Microbial Control of Whitefly *(Bemisia Tabaci)* and Thrips Palmi in Trinidad and Tobago

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Introduction

Problems with chemical control, whether due to resistance of insect pests to pesticides, the enormous cost of bringing new chemicals onto the market, or to environmental considerations, are beginning to dictate the pace at which the development of biocontrol agents is moving. The Government of Trinidad and Tobago has recently invested in several research programmes to develop alternative strategies to chemical control on vegetables and other edible crops. These research projects take the form of training programmes, the aim being to establish a permanent biocontrol capability in the region.

The training is accomplished through post-graduate research programmes targeted at the major pests of food crops in Trinidad and Tobago, as follows:

(i) control of whitefly by pathogens;
(ii) control of thrips by pathogens;
(iii) control of thrips by predators;
(iv) control of sugarcane froghopper by pathogens;
(v) control of caterpillar pests on vegetables; and
(vi) control of locusts by pathogens.

These projects are being financed and assisted by various organisations in Trinidad and Tobago, notably the National Institute of Higher Education, Research, Science and Technology (NIHERST), Caroni (1975) Limited, the Ministry of Agriculture, the International Institute of Biological Control and the Commonwealth Secretariat.

Since this workshop focuses on the problems posed by *Thrips palmi* and *Bemisia tabaci* (whitefly), I shall be talking exclusively about the potential of fungal pathogens of these pests.

Why only fungal pathogens?

Thrips and whitefly feed on plant juices which, being virtually sterile, contain no micro-organisms capable of causing disease in insects. Therefore, in order to provoke disease in such an insect and kill it, a disease causing micro-organism must invade via the cuticle, natural orifices or wounds. Invasion via the latter route would be expected to be comparatively rare and so will not be considered here. Of the various groups of micro-organisms (bacteria, viruses, protozoa and fungi) only fungi possess the ability to literally bore a hole through the insect cuticle thereby achieving infection. Thus, microbial control of pests such as thrips and whitefly means, exclusively, control by fungi.

Fungi Attacking Insects

Fungi from many taxonomic classes infect insects. However, we are concerned chiefly with the class Deuteromycetes. This is mainly because this class contains species which are highly infective for insects, and easy to culture and produce in both. The species with which we are concerned are, in addition, considered to be safe for plants, animals and humans and, due to strain specificity, also for non-target insect pests. Deuteromycete species reproduce asexually by spores called conidia or conidiospores.

Mention in this paper is also made of some species of the class Zygomycetes - the (Order) Entomophthorales. Species in this class tend not to be easily manipulable but I refer to them briefly as they are amongst the most effective of insect pathogens in nature.

Do Fungal Pathogens Work?

With very few exceptions, all fungi have an absolute requirement for high humidity (>93 per cent) for spore germination and growth. It does not matter whether this humidity requirement is satisfied at a micro-climatic or macro-climatic level. Although it appears that the microclimate humidity may be high on certain parts of the cuticle on some insects (particularly the supple connective tissue between sclerites), thereby possibly permitting germination of insect pathogens at low ambient humidities (Ramoska, 1984), there can be no doubt that infection will occur most efficiently on an insect cuticle during a period of high ambient humidity. The best successes have been recorded where humidity was expected to be high, e.g., in greenhouses, and we may cite the example of *Verticillium lecanii*, a pathogen of aphids and whiteflies. Several years' research by the author on many strains of this fungus culminated in the commercialisation of two such strains - an 'aphid strain'

('VERTALEC') and a 'whitefly strain' ('MYCOTAL') (Hall and Papierok, 1982). These two products are currently produced by the world's largest commercial producer of biological control agents, Koppert B V in Holland. There are not many other examples of commercialised fungal pesticides ('mycopesticides'). Bayer in Germany has recently brought a preparation of *Metarhizium anisopliae* ('BIO 1020') onto the market for the control of a subterranean pest, vine weevil (*Otiorhynchus sulcatus*). The same species is produced commercially for the control of froghopper (*Aeneolamia varia saccharina*) on sugarcane in Venezuela, and has enjoyed considerable success. M. *anisopliae* is used on a large scale in Brazil for the control of other sugarcane and pasture pests and elsewhere in South and Central America. There are numerous reports emanating from the former Soviet Union and China of fungi being used on a commercial scale.

The paucity of economic applications of entomogenous fungi is a little deceptive, as many research efforts failed to focus on those insect pests occupying a habitat where the essential physical requirements, notably temperature and humidity, would be adequately met. In addition, the relationship between the fungal pathogen and the population dynamics of the pest must be in favour of the fungus if the pest is a rapidly-reproducing one - such as whitefly or thrips. In those regions where fungi might be expected to have most potential, such as in the humid tropics, it is true to say that comparatively few in-depth studies have been carried out.

A few years ago, the answer to the question 'do fungi work?' might have been yes, as long as we did not stray too far from the ecological niche and host range within which these pathogens are naturally very (and sometimes dramatically) effective. However, very recently some developments have indicated that it might be possible to stretch the capabilities of fungi beyond their traditionally perceived limits. Instead of aqueous sprays, spores may be applied in oil-based formulations. Fungal spores have been shown to kill locusts at ambient humidities of 35 per cent (Goettel, 1992). Also, oil-based formulations appear to lower LC50s in bioassays (Prior et al., 1988). However, such formulations would not help a fungal pathogen spread from an infected insect to a healthy one if the humidity were not sufficiently high for a reasonable length of time to permit reproduction of the infectious propagule, i.e., sporulation on the insect body. Such spread of disease may be crucial in obtaining economic control of rapidly reproducing insect pest populations. To overcome this constraint, repeated applications at judiciously chosen intervals would be necessary to bring about the control of such insects.

Another strategy whereby fungi may be rendered more effective focuses on accelerating the infection process so that spores may germinate during a shorter 'window' of high humidity, or before they become dislodged, preened off or inactivated for whatever reason. At least two phenomena have been discovered by our group in Trinidad and Tobago which may lead to methods of accelerating the infection process. One discovery relates to the fact that spores produced in very young cultures germinate significantly faster than those from older (but still young) cultures. (These spores are probably the first-formed spores on a phialide). The elucidation of the reasons for faster germination may eventually result in more efficient spore preparations and possibly genetically modified strains with greatly improved epizootic potential.

Such improvements in application technology and a greater understanding of the fundamental key processes in the infection cycle will probably usher in a new and exciting era of research into pest control by fungi.

Pathogens of whiteflies

The following species have been and are being investigated as whitefly control agents:

Aschersonia aleyrodis
Verticillium lecanii
Paecilomyces fumoso-roseus
Bauveria bassiana

Their potential for controlling *Bemisia tabaci* will be discussed.

Aschersonia aleyrodis

This species is frequently found on whiteflies in lowland tropical zones, though we have yet to find it on *B. tabaci* in Trinidad. However, the species is common on citrus scale in Trinidad, as is *Aschersonia turbinata*. Apparently, *A. aleyrodis* has been shown to attack *B. tabaci* in Florida.

A. aleyrodis is an intriguing fungus. Being slow to germinate, it would be expected to infect insects rather more slowly than other pathogens such as the faster-germinating *V. lecanii*. In reality, against the glasshouse whitefly (*Trialeurodes vaporariorum*), the speed at which sprayed *A. aleyrodis* pores infect the pest is quite surprising - faster than *V. lecanii* - even when humidity appears to be sub-optimal. *A. aleyrodis* infects only the larval stages of whitefly, adults and eggs rarely becoming infected. Consequently, in European glasshouses, it does not spread from infected to healthy insects and so must be sprayed many times to achieve control of this

rapidly-reproducing pest. However, on citrus whitefly the fungus spreads extremely efficiently, as evidenced by virtually 100 per cent natural control of this pest each season. Osborne and Landa (unpublished) have recently made observations which may explain this apparent contradiction.

A. aleyrodis is highly specific for whiteflies and does not harm natural enemies. Therefore, in glasshouses, it may be used against whitefly in conjunction with the parasitoid *Encarsia formosa*, and is especially useful as a 'knock down' agent when whitefly populations have become too large for *E. formosa* to control. There are some outstanding problems with this fungus relating to mass-production and stabilisation of spores of this fungal species.

In Trinidad, we have tested in the laboratory a strain of *A. aleyrodis* which gave good results against *T. vaporariorum* in the UK (Hall, unpublished observations). However, we have not been able to achieve any infection of *B. tabaci* in Trinidad with this strain. We have recently acquired, from Dr Lance Osborne (University of Florida), a strain known to infect *B. tabaci*. If the results which I obtained in the UK on the glasshouse whitefly can be repeated on *B. tabaci*, then we shall be focusing a good deal of attention on this fungus.

Verticillium lecanii

There are many strains of *V. lecanii* attacking a wide variety of insect hosts and plant pathogens. The classification of this fungus is open to question, some workers preferring to split the complex into further species. Certainly *V. lecanii* can be divided into large-spored and small-spored strains. Large-spored strains are found exclusively infecting aphids, though in the laboratory they will also infect other pests including whitefly (Hall, 1982). The commercial aphid-killing product VERTALEC is based on such a large-spored strain (Hall, 1982). Strains from thrips, whitefly, mites and phytopathogenic fungi are always small-spored strains and the commercial product for whitefly and thrips (MYCOTAL) is based upon a small-spored strain (Hall, 1982). However, small-spored strains are sometimes found in nature on aphids also. To simplify the situation, small strains perform best against whitefly and thrips while large-spored strains do best against aphids. From the point of view of whitefly control, therefore, we are interested in small-spored strains. These have a temperature optimum of about 24°C which may limit their potential in the humid tropics where average temperatures are often higher than this. In general, constant temperatures a few degrees beyond the optimum have a dramatically negative effect on the performance of insect pathogenic fungi. While *V. lecanii* is the only fungus found infecting the glasshouse whitefly (*Trialeurodes vaporariorum*) in glasshouse environments in temperate regions and at high altitude in Colombian plastic houses, it is very rarely found infecting whitefly in lowland tropical areas. For this reason, I feel that *V. lecanii* has little potential against *B. tabaci* in such areas.

V. lecanii infects both larval and adult stages of whitefly but not eggs. This fungus may be mass-produced on solid-substrate or in liquid medium.

Paecilomyces fumoso-roseus

This species has a rather wide insect host range including whiteflies. Amongst the latter, both larval and adult stages are attacked and to a certain extent, if humidity is very high, the eggs. It has been investigated to some extent in Florida as a control agent of *B. tabaci* and by our group in Trinidad. This fungus has a temperature optimum of 28°C - considerably higher than the small-spored strains of *V. lecanii*. For this reason, I believe that from an ecological standpoint, *P. fumoso-roseus* is better adapted than *V. lecanii* to exploit the lowland tropical niche. It is not surprising therefore that *P. fumoso-roseus* is the only fungus found in Trinidad on *Bemisia*. By the same token, this species has, to my knowledge, never been found in European glasshouses or high altitude plastic houses in Colombia.

Since *P. fumoso-roseus* is amenable to mass-production and displays high virulence to *B. tabaci*, we are concentrating much of our research effort on this fungus.

Beauveria bassiana

The fungus *B. bassiana* is not considered to be a natural pathogen of whiteflies. However, it is included in this list because scientists in the USA claim that a strain of this fungus (isolated from the cotton boll weevil), provides very good control of *B. tabaci* in greenhouse and field vegetables. In arid areas in Arizona and California, they reported that oil-based formulations of *Beauveria bassiana conidia* gave adequate control. Eggs, larvae and adult stages are reported to be infected by this strain.

Pathogens of Thrips Species

Many of the species which attack whitefly also infect thrips. The fungi known to attack thrips species are as follows:

Verticillium lecanii

Paecilomyces fumoso-roseus

Beauveria bassiana

Neozygites parvispora (Entomophthorales)

Entomophthora thripidum (Entomophthorales)

Metarhizium anisopliae

Hirsutella spp

Verticillium lecanii

This species has been described above. In temperate zones, *V. lecanii* may easily be found infecting thrips species in glasshouses and, in both temperate and tropical zones, laboratory cultures. Small-spored isolates of the fungus are exclusively associated with these insects. The commercial product MYCOTAL also possesses the best thrips activity of any strain of *V. lecanii* so far examined. We have established that *Thrips palmi* is susceptible to the MYCOTAL strain but, for the reasons given above, it is not likely that the known strains of this fungus will be of much value in controlling *T. palmi* in lowland tropical areas.

Paecilomyces spp

Paecilomyces spp have rarely been associated with field infections of thrips. However, there is at least one report from Puerto Rico of a *Paecilomyces sp* infecting *Gynaikothrips ficorum*. Furthermore, we have encountered *Paecilomyces fumoso-roseus* killing *Thrips palmi* in laboratory cultures, and on one occasion in the field.

Beauveria bassiana

There are several reports in the literature of this species attacking thrips species, most notably *Selenothrips rubrocinctus* on cocoa. In the laboratory, *B. bassiana* is pathogenic to *Thrips palmi*.

Metarhizium anisopliae

There is only one report of this fungus attacking thrips *T. palmi*, in a greenhouse in Puerto Rico.

Neozygites parvispora and *Entomophthora thripidum*

There are numerous reports of these fungi attacking a range of thrips species. *N. parvispora* has been reported from Japan on *T. palmi*. These fungi provoke spectacular epizootics in thrips populations. However, neither of these fungi has been successfully cultured and so the prospects of producing sufficient infective material for wide-scale application are, at present, poor.

Hirsutella spp

Recently, several isolations of new *Hirsutella* species have been made. At least two have been made in Trinidad - one by Prior from *Limothrips* sp and a new species from *Thrips palmi* (Hall, 1992). The latter has been cultured and its potential is being investigated in the laboratory by our group in Trinidad.

To summarise, there are only two reports of fungi attacking *T. palmi* in natural field conditions - *Neozygites parvispora*, and *Hirsutella* spp *Metarhizium anisopliae* was found only once in a greenhouse in Puerto Rico. Of the fungi found consistently on thrips in general, we may cite *Beauveria bassiana*, *Verticillium lecanii*, *Hirsutella* spp and members of the Entomophthorales.

Research Strategy

Once we have a collection of pathogenic isolates of fungi, how do we decide which performs best against the target pest? The definitive way is to spray infective material in the field. However, when there are more than a very few such pathogens to be tested, this is impractical because field trials are expensive and time-consuming. Therefore, prior to investing scarce resources in field trials, one must develop laboratory systems which provide information which is reasonably predictive. Such a laboratory system for quantifying the ability of a pathogen to infect its host is called a bioassay. A well-designed bioassay system will provide information which will enable the researcher to predict which strains of pathogens will perform best under field conditions. An ill-conceived system will yield practically no information at all. For example, the literature abounds with examples of bioassay systems which do no more than demonstrate how insects react to a stressor (in this case a pathogen) in already stressful conditions e.g. continuous high humidity - which, of course favours the fungal pathogen. Some insects may react negatively to continuous high relative humidity and this is revealed by a high control mortality. Furthermore, small, highly mobile insects such as thrips may drown in small condensation droplets or they may escape or become trapped or hidden in crevices all of which contribute to a high control mortality. Whatever the reason, a high control mortality prevents an accurate assessment of the infective ability of a pathogen. Very little work has been published on the bioassay of fungi against thrips. However, high control mortalities have been a feature in many of these and, consequently, the significance of the results is questionable.

Therefore, it is important to develop a bioassay system which permits almost all control insects to survive apparently healthily to the end of the assay period. Us-

ing previously developed systems for thrips species, we first encountered unacceptably high control mortalities sometimes as high as 90 per cent. Consequently, we had to develop a totally new bioassay system which fulfilled the following important criteria:

- the insects must be individually contained to eliminate the risk of cross-contamination;
- the insect should be able to move freely within the bioassay chamber;
- the system should permit clear observation of the insects at all times;
- there should be sufficient air exchange to supply the needs of the insect and leaf tissue and to prevent condensation (in which small insects such as thrips may drown) on the walls of the chamber or on the leaf discs;
- the substrate, normally leaf tissue, must be maintained in a palatable form for the duration of the bioassay;
- there should be minimal handling of the insect;
- the control mortality must be within acceptable limits (below 10 per cent);
- the cages must be escape-proof; and
- the system must be reasonably simple to set up so that large numbers of assays may be performed without undue difficulty.

Our new system relies on using a Plaster of Paris matrix for supporting leaf discs, the whole contained in a compartmented square Petri dish. Control mortalities, including escapes, have been reduced from a maximum of 90 per cent using other systems to a maximum of 8 per cent using the Plaster of Paris system (Hall et al., 1993). Furthermore, a much simplified version of this system is suitable for sessile insects such as *Bemisia* immatures.

The function of a laboratory bioassay in terms of the epizootiological field characteristics of a pathogen requires further definition. If a pathogen is to infect only those insects which are hit by a spore spray - such as dry-season multiple application of oil-based spore sprays - then the sort of infectivity assay which has been described will probably be adequate. However, it is necessary to look at a new factor, the epizootic potential when we consider post-application spread of the pathogen from diseased insects hit by a spray to healthy insects. Epizootic potential becomes very important when, within rapidly reproducing pest populations, in conditions which are favourable for the fungal pathogen, we expect the disease to achieve control after only one or a very few applications. How do we measure epizootic potential? It is difficult conceptually to standardise and quantify the ability of a pathogen to spread. A good epizootic potential probably cannot be predicted from high infectivity as measured by bioassay. In the case of the fungus *Verticillium lecanii* on aphids, while strains with good epizootic potential always exhibited high infectivity, the reverse was most certainly not true (Table 1). We cannot measure epizootic potential but we can certainly compare epizootic potentials of pathogens, as the data in Table 1 adequately demonstrate.

TABLE 1: **Mortalities of adult aphids treated with pores of different strains of *V. lecanii* and their progeny acquiring disease from the adults through contagion**

Strain	Adult mortality per cent	Progeny mortality per cent	Epizootic potential
1-72	100	84	good
93-82	100	77	good
57-81	91	9	poor
79-82	100	0	poor
18-78	91	8	poor

Recently, there have been reports that bioassays at sub-optimal relative humidities may serve to select the most aggressive strains of fungi, and possibly those with good epizootic potential also. This may stem from the fact that, as I mentioned before, some insects are stressed under conditions of continuous high humidity, probably because they cannot get rid of excess moisture sufficiently rapidly. At sub-optimal humidities, insects may be in much better shape and only the most pathogenic organisms may be able to provoke significant mortality. In our laboratory we are modifying the Plaster of Paris assay system along these lines to test this possibility. To summarise, there are no hard and fast rules as to what sort of assay system one should employ to screen fungal pathogens prior to testing them in the field. What is important is that the researcher should have a very clear idea of what the objectives are, and therefore what pathogenic characteristics should be focused upon.

Progress of Research in Trinidad

Our microbial research projects in Trinidad have only been in progress for just over one year. However, what can we conclude so far about the potentials of the fungi at our disposal?

Bemisia tabaci

This pest is most serious in the dry season. Fungi are not going to be in an 'epizootic phase'. Therefore, in this scenario, only oil- or emulsion-based spore sprays would have any impact on populations at this time. At other times during the wet season, a strategy of wide-scale applications of fungal strains with good epizootic potential may eliminate populations from an area enabling farmers to have a 'clean start' at the beginning of the dry season. In the context of sound integrated pest management practices, this 'clean start' could be expected to last well into the dry season.

The candidate fungi likely to be developed successfully for whitefly control in the short term are *Paecilomyces fumoso-roseus* and, in the light of the favourable reports from the USA, one cannot ignore *Beauveria bassiana*. *Verticillium lecanii*, which can so spectacularly control whitefly in glasshouses in temperate regions (Hall, 1982), is not likely to have a significant role in lowland tropical areas. The fungus *Aschersonia aleyrodis* should be tested against *Bemisia* as it has given good results against other whitefly species.

Thrips palmi

We still have little information on the abilities of the pathogens mentioned earlier to control *T. palmi*, either in the field or in the laboratory. Now that we have developed a good bioassay system, the laboratory evaluation phase is well under way. Again, *T. palmi* is mainly a dry season pest and the principles propounded above for whitefly are also appropriate for the control of this pest.

Conclusions

We probably already have in our collections the best species and strains of pathogens of *Bemisia tabaci* and *Thrips palmi*. In the past, we would have been restricted to wet-season application of infectious spores of these fungi in high-volume aqueous suspensions. Oil-based spraying technologies now also open up the possibility of dry-season application. Further advances hold the promise of increasing the natural efficiency of these pathogens. For example, our work in Trinidad and Tobago has revealed two methods of accelerating spore germination, and by implication the infection process. Given that in the field, fungal spores can only germinate during a 'window' of favourable humidity and temperature which occurs usually at night and is usually of limited duration, such discoveries could lead to a disproportionately high increase in infectivity or epizootic potential. These developments give cause for pathogenic fungi. It is highly likely that fungi will have an important role in future integrated pest management programmes for whitefly and thrips control.

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