Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops

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Abstract   The spider mites *Tetranychus urticae* Koch and *Tetranychus evansi* Baker and Pritchard are important pests of horticultural crops. They are infected by entomopathogenic fungi naturally or experimentally. Fungal pathogens known to cause high infection in spider mite populations belong to the order Entomophthorales and include *Neozygites* spp. Studies are being carried out to develop some of these fungi as mycoacaricides, as stand-alone control measures in an inundative strategy to replace the synthetic acaricides currently in use or as a component of integrated mite management. Although emphasis has been put on inundative releases, entomopathogenic fungi can also be used in classical, conservation and augmentative biological control. Permanent establishment of an exotic agent in a new area of introduction may be possible in the case of spider mites. Conservation biological control can be achieved by identifying strategies to promote any natural enemies already present within crop ecosystems, based on a thorough understanding of their biology, ecology and behaviour. Further research should focus on development of efficient mass production systems, formulation, and delivery systems of fungal pathogens.

Keywords   Tetranychidae · Spider mites · *Tetranychus evansi* · *Tetranychus urticae* · Entomopathogenic fungi · Biological control · Integrated pest management · Horticulture

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, and the tomato red spider mite, *Tetranychus evansi* Baker and Pritchard, are among the most important pests of horticultural crops, such as tomato, cut flowers, French beans, eggplants and cucumber. Whereas *T. urticae* has been known as a worldwide pest of a wide range of horticultural crops both

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outdoors and in the greenhouses, the importance of *T. evansi* has dramatically increased during the last decade. *Tetranychus evansi* is probably of South American origin (Gutierrez and Etienne 1986) and invaded Africa in the late 1970s (Blair 1983; Meyer 1987; Knapp et al. 2003), Europe in the mid 1990s (Ferragut and Escudero 1999; Migeon 2005) and parts of Southeast Asia in 2004 (Ho et al. 2004). It is a major pest of tomatoes in eastern and southern Africa (Knapp et al. 2003; Saunyama and Knapp 2003). If left uncontrolled under hot and dry conditions, *T. evansi* can destroy tomato plants within 3–5 weeks (Qureshi et al. 1969), and the farmer can lose his production within a week's time. The economic importance of *T. urticae* and its control were extensively reviewed by Helle and Sabelis (1985a, b). Biological control of *T. urticae* with phytoseiid predatory mites has been successful mainly in protected environments but also in open fields in many parts of the world (e.g., Gerson et al. 2003; Zhang 2003). Farmers in Africa largely rely on expensive synthetic pesticides to control *T. evansi*, but pesticide applications are frequently ineffective (Sibanda et al. 2000; Saunyama and Knapp 2003). Although no effective biological control strategy has yet been developed, a Brazilian strain of the predatory mite *Phytoseiulus longipes* Evans and the pathogenic fungus *Neozygites floridana* Weiser and Muma have recently shown promising results in laboratory experiments (Furtado et al. 2007; Wekesa et al. 2007). Detailed information on the distribution of both mite species, including maps and many relevant references, are in Migeon and Dorkeld (2006). Alternatives to chemical control need to be developed because spider mites can rapidly develop resistance to acaricides, and due to the growing concern about environmental and health risks associated with pesticide use.

Research and development of biological control options for spider mites has largely concentrated on the conservation of natural enemies and releases of predatory mites (Nyrop et al. 1998; Gerson et al. 2003; Zhang 2003). However, this is often not sufficient and supplementary sprays of acaricides are needed. Entomopathogenic fungi may play a major role in the natural regulation of spider mite populations and could be used in biological control programme, either as a stand-alone solution in replacement of synthetic acaricides that are currently in use, or as a component of integrated mite management. The diseases caused by entomopathogenic fungi in mites and spider mites were reviewed by van der Geest (1985) and by van der Geest et al. (2000). Chandler et al. (2000) reviewed opportunities of exploiting fungal pathogens for biological control of Acari, including mites. In this paper we review the use of fungal pathogens in the inundative, conservation and classical biological control of *T. urticae* and *T. evansi*.

Entomopathogenic fungi associated with *Tetranychus evansi* and *Tetranychus urticae*

Natural incidence

Entomopathogenic fungi can play an important role in the regulation of arthropod pest populations. Many reports have been published on natural incidence of entomopathogenic fungi on tetranychid mites, including *T. evansi* and *T. urticae,* and were reviewed by van der Geest (1985); Chandler et al. (2000) and by van der Geest et al. (2000) (Table 1). The fungi known to cause high infections in *T. evansi* and *T. urticae* populations belong to the order Entomophthorales and include *Neozygites* spp., the mitosporic fungi *Hirsutella thompsonii* Fisher and members of the *Lecanicillium* (=*Verticillium*) *lecanii* complex.

Identification of mite pathogenic species of *Neozygites* is still unresolved. Four species of *Neozygites* were described from spider mites: *N. floridana*, *N. tetranychi*, *N. adjarica* and more recently *N. tanajoae*. Both *N. tetranychi* and *N. adjarica* are only known from a

Table 1 Entomopathogenic fungi reported naturally on *Tetranychus evansi* and *Tetranychus urticae*

Fungal species	Host	Reference
Zygomycota		
Basidiobolus sp.	*T. urticae*	Jegina (1976) (cited by van der Geest 1985)
Conidiobolium obscurus	*T. urticae*	Jegina and Cinowski (1970) (in van der Geest 1985), Andreeva and Shternshis (1995) (cited by Chandler et al. 200
Entomophthora sp.	*T. urticae*	Kenneth et al. (1971) (cited by van der Geest 1985)
Neozygites floridana	*T. urticae*	Weiser and Muma (1966), Kenneth et al. 1972 (cited by van der Geest 1985), Smitley et al. (1986), Keller (1991), Mietkiewski et al. (1993), Shih and Shiue (1994), Dick and Buschman (1995) (cited by Chandler et al. 2000), Smith and Furr (1975) (cited by van der Geest et al. 2000)
	T. evansi	Fiaboe (2007)
Neozygites sp. near *floridana*	*T. urticae*	Ramaseshiah (1971) (cited by van der Geest 1985)
Neozygites tetranychi	*T. urticae*	Weiser (1968) (cited by van der Geest 1985)
Neozygites sp.	*T. urticae*	Cincadze et al. (1976) (cited by van der Geest 1985), Carner and Canerday (1968), Carner (1976), Brandenburg and Kennedy (1981), Klubertanz et al. (1991) (cited by Chandler et al. 2000)
	T. evansi	Humber et al. (1981)
Ascomycota		
Beauveria bassiana	*T. urticae*	Wright and Kennedy (1996) (cited by Chandler et al. 2000)
Lecanicillium (= *Verticiliium*) *lecanii*	*T. urticae*	Gams (1971) (cited by van der Geest 1985), Gillespie et al. (1982), Andreeva and Shternshis (1995) (cited by Chandler et al. 2000), Chandler et al. (2005).
Metarhizium anisopliae	*T. urticae*	D.M. Bugeme (unpubl. data)

single collection. A comparison of these four species indicates a considerable overlap of taxonomic characteristics, such as spore sizes. *Neozygites adjarica* is considered a synonym of *N. floridana* (Keller 1991; Balazy 1993). *Neozygites tetranychi* also might be *N. floridana* (Balazy 1993). In the past, *N. floridana* was called *Triplosporium floridana* (Kenneth et al. 1972), *Triplosporium* sp. (Humber et al. 1981), and *Entomophthora floridana* (Nemoto et al. 1975). More recently, authors have referred to the pathogens associated with spider mites as *N. floridana* (Keller, 1997; Kennedy and Smitley 1988; Mietkiewski et al. 2000). In this paper, we will consider all *Neozygites* species pathogenic to *T. urticae* and *T. evansi* as *N. floridana,* although we think that more studies are needed to clarify the taxonomy of this species complex.

Susceptibility of *Tetranychus urticae* and *Tetranychus evansi* to fungal infections under laboratory conditions

Fungi that are not associated with arthropod hosts in nature can be tested for their pathogenic activity against different target species in the laboratory (Hall and Papierok 1982). Experimental infections, induced under controlled conditions, allow testing of the pathogenic activity of different fungal isolates with the aim of developing them as biological control agents or biopesticides. The fungal pathogens that have been tested against *T. evansi* and *T. urticae* in the laboratory are listed in Table 2. The report in this section focuses on recently published articles published after the reviews by Chandler et al. (2000) and van der Geest et al. (2000).

Tamai et al. (2002a) screened 45 isolates of mitosporic fungi against *T. urticae*, including 32 isolates of *Beauveria bassiana* (Balsamo) Vuillemin, 10 isolates of *Metarhizium*

Table 2 Entomopathogenic fungi tested against *Tetranychus evansi* and *Tetranychus urticae* in the laboratory

Fungal species	Host	Reference
Basidiobolus sp.	*T. urticae*	Jegina (1976) (cited by van der Geest 1985)
Beauveria bassiana	*T. urticae*	Tamai et al. (1999) (cited by Irigaray et al. 2003), Alves et al. (2002), Irigaray et al. (2003), Bugeme et al. (unpubl. data)
	T. evansi	Wekesa et al. (2005), Bugeme et al. (unpubl. data)
Hirsutella thompsonii	*T. urticae*	Gerson et al. (1979), Gardner et al. (1982) (cited by Chandler et al. 2000), Chandler et al. (2005)
Lecanicillium lecanii	*T. urticae*	Gillespie et al. (1982), Chandler et al. (2005)
L. muscarium	*T. urticae*	Mineiro et al. (2004)
Metarhizium anisopliae	*T. urticae*	Chandler et al. (2005), Bugeme et al. (unpubl. data)
	T. evansi	Wekesa et al. (2005), Bugeme et al. (unpubl. data)
Neozygites floridana	*T. urticae*	Smitley et al. (1986)
Meira geulakonigii	*T. urticae*	Paz et al. (2007)
Meira argovae	*T. urticae*	Paz et al. (2007)
Acaromyces ingoldii	*T. urticae*	Paz et al. (2007)

anisopliae (Metchnikoff), one each of *Aschersonia aleyrodis* Webber, *Hirsutella* sp. and *Isaria farinosa* (= *Paecilomyces farinosus*) (Holmsk.) Fr. Among these isolates, eight *B. bassiana* and four *M. anisopliae* isolates caused >80% and 90% mortality, respectively, at concentration of 5×10^7 conidia ml^{-1}, 5 days postinfection. Applied at concentration of 1.7×10^7 conidia ml^{-1}, *Hirsutella* sp. caused 73% mite mortality. *Aschersonia aleyrodis* and *I. farinosa* were not pathogenic to the two-spotted spider mite. The authors concluded that *M. anisopliae*, *B. bassiana* and *Hirsutella* sp. were the most promising fungi to be formulated as mycoacaricides for *T. urticae* control. The authors also noted that conidia, blastospores and yeast-like cells of five isolates of *B. bassiana* were pathogenic against the same mite. Lethal concentration to 50% mortality (LC$_{50}$) values ranged from 4.95 to 82.1×10^6 cells ml^{-1}.

Irigaray et al. (2003) evaluated the efficacy of Naturalis-L® (a *B. bassiana*-based commercial biopesticide) against the two-spotted spider mite and obtained lethal concentration values (LC$_{50}$) of 3184 viable conidia ml^{-1} for the juvenile stages and 1949 viable conidia ml^{-1} for the adults. Naturalis-L® caused significant egg mortality compared to the control, with no significant differences amongst egg age classes (24-, 48-, 72-, and 96-h-old eggs) at the tested concentrations of 1,400–22,800 viable conidia ml^{-1}. In another study, Simova and Draganova (2003) evaluated the virulence of four isolates of *B. bassiana*, one isolate each of *M. anisopliae*, *I. farinosa* and of *L. lecanii*, to *T. urticae* at conidial concentrations of 2×10^9 conidia ml^{-1}. Six out of the seven isolates tested were virulent to *T. urticae* and one isolate of *B. bassiana* outperformed the others with lethal time to 50% mortality (LT$_{50}$) values of between 1.3 and 1.4 days. Except for *I. farinosa,* which was less virulent, the other isolates were equally virulent to *T. urticae*. Chandler et al. (2005) tested 40 isolates of anamorphic entomopathogenic fungi from six genera against *T. urticae,* along with three commercial fungus-based products: Naturalis-L®, Mycar® (*H. thompsonii*) and Mycotal® (*Lecanicillium (=Verticillium) muscarium*). Three isolates, *M. anisopliae*, *Hirsutella* spp. and *L. muscarium*, and the three commercial isolates, were pathogenic to *T. urticae*. Koike et al. (2005) also demonstrated that four isolates of *L. lecanii* (Vertalec, Mycotal, A-2, B-2) were pathogenic against *T. urticae*, but the levels of virulence differed among the isolates. Alves et al. (2002) compared the virulence of yeast-like cells and conidia of *B. bassiana* against *T. urticae* and found that their virulence was similar, causing

mortalities of 42.8–45.0% at a concentration of 10^7 cells ml^{-1}, and of 74.4–77.8% at 10^8 cells ml^{-1}.

Recently, new taxa of fungi were described as Exobasidiomycetidae of the class Ustilaginomycetes (Basidiomycota) (Boekhout et al. 2003), that include *Meira geulakonigii* gen. nov., sp. nov.*, Meira argovae* sp. nov. and *Acaromyces ingoldii* gen. nov., sp. nov. Their pathogenicity was evaluated in the laboratory against herbivorous mites including *T. urticae* (Paz et al. 2007). With the exception to *M. argovae* which was not virulent, *M. geulakonigii* and *A. ingoldii* caused mortalities of 81.9–90.0% to *T. urticae,* 7 and 14 days post-application, respectively. Some isolates of *H. thompsonii* produce metabolites in broth cultures and exudates over the surface of solid cultures (Cabrera and Lopez 1977; Samson et al. 1980). These metabolites have been reported to be toxic to mites (Omoto and McCoy 1998). Rosas-Acevedo et al. (2003) recently tested the effect of an exudate of *H. thompsonii* Mexican strain HtM120I on *T. urticae* egg production and obtained 100% reduction in mite fecundity after topical application of the metabolite over the initial 6 days of the experiment. They also observed that depending on the exudate dosage, mites partially recovered within 3 and 6 days post-treatment, but produced fewer eggs. Further studies are needed to identify metabolites and quantify exudate concentration.

Wekesa et al. (2005) evaluated 17 isolates of *M. anisopliae* and two isolates of *B. bassiana* against *T. evansi.* All isolates were pathogenic to adult female mites, causing 22.1–82.6% mortalities. Isolates causing more than 70% mortality were subjected to dose-response bioassays. The LC$_{50}$ values ranged between 0.7×10^7 and 2.5×10^7 conidia ml^{-1} and the LT$_{50}$ values of the most active isolates of *B. bassiana* and *M. anisopliae* strains varied between 4.6 and 5.8 days. Adults were more susceptible to *B. bassiana* and *M. anisopliae* infections than immatures. Both isolates also caused egg mortality of >80% at a concentration of 1.0×10^8 conidia ml^{-1}. When deutonymphs were treated with a sub-lethal concentration of 1.0×10^5 conidia ml^{-1} of either isolate, the adults that developed from these deutonymphs laid significantly fewer eggs than untreated mites (Wekesa et al. 2006).

Field and glasshouse assessment of fungi for mite management

Relatively few field trials have been undertaken to evaluate entomopathogenic fungi against *T. urticae* and, even to a lesser extent, *T. evansi.* Dresner (1949) treated *T. urticae* with a dust formulation of conidia containing 0.5% spores of *B. bassiana* in the field and obtained 71% mortality. In a semi-field experiment using *B. bassiana* against *T. urticae* infesting chrysanthemum (*Dendranthema grandiflora*) (Ramat), Alves et al. (1998) obtained results that were better than the chemical pesticide used. Tamai et al. (2002a) reported similar results with *B. bassiana* against *T. urticae* on chrysanthemum when the fungus was applied at a concentration of 2×10^8 conidia ml^{-1}. They also observed that with four fungal sprays within 14 days, mite density was reduced from 1.8 to 0.1 mites/leaf. However, the reduction was lower in strawberry (*Fragaria* sp.) than in chrysanthemum, with a mean density of 13 mites/leaflet 21 days after application of 1×10^8 or 5×10^7 conidia ml^{-1}, compared to 43 mites/leaflet in control plots. The authors also observed an effect of strawberry varieties on the pathogen performance, with the varieties 'Campinas' and 'Princesa Isabel' having the lowest mite densities. Tamai et al. (2002a) concluded that *M. anisopliae*, *B. bassiana* and *Hirsutella* sp. were the promising fungi to be formulated as mycoacaricides for *T. urticae* control.

Chandler et al. (2005) obtained reductions of *T. urticae* populations in a glasshouse following spray applications of *B. bassiana*, *H. thompsoni*, *M. anisopliae*, *L. lecanii* and Naturalis-L®. Naturalis-L® reduced *T. urticae* numbers by up to 97%. In another glasshouse

experiment, single sprays of Naturalis-L® resulted in 98% reductions of adults, nymphs and eggs of *T. urticae* (Chandler et al. 2005). However, attempts to use an entomopathogenic fungus to control *T. urticae* in greenhouses by Andreeva and Shternshis (1995) (cited by Irigaray et al. 2003) were unsuccessful.

Wekesa et al. (2005) treated potted tomato plants artificially infested with *T. evansi* with oil and aqueous formulations of *B. bassiana* and *M. anisopliae,* and obtained reductions of the mite population in comparison with the untreated controls at 7 and 14 days post-treatment. Conidia formulated in oil outperformed the ones formulated in water. For instance, the number of mites/leaf on middle leaves was 24.2 and 5.3 in the aqueous formulations of *M. anisopliae* and *B. bassiana*, respectively, compared to 2.3 and 0.3 mites/leaf in oil formulations of *M. anisopliae* and *B. bassiana*, respectively, 14 days post-treatment.

Strategic options in the use of entomopathogenic fungi for spider mite control

Research efforts intended to develop entomopathogenic fungi as mycoinsecticides in general, and acaricides in particular, have markedly increased in recent years (Faria and Wraight 2007). Although entomopathogenic fungi can be used in classical, conservation and augmentative biological control, emphasis has been placed on their development as inundative augmentative control agents (Goettel and Hajek 2001).

Classical biological control

Classical biological control aims at the permanent establishment of an exotic agent in a new area. Pathogens used for classical biological control are extremely host specific and have great potential to persist in the environment and cause epizootics. While examples of the use of parasitoids and predators in classical biological control abound in literature, there are only few reported examples about entomopathogenic fungi (Hajek et al. 2007). Nineteen species of entomopathogenic fungi have been used in 57 classical biological control programs, but only three mite species were targets for the classical approach. *Hirsutella thompsonii* Fisher var. *synnematosa* Samson, McCoy & O'Donnell was introduced from Zimbabwe into Argentina against *Eriophyes sheldoni* (Ewing) and *Phyllocoptruta oleivora* (Ashmead) in 1985. At the same time, *H. thompsonii* Fisher var. *vinacea* Samson, McCoy & O'Donnell from North Carolina was also released in Argentina. Infection after release was high but persistence is unknown and the project was discontinued (Hajek et al. 2005).

Neozygites tanajoae Delalibera, Hajek and Humber was introduced into Benin against the cassava green mite, *Mononychellus tanajoa* Bondar, in 1998–2000 (Yaninek and Hanna 2003), where it is established (Hountondji et al. 2002b). Indeed, preliminary surveys conducted in the semi-arid region of North-eastern Brazil have identified *N. floridana* as a pathogen of *T. evansi* (Furtado et al. 2007). This pathogen has previously been reported from *T. evansi* over 25 years ago (Humber et al. 1981) and plans are underway to import this fungus to Africa as a possible agent for the classical biological control of *T. evansi* (V. Wekesa and M. Knapp, unpublished).

Inundative augmentative biological control

Use of fungi as biopesticides is considered an attractive strategy in inundation biological control, not only in the control of mites but also for the control of several agricultural pests, because the effect on the targets with this strategy is relatively fast. From a commercial

point of view, this strategy is similar to a 'chemical approach' where the fungal inoculum is applied directly to the crop or the target pest and control is achieved exclusively by the released propagules themselves (Eilenberg et al. 2001). Entomopathogenic fungi in the Ascomycota, order Hypocreales, are well suited for this inundative strategy based on their relatively wide host range, ease of production, formulation and application (Wraight et al. 2001). A major disadvantage of using fungi in this strategy is the dependence of most species on high relative humidity and success may only be guaranteed therefore where optimum humidity conditions are met. However, the fact that dry and hot conditions normally favour development of spider mites may compromise control efficiency, but this could be overcome by high relative humidity during night, favouring fungal sporulation and germination. Moreover, since epizootic development is density dependent and high mite density is common on crops, this makes fungi good candidates for spider mite control.

Because of the high strain variability and wide host range of Hypocreales, most members of this group have the potential to be developed as mycoinsecticides and mycoacaricides. However, they require many steps in their development as mycoinsecticides, including isolation, identification, strain selection, mass production and formulation, field trial, registration and commercialization (Zimmerman 1986). Strain selection is considered an essential starting point in their successful development (Soper and Ward 1981). Consequently, many isolates of entomopathogenic fungi have been screened against *T. urticae* and *T. evansi* and hold potential for their development as mycoacaricides (see Table 2). A recent review (Faria and de Wraight 2007) provides a table showing the list of the mycoacaricides, of which 17 formulations were developed to control mites of the family Tetranychidae.

Entomophthoralean fungi, on the other hand, possess very few characteristics that can fit them into the inundation biological control paradigm. A strong positive attribute of this group is their general high virulence, an attribute that makes them desirable for the inundation strategy. However, the major drawback of this group is that their infective stages are rather short-lived, making their development and use far more difficult. Another shortcoming is the difficulty associated with mass production. Recent attempts suggest that entomophthoralean fungi have high prospects in inundative strategy under greenhouse conditions (Shah et al. 2000). Production of high value crops, including several horticultural crops, is increasingly been undertaken in greenhouses, and the use of entomophthoralean fungi might become more attractive under these conditions, because environmental conditions that normally favour the efficacy of these fungi can be easily manipulated. The use of entomophthoralean fungi under greenhouse conditions has another advantage over alternative control agents in that efficient horizontal transmission, which relies on availability of susceptible hosts, may be increased and repetitive application may be unnecessary. However, reliance on horizontal transmission implies that these fungi are dependent on host population density for survival and dispersal, which means that their efficacy may be compromised at low host densities (Fuxa 1987). Intrinsic differences in mite susceptibility to *N. tanajo*ae and *N. floridana* is associated with the mite life stages, size and behaviour, as well as age (Elliot et al. 2002). Host death caused by these fungi normally occurs at night, when relative humidity is high, favorable for sporulation (Hajek and St Leger 1994). Sporulated conidia germinate to form infective capilliconidia (Fig. 1) that infest arthropod hosts during the day, when their activities are accelerated following increased daytime temperatures.

Another limitation in the use of fungal pathogens to control spider mites is the lack of appropriate formulation and application strategies for the target host. While progress has been made in the formulation of Hyphomycetes fungi, whose aerial spores can be produced
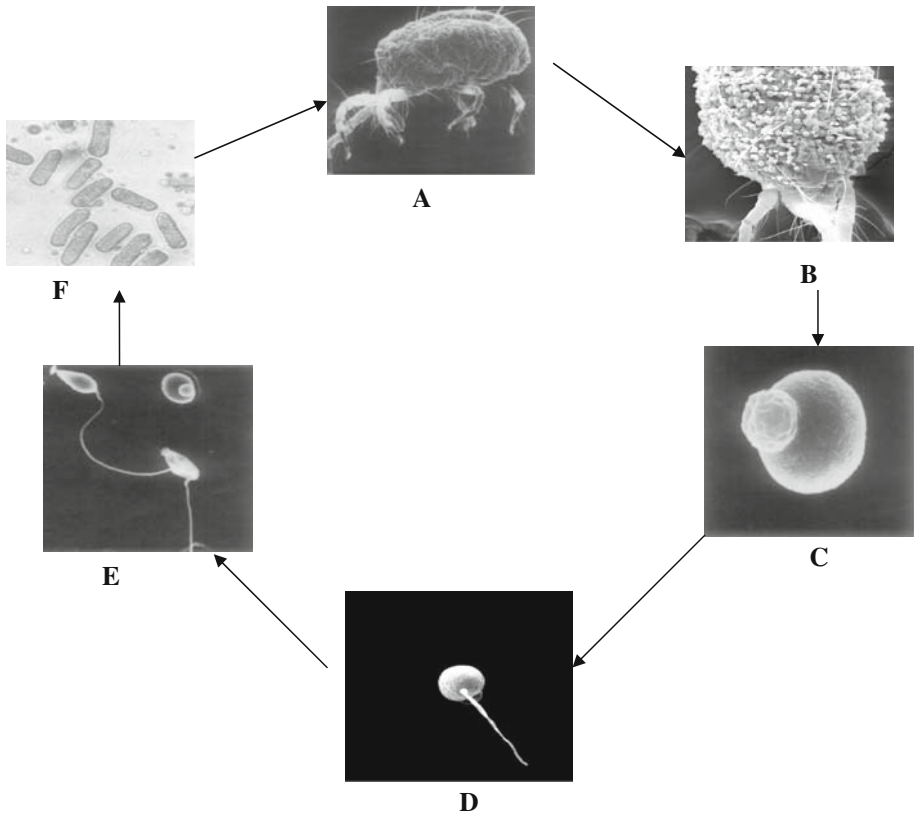
Fig. 1 Schematic diagram of the life cycle of *Neozygites floridana* infesting the tomato spider mite, *Tetranychus evansi*: mummified mite (**a**), sporulated mummy (**b**), primary conidium (**c**), germinating primary conidium (**d**), capilliconidium (**e**), and hyphal bodies (**f**). Courtesy of V.W. Wekesa

easily on common media, little progress has been made with the Entomophthorales whose members include major pathogens of spider mites. Formulation of fungal pathogens can extend shelf life, facilitate handling and application, aid in persistence due to protection from harmful environmental factors and enhance efficacy by increasing contact with the target pests (Jones and Burges 1998). The use of oil formulations in ultra low volume (ULV) spraying has recently helped to overcome many problems on the use of *M. anisopliae* to control locusts in Africa (Bateman 1997). Application of *M. anisopliae* and *B. bassiana* in oil emulsion has been tested against *T. evansi* with very promising results (Wekesa et al. 2005).

The fragility of the hyphal bodies and protoplasts from members of the Entomophthorales has made formulation difficult. Dried mycelia of *Zoophthora radicans* (Brefeld) Batko were formulated with sugar coating as a method for their long-term storage (McCabe and Soper 1985) and algination of mycelia of *Erynia neoaphidis* Remaudière & Hennebert has been demonstrated as a promising method for formulating conidia (Shah et al. 1998). Sugar coating of dry mycelia and algination of the hyphal matrix was facilitated by the in vitro culturing of the fungal species.

Conservation biological control

Conservation biological control involves "modification of the environment or existing practices to protect and enhance natural enemies … to reduce the effect of pests" (Eilenberg et al. 2001; Fuxa 1998). It does not rely on the addition of natural enemies but rather on identifying strategies to promote those natural enemies already present within crop ecosystems, based on a thorough understanding of their biology, ecology and behaviour (Gurr et al. 1998; Landis and Menalled 1998). Despite the important role played by Entomophthorales in the natural regulation of arthropod pests, little consideration has been given to understanding their ecology and function in crop ecosystems (Pell 2007). Because of their ability to persist in the target pest populations, entomophthoralean fungi may fit well in these cropping systems. Research in the UK is evaluating the potential use of arable field margins as habitat refugia in order to encourage early season multiplication of natural enemies, including entomophthoralean fungi, for dispersal into field crop to suppress pest aphids (Pell et al. 2001; Ekesi et al. 2005). The success of this approach largely depends on the presence of a succession of different pest and non-pest insects feeding on non-crop plants in the field boundaries that provide sufficient host densities for continuous infection transmission and dispersal of inoculum into the crop. Future studies should consider these practices and their influence on the biological control of *T. evansi* and *T. urticae*.

Entomopathogenic fungi as a component of spider mite IPM

Although effective in the management of many arthropod pests, the use of entomopathogenic fungi will not supersede the use of synthetic pesticides in all commercial production systems, but in many instances may be applied in conjunction with pesticides in integrated pest management (IPM) programmes. Regardless of whether an entomopathogenic fungus is to be classically introduced, conserved or augmented in an environment as part of an IPM programme, it is crucial to know how it might be affected by the synthetic pesticides commonly used in that environment, in order to determine whether the pesticide application needs to be momentarily or spatially separated from the most susceptible life stages of the fungal pathogen (Pell et al. 2001). It is therefore essential to be aware of the adverse effects that chemical pesticides may have on the efficacy of fungal biological control agents, or the adverse effects the entomopathogenic fungi can have on other natural enemies, especially on predatory mites.

Since the interactions between different pests and disease control methods constitute a key factor in IPM strategies, we shall concentrate in this section on the interactions between entomopathogenic fungi and pesticides, and on the interactions between entomopathogenic fungi and other natural enemies that are used in the same agro-ecosystems while controlling *T. urticae* and *T. evansi*.

Interactions between entomopathogenic fungi and pesticides

Several studies showed negative or positive interactions between entomopathogenic fungi and pesticides used in the same environment for controlling mite populations. While studying the effects of four concentrations of the insecticide imidacloprid (50, 100, 200 and 500 ppm) on two spider mite pathogens, *N. tanajoae* and *H. thompsonii,* Dara and Hountondji (2001) found that the insecticide significantly reduced the germination of primary conidia and the formation of infective capilliconidia in *N. tanajoae,* thus significantly reducing its

infectivity on mites. In contrast, the same insecticide, at a concentration of 100 ppm and above, increased conidial germination in *H. thompsonii*. However, no synergism between imidacloprid and *H. thompsonii* was detected on the mortality of the cassava green mite.

Suppression and reduction of the infection level of *N. floridana* in *T. urticae* populations by the fungicide benomyl (a benzimidazole) have been reported in bean and corn fields (Brandenburg and Kennedy 1982, 1983). Other fungicides, such as chlorothalonil, mancozeb and maneb also reduced the infection level of *N. floridana* in *T. urticae* populations infesting corn and peanut fields (Brandenburg and Kennedy 1982; Boykin et al. 1984; Smitley et al. 1986). Klingen and Westrum (2007) compared the effect of different pesticides (fungicides, insecticides, acaricides and molluscicides) used in strawberry plantation on *N. floridana.* Although their negative effects varied with fungicide, all fungicides tested (tolylfluanid, fenhexamid, cyprodinil + fludioxonil) were harmful to *N. floridana* and could potentially reduce its survival and efficacy, while the acaricide/insecticide/molluscicide, methiocarb, appeared to have a stimulating effect on the fungus.

Apart from *N. floridana* and *H. thompsonii*, the two major mite-pathogenic fungi, the entomopathogenic *B. bassiana, M. anisopliae* and members of the *L. lecanii* complex are also potential biological control agents of tetranychid mites. Tamai et al. (2002b) studied the toxicity of 93 products (three stickers, 36 fungicides and 54 insecticides/acaricides), normally used to control insects and diseases, to the fungus *B. bassiana*, and found much variability in the toxicity of different classes of products. All stickers were very toxic to the fungus, whereas only three out of 36 fungicides (propamocarb hydrochloride, sulphur and kasugamycin) and 24 out of 54 insecticides/acaricides (including those with the following active ingredients: abamectin, acephate, acetamiprid, betacyfluthrin, bifenthrin, ciromazine, deltamethrin, diafenthiuron, diflubenzuron, dimethoate, fenpropathrin, fenpyroximate, fenvalerate, imidacloprid, metamidophos, propargite, and tebufenozide etriclorfon) were compatible with *B. bassiana*. Wenzel et al. (2004) evaluated the compatibility of the insecticides Provado (imidachloprid) and Trigard700PM (cyromazine) to the entomopathogenic fungus *L. lecanii,* in terms of vegetative growth, sporulation, conidial viability and pathogenicity against *T. urticae*. All the insecticides tested were compatible with *L. lecanii* in all evaluated parameters and mite mortality was above 68% in all treatments. Compatibility of *B. bassiana* with triflumuron was investigated for the control of *T. urticae* by Irigaray et al. (2003). Combination of *B. bassiana* with 0.25 g Alsystin (25% triflumuron as a wettable powder) l^{-1} resulted in a significant decrease of *T. urticae* egg mortality. Triflumuron reduced mycelial growth but not conidial germination of *B. bassiana*. They concluded that *B. bassiana* is a possible candidate to be included in IPM programs of *T. urticae* with triflumuron. Shi et al. (2005) investigated the effects of 10 acaricides on *B. bassiana* in the laboratory. At field concentration rates, dicofol, chlorpyrifos, abamectin, liuyangmycin, and azocyclotin significantly reduced the germination rate of *B. bassiana* conidia, whereas pyridaben, propargite, hexythiazox, amitraz and matrine had no effect. Combinations of *B. bassiana* with pyridaben, the acaricide with the least effect on conidia germination, significantly increased the mortality of *Tetranychus cinnabarinus* (Boisduval) eggs compared to *B. bassiana* alone. According to Inglis et al. (2001), pesticides that are inhibitory in the laboratory do not always exhibit the same action in field conditions. This may be due to the concentration of the pesticide used in the field or to applying the pesticide in a manner that minimizes contact with the fungus.

Interactions between entomopathogenic fungi and other natural enemies

Pathogens may contribute to the suppression of spider mite populations in combination with other arthropod natural enemies. However, because natural enemies of spider mites have

evolved and function in a multitrophic context, it is important to assess interactions within complexes of natural enemies if they are to be exploited effectively in pest management (Ferguson and Stiling 1996; Roy and Pell 2000). Fungal natural enemies can interact either synergistically/additively (e.g., enhanced transmission and dispersal of spider mite pathogens) or antagonistically (e.g., parasitism/infection, predation and competition) (Ferguson and Stiling 1996; Roy and Pell 2000).

Only a few reports are available on the interactions between entomopathogenic fungi and other spider mite natural enemies. Ludwig and Oetting (2001) studied the susceptibility of *Phytoseiulus persimilis* Athias-Henriot and *Iphiseius degenerans* (Berlese) to *B. bassiana* (strain JW-1). The natural enemies were highly susceptible to infection by *B. bassiana* under laboratory conditions, whereas lower infection rates were observed in greenhouse trials. Ludwig and Oetting (2001) also evaluated the susceptibility of *I. degenerans* to the entomopathogenic fungi *B. bassiana* (strain GHA), *L. lecanii* and *M. anisopliae* in a greenhouse and observed that the predatory mite was least susceptible to *M. anisopliae*, followed by *L. lecanii* and *B. bassiana*. Studying the effect of *B. bassiana* on the predatory mite *Neoseiulus cucumeris* Oudemans, Jacobson et al. (2001) found that *B. bassiana* had no detrimental effect on the mite when sprayed onto excised cucumber leaves in a laboratory bioassay, or when sprayed onto glasshouse-grown cucumbers. They suggested that a mycopesticide based on *B. bassiana* could be used as a second line of defence to support preventative pest management with *N. cucumeris*. Chandler et al. (2005) evaluated the efficacy of Naturalis-L® as a supplementary treatment to *P. persimilis* for the control of *T. urticae* populations on tomato in a glasshouse. Application of *P. persimilis* on its own did not reduce numbers of *T. urticae* adults, nymphs or eggs. In contrast, application of *P. persimilis* + Naturalis-L® reduced numbers of *T. urticae* adults, nymphs and eggs compared with all other treatments. Fewer *P. persimilis* were recorded from the *P. persimilis* + Naturalis-L® treatment than from the *P. persimilis* treatment alone. Since it was not clear if this was caused by Naturalis-L® directly killing *P. persimilis,* or by lack of prey causing the predatory mites to migrate, the authors suggested that further work on the mode of action of Naturalis-L® and its compatibility with *P. persimilis* be conducted.

Recent studies on the effect of *N. floridana* on *T. evansi* and its predator *P. longipes* showed that, despite being able to attach to the body of *P. longipes*, *N. floridana* is unable to infect it (Wekesa et al. 2007). Although *N. floridana* and *N. tanajoae* have been reported to be non-pathogenic to some phytoseiids and several non-target insects (Moraes and Delalibera 1992; Hountondji et al. 2002a; Wekesa et al. 2007), Furtado et al. (1996) reported that the fungus *Neozygites acaricida* (Petch) is pathogenic to the phytoseiid mite *Euseius citrifolius* Denmark and Muma.

Conclusions and prospects for future development

Compared to other biological control agents (e.g., predators, parasitoids, *Bacillus thuringiensis*), the development of entomopathogenic fungi for inoculative or inundative and classical biological control programs is still far from satisfactory. However, entomopathogenic fungi have a considerable potential to become major components of sustainable IPM, provided there is continued investment in research, technology transfer and education (Shah and Pell 2003). There is a great potential for their use in conservation and classical biological control programs, as public pressure is growing to adopt sustainable agricultural practices, reduce synthetic pesticides and protect the environment. Successful use of entomopathogenic fungi as microbial control agents of mites will ultimately depend on

how well the strains are selected (virulence, persistence), on large scale production, formulation, compatibility with other control agents and on better systems of delivery, including timing of applications.

More research is required to make in vitro production of entomophthoralean fungi (hyphal bodies, conidia or resting spores) possible, together with development of appropriate formulations for better delivery to target spider mites. Some entomophthoralean fungi produce resting spores in submerged culture and these spores can be harvested, formulated and applied in the field for control of pests in inoculative releases (Kogan and Hajek 2000). The potential of conidia as the basis of a commercial product is limited by their rapid environmental desiccation. In contrast, resting spores are long-term survival structures that are thick-walled and robust, long-lived and environmentally stable. Resting spores, therefore, have potential as alternative commercial inocula for use in augmentation (inoculative and mycoacaricide use) approach. No studies have been undertaken with the mite-specific Entomophthorales to apply their spores in the aforesaid manner for biological control. For this group of fungi, resting spores seem to be the best stage that can be easily manipulated and attempts at their mass production should primarily be of this stage. Future studies should investigate methods for the induction of resting spore formation in *N. floridana,* either through nutritional or physical stress, followed by other tests that can enable the use of this fungus as a mycoacaricide.

Difficulties associated with the establishment of in vitro cultures of these pathogens are likely to be circumvented through selection and development of new inexpensive cell culture media. The use of genetic manipulation to overcome other limitations is promising. For instance, recombinant DNA technology can be used to expand the host range of the promising host-specific strains so that they can be used to target multiple pest mite species, as has been done with other fungal pathogens (St. Leger 2001). Bioprospecting for the discovery of fungal isolates with new traits should also be considered. For instance, surveys for more strains with varied virulence from different geographic regions and hosts will increase the possibility of obtaining a wide range of strains for use in different agroecoystems.

For the development of mycoacaricides based on entomopathogenic fungi in the Ascomycota, order Hypocreales, screening for more efficient strains is still necessary. Efficient mass production methods and formulations also need to be developed. A major problem for open field applications is the requirement of high ambient humidities for successful infection. The commercial products Vertalec and Mycotal, based on the *L. lecanii* complex and used in the control of aphids, thrips and whiteflies, are exclusively used in greenhouses where humidity can be modified to favour infection (Milner 1997). However, recent advances in formulation technology have resulted in an adjuvant that enhances the activity of Mycotal at low humidities (Shah and Pell 2003).

Entomopathogenic fungi can play an important role in IPM if used in conjunction with other strategies for sustainable pest control (Shah and Pell 2003). To achieve this, the compatibility of the mycoacaricides with other mite biocontrol agents, especially phytoseiid mites, as well as with synthetic pesticides, needs to be investigated.

The further development of entomopathogenic fungi as control agents for spider mites needs considerable investment in multidisciplinary research by the public and private sectors. When commercial interests are absent, as in the development of classical biological control and conservation strategies, especially in developing countries, long-term government support is essential.

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