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Review Article

Biological control of weed and insect pests using fungal pathogens, with particular reference to Sri Lanka[1]

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Abstract

The principles and practice of using fungal pathogens to control both weed and insect pests are outlined. It is emphasized that a clear distinction should be made between natural control, resulting from the direct action of natural enemies, and biological control, achieved by the deliberate manipulation of natural enemies by man.

The past history of research on entomopathogenic fungi in Sri Lanka is traced and discussed, highlighting the pivotal role played by early colonial scientists, such as Tom Petch, in elucidating their ecology and systematics. The actual and potential exploitation of entomopathogenic fungi as biological control agents is reviewed.

Several plant species, such as *Dichrostachys cinerea*, *Ligustrum robustum* and *Rottboellia cochinchinensis*, which are indigenous to but generally uncommon in Sri Lanka, have become major invasive weeds in exotic situations, particularly in the Neotropics. In contrast, a number of native, neotropical plant species, such as *Chromolaena odorata*, *Eichhornia crassipes, Lantana camara* and *Mimosa pigra*, which are typically only minor weeds in Latin America, have become invasive in Sri Lanka, posing a threat to both agricultural and natural ecosystems. These examples are discussed in relation to the role of natural enemies, with particular reference to fungal pathogens, in the regulation of plant populations and their use as classical biological control agents.

Introduction

Fungal pathogens can be exploited as biological agents for the management of agricultural pests, including the most problematic groups: weeds, insects (and invertebrates in general) and diseases. This paper will be restricted to the biological control of weed and insect pests.

In its purest form, biological control is “the use of living natural enemies to control noxious organisms (pests)”. By definition, therefore, it involves the manipulation of biological systems (in one way or another), by man, in an attempt to achieve control, and should not be confused with natural control, which occurs without man’s deliberate intervention (Evans, 1974).

In natural ecosystems, a state of equilibrium has been reached whereby plants and animals are held in check by a combination of factors, including natural enemies. In tropical forest ecosystems, for example, it has been proposed that pressures from fungal pathogens may have played an important role in the evolution of both plants and arthropods (Harlan, 1976; Evans, 1988).

The great diversity of climate, geology, topography and soils in Sri Lanka is reflected in its flora, fauna and ecosystems. There is a high degree of endemism in Sri Lanka, particularly in rain forest ecosystems, and it has been calculated that nearly 28% of the flowering plants are endemic (Erdelen, 1988). No such estimates exist for the fungi or insects, although there is no reason to believe that these would differ significantly from this figure. Each plant and insect genus or species has a guild of natural enemies, including fungi, a proportion of which is highly specific or coevolved. Thus, Sri Lankan ecosystems could serve as a source of fungal pathogens for use in the management of weed and insect pests. However, such finely balanced ecosystems are prone to invasions by exotic or alien species, which typically lack the coevolved natural enemies and thus are better fitted to compete against the endemic flora and fauna. The present paper aims to: identify past examples of biological

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control involving fungal pathogens in Sri Lanka, especially within an historical context; identify potential sources of both plant and insect fungal pathogens, which could be exploited in biological control strategies; identify species exotic to Sri Lanka, particularly weeds, which could be managed using the classical biological control approach.

It is relevant here to define the two broad approaches to biological control: classical and inundative. Classical biological control involves the introduction of a natural enemy of the target pest from its native range into the exotic range, ideally, resulting in a self-sustaining, balanced system in which the pest population is maintained at a non-damaging or sub-economic level, as its fitness or competitive edge is eroded (Evans & Ellison, 1990). Inundative control, in the case of fungal pathogens, involves the mass production, formulation and application of a product (mycoinsecticide, mycoherbicide) that can be marketed and employed in much the same way as a conventional chemical pesticide.

Fungal Pathogens of Insects

History in Sri Lanka

In Sri Lanka, there has been a long history of research on the fungal pathogens of insects, the so-called entomogenous or, more correctly, entomopathogenic fungi (Samson *et al.*, 1988), which dates back to the last century when specimens of fungal diseased insects were sent by G. Thwaites (Superintendent, and then Director of the Royal Botanic Gardens (RBG), Peradeniya, from 1849-1880) to the Royal Botanic Gardens at Kew. Descriptions of these entomopathogenic fungi appeared in a series of papers on “Ceylon Fungi”, edited by the foremost mycologists of the time, M. Berkeley and B. Broome, and published in the *Journal of the Linnaean Society* from 1870 onwards. These were later included in the classic publication by Cooke (1892) on entomopathogenic fungi, or “Vegetable Wasps and Plant Worms” as they were then popularly known. One of these fungi, *Cordyceps barnesii* Thw., a pathogen of cockchafer or lamellicorn larvae, reported as feeding on coffee roots in Peradeniya and Bolagodde (Massee, 1895), has since been recorded from East Africa and is currently being evaluated as a potential biocontrol agent of cockchafer pests of sugarcane (Evans *et al.*, 1999). Another *Cordyceps* species, *Cordyceps dipterigena* Thw., a widespread pathogen of dipteran hosts, was also typified from a Sri Lankan specimen (Cooke, 1892; Massee, 1895). Later, the Government Entomologist and Scientific Assistant to the Director of RBG Peradeniya, E. Green, author of the “Coccidae of Ceylon”, assembled a collection of fungal pathogens of scale insects which was identified provisionally by J. Parkin, a visiting mycologist to the Gardens in 1899. This resulted in a monograph on the fungal pathogens of scale insects (Coccidae and Aleyrodidae), published by the Gardens (Parkin, 1906). This work was later expanded upon by Tom Petch in two classic monographs on the fungal pathogens of soft scale and whiteflies, especially the genus *Aschersonia* (Petch, 1921a, 1921b). Petch occupied the post of mycologist at the Gardens from 1905 to 1925 and subsequently became the first Director of the Tea Research Institute (1925-1928). He was regarded as one of the outstanding tropical plant pathologists and mycologists of his generation (Ainsworth, 1976) and published standard texts on “The Diseases and Pests of the Rubber Tree” and “Diseases of the Tea Bush”. In addition to his many published works on tropical plant pathology, Petch also published a series of papers on entomopathogenic fungi over a 40-year period, mostly based on his collections in Sri Lanka, many of which are now housed at RBG, Kew. Both Parkin and Petch commented on the widespread occurrence of fungi parasitic on or pathogenic to scale insects, particularly in the humid cloud forests of Sri Lanka, such as Hagkala. As a rule, these fungi induce diseases of epidemic proportions (epizootics) amongst the scale insects they attack; “...few scales on a plant or group of plants escaping” (Parkin, 1906). In the latter publication, Parkin noted, in particular, the impact of the plurivorous pathogen, *Verticillium lecanii* (Zimm.) Viégas, on the coffee green scale pest, *Coccus viridis* (Green) (Hom., Coccidae). However, his attempts to artificially inoculate green scales, using conidial suspensions, in order to initiate epizootics in the field, all failed and he reflected that more research was needed before *V. lecanii*, and entomopathogenic fungi in general, could be used to control insect pests, such as scale insects. He further commented: “As moisture and warmth naturally favour their growth, Ceylon should be a suitable country for testing their efficacy as a remedy for scale attacks” (Parkin, 1906). This recommendation appears to have been taken up when inoculum of *V. lecanii* was distributed within coffee plantations in Sri Lanka to combat green scales, either by spraying spore suspensions or by hanging leaves colonized by naturally-infected green scales within ‘disease-free’ coffee bushes heavily attacked by the pest. Nevertheless, Petch was extremely sceptical about the methodology used: “There is no doubt that *Cephalosporium* [*Verticillium*] *lecanii* kills enormous numbers of green bug in Ceylon. At the beginning of each rainy period the green bug on coffee will generally be found to be covered by the fungus, and it is surprising that any manage to survive. The fungus is so generally distributed that artificial distribution could not make any appreciable difference” (Petch, 1925). Since the pioneering work of these colonial scientists, relatively little attention appears to have been paid to either systematic or applied research on entomopathogenic fungi in Sri Lanka.

Biological control potential

“Entomogenous fungi in nature cause a regular and tremendous mortality of many pests in many parts of the world and do, in fact, constitute an efficient and extremely important natural control factor” (Steinhaus, 1949). More recently, quantitative evidence of the importance of entomopathogenic fungi on arthropod populations has been presented for tropical forest ecosystems (Evans, 1974), as well as for agroecosystems (Samson *et al.*, 1988). However, as noted above, Petch’s experience in Sri Lanka left him with a somewhat pessimistic view of biological control: “No fungus disease has ever exterminated an insect or prevented an epidemic [outbreak]. That such diseases do kill off large numbers of insects periodically and so exercise a considerable natural control is undoubted but it has not yet been possible to improve on nature in this respect. It is quite possible that a study of the insects in relation to the fungi might disclose facts which would throw some light on the conditions which govern the incidence of these diseases and that in consequence of such discoveries it might be possible to utilize them in controlling certain pests, but at the present time the available evidence is decidedly opposed to the idea that any practical use can be made of them” (Petch, 1925). His negativism seems to have been based on contact with non-scientists whom he considered to be enthusiastic but misguided and unfamiliar with the past failures in the field of biological control (“...proposals of this nature are still periodically put forward with no better foundation than the discovery of another fungus on another insect”), many of which he critically analysed (Petch, 1925). Was Petch correct in his interpretation of these early attempts at biological control using entomopathogenic fungi and what advances have been made over the past 70 years?

To some extent, Petch’s scepticism has proven to be prophetic, since, over the intervening years, there have been many attempts to realise the potential of entomopathogenic fungi for the management of insect pests, most of which have ended in failure and, out of those which have reached the product stage, few are currently on the market. The most probable cause of this poor performance has been insufficient investment in both time and money in order to interpret the complex host-pathogen interactions and to develop appropriate technology to overcome the well-documented constraints to successful implementation in the field as compared to the laboratory situation. Poor formulation and application strategy led to inconsistent control, mainly due to insufficient inoculum hitting the target pest and a rigid dependence on favourable environmental conditions, especially high humidity. Through a combination of good science, and generous and committed funding over a reasonable period of time, it has now been shown to be possible to overcome these constraints and to offer a viable alternative to conventional chemical insecticides. A collaborative, international project, with major inputs from CABI Bioscience, has recently successfully developed and launched a mycoinsecticide that has the potential to control both locusts and grasshopper pests (Acrididae), and which has been specifically targeted at subsistence agriculture in Africa (Bateman, 1997). The product, Green Muscle®, based on an acridid-specific variety of the well-known entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorok., offers an environmentally friendly and cost-effective method of control of these pests which are a constant threat to resource-poor farmers in Africa. The breakthrough has involved the use of an oil-based formulation applied with ultra-low volume spraying equipment which has enabled the product to be used not only successfully at extremely low humidities (down to 30 %), typical of the semi-arid habitats of locusts, but also economically over wide areas. Thus, the critical reliance on free water or humidity has been circumvented. The mycoinsecticide is now filling a gap in the locust management armoury, which followed the banning of the toxic and persistent organochlorine and organophosphorus insecticides, such as dieldrin and malathion. Hopefully, the scene has been set to allow the potential of entomopathogenic fungi to be more fully realised. Perhaps the *Aschersonia*-rich forests of Sri Lanka could be exploited as potential sources of biocontrol agents to combat the ever-increasing menace of whitefly pests. Similarly, coevolved pathogens may occur on red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Col., Curculionidae), which is endemic in Sri Lanka but now a major pest in the Gulf States (Murphy & Briscoe, 1999).

Fungal Pathogens of Weeds

Background

Typically, plant species become weedy due to an imbalance whereby they develop an increased fitness or aggressiveness which makes them more competitive and thus more able to exploit a habitat or ecosystem. There may be a number of widely different reasons for this increased competitiveness. A major cause results from the arrival or introduction of an alien or exotic species lacking its full complement of natural enemies. In the absence of the guild of coevolved pathogen and insect natural enemies, the exotic species may become invasive and dominant within the 'new' ecosystem since any controlling effect has been lost. Thus, the alien has an immediate competitive advantage over the indigenous flora. Members of this native flora may also become weedy when the delicate balance of power within the ecosystem has been disturbed, generally due to the activities of man. Obviously, cultivation of the land has created these disturbances, which usually favour plant species with rapid growth rates and high seed production. Thus, although natural enemies may still be present, they may survive poorly and thereby operate slowly and inefficiently within the agricultural ecosystem, favouring the rapid build-up or population explosion of the host plant.

Potentially, the exotic or alien species can be controlled by introducing all or a part of the guild of highly specific or coevolved natural enemies from its centre of origin or native range. This is the classical biological control approach and has been exploited by entomologists for over a century but only relatively recently by plant pathologists. In the case of indigenous weed species, the strategy is to mass produce a selected natural enemy, which may be either coevolved or adapted, and apply it early in the growing season in order to induce suppressive epidemics (epiphytotics) within the still expanding population. This is the inundative or augmentative approach.

Classical biological control

In Sri Lanka, there appear to be no examples of using fungal pathogens in a classical strategy. There have, however, been several successful attempts to employ this approach with insect natural enemies, involving two weeds of neotropical origin, *Salvinia molesta* (Salviniaceae) and *Chromolaena odorata* (Asteraceae). For the former weed at least, the results have been spectacular (Doeleman, 1989; Room & Fernando, 1992). Several other neotropical plant species that have become weedy invasives can be identified in Sri Lanka which, based on the results of recent research, potentially could be controlled by the introduction of coevolved fungal pathogens. In addition to *C. odorata* (Barreto & Evans, 1994), these include *Ageratina riparia* (Asteraceae) (Barreto & Evans, 1988), *Lantana camara* (Verbenaceae) (Barreto *et al.*, 1995), *Mikania micrantha* (Asteraceae) (Barreto & Evans, 1995), *Mimosa pigra* (Mimosaceae) (Evans *et al.*, 1995), *Euphorbia* spp. (Euphorbiaceae) (Barreto & Evans, 1998), and *Eichhornia crassipes* (Pontederiaceae) (Barreto & Evans, 1996). The above references detail the fungal mycobiota associated with these weeds in their proven or purported centre(s) of origin in the Neotropics. For example, in the case of mistflower (*A. riparia*), spectacular control has been achieved in Hawaii through the introduction and release of a white smut fungus, *Entyloma ageratinae* Barreto & Evans, from the Caribbean and the same agent has recently been released in New Zealand; whilst two fungal pathogens, the rust *Diabole cubensis* (Arth.) Arth. and the coelomycete *Phloeospora mimosae-pigrae* Evans & Carrion, have recently been introduced from Mexico by the International Institute of Biological Control (now incorporated into CABI Bioscience), into the Northern Territory of Australia for control of the giant sensitive plant, *M. pigra* (Seier & Evans, 1996). Both these neotropical plant species are becoming increasingly invasive in Sri Lanka (B. Marambe, pers. comm.; H. C. Evans, pers. obs.). However, although the other neotropical weeds, *C. odorata* and *Mikania micrantha,* are widespread in Sri Lanka, they do not appear to be posing a serious economic or ecological threat. The moth *Pareuchaetes pseudoinsulata* (Walker) (Lep., Arctiidae) may have had an impact on *C. odorata* (Dharmadhikari *et al.*, 1977), but why *M. micrantha* is not more invasive remains puzzling. In particular, it is generally not regarded as a weed of tea in Sri Lanka (B. Marambe, pers. comm.), whereas in Assam, *M. micrantha* is the most problematic weed in tea plantations (H. C. Evans & S. T. Murphy, pers. obs.). Clearly, the reasons for this warrant further investigation. Conversely, Sri Lanka may also be a source of fungal pathogens that could be exploited for the control of invasive, exotic weeds in other parts of the world. *Dichrostachys cinerea* (Leguminosae), *Rottboellia cochinchinensis* (Gramineae) and *Ligustrum robustum* (Oleaceae) are all indigenous in Sri Lanka but are never dominant members of the flora, and can even be described as rare to sporadic. However, *D. cinerea* (or 'marabu') in Cuba, where it has been known as an invasive pest for some considerable time (Weir, 1927), now occupies a significant part of the island affecting both its agriculture and biodiversity. It is even included in the local vocabulary to signify a disastrous event – 'más malo de que marabú' – 'even worse than *Dichrostachys*'! The plant is also native to India from where several potential biocontrol agents have been identified; including a rust fungus, *Uredo deformis* (Tyagi & Prasad) Bagyanarayana & Ravinder (in Bagyanarayana &

Ravinder, 1988), which attacks the growing points inducing tissue malfunction and resulting in the formation of spectacular witches' brooms. The same disease has recently been observed in southern Sri Lanka (H. C. Evans, pers. obs.). Almost certainly, more comprehensive surveys in Sri Lanka would reveal an even greater range of natural enemies that could be exploited for the control of *Dichrostachys* in Cuba.

The fungal pathogens of *R. cochinchinensis* (itch grass) have been evaluated by CABI Bioscience over the past decade and a head smut, *Sporisorium ophiuri* (P. Henn.) Vanky, has been found to be present and damaging on this relatively uncommon plant in Sri Lanka (Evans, 1991). This Sri Lankan strain or pathotype, in addition to other palaeotropical strains, is currently being screened in the UK for possible introduction into Latin America where itch grass (or 'caminadora') has become a highly problematic weed in graminaceous crops, such as maize, sugarcane and upland rice. Finally, the natural enemies associated with Ceylon privet (*Ligustrum robustum* ssp. *walkeri* or 'bora-bora') are currently being studied in Sri Lanka and the UK through a collaborative project with CABI Bioscience, the government of La Réunion and the University of Peradeniya. This plant species was introduced into the Indian Ocean island of Mauritius early in the 20th century, probably as part of a botanical exchange between Peradeniya and Pamplemousse Botanical Gardens, where it rapidly developed into a highly invasive weed and a threat to native forests (Lorence & Sussman, 1986; Cronk & Fuller, 1995). Subsequently, it was taken to La Réunion, probably as a hedge plant, and now poses a major threat to the island's ecosystems, especially montane forest (Macdonald *et al.,* 1991).

These examples clearly demonstrate that non-weedy plant species, once separated from their natural enemies, can become dominant and highly invasive in exotic situations. The vigour, seed production and seedling establishment of *Ligustrum* in La Réunion are all considerably greater than in Sri Lanka (C. Lavergne, pers. comm.), as is the case for *Dichrostachys* in Cuba and *Rottboellia* in Latin America.

Significantly, no coevolved pathogens or insects of these weeds have been identified in their exotic ranges, a striking testament to the pressures exerted by natural enemies.

Inundative biological control

The exploitation of mycoherbicides is still in an early or even experimental phase and, although a relatively large number of pathogens have been assessed, especially in North America, few have been commercialized or are currently on the market.

The constraints to their exploitation have been reviewed recently (Auld & Morin, 1995; Mortensen, 1997; Greaves *et al.,* 1998), and, although sophisticated technology is usually required to produce them (Green *et al.,* 1997), which makes mycoherbicides more suitable for advanced agricultural systems, scientists at the International Rice Research Institute (Philippines) are assessing their potential for use against grassy weeds in rice using low technology production methods (Zhang & Watson, 1997a, 1997b). Such technologies may be relevant to Sri Lanka in the future, especially for the management of problematic graminaceous weeds in rice ecosystems (Watson, 1999).

General Discussion

Sri Lanka has played an important and pioneering role in our understanding of the ecology and systematics of entomopathogenic fungi. The island is rich in these fungi, especially in those regions where humid forests still occur, and, potentially, such ecosystems could be exploited as sources of biocontrol agents of insect pests. Improved technology, particularly in production and application techniques, has made the increasing use of mycoinsecticides, and perhaps of mycoherbicides, more feasible as we move into the next millennium. More importantly, the value of natural control must be recognized so that entomopathogenic fungi can best be exploited in an integrated pest management strategy. There is no doubt that many potential pest species are kept in check by entomopathogenic fungi in humid tropical regions (Evans, 1982). However, the degree of natural control is never obvious, nor indeed appreciated, until the ecological balance is disturbed. Such a scenario can occur when pesticides are applied. The ill-timed application of a fungicide, for example, could favour pest outbreaks by suppressing the entomopathogenic fungi naturally present within the ecosystem. In rice ecosystems in Sulawesi (Indonesia), where no pesticides are now applied, a complex of fungal pathogens can readily be identified on potential insect pests (Evans, 1982, 1988). The author has also observed epizootics of fungal pathogens (*Hirsutella citriformis* Speare; *Metarhizium flavoviride* Gams & Roszypal) on the brown planthopper (*Nilaparvata lugens* Stål; Hom., Delphacidae) in Vietnam, the presence and value of which both the local farmers, extension officers and local scientists were unaware; a clear demonstration of the cryptic but significant role that natural enemies play in regulating populations of this insect, and of how its pest status has been achieved mainly through the misuse of pesticides within rice ecosystems.

Similarly, in Sri Lanka there are many examples of the pivotal role that natural enemies play in regulating not only insect but also plant populations. Plants which have become major invasive weeds in the Neotropics, such as *Rottboellia cochinchinensis* and *Dichrostachys cinerea*, and which are native to but relatively uncommon in Sri Lanka, are undoubtedly held in check within their region of origin by coevolved natural enemies, including fungal pathogens. Conversely, a number of plant species of neotropical origin, such as *Ageratina riparia*, *Mimosa pigra*, *Lantana camara,* and *Eichhornia crassipes*, have become alien invaders within Sri Lanka and may be posing a threat not only to agriculture but also to the flora and fauna, particularly in national parks. The weeds have been introduced, accidentally or deliberately, without their coevolved natural enemies and thus classical biological control offers an environmentally friendly, sustainable and cost effective method for managing such invasives, although this strategy has been underexploited in Sri Lanka and has yet to be attempted with fungal pathogens.

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