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PROSPECTS FOR BIOPESTICIDES FOR APHID CONTROL

R. J. MILNER

CSIRO, Division of Entomology, Canberra, Australia.

Diseases form an important component of the natural enemy complex of aphids. The most common and obvious of these diseases are entomophthoran fungi such as *Erynia neoaphidis* Remaudiere & Herbert, *Entomophthora planchoniana* Cornu, *Zoophthora radicans* (Brefeld) Batko and *Conidiobolus obscurus* (Hall & Dunn) Remaudiere & Keller. The pest status of some aphids such as the pea aphid, *Acyrthosiphon pisum* (Harris), is considerably reduced by natural epizootics of fungal disease. However, disease may contribute little to practical control as it is mainly effective in high density populations when weather conditions are suitable. Introduction of exotic diseases for classical biological control is only rarely possible since most diseases, like their aphid hosts, are distributed world-wide. One exception was the successful introduction into Australia of a strain of *Z. radicans* for control of spotted alfalfa aphid, *Therioaphis trifolii* (Monell) f. *maculata* in 1979. Attempts to manipulate entomophthoran fungi have had limited success because of problems with mass production, the fragility of the conidia and the need for suitably moist conditions. Hyphomycete fungi such as *Verticillium lecanii* (Zimm.) Viegas, *Metarhizium anisopliae* (Metsch.) Sorokin, *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces* spp. are more suitable for development as mycoinsecticides as they are cheap to mass produce and form stable conidia. "Vertalec™", a formulation of *V. lecanii*, has been sold in small quantities commercially in Britain and parts of Europe for many years and used mainly in glasshouses. Recently promising results have been obtained with use of *M. anisopliae* for control of lettuce root aphid, *Pemphigus bursarius* (L.) in the UK. Laboratory studies on selected isolates of *B. bassiana* and *Paecilomyces* spp. show a promising level of activity. Problems may occur with these species as they can kill aphid predators such as coccinelids. In addition, more research is needed on developing improved formulations which enable control to be achieved under low humidity conditions.

KEY-WORDS: aphids, bioinsecticides, pathogens, biological control, fungi, review.

Aphids are one of the most important groups of insect pests in all parts of the world. They attack a wide range of crops especially cereals, pastures, vegetables, fruit crops and forest trees, and while they are more significant in temperate climates, they can cause substantial damage in the tropics. They can attack both the foliage and the roots of plants and, besides inflicting direct damage, they transmit many plant viruses. A large number of pathogens are known to kill aphids (Latgé & Papierok, 1988; Laubscher & von Wechmar, 1993) and the dramatic effects of natural epizootics have encouraged the development of biopesticides. As stated by Hajek and St Leger (1994): "Naturally occurring epizootics of insects are ever-present reminders of the potential of these pathogens for insect control.

However, epizootics are complex phenomena; a profusion of interacting processes in specific states are necessary for the understanding of epizootics... We must understand which factors are critical determinants of pathogenicity and epizootic development."

Research over the past 20 years has substantially increased our knowledge and understanding of epizootics in aphids but has so far led to the development of only one commercial bioinsecticide, "Vertalec"™ (based on *Verticillium lecanii* (Zimm.) Viegas, which has only limited sales for use in glasshouses in a small number of European countries (Dr. Ravensberg, pers. comm.). The 3 main areas of research have been: to elucidate the role of disease in natural control, to develop entomophthoran fungi as bioinsecticides and to develop Hyphomycete fungi as bioinsecticides. This paper aims to review recent progress and to highlight the prospects for developing Hyphomycete fungi for control of aphids over a wider range of crops and environmental conditions.

There have been many reviews notably general reviews by Hall and Papierok (1982) and Ferron *et al.* (1986), on *V. lecanii* by Hall (1981), and on entomophthoran fungi by Remaudière (1971), Latgé *et al.* (1978), Wilding (1981a), Wilding *et al.* (1986), Zimmermann (1978) and Verberne & Zadoks (1984).

FUNGAL PATHOGENS OF APHIDS

Aphid populations, possibly more than any other group of insects, suffer from epizootics of fungal diseases and there have been many studies documenting the incidence of naturally occurring diseases. Most of these fungal pathogens, such as *Erynia neoaphidis* Remaudiere & Hennebert, are entomophthoran fungi (Zygomycetina: Entomophthoraceae) which have a number of characteristics which make them successful pathogens of aphids. These characteristics include rapid sporulation and germination enabling the infection process to be completed often in a few hours, active discharge of conidia which maximises the dispersal of this infectious stage, high virulence whereby only a very small number of conidia are needed to infect, short generation time and, in some species, the production of resting spores so that the fungus persists through periods when aphids are absent or weather coniditions unsuitable for transmission. In contrast, the Hyphomycete fungi (such as *V. lecanii* and *Beauveria bassiana* (Bals.) Vuill. require several days of high humidity for sporulation and infection, depend on aphids moving to contact the conidia, and require a much larger number of conidia to infect. For these reasons, they are much less important as natural pathogens of aphids.

Under natural conditions, the same species of entomophthoran fungi dominate in all parts of the world - *E. neoaphidis*, *Entomophthora planchoniana* Cornu, *Neozygites fresenii* (Nowakowski) Remaudiere & Keller, *Zoophthora radicans* (Brefeld) Batko, and *Conidiobolus obscurus* ((Hall & Dunn) Remaudiere & Keller. The only Hyphomycete fungus commonly found as a natural pathogen of aphids is *V. lecanii*. Only a few pathogens have a restricted geographical range for example *Entomophthora chromaphis* Humber & Feng has only been described from North America (Humber & Feng, 1991), *Metarhizium anisopliae* (Metsch.) Sorokin only from root aphids in the UK (Foster, 1975) and *Erynia kondoiensis* Milner only in Australia and China (Milner *et al.*, 1980; Fan *et al.*, 1991). More rarely aphids are attacked by other fungi such as the entomophthorans *Zoophthora phalloides* Batko and *Conidiobolus thromboides* Dreschler, as well as more general Hyphomycete fungi such as *Fusarium* spp., *Paecilomyces* spp. and *B. bassiana*. Certain aphids seem particularly susceptible to certain fungi; for example, cotton aphids are most frequently attacked by *N. fresenii*, pea aphids, cereal aphids and cabbage aphids by *E. neoaphidis*, and spotted alfalfa aphid, *Therioaphis trifolii* (Monell) f. *maculata*, by

Z. radicans. *E. neoaphidis* is the most widespread and common (Glare & Milner, 1986). For example, Sivcev (1992) in Yugoslavia found that 73.5% of diseased cabbage aphids were infected by *E. neoaphidis*, and Feng *et al.* (1991) found that 43.9% of diseased cereal aphids in the USA were attacked by *E. neoaphidis*. Other studies from tropical countries such as Chad (Silvie & Papierok, 1991) and Mexico (Remaudiere & Latgé, 1985) and from cooler countries such as Finland (Papierok, 1989; Kariluoto, 1982) have found remarkably similar occurrence of entomophthoran fungi.

The development of epizootics of entomophthoran fungi depends on many factors which are only partially understood. Obviously the pathogen has to be present and fungi have developed a number of strategies to persist at a site or to disperse into a site. Some such as *Z. radicans* form resting spores which can persist in the soil, probably for several years, and certainly from season to season. A period of rainfall is often needed to break the dormancy of these spores so that they germinate to conidia which then infect if they land on a susceptible host. Other species may survive as mycelium within the diseased cadaver, or (more controversially) as conidia in protected sites such as the soil surface. In more equitable climates the pathogen may persist by infecting non-pest aphids on weeds. For example, in Australia *E. neoaphidis* infects *Hypomyzus lactucae* on sowthistle aphids at times when its main host *Acyrthosiphon pisum* Harris is absent (Milner, unpubl.). Once the pathogen and aphid host are present then environmental factors especially moisture become critical. Entomophthoran fungi require very high humidity (close to 100%) to sporulate, germinate and infect (Milner & Bourne, 1983). However they are well adapted to take advantage of short periods of high humidity at night. Aphids normally die in the late afternoon and sporulate rapidly enabling the infection process from death to new infections to take place overnight under warm, moist conditions (Milner & Bourne, 1983). Under cooler conditions, the infection process can be interrupted during the day and recommence when the humidity increases the next night (Glare & Milner, 1986). Numerous studies have shown that aphids in moist climates, or on irrigated crops, are much more likely to be diseased that aphids on non-irrigated crops or in dry conditions. For example, Wraight *et al.* (1993) found over 50% disease in populations of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), in irrigated fields while in non-irrigated fields the level of disease did not exceed 2.5%. Another important factor, which is often underestimated, is aphid population density. High density populations favour disease by being forcing the aphids into more exposed positions on the plant, increasing the inoculum level and promoting contact between conidia and aphid. Disease interactions with parasitoids may be complex and counter-intuitive. For example, parasitoid activity may stimulate the production of alarm pheromones which in turn leads to aphid dropping off the plant onto the soil where they are more likely to pick up an infectious dose of conidia often some pathogens such as *C. obscurus*. Also since fungi cause aphids to die more quickly than parasitoids, it is thought that entomophthoran fungi would outcompete the parasitoids leading to an overall reduction in parasitoid abundance and hence a resurgence of the aphid population (Powell *et al.*, 1986). However it may be that the combination is more additative or even synergistic (Milner *et al.*, 1984) since parasitoids are more effective than pathogens at low population densities and the entomophthoran fungi are unlikely to directly infect parasitoids (or predators). Finally, parasitoids may avoid infected aphids thus minimising the "wastage" of eggs.

STRATEGIES FOR UTILISATION OF APHID PATHOGENS

There are essentially 4 strategies for exploiting pathogens: taking account of epizootics as part of natural control; introduction into a crop to induce an epizootic; classical biological control; and as a sprayable bioinsecticide. While this review is mainly concerned with the last of these strategies as it offers most scope for aphid control in the future, I will give some examples of the other approaches. The exploitation of natural epizootics has been suggested by Hollingsworth *et al.* (1995) who reported that cotton farmers in the Mississippi Delta, USA, delayed application of chemical pesticides for control of cotton aphids, *Aphis gossypii* Glover, as infestations were often devastated by epizootics of *Neozygites fresenii* (Nowakowski) Remaudier & Keller. As little as 15% infection in the aphid population resulted in some population reduction. The pathogen is able to complete its life-cycle in 3 days and infection rates in one field increased from 5.9% of apterae to 70.1% in just 6 days. It is suggested that farmers could scout their fields for diseased aphids and thereby reduce the number of insecticide sprays required.

Attempts at early induction of epizootics by means of introduction of infected aphids or spraying of mycelium of pathogens such as *E. neoaphidis*, have generally been unsuccessful (Dedryver & Rabasse, 1982; Wilding, 1981b; Wilding *et al.*, 1986; Wilding *et al.*, 1990). These preparations generally were effective in producing conidia and resulted in some aphids becoming diseased. However factors such as environmental conditions and aphid density limited the efficacy and useful population reductions were not obtained. Other factors which inhibit further development of this strategy include the difficulty of mass production of entomophthoran fungi, problems of storage and the need for large amounts of mycelium. Thus this strategy is unlikely to be cost effective unless there are some breakthroughs in research on mass production.

The world distribution of aphid pathogens is now well established and seems to offer very little scope for introductions as classical biological control agents. For example, the Russian wheat aphid, *D. noxia*, has recently spread into the USA where it is commonly infected by *E. neoaphidis* (Feng *et al.*, 1991, Wraight *et al.*, 1993) without any intervention by man. This pathogen and several others which also occur in the USA were also found to be part of the natural enemy complex in Eurasia (Gruber *et al.*, 1991). A possible exception to this rule is *E. chromaphis* recently described from North America (Humber & Feng, 1991), which apparently does not occur elsewhere. The only successful case of a pathogen for classical biological control of an aphid is the introduction of a spotted alfalfa aphid adapted strain of *Z. radicans* into Australia for control of *T. maculata* f. *maculata* (Milner & Soper, 1981; Milner *et al.*, 1982).

HYPHOMYCETES AS BIOINSECTICIDES FOR APHIDS

The ideal biopesticide for aphid control would have the following characteristics:

1. cheap to mass produce
2. easy to store
3. effective over a wide range of temperature and humidity conditions
4. provide rapid kill at economical doses
5. wide host range within aphids
6. minimal non-target effect especially on parasites and predators of aphids.

While no pathogen is presently known with all these characteristics, Hyphomycete fungi meet most of these criteria and thus are the most likely to be effective as commercial products.

V. lecanii is the only Hyphomycete fungus commonly found attacking aphids under natural conditions and a bioinsecticide for aphids based on this pathogen has been available for many years under the trade name Vertalec (Koppert, Netherlands). The other Hyphomycete fungi being considered as aphid bioinsecticides are *M. anisopliae, B. bassiana, Paecilomyces fumosoroseus* (Wize) Brown & Smith and *P. farinosus* (Holm) Brown & Smith.

In recent years, research on mycoinsecticides has been dominated by a small number of species of Hyphomycete fungus. Two of these, *B. bassiana* and *M. anisopliae,* have been used as bioinsecticides in countries such as China and Brasil for many years. Now there are several products based on Hyphomycete fungi registered in the developed world (table 1). This group of fungi is amenable to mass production, usually by solid substrate fermentation to give conidia but also by liquid fermentation to give blastospores or more rarely conidia. Both these spore types are directly infectious and can be formulated to optimise field efficacy and to give a reasonable storage capacity. Two key developments of recent research has been the increase in knowledge and understanding of the genetic diversity within species such as *V. lecanii* (Mor *et al.,* 1996), *M. anisopliae* (Curran *et al.,* 1994) and *B. bassiana* (St Leger *et al.,* 1992), and the development of oil-based formulations which increase field efficacy and reduce (or eliminate) the need for a high humidity environment for germination and infection (Bateman *et al.,* 1993). Thus it is now possible to develop mycoinsecticides for insect pests such as locusts and grasshoppers based on isolates which are highly virulent and quite specific, and formulated in oil to ensure good storage and field efficacy over a wide range of environmental conditions. With the exception of products based on *V. lecanii,* research on these fungi is mainly directed at pests other than aphids.

VERTICILLIUM LECANII

This is the only Hyphomycete which regularly causes mortality of aphids under natural conditions. Other susceptible insects include scale insects, thrips, mites and white-fly. In addition, some isolates of this fungus are known to be secondary pathogens of fungal plant pathogens such as rusts, mildew and leafspot (Subrahamyam *et al.,* 1990; Heintz & Blaich, 1990), and to plant parasitic nematodes (Meyer & Meyer, 1995). The product "Vertalec"™ was first introduced for control of aphids on chrysanthemums in 1981, while another strain for control of whitefly on cucumbers and tomatoes, "Mycotal"™ was first used in 1982 (Quinlan, 1988). These products are still available and are manufactured by Koppert in the Netherlands. "Vertalec"™ is produced by liquid fermentation and the blastospores are formulated to improve stability and also to provide a nutrient base so that secondary growth and conidiation can take place on the leaf surface. The product is a wettable powder containing 10^9 blastospores/g and it is normally diluted with water and applied as a high volume spray containing 10^6 blastospores/ml. The product is registered in the UK and Switzerland, and allowed in Denmark and Sweden. It is exclusively used where the humidity can be modified either in a glasshouse or on chrysanthemums where blackout covers are used to control day length. Crops include other ornamentals, tomatoes, cucumbers and other vegetables (Dr Ravensberg, pers. comm.). A wide range of aphids are attacked, though mobile aphids such as *Myzus persicae* (Sulzer) are more easily controlled than sedentary species such as *Macrosiphonella sanborni* (Gillette) and *T. trifolii* f. *maculata* (Quinlan, 1988; Harper & Huang, 1986). Problems of requiring high humidity (Milner & Lutton, 1986) and competition from imidocloprid limit the market size for this product (Dr Ravensberg, pers. comm.).

There have been many studies aimed at improving *V. lecanii* for aphid control. For example frequent low dose applications of Vertalec gave good control of *M. persicae* and *A. gossypii* on chrysanthemums (Helyer & Wardlow, 1987). Another approach to increasing the efficacy of Vertalec is to use an electrostatic sprayer which provides better coverage and improved aphid control on chysanthemums (Sopp *et al.,* 1989; Sopp *et al.,* 1990). These authors also found that the addition of nutrients (as in the "Vertalec" formulation) did not improve efficacy. An experimental formulation of 2 strains (from aphids and whitefly), "Microgermin" (pers. comm. Dr Adrian Gillespie, Chris. Hansens, Denmark) gave good control of aphids and thrips on chrysanthemums provided the humidity was raised 4 nights per week (Helyer *et al.*, 1992). In an unusual application, *V. lecanii* is used as an introduction on aeroponically grown squash in Florida to control the rice root aphid, *Rhopalosiphum rufiabdominalis* (Sasaki) and is claimed to be so effective that this control method is now routine (Etzel & Petitt, 1992).

V. lecanii is normally ineffective under field conditions (e.g. Khalil *et al.*, 1985), but Pfrommer & Mendgen (1992) found that by adding various substances, such as polysaccharides and phospholipids, control of *Brevicoryne brassicae* (L.) could be achieved on field cabbages. However some of these additives were themselves aphidocidal. Oil formulations are difficult as the spores are lipophobic; however Helyer (1993) claimed improved control with the addition of rape seed oil and John Curtis, a student at La Trobe University, Melbourne, Australia, has developed a sophisticated oil formulation which enables the fungus to infect and kill 80% of *M. persicae* at 40-50% RH (J. Curtis, pers. comm.).

BEAUVERIA BASSIANA

B. bassiana is a common and ubiquitous pathogen of many insects including Coleoptera, Diptera, Orthoptera and Lepidoptera. Under natural conditions, it rarely attacks aphids. The fungus can be mass produced by liquid fermentation to form blastospores or conidia, and by solid-substrate fermentation to form conidia (Feng *et al.*, 1994). These spores can be formulated into stable and effective mycoinsecticides (for example see Knudsen *et al.*, 1990). Products for whitefly, cotton boll weevil, European corn borer are now produced in the USA and France while other products are used in countries such as China and Colombia against a variety of pests. Feng *et al.* (1990a) reported a natural infection of cereal aphids and subsequently found that this aphid-derived isolate killed aphids more rapidly in the laboratory than *V. lecanii* (Feng *et al.*, 1990b). In a comparison with 5 other isolates, the aphid derived isolate was found to be the most virulent for Russian wheat aphid (Feng & Johnson, 1990; Wang & Knudsen, 1993) and highly virulent for the hop aphid, *Phorodon humuli* (Schrank) (Dorschner *et al.*, 1991). A field trial against *M. persicae* on canola gave up to 86% mortality in plots treated with *B. bassiana* and it was suggested that the aphid-derived isolates were promising for aphid control (Miranpuri & Khachatourians, 1993). Subsequent genetic analysis however has suggested that the aphid- derived isolate is not distinct from isolates obtained for non-homopteran hosts (St Leger *et al.*, 1992) and further field trials (James *et al.*, 1995) have shown that the isolate is more virulent for coccinellid predators than for pea aphids when sprayed onto lucerne. However weather conditions, during this field trial, were probably too dry for the water-based preparation to be fully effective. More recently Vandenberg (1996) has shown that *B. bassiana* is highly virulent for Russian wheat aphid both in the laboratory and in the field where significant reductions in populations were obtained 28 days after spraying a suspension of conidia (Dr J.Vandenberg, pers. comm.).

These results show that there are isolates of *B. bassiana* that can be mass produced and formulated as aphid mycoinsecticides. Further work needed to develop formulations effective over a range of weather conditions and to assess the likely impact on predators and parasites.

METARHIZIUM ANISOPLIAE

The only recorded isolates of *Metarhizium* sp. from aphids have come from *Pemphigus* in Norfolk, UK. These isolates are genetically distinct from all other isolates of *Metarhi-*

zium sequenced to date (Curran *et al.*, 1994; Driver, pers. comm.) and represent an undescribed variety of *M. flavoviride* Gams & Roszypal. Laboratory bioassays have found them to be the more virulent for lettuce root aphid, *Pemphigus bursaris* (L.), than other isolates of *V. lecanii* or *Metarhizium* spp. (Chandler, 1992; Chandler, 1997). Two seasons of field trials in which lettuces were grown in potting mix containing conidia of *M. flavoviride* have given promising results (Chandler, pers. comm.). Tests on other aphids with these *M. flavoviride* isolates have not given promising results (Hall, 1980; Milner unpublished) Some isolates of *M. anisopliae* are also pathogenic for aphids in the laboratory (Butt *et al.*, 1994), but no field trials have been reported to date. As with *B. bassiana,* the effect on natural enemies may be a disadvantage of *Metarhizium* for aphid control though in field trials against lettuce root aphid no infections have been detected in non-targets (Chandler, pers. comm.).

PAECILOMYCES spp.

Two species of *Paecilomyces* are commonly recorded from insects, occasionally from aphids, *P. farinosus* and *P. fumoroseus.* As with *B. bassiana* and *Metarhizium* spp., these fungi can be mass produced in liquid or solid fermentation and formulated as mycoinsecticides. One product, Per-97, with *P. fumosoroseus* as the active ingredient is registered for white-fly control in the USA (table 1).

TABLE 1

Some examples of registered mycoinsecticides

Product name	Pathogen	Main target	Company name	Country	Comments
Naturalis	*Beauveria bassiana*	boll weevil	Troy Biosciences	USA	available in USA
?	*B. bassiana*	whiteflies, grasshoppers	Mycotech	USA	not yet available
Ostrinal	*B. bassiana*	European corn borer	Calliope	France	available in France
Engerlinspilz	*B. brogniartii*	cockchafers	Andermatt	Switzerland	available in Switzerland
BioPath	*Metarhizium anisopliae*	cockroaches	EcoScience	USA	no longer available
BioBlast	*M. anisopliae*	termites	EcoScience	USA	available in USA
Biogreen	*M. anisopliae*	cockchafers	BioCare	Australia	available in Australia
Per-97	*Paecilomyces fumosoroseus*	whiteflies	Grace	USA	available in USA
Vertalec	*Verticillium lecanii*	aphids	Koppert	Netherlands	available in some countries in Europe
Mycotal	*V. lecanii*	whitefly	Koppert	Netherlands	available in some countries in Europe
Betel	*B. brogniartii*	scarabs	Calliope	Reunion	available in Reunion

Hayden *et al.* (1992) found that natural isolates of *P. farinosus* had only a low level of virulence for *Sitobion avenae* (Fabricius) but passage on aphids or in an agar medium containing aphid-cuticle for 3 generations reduced the LT_{50} from 11.1 days down to about 5 days. However *P. fumosoroseus* are usually more effective against aphids and in a detailed study Vandenberg (1996) has found that some isolates have an LTso as low as 3.3 days for Russian wheat aphid. This discovery of highly virulent isolates, together with improved methods of mass production (Jackson *et al.,* 1996) and formulation (Sosnowska, 1992; Vega *et al.*, 1996) suggest that *P. fumosoroseus* is probably the most promising pathogen for development as a mycoinsecticide against aphids. As with *B. bassiana*, the pathogen may well infect and kill non-targets including predators and parasitoids (Lacey *et al.*, 1996). Recent field trials in the USA, have shown significant population reductions on aphid-susceptible wheat following application of *P. fumosoroseus* aerial conidia or blastospores (Dr J. Vandenberg, pers. comm.)

OTHER PATHOGENS

Other pathogens of aphids described include species of *Mucor* (Mathai *et al.*, 1990), *Penicillium* (Santamarina *et al.*, 1988) and *Fusarium* (Hareendranath *et al.*, 1987); Nagalingam & Jayaraj, 1986; Ozino *et al.*, 1987), as well as a "lethal paralysis virus" (Laubscher & von Wechmar, 1993) and the *Rhopalosiphon padi* virus (Williamson *et al.*, 1989). In addition, it is known that certain strains of *Bacillus thuringiensis* Berliner can be toxic to aphids (Walters & English, 1995). While none of these pathogens are likely to be developed as biological insecticides, they may provide candidate genes for insertion into transgenic plants to provide resistance to aphids.

TABLE 2

Advantages and disadvantages of hyphomycete fungi for aphid control

ADVANTAGES	DISADVANTAGES
* spores can be produced by fermentation	* wide host range
* can be formulated to overcome moisture limitations	* slow to kill
* genetically variable - some isolates virulent for aphids	* may be inhibited by low temperatures
* being developed as mycoinsecticides against other pests	

CONCLUSIONS AND FUTURE RESEARCH

Pathogens often provide a useful level of natural control which may be disrupted by, for example, the use of chemical fungicides (Smith & Hardie, 1996), and probably the most cost-effective use of pathogens is as a component of natural control in an integrated control program.

Substantial progress has been made over the past few years in developing biological insecticides for use against aphids. Two significant driving forces have been the introduction into the USA of the Russian wheat aphid and the need to develop environmentally friendly control strategies, and the development of Hyphomycete fungi as biological insecticides against a range of other pests. A major impediment to developing aphid-specific mycoinsecticides is the high cost of registration in Europe (van Schelt, pers. comm.) as well as the capital costs of establishing a facility for production and formulation. If this investment can be used on developing a product for a range of pests which includes aphids then clearly it is more attractive commercially. Problems of mass production and formulation are now being overcome and it is likely that mycoinsecticides will increase their market share over the next decade. For aphid control, major disadvantages may be the non-target effects on parasites and predators, and the need for repeated applications as epizootics are unlikely to develop (table 2). Nevertheless mycoinsecticides based on fungi such as *B. bassiana* and *P. fumosoroseus* may be a key component of integrated aphid control in the future, while other products such as *M. flavoviride* for lettuce root aphid, and *V. lecanii* for aphids on chrysanthemums, may have important niche markets. While the search for better strains will continue, the most significant advances will come for further research on formulation and application technologies.

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RÉSUMÉ

Perspectives d'utilisation des biopesticides dans la lutte contre les pucerons

Les maladies constituent une part importante du complexe des ennemis naturels des pucerons. Les plus communes et les plus connues sont des champignons entomophthorales tels que *Erynia neoaphidis* Remaudiere & Herbert, *Entomophthora planchoniana* Cornu, *Zoophthora radicans* (Brefeld) Batko et *Conidiobolus obscurus* (Hall& Dunn) Remaudiere & Keller. La nuisibilité de certains ravageurs comme le puceron du pois *Acyrthosiphon pisum* (Harris), est considérablement réduite par des épizooties naturelles de maladies fongiques. Cependant, les maladies ne contribuent peut être que faiblement à la régulation naturelle au champ, vu qu'elles sont surtout efficaces sur des populations de densité élevée et dans des conditions climatiques particulières. L'introduction de maladies exotiques dans le cadre d'une lutte biologique classique n'est que rarement possible, puisque la plupart des maladies, à l'instar des pucerons-hôtes, sont cosmopolites. Une exception a été celle de l'introduction réussie en Australie d'une souche de *Z. radicans* pour contrôler *Therioaphis trifolii* (Monell) f. *maculata* en 1979. Les tentatives de manipulation des entomophthorales ont eu un succès limité en raison des problèmes de production de masse, de la fragilité des conidies et de la nécessité de conditions d'humidité convenables. Les champignons hyphomycètes tels que *Verticillium lecanii* (Zimm.) Viegas, *Metarhizium anisopliae* (Metsch.) Sorokin, *Beauveria bassiana* (Bals.) Vuill. et *Paecilomyces* spp. sont plus à même d'être utilisés comme mycoinsecticides, étant donné que le coût de leur production de masse est faible et qu'ils forment des conidies stables. Le « Vertalec® », une formulation de *V. lecanii*, est vendu dans le commerce, en petites quantités, en Grande-Bretagne et en d'autres parties de l'Europe depuis de nombreuses années, pour être utilisée prinicipalement sous serre. Récemment des résultats prometteurs ont été obtenus avec l'utilisation de *M. anisopliae* pour lutter contre le puceron des racines de la laitue, *Pemphigus bursarius* (L.) en Grande-Bretagne. Des études en laboratoire d'isolats choisis de *B. bassiana* et de *Paecilomyces* spp. montrent un niveau d'activité prometteur. Des problèmes peuvent apparaître avec ces espèces car elles peuvent aussi tuer les prédateurs de pucerons tels que les coccinelles. Par ailleurs, des recherches supplémentaires sont nécessaires pour développer des formulations améliorées permettant l'emploi de ces champignons dans des conditions d'humidité basses.

REFERENCES

Bateman, R. P., Carey, M., Moore, D. & Prior, C. — 1993. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. — *Ann. Appl. Biol.*, 122, 145-152.

Butt, T. M., Ibrahim, L., Ball, B.V. & Clark, S. J. — 1994. Pathogenicity of the entomogenous fungi *Metarhizium anisopliae* and *Beauveria bassiana* against crucifer pests and the honey bee. — *Biocontr. Sci. Tech.*, 4, 207-214.

Chandler, D. — 1992. The potential of entomopathogenic fungi t° control the lettuce root aphid, *Pemphigus bursarus*. — *Phytoparasitica*, 20, 11-15.

Chandler, D. — 1997. Selection of an isolate of the insect pathogenic fungus *Metarhizium anisopliae* virulent to the lettuce root aphid, *Pemphigus bursarius*. — *Biocontr. Sci. Tech.*, 7, 95-104.

Curran, J., Driver, F., Ballard, J. W. O. & Milner, R. J. — 1994. Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequence data. — *Mycol. Res.*, 98, 547-552.

Dedryver, C. A. & Rabasse, J. M. — 1982. Attempt of biological control of the lettuce aphids in glasshouses with resting spores of *Conidiobolus obscurus* Hall and Dunn and mycelium of *Erynia neoaphidis* Remaud. and Henn. — *Proc. III'rd Int. Coll. Invert. Pathol.*, Brighton, UK, p. 103.

Dorschner, K. W., Feng, M. G. & Baird, C. R. — 1991. Virulence of an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) to the hop aphid, *Phorodon humuli* (Homoptera: Aphididae). — *Environ. Entomol.*, 20, 690-693.

Etzel, R. W. & Petitt, F. L. — 1992. Association of *Verticillium lecanii* with population reduction of red rice root aphid (*Rhopalosiphum rufiabdominalis*) on aeroponically grown squash. — *Fla. Entomol.*, 75, 605-606.

Fan, M. Z., Guo, C. & Li, Z. Z. — 1991. New species and a new record of the genus *Erynia* in China. — *Acta Mycologia Sinica*, 10, 95-100.

Feng, M. G. & Johnson, J. B. — 1990. Relative virulence of six isolates of *Beauveria bassiana* on *Diuraphis noxia* (Homoptera: Aphididae). — *Environ. Entomol.*, 19, 785-790.

Feng, M. G., Johnson, J. B. & Halbert, S. E. — 1991. Natural control of cereal aphids (Homoptera: Aphididae) by entomopathogenic fungi (Zygomycetes: Entomophthorales) and parasitoids (Hymenoptera: Braconidae and Encyrtidae) on irrigated spring wheat in southwestern Idaho. — *Environ. Entomol.*, 20, 1699-1710.

Feng, M. G., Johnson, J. B. & Kish, L. P. — 1990a. Survey of entomopathogenic fungi naturally infecting cereal aphids (Homoptera: Aphididae) of irrigated grain crops in southwestern Idaho. — *Environ. Entomol.*, 19, 1534-1542.

Feng, M. G., Johnson, J. B., & Kish, L. P. — 1990b. Virulence of *Verticillium lecanii* and an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) for six species of cereal-infesting aphids (Homoptera: Aphididae). — *Environ. Entomol.*, 19, 815-820.

Feng, M. G., Poprawski, T. J. & Khachatovrians, G. G. — 1994. Production, formulation and application of the entomopathogenic fungus, *Beauveria bassiana* for insect control: Current status. — *Biocontr. Sci. Tech.*, 127, 3-34.

Ferron, P., Fargues, J. & Riba, J. — 1986. Fungi as microbial insecticides against pests. In: Handbook of Applied Mycology (**D. K. Arora, L. Ajello, & K. G. Mukeji,** eds). — *Marcel Dekker*, New York, vol. 2, pp. 665-706.

Foster, W. A. — 1975. Life history and population biology of an intertidal aphid, *Pemphigus trehernei* Foster. — *Trans. Roy. Entomol. Soc.*, 127, 192-207.

Glare, T. R. & Milner, R. J. — 1986. Ecology of Entomopathogenic fungi. In: Handbook of Applied Mycology (**D. K. Arora, L. Ajello, & K. G. Mukeji I.,** eds). — *Marcel Dekker*, New York, vol. 2, pp. 547-612.

Gruber, F., Poprawski, T. J. & Rey, E. — 1991. Survey for natural enemies of *Diuraphis noxia* (Mordvilko) in Eurasia. — *Bulletin SROP*, 14, 102-109.

Hajek, A. E. & St Leger, R. J. — 1994. Interactions between fungal pathogens and insect hosts. — *Ann. Rev. Ent.*, 39, 293-322.

Hall, R. A. — 1980. Comparison of laboratory infection of aphids by *Merarhizium anisopliae* and *Verticillium lecanii*. — *Ann. Appl. Biol.*, 95, 159-162.

Hall, R. A. — 1981. The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. In: Microbial Control of Pests and Plant Diseases 1970-1980 (**H. D. Burges**, ed.). — *Academic Press*, London, pp. 483-498.

Hall, R. A. & Paperiok, B. — 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. — *Parasitology*, 84, 205-240.

Hareendranath, V., Nair, K. P. V., & Paulose, S. — 1987. *Fusarium pallidoroseum* (Cooke) Sacc. as a fungal pathogen of *Aphis craccivora* (Koch). — *Entomon.*, 12, 392-394.

Harper, A. M., & Huang, H. C. — 1986. Evaluation of the entomophagous fungus *Verticillium lecanii* (Moniliales: Moniliaceae) as a control agent for insects. — *Environ. Entomon*, 15, 281-284.

Hayden, T. P., Bidochka, M. J. & Khachatourians, G. G. — 1992. Entomopathogenicity of several fungi toward the English grain aphid (Homoptera: Aphididae) and enhancement of virulence with host passage of *Paecilomyces farinosus.* — *J. Econ. Entomol.*, 85, 58-64.

Heintz, C. & Blaich, R. — 1990. *Verticillium lecanii* as a hyperparasite of grapewine mildew (*Uncinula necator*). — *Vitis*, 29, 229-232.

Helyer, N. — 1993. *Verticillium lecanii* for control of aphids and thrips on cucumber. — *Bull. OILB SROP*, 16, 63-66.

Helyer, N. & Wardlow, L. R. — 1987. Aphid control on chrysanthemum using frequent, low dose applications of *Verticillium lecanii.* — *Bull. OILB SROP*, 10, 6265.

Helyer, N., Gill, G., Bywater, A. & Chambers, R. — 1992. Elevated humidities for control of chysanthemum pests with *Verticillium lecanii.* — *Pest. Sci.*, 36, 373-378.

Hollingsworth, R. G., Steinkraus, D. C. & McNew, R. W. — 1995. Sampling to predict fungal epizootics in cotton aphids (Homoptera: Aphididae). — *Environ. Entomol.*, 24, 1414-1421.

Humber, R. A. & Feng, M. G. — 1991. *Entomophthora chromaphidis* (Entomophthorales): the correct identification of an aphid pathogen in the Pacific northwest and elsewhere. — *Mycotaxon*, 41, 497-504.

Jackson, M. A. & Cliquet, S — 1996. Improved culture conditions for production of desication tolerant blastospores of the bioinsecticidal fungus, *Paecilomyces fumosoroseus.* — *Soc. Invert. Pathol.*, Abstracts from 29th annual meeting, p. 39.

James, R. R, Shaffer, B. T., Croft, B. & Lighthart, B. — 1995. Field evaluation of *Beauveria bassiana*: its persistence and effects on pea aphid and a non-target coccinellid in alfalfa. — *Biocontr. Sci. Tech.*, 5, 425-437.

Kariluoto, K. T. — 1982. New records of the genus *Entomophthora* Fres. infesting insects in Finland. — *Annal. Entomol. Fenn.*, 48, 49-50.

Khalil, S. K., Bartos, J. & Landa, Z. — 1985. Effectiveness of *Verticillium lecanii* to reduce populations of aphids under glasshouse conditions. — *Agric. Ecosys. Environ.*, 12, 151-156.

Knudsen, G. R., Johnson, J. B. & Eschen, D. J. — 1990. Alginate pellet formulation of a *Beauveria bassiana* (Fungi: Hyphomycetes) isolate pathogenic to cereal aphids. — *J. Econ. Entomol.*, 83, 2225-2228.

Lacey, L. A., Mercadier, G. & Mesquita, A. L. M. — 1996. The acute and sub-lethal activity of *Paecilomyces fumosoroseus* on *Aphelinus asychis* a primary parasite of *Diuraphis noxia.* — *Soc. Invert. Pathol.*, Abstracts from 29th annual meeting, p. 46.

Latge, J. P. & Papiereok, B. — 1988. Aphid pathogens. In: Aphids their Biology, Natural Enemies and Control, vol. 2B (**A. K. Minks & P. Harrewijn**, eds). — *Elsevier*, Amsterdam, pp. 323-335.

Latge, J. P., Remaudiere, G. & Papierok, B. — 1978. An example of research into biological control: the Entomophthora fungi pathogenic to aphids. — *Bull. Soc. Pathol. Exot.*, 71, 196-203.

Laubscher J. M. & Von Wechmar, M. B. — 1993. Assessment of aphid lethal paralysis virus as an apparent population growth-limiting factor in grain aphids in the presence of other natural enemies. — *Biocontr. Sci. Tech.*, 3, 455-466.

Mathai, S., Kumari, G. P., Kumari, T. N. & Hebsybai. — 1990. *Mucor circinelloides* Van Tiegham, an entomogenous fungus of the banana aphid, *Pentalonia nigronervosa* Coq. (Aphididae: Hemiptera). — *J. Biol. Contr.*, 4, 124.

Meyer, S. L. F. & Meyer, R. J. — 1995. Effects of a mutant strain and a wild type strain of *Verticillium lecanii* on *Heterodera glycines* populations in the glasshouse. — *J. Nematol.*, 27, 409-417.

Milner, R. J., Soper, R. S. & Lutton, G. G. — 1982. Field release of an Israeli strain of the fungus *Zoophthora radicans* (Brefeld) Batko for the biological control of *Therioaphis trifolii* (Monell) f. *maculata.* — *J. Aust. Ent. Soc.*, 21, 113-118.

Milner, R. J. & Bourne, J. — 1983. Influence of temperature and duration of leaf wetness on infection of *Acyrthosiphon kondoi* with *Erynia neoophidis.* — *Ann. Appl. Biol.*, 102, 19-27.

Milner R. J. & Lutton, G. G. — 1986. Dependence of *Verticillium lecanii* (Fungi: Hyphomycetes) on high humidities for infection and sporulation using *Myzus persicae* (Homoptera: Aphididae) as host. — *Environ. Entomol.*, 15, 380-382.

Milner, R. J.. & Soper, R. S. — 1981. Bioassay of *Entomophthora* against the spotted alfalfa aphid *Therioaphis trifolii* f. *maculata.* — *J. Invertebr. Pathol.*, 37, 168-173.

Milner, R. J., Glutton, G. G. & Bourne, J. — 1984. A laboratory study of the interaction between aphids, fungal pathogens, and parasites. — *Proc. 4th Austral. Appl. Ent. Res. Conf.*, pp. 375-381.

Milner, R. J., Teakle, R. E., Lutton, G. G. & Dare, F. M. — 1980. Pathogens (Phycomycetes: Entomophthorales) of the blue-green aphid *Acyrthosiphon kondoi* Shinji and other aphids in Australia. — *Aust. J. Bot.*, 28, 601-619.

Miranpuri, G. S. & Khachatourians, G. G. — 1993. Application of entomopathogenic fungus, *Beauveria bassiana* against green peach aphid, *Myzus persicae* (Sulzer) infesting canola. — *Ins. Sci.*, 6, 287-289.

Mor, H., Gindin, G., Ben-Ze'Ev, I. S., Raccah, B., Geschtovt, N. U., Askhozegna, N. & Barash, I. — 1996. Diversity among isolates of *Verticillium lecanii* as expressed by DNA polymorphism and virulence towards *Bemisia tabaci.* — *Phytoparasitica*, 24, 111-118.

Nagalingam, B. & Jayaraj, S. — 1986. First record of *Fusarium semitectum* Berk and Rav as an entomophagous fungus. — *Curr. Sci. India*, 55, 377-378.

Ozino, O. I., Arzone, A. & Alma, A. — 1987. Action of *Verticillium lecanii* (Zimm.) Viegas and three species of *Fusarium* Link. ex Fr. on *Sitobion avenae* (F.). — *Difesa delle Piante*, 10, 331-337.

Papiero, B. — 1989. On the occurrence of Entomophthorales (Zygomycetes) in Finland I. Species attacking aphids (Homoptera, Aphididae). — *Ann. Entomol. Fenn.*, 55, 63-69.

Pfrommer, W. & Mendgen, K. — 1992. Control of the cabbage aphid (*Brevicoryne brassicae*) with the entomopathogenic fungus *Verticillium lecanii* in the laboratory and field. — *Z. Pflanzenkr. Pflanz.*, 99, 209-217.

Powell, W., Wilding, N., Brobyn, P. J. & Clark, S. J. — 1986. Interference between parasitoids (Hym.: Aphidiidae) and fungi (Entomophthorales) attacking cereal aphids. — *Entomophaga*, 31, 293-302.

Quinlan, R. J. — 1988. Use of fungi to control insects in glasshouses. In: Fungi in Biological Control Systems (**M. N. Burge**, ed.). — *Manchester University Press*, Manchester, pp. 19-36.

Remaudiere, G. & Latgé, J. P. — 1985. Importance of fungi pathogenic to insects (especially Aphididae and Cercopidae) in Mexico and prospects for their use. — *Bol. Serv. Defensa Plagas Inspec. Fitopatologica*, 11, 217-225.

Remaudiere, G. — 1971. Towards the practical use of *Entomophthora* spp. parasitic on aphids. — *Parasitica*, 27, 115-126.

Santamarina, M. P., Sanchis, V., Jimenez, M., Garcia, F. & Hernandez, E. — 1988. A strain of *Penicillium funiculosum* Thom with activity against insects and mites. — *J. Appl. Entomol.*, 105, 167-173.

Sileve, P. & Papierok, B. — 1991. Natural enemies of insect pests on cotton in Chad: preliminary data on entomophthoralean fungi. — *Coton et Fibres Tropicales*, 46, 304-308.

Sivcev, I. — 1992. Seasonal dynamics and density of the entomogenous fungi of the cabbage aphid (*Brevicoryne brassicae* L.). — *Zastita Bilja*, 43, 181-195.

Smith, M. T. & Hardie, D. D. — 1996. Influence of fungicides on development of an entomopathogenic fungus (Zygomycetes: Neozygitaceae) in the cotton aphid (Homoptera: Aphididae). — *Environ. Entomol.*, 25, 677-687.

Sopp, P. I., Gillespie, A. T. & Palmer, A. — 1989. Application of *Verticillium lecanii* for the control of *Aphis gossypii* by a low-volume electrostatic rotary atomiser and a high-volume hydraulic sprayer. — *Entomophaga*, 34, 417-428.

Sopp, P. I., Gillespie, A. T. & Palmer, A. — 1990. Comparison of ultra-low-volume electrostatic and high-volume hydraulic application of *Verticillium lecanii* for aphid control on chrysanthemums. — *Crop Protection*, 9, 177-184.

Sosnowska, D. — 1992. Adjuvants increasing the effectiveness of *Paecilomyces fumosoroseus* in the biological control of the aphid *Phorodon humuli*. — *Materialy Sesji Instytutu Ochrony Roslin*, 32, 223-226.

St Leger, R. J., Allée, L. L., May, B., Staples, R. C. & Roberts, D. W. — 1992. World-wide distribution of genetic variation among isolates of *Beauveria* spp. — *Mycol. Res.*, 96, 1007-1015.

Subrahamyam, P. Reddy, P. M. & McDonald, D. — 1990. Parasitism of rust, early and late leafspot pathogens of peanuts by *Verticillium lecanii*. — *Peanut Sci.*, 17, 1-3.

Vandenberg, J. — 1996. Standardized bioassay and screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against Russian wheat aphid (Homoptera: Aphididae). — *J. econ. Entomol.*, 89, 1418-1423.

Vega, F. E. & McGuire, M. R. — 1996. Formulation of the fungal entomopathogen *Paecilomyces fumosoroseus*. — *Soc. Invert. Pathol.*, Abstracts from 29th annual meeting, p. 86.

Verberne, F. & Zadoks, J. C. — 1984. Some epidemiological considerations on the utilization of entomopathogenic fungi (Entomophthorales). — *Gewasbescherming*, 15, 135-140.

Walters, F. S. & English, L. H. — 1995. Toxicity of *Bacillus thuringiensis* endotoxins toward the potato aphid in artificial diet bioassay. — *Entomol. Exp. Appl.*, 77, 211-216.

Wang, Z.G. & Knudsen, G. R. — 1993. Effect of *Beauveria bassiana* (Fungi: Hyphomycetes) on fecundity of the Russian wheat aphid (Homoptera: Aphididae). — *Environ. Entomol.*, 22, 874-878.

Wilding, N. — 1981. Pest control of Entomophthorales. In: Microbial Control of Insect Pests and Plant Diseases 1970-1980 (**H. D. Burges**, ed.). — *Academic Press*, London, pp. 539-554.

Wilding, N. — 1981. The effect of introducing aphid-pathogenic Entomophthoraceae into field populations of *Aphis fabae*. — *Ann. Appl. Biol.*, 99, 11-23.

Wilding, N., Latter, G. & Dedryver, C. A. — 1986. Evaluation of entomophthorales for aphid control: laboratory and field data. In: Fundamental and Applied Aspects of Insect Pathology (**R. A. Samson, J. M. Vlach & R. Peters**, eds), Foundation of the IVth Int. Coll. Invert. Pathol., Wageningen, The Netherlands, pp. 159-162.

Wilding, N., Mardell, S. K. & Brobyn, P. J. — 1986. Introducing *Erynia neoaphidis* into a field population of *Aphis fabae*: form of the inoculum and effect of irrigation. — *Ann. Appl. Biol.*, 108, 373-385.

Wilding, N., Mardell, S. K., Brobyn, P. J., Wratten, S. D. & Lomas, J. — 1990. The effect of introducing the aphid-pathogenic fungus *Erynia neoaphidis* into populations of cereal aphids. — *Ann. Appl. Biol.*, 117, 683-691.

Williamson, C., Von Wechmar, M. B. & Rybicki, E. P. — 1989. Further characterization of *Rhopalosiphon padi* picorna-like virus of aphids and comparison of isolates from South Africa and Illinois. — *J. Invertebr. Pathol.*, 53, 85-96.

Wraight, S. P., Poprawski W. L., Meyer, W. L. & Peairs, F. B. — 1993. Natural enemies of Russian wheat aphid (Homoptera: Aphididae) and associated cereal aphid species in spring-planted wheat and barley in Colorado. — *Environ. Entomol.*, 22, 1383-1391.

Zimmermann, G. — 1978. On biology, research methods and determination of Entomophthoraceae (Phycomycetes: Entomophthorales) infecting aphids. — *Z. Angew. Ent.*, 85, 241-252.