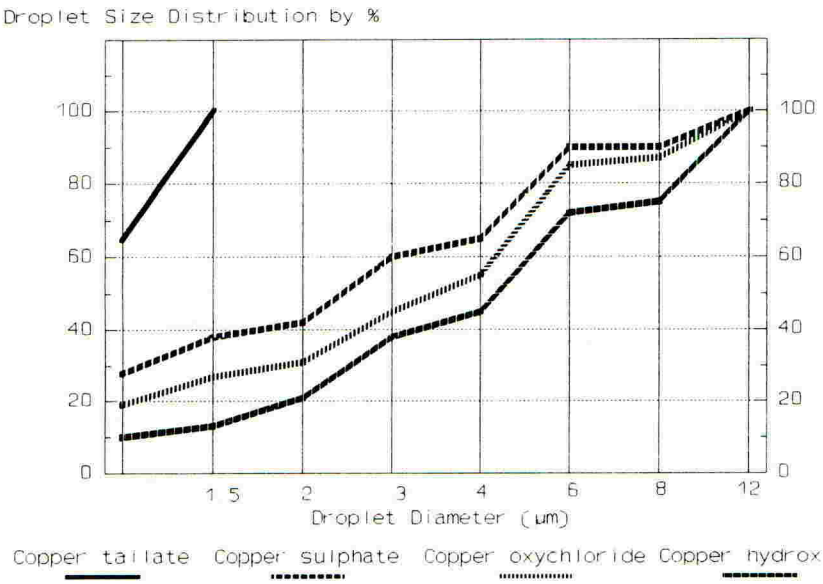
The acid distillation of pine or other resinous trees results in an acid liquid, tall oil, which reacts with copper derivatives. Tall oil contains two types of products, fatty acids, mainly oleic, linoleic and linolenic, and resin acids, mainly abietic. Varying the conditions of the distillation affects the exact ratios obtained of the different fatty and

resin acids. Fatty acids help the penetration of many molecules into animal or vegetal cells. Resin acids aid the sticking of the spray mixture on the plant. The copper tallate described in this paper is a proprietary formulation that has been developed by Proval S.A.R.L, derived by acid distillation of *Pinus maritimus* from the Les Landes region of France. It contains 50g copper metal/l, typically 650g copper tallate /l. Copper tallate has a strong terpene odour and is an eye irritant. It can leave green stains on plastic but these can be removed by aromatic solvents. The formulation has a long shelf life and is not sensitive to low temperatures.

Surfactant Properties

As shown in Figure 1, when diluted at registered rates, droplets of copper tallate are much smaller than those formed by other copper fungicides. Copper tallate droplets are typically in the range 0.3-0.9μm, droplets of inorganic copper derivatives in the range 2.5-4.3μm, giving clear benefits in terms of biological efficacy.

FIGURE 1. Comparison of droplet sizes of copper fungicides.



Preliminary, unpublished results of a study currently being conducted by Dr. Doux, ENSA, Toulouse, show that
(1) the diameter of water droplets is doubled by addition of copper tallate, thereby giving better surface coverage.
(2) copper tallate greatly increases the time for spray droplets to dry, i.e. it is a desiccation retardant.
(3) copper tallate prevents copper sulphate crystals from forming, thus avoiding loss of efficacy of copper sprays.
(4) a drop of copper tallate spray at usual dose rates shows a contact angle of 20-25° versus ±90° for copper sulphate.

Environmental Characteristics and Compatibility

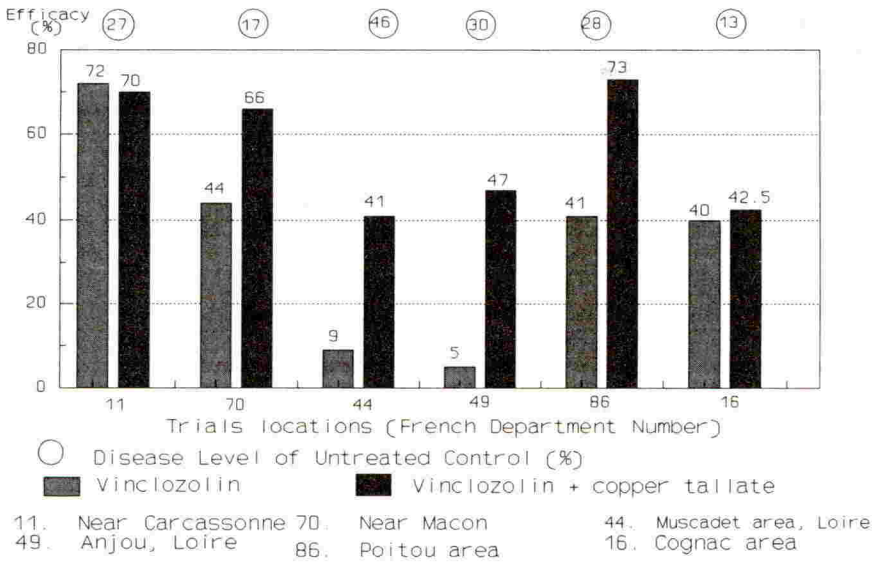
Applications of copper tallate in crop protection use much smaller quantities of copper metal per hectare, 50-100g/ha for use as an active adjuvant, 175-200g/ha as a fungicide, when compared with traditional, inorganic copper derivatives. One application of Bordeaux mixture will use up to 10 times more copper metal per hectare than copper tallate for an equivalent level of efficacy. It is generally accepted that thiram and copper fungicides should not be sprayed together. This also applies to copper tallate. High-nitrogen liquid fertilizers are not compatible with copper tallate.

APPLICATIONS IN VINES

Botrytis

Copper tallate was first registered as an active adjuvant for foliar fungicide treatments on vines to help solve the Botrytis resistance problems experienced with dicarboximide fungicides. INRA Bordeaux established the synergy between copper tallate and vinclozolin (Soyez, 1992). An application rate of copper tallate equivalent to 100g Cu metal/ha is the optimum required, although 175g Cu/ha is necessary in areas where there is appreciable resistance to dicarboximides such as the vineyards of North East France and in some areas of Bordeaux. In 1991, the Service de la Protection des Végétaux conducted trials to establish the benefits brought by copper tallate when applied with dicarboximide fungicides. This is summarised in Figure 2.

FIGURE 2. Comparison of vinclozolin alone and with copper tallate for Botrytis control at various sites in France.

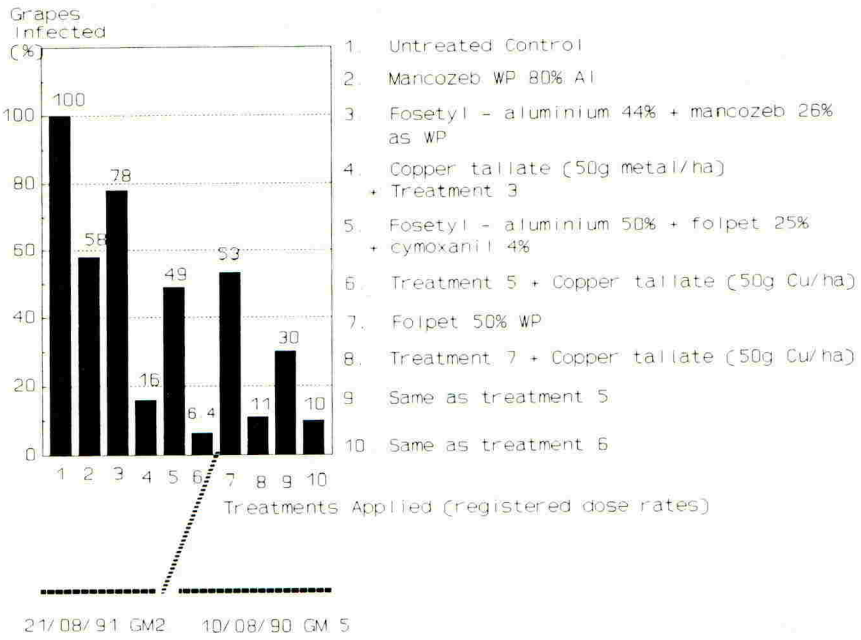


In 4 out of 6 vineyards, a marked improvement in efficacy was reported. Another part of the trials programme showed that copper tallate (100-175g Cu metal/ha) could replace thiram at 3.2kg a.i./ha without reduction in efficacy against Botrytis.

Downy Mildew

The potential uses of copper tallate in programmes for the control of downy mildew are considerable. A rate of 1l/ha copper tallate (50g copper metal/ha) is sufficient to increase the efficacy of ethylenebisdithiocarbamates, phthalimides, cymoxanil and fosetyl-aluminium, with or without inorganic copper compounds. It is especially noteworthy that the improvement in efficacy is evident on grape berry downy mildew as well as on leaf downy mildew. This is shown in Figure 3, the summarised results of field trials conducted by INRA Bordeaux in 1990 and 1991 with spray intervals of 14 days.

FIGURE 3. Downy mildew trials with copper tallate as adjuvant.



APPLICATIONS IN CEREALS - RESEARCH IN PROGRESS

Abstracts from a test programme conducted over the last six years illustrate the progress that has been made so far:

Powdery Mildew

The potential use of copper tallate in cereals was first studied by Japanese co-operators in greenhouse tests in 1986.

Complete efficacy was shown against *Erysiphe graminis* on barley with copper tallate at a concentration of 125mg/l (9.6mg copper metal/l) and triadimefon at 50mg/l.

Glasshouse tests carried out in the UK in 1989 showed enhanced performance of registered mildewicides when 2.5l copper tallate (50g copper/l) was added. Copper tallate itself showed some activity against mildew for a period of at least 14 days. Similar tests were repeated in the UK in 1992 using a reduced rate of copper tallate, 1l/ha (50g copper metal/ha), together with fungicides used at half the commercially recommended rates. The conclusions were:
1. Copper tallate, alone at both 50 and 250g copper metal/ha, was comparable with a reduced rate of tridemorph at 14 and 21 days after treatment on barley under low disease pressure.
2. The addition of 1l/ha copper tallate to reduced rates of carbendazim or prochloraz showed a trend towards increasing efficacy against powdery mildew on two barley cultivars over a two-week period. The carbendazim improvement was maintained on one cultivar for three weeks. These effects now need to be confirmed under field conditions.

Septoria tritici

Prochloraz and copper tallate

Two tests carried out by ITCF in 1988 showed that a programme of two treatments with prochloraz, 750g AI/ha followed by 450g AI/ha, could be replaced by two treatments with an experimental formulation with prochloraz at 225g AI/ha, copper tallate at 125g copper/ha, and carbendazim at 200g AI/ha. The efficacy was 56% in both trials and the wheat yields obtained with the experimental formulation exceeded those from the prochloraz treatments alone.

Chlorothalonil and copper tallate

Four tests carried out in 1990 were designed to study the activity of chlorothalonil, at reduced rates, in combination with copper tallate. For the first treatment, chlorothalonil was sprayed alone at 1100g AI/ha and at 750g AI/ha with copper tallate at 125g copper metal/ha. For the second treatment, chlorothalonil was applied at 1100g AI/ha together with fenpropimorph at 750 g AI/ha. The competitive programme was chlorothalonil at 750g AI/ha with copper tallate at 125g copper metal/ha. On ten ratings taken on various leaves, the efficacy, 60%, was the same for both programmes. From these four tests, it was concluded that:
(1) chlorothalonil at 750g AI/ha in presence of copper tallate at 125g copper metal/ha was equivalent to chlorothalonil at 1100g AI/ha.
(2) in presence of copper tallate the second fenpropimorph treatment was not needed.

Tapesia yallundae

INRA, Rennes, tested copper tallate in 1989 and 1990 against eyespot and concluded that it was not active against this disease. However, five other separate trials were

conducted in 1989 to investigate the activity of reduced rates of carbendazim with copper tallate in areas where there was disease resistance to carbendazim. Carbendazim at 125g AI/ha with copper tallate at 125g copper/ha was compared against the registered rate of carbendazim, 200g AI/ha, and, overall, proved to be more effective. Moreover, the proprietary formulation used for *Septoria tritici* control, using only 225g AI prochloraz/ha, in two application, gave 72% control of eyespot, equivalent to prochloraz alone at 750g AI/ha followed by 450g AI/ha.

POTENTIAL FOR USE AS AN ACTIVE ADJUVANT

The experimental results suggest that copper tallate has wide potential. As general guide, copper tallate should be considered for use in the following cases:

1. As with other copper fungicides.
2. Where a disease can be controlled by fungicides that are synergised by copper tallate, e.g. benzimidazoles, dicarboximides, ethylenebisdithiocarbamates, chlorothalonil, cymoxanil, fosetyl-aluminium. The use of copper tallate in these cases allow application rates to be reduced.
3. In areas of disease control where there are problems of fungicide resistance, toxicology or economics. Many fungal and bacterial diseases of crops such as potato, tomato, hops, apple, stone fruit, beet and banana could be treated by adding an active adjuvant, such as copper tallate, to the basic fungicide treatment.

Rates of 1l/ha copper tallate (50g copper metal/ha) should be studied in cases (2) and (3), for use as an active adjuvant. Rates of 1l/ha and 2.5l/ha should be studied in case (1), where copper tallate is used as a fungicide and where increasing the rate may increase the control.

CONCLUSIONS

The concept of *active adjuvant* is opening up new areas of research and could:

- complement the registered rates of many fungicides while increasing their biological efficacy
- help reduce the number of fungicide applications in the same season or, in certain cases, the application rates of fungicides necessary to give good control
- assist in resolving situations where fungicide residues in crops must be lowered
- contribute to overcoming disease resistance problems

REFERENCES

Soyez, J-L. (1988) European patent number 0364529, based on French patent number 8803163.

Soyez, J-L. (1992) Le tallate de cuivre - un adjuvant utile en viticulture. *Phytoma*, ***439***, 36-38.