

BIOLOGICAL CONTROL IN TERMITE MANAGEMENT: THE POTENTIAL OF NEMATODES AND FUNGAL PATHOGENS

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Abstract A brief overview on the options for biological control of termites is presented. Many organisms have been identified as being able to kill termites. However, we do not know their real impact on field populations of termites. Research has focused on some entomopathogenic nematodes and the fungi *Beauveria bassiana* and *Metarhizium anisopliae*. Only a limited number of field studies have been conducted using both groups of organisms as control agents for termites. Work with *M. anisopliae*, notably from Australia, is discussed in more detail in this paper. Strains selected for field trials have to: be virulent; be able to tolerate temperatures above 30° C; pose no health threats to humans and higher animals; be easily mass produced; and have long-lived spores that are robust enough for easy formulation and storage. Spores from virulent isolates of *M. anisopliae* are repellent to termites and behavioural defence mechanisms by termites can limit the effectiveness of conidia applications. A number of options are available to formulate the spore product thus rendering it less repellent. Applications of conidia as inundative treatments to termite sites, or within an attractive bait matrix, are options for termite control with *M. anisopliae*. Microbial pathogens will solve certain termite problems but may not help with others. However, they have their place as one of the tools in integrated termite (pest) management.

Key Words *Heterorhabditis*, *Metarhizium anisopliae*, *Beauveria bassiana*, subterranean termites

INTRODUCTION

For many decades organochlorines formed the backbone of termite management worldwide. However, these pesticides were banned or withdrawn from the market for human health and environmental reasons from an increasing number of countries in the late eighties and the nineties. The move away from organochlorines is being further accelerated in recent years by efforts by the United Nations Environment Program (UNEP) and Food and Agriculture Organization (FAO) to eliminate globally the production and use of certain persistent organic pollutants (POPs) which include the organochlorine pesticides (UNEP/FAO/Global IPM Facility 2000). As a consequence of these developments, the focus in termite management has shifted increasingly to alternative methods in dealing with termite problems. Among the diversity of practiced and potential methods, the option of using biological control agents against termites continues to attract a great deal of attention. In this paper some of the issues in using such entomopathogens in termite management are discussed. Only two groups of organisms, nematodes and fungi have been investigated in the field to see whether they could cause an epizootic among termites. The main emphasis in this paper will be on fungi as termite control agents.

MICRO-ORGANISMS WITH AN IMPACT ON TERMITES

The literature contains numerous reports of organisms that may have potential to cause the death of termites. A partial review by Myles (2002a) lists 2 viruses, 5 bacteria, 17 fungi, 5 nematodes and 4 mites. The full list of such organisms is no doubt larger. Diseased termite colonies are rarely encountered in the field, although at any time even a healthy, vigorous termite colony will harbour some pathogenic organisms. However, sanitary measures within a colony, such as allogrooming, removing, entombing or feeding on cadavers, and the production of antibiotics ensure that disease outbreaks are kept in check. Only when colony vigour is weakened by age or chemical control measures, can epizootics readily develop and colonies may perish from diseases. Some of the modern termiticides are even known to act synergistically with soil micro-organisms to cause a more rapid decline in termite populations. For example, exposure of termites to sublethal doses of the insecticide imidacloprid triggers a high rate of fungal infestations in stressed termites, leading to a faster colony collapse than either agent could achieve on its own (Boucias et al., 1996; Neves and Alves, 1999; Zeck and Monke, 1992).

In many instances, conclusions on the control potential of any of the entomopathogenic micro-organisms for termites is tentative and based on either laboratory studies with limited numbers of termites in a restrictive environment (Petri dish) or on observations of organisms isolated from termites in the field without much knowledge of the impact of such organisms at the population level of field colonies.

NEMATODES

A number of factors have contributed to the growing interest in nematodes in the management of insect pests: successes as biocontrol agents against a number of insect species; ready availability of some nematode species in Integrated Pest Management (IPM); and, for inundative or inoculative release programs (Bedding, 1998; Kaya et al., 1993).

Field studies with termites are limited. Populations of the dampwood termite *Glyptotermes dilatatus* that form colonies of only several thousand members have been successfully managed in tea plantations on Sri Lanka with *Heterorhabditis* sp. (Dhanthararyana and Vitarana, 1987). Likewise, for species of the dampwood termite in the genus *Neotermes* on islands of the South Pacific, nematodes showed potential in eliminating infestations in the unbranched trunks of coconut palms, but their effectiveness was less guaranteed in branched trees of *Citrus*, cocoa or American Mahogany (*Swietenia macrophylla*) (Lenz and Runko, 1992; Lenz et al., 2000). These branches allowed parts of the population occasionally to retreat into them and block off the connection to the main trunk which had received injections of infective nematode larvae, thus preventing the spread of the nematodes to all areas occupied by a colony.

In Australia, *Heterorhabditis* sp. have also been used to eliminate residual populations of active infestations by subterranean *Coptotermes* sp. trapped in buildings after a perimeter barrier with a repellent chemical has been applied. Infective nematode larvae will kill the trapped termites and move from the site of application inside the building to the nest of the colony. The reported temperatures of above 30°C in the centre of nests of *Coptotermes* where reproductives and brood are housed prove lethal for the nematodes. Hence the impact with currently used isolates of the nematode may never go beyond killing termites in the outer parts of the nest or within the tunnel system in the soil, although some cases of apparent colony elimination have been reported (R. A. Bedding, personal communication). Different isolates or species of entomogenous nematode species that are tolerant to higher temperatures are required for control of subterranean termite species with central compact nests such as species of *Coptotermes*.

After injections of larvae of a *Heterorhabditis* isolate from tropical Australia into eucalypt trunks in which *Mastotermes darwiniensis* foragers were active, masses of dead termites were found. However, due to the complex biology of *M. darwiniensis*, including its diffuse nest system, the presence of multiple sets of reproductives, large territory size and simultaneous use of many feeding sites, it remained uncertain what the impact of the treatment on the colonies as a whole was (R. A. Bedding, *pers. commun.*). Two types of nematodes sold commercially in the US failed to eliminate colonies of another diffuse-nesting subterranean termite, *Reticulitermes flavipes*, in controlled field experiments (Mauldin and Beal, 1989).

FUNGI

More than 700 species of fungi have been reported as pathogens of insects (Milner, 2000). Fungi invade their host directly through the cuticle; the spores do not have to be ingested. It is no surprise then that fungi have had a place in the management of a wide range of insect pests for some time (Ferron, 1978; Glare and Milner, 1991; Lacey and Goettel, 1995; Lacey et al., 2001; Milner, 1991; 2000; Milner and Pereira, 2000). Under field conditions, pathogenic fungi are commonly encountered. For example, it is often possible to collect isolates from termites and from the materials they have been in contact with such as galleries or nest carton and attacked wood (examples: Milner et al., 1998; Sun et al., 2003; Zoberi and Grace, 1990).

Investigations with termites have largely focused on two fungal species, *Beauveria bassiana* and *Metarhizium anisopliae* and recently also *Paecilomyces fumosoroseus* (Wright et al., 2005) (for further references see for example: Milner, 2000; Myles, 2002; Sun et al., 2003; Wang and Powell, 2003), although the favoured candidate in the majority of studies, and notably in field experiments, is *M. anisopliae*. One commercial product in the US and Japan, Bioblast™, relies on this fungus as the control agent against subterranean termites. The most intensive field study program to date was run by R J Milner and his group at CSIRO Entomology in Australia.

The following comments do not aim to review the comprehensive literature but rather point to some of the issues that have to be considered when developing a mycoinsecticide for termite management. The comments refer mostly to work with *Metarhizium* and link to an earlier presentation on the subject (Milner et al., 1996).

Selection of Virulent Isolates. As indicated earlier, different isolates can display very different characteristics. When finally selecting the biocontrol agent, the availability of a suitable isolate may be more important than the species of fungus (Sun et al., 2003). The isolate of the fungus that was used in the early studies in Australia originated from an infected homopteran insect in Mexico (Hänel, 1982; Hänel and Watson, 1983). Isolates of *Metarhizium* vary in their pathogenicity for specific hosts; the most effective isolates are often obtained from naturally infected target hosts (Glare and Milner, 1991). The library of nearly 100 isolates Milner et al. (1998) established as part of the termite control project in Australia contained hardly any collected directly from termites, and those found in termite workings were thought to have originated from surrounding soil rather than being associated specifically with termites. The isolates were typical *M. anisopliae* and did not represent a specific pathotype.

A number of laboratory screening methods have been developed to select the most virulent isolates from the survey. The methods rely mostly on the social nature of termites, i.e. the grooming or the mutual cleaning behaviour of termites, to ensure that the infection is spread through an experimental group via spore-covered individuals released into the group. Effective dose, survival time, ratio between number of spore donors to recipients, and even growth characteristics of the fungus cultures are measures that assist in selecting the most virulent strains (Jones et al., 1996; Lai et al., 1982; Milner, 1991; Myles, 2002a, b; Sun et al., 2003; Wang and Powell, 2003; Wells et al., 1995). Other traits, such as: growth rates over a range of temperatures (notably growth at temperatures above 30°C); posing no health issues for humans or higher animals; ease and cost of mass production; robustness of spores permitting easy formulation and storage; and longevity of spores, have to be investigated before an isolate could be taken into the field (Milner and Staples, 1996; Milner et al., 1998).

Field Assessment. Termite colonies can be destroyed when large quantities of pure, dry conidia of *M. anisopliae* are blown directly into the nursery region of a nest. This approach has been trialled intensively with many colonies of Australian mound-building and tree-nesting species of termite (*Coptotermes*, *Nasutitermes*) (Milner, 2003; Milner and Staples, 1996). In a very different environment, on islands in the South Pacific with tree-dwelling colonies of *Neotermes* which usually restrict their activity to single trees, applications of *M. anisopliae* can readily eradicate infestations (Lenz, 1996; Lenz and Runko, 1992; Lenz et al., 2000).

Termite Behaviour that Limits the Impact of *Metarhizium*. Several authors have reported that the conidia of virulent strains of *M. anisopliae* are repellent to termites (Myles, 2002b; Rath and Tidbury, 1996; Staples and Milner, 2000), triggering alarm and aggregation around spore-dusted individuals. Such termites will be groomed by nest mates, but may also be bitten and defecated upon. Dead individuals will be buried (Myles, 2002b). The demography of the colony or test group may have a great impact on the effectiveness of allogrooming in removing pathogenic spores from the bodies of nest mates. The larger the groups and the more natural the caste composition, the more likely it is that an individual carrying spores on its exoskeleton will be freed from adhering spores, and thus will have increased chances of survival (Rosengaus and Traniello, 2001; Rosengaus et al., 1998). The behavioural defence mechanisms can be rendered less effective, even ineffective by inundating a nest with conidia.

Metarhizium isolates with less repellent conidia are as a rule, less virulent and may not be as effective in causing an epizootic in the field (Milner, 2003). However, it is possible to reduce the repellency of conidia and overcome behavioural defences by formulating the conidia in attapulgitic clay and surfactant (Rath and Tidbury, 1996), by adding attractants, or by reducing spore doses and the like (Milner, 2003; Myles, 2000b). Field observations with *Nasutitermes exitiosus* showed that foragers that were dusted with repellent spores at a feeding site were largely excluded from entering the nest, but when applying conidia from a less repellent strain or applying repellent spores together with a masking formulation, return rates of treated individuals were high (Milner, 2003).

Interestingly, observations by P. Stamets, reported by Coghlan (2004), indicate that the mycelium of *M. anisopliae* pre-sporulation can be 'irresistible' to termites and ants which carry it back to the nest where it will then produce spores. This way, the pathogen is introduced to the nest before it becomes repellent to termites and would be avoided. One approach based on these observations is to select strains of the fungus with a longer pre-sporolytic phase (Coghlan, 2004), thus attractive material would be on offer to termites for longer in control programs with *Metarhizium* increasing the chances for success. However, for this strategy to work, termites would need to collect the mycelium and not consume and digest it, but deposit it within the nest where it can produce spores. Testing of such a control strategy with field colonies of termites is now needed.

Bait formulations with *Metarhizium* conidia in them have been developed. While termites could be readily enticed to consume this treated matrix (Milner, 2003), it has to be kept in mind that spores that are ingested

will pass the gut without causing harm to the termites. The fungus enters termites only through the cuticle. Spores will remain viable after being passed, however, they are then encased in faecal material and hence also lost as a source for further infections. Termite faeces can even have antifungal properties (Rosengaus et al., 1998) thus reducing further, the chances of such spores infecting a host. In baiting with a spore-treated matrix, only the spores that manage to attach themselves to the exoskeleton of termites as the insects move through the matrix will have an impact. These limitations to the impact of the spores were reflected in relatively long times (up to a year) before the population in mounds of *N. exitiosus* was significantly reduced (Milner, 2003).

Fungus-affected workers in colonies of mound-building termites, and even in laboratory-held larger groups of the same termites, tended to move to the bottom layers of the nest or near the container base respectively. If cadavers have a more stratified and restricted distribution rather than being dispersed throughout the nest, the bulk of survivors may have less exposure to the masses of spores being produced on the cadavers. Further, healthy workers tended to encase these clusters of cadavers with faecal and other building material, thus reducing further contact with spores.

Inundative treatments of nests with conidia have to reach the central nest area to ensure elimination of reproductives and the brood, in addition to large numbers of the worker, nymph and soldier population. However, in species of *Coptotermes*, surviving nymphs will readily moult into replacement reproductives after the death of the colony-founding king and queen. The replacement reproductives can commence breeding within a few months (Lenz and Runko, 1993). During experiments over a range of conidia doses applied to mounds of *C. lacteus*, all primary reproductives and their brood were killed. Colony re-establishment with the help of newly formed replacement reproductives occurred in most instances, but a significant build-up in termite numbers was noted only in colonies that had originally been exposed to lower spore doses (Lenz, Staples and Milner, unpubl.; Milner, 2003). Colonies treated with larger amounts of conidia eventually succumbed or at least showed greatly reduced vigour even though they may have initially started to breed again after spore application.

In recent years, data with the dampwood termite *Zootermopsis angusticollis* have indicated that termites may even develop a level of immunity to various pathogens (Rosengaus et al., 1999). Relevant observations for species of subterranean termites are not available.

OPTIONS FOR TERMITE MANAGEMENT

Control of colonies of pest species of termite can be achieved within three months with a single treatment of between 1 to 10 g of conidia applied directly to the nest, although the time to elimination may vary depending on factors such as the target species, time of year and colony vigour. Spores will remain active in nests for at least two years. The repellency of conidia can be used to protect timber. Spores can be sprayed directly onto sound timber or into termite-infested timber to provide protection at least for a period of time. Conidia are capable of providing protection from termite attack for timber in ground contact. A soil barrier created by mixing conidia of *M. anisopliae* at a rate of 10^8g^{-1} with soil (about 2 g of conidia kg^{-1} of soil) has given protection to susceptible timber for up to three years under cool, dry conditions in the Canberra region, but only for less than six months at a site near Darwin in the tropics.

With a “trap-and-treat” system, one of the approaches in bait technology, it is possible to introduce the conidia to a termite colony. The major factor limiting the efficacy of *M. anisopliae* with the currently available isolates is the behavioural response of healthy termites to the applied conidia, to foraging termites bringing conidia into the nest, and to termites infected with the disease.

FINAL COMMENTS

In an overview paper discussing the future of insect pathogens as biological control agents Lacey et al. (2001) listed a number of requirements that have to be met: increased pathogen virulence and speed of kill; Improved pathogen performance under challenging conditions (lower, higher temperatures); greater efficiency in their production; improvements in formulation that enable ease of application, increased environmental persistence, and longer shelf life; better understanding of how they will fit into integrated systems and their interactions with the environment and other integrated pest management (IPM) components; greater appreciation of their environmental advantages; and acceptance by growers and the general public. To these one further requirement is necessary: surety that the pathogens are non-toxic to humans and non-target species.

Several of these points have been touched upon in considering the options for biocontrol agents in the management of termites. However, there remains the discrepancy between the numerous laboratory data indicating a high potential of insect pathogens in controlling termites and the limited field observations, (currently mainly from Australia) which demonstrate that both the termites themselves and the environment can significantly

limit the success of pathogens. This means that a more complex application system rather than simple spread of pathogens will be required in order to achieve control. It is clear that use of microbial pathogens will solve certain termite problems, but may not help with others. Pathogens may be effective against certain species and less so against others. Biocontrol agents should be seen as one tool amongst others in an integrated approach to managing termite problems.

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