CHAPTER III

HYPERPARASITES

1. ECONOMIC IMPORTANCE

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Most reports of hyperparasites suggest that they might be useful for biological control. But hyperparasites are rarely used in the pest management programmes. Hyperparasites have provided biological control of plant diseases in experimental plots.

Hyperparasites can be used to develop control measures. Several attempts have been made to utilise fungi for the control of nematodes such as Meloidogynes which attack pineapple in Hawaii for nematodes parasitize on animals in France and for controlling the golden nematodes of the potato in England. Some encouraging results were obtained but practical utilisation of these fungi in agriculture is still a remote possibility. In Hawaii for control of root-knot of pineapple has been secured by the application of the infected soil of pineapple refuge at the rate of 100-150 tons/acre. The application of organic matter favours the growth and development of nematode trapping fungus. Dactyella bemibicoides which kills the soil nematodes and thus makes the infected soil land for further cropping. Nematode trapping fungi (Cooke, 1968), fungal parasites of cysts (Burshall and Tribe, 1974) and endozoic fungi (Giuna and Cooke, 1974) all have been considered for potential to reduce populations of plant pathogeneic nematodes but results in the field have been disappointing.

Many genera of fungi such as Arthrobotrys, Dactylella, Dactylaria, Harposporium, Nematoctonus have species that predacious on free living or plant parasitic nematodes. They do not form any host parasite relationship with the nematode, but entrap them with their special organs and feed on their body contents.

Ciccinobolus seldom reduces the attacks of the powdery mildews in nature because it rarely occurs in large quantities. Several workers have tried to utilise Eudarluca australis (pycnidial stage is known as Darluca filum) for controlling rust. The fungus does control the disease under certain natural climatic conditions over which man has little control. As discussed by Chester (1946), extra innoculum of the hyperparasites will not be much use as the weather, unless very favourable to the development of this hyperparasite is unlikely to permit any marked control of disease. There are other several fungal species which attack rusts such as Tubercularia species which attack aecia and spermogonia of several rusts and Verticillium hemileiae on Hemiliea vastatrix. It is known that in nature, fungi causing powdery mildews, rusts and ergot are parasitized by hyperparasite like species of Darluca and Cerebella. The control of root rot of tea caused by Armillaria melea has been attempted by use of species of Lasidiplodia in Nysilland.Remarkable control of Rhizctonia damping off of sugarbeets has been recently obtained through the additions of five cultures of Bacillus subtilis to the infected soil, in the U.S.A. (Dunleavy, 1952). Hyperparasites of rust pustules may prevent sporulation or cause the pathogen to form teliospores instead of uredospores (Biali et al., 1972).

Hyperparasites of fungi were first described in the 1800s by mycologists with interest in plant diseases. Most reports of hyperparasites suggest that they might be useful for biological control but hyperparasites are rarely used in pest management programmes. Hyperparasites have provided biological control of plant diseases in experimental plots. An unusual recent example is the control of Claviceps purpurea with Fusarium roseum 'Sambucinum' (Mower et al., 1975) in California. Several other sclerotium forming pathogens are parasitized by fungi. These hyperparasites have controlled plant diseases in special situations. Trichoderma harzianum isolated from decomposing sclerotia of S. rolfsii in green house and field experiments (Wells et al., 1972). In green house pycnidial dust reduced damage by Sclerotium cepivorum on onion plants (Ahmed and Tribe, 1977). The disease potential of Phomopsis sclerotioides, the cause of cucumber black rot in green house soils, was considerably reduced by innoculum of Gliocladium roseum (Moody and Gindrat, 1977).

Dormant sclerotia are killed off by Trichoderma species, Coniothyrium minitans, Fusarium roseum and other fungi (Coley-Smith and Cooke, 1971) and bacteria (Dhingara and Sindair, 1975). Sclerotia with well developed rind are apparently no more resistant to attack than those without a rind.

The protection of wounds or cut surfaces of trees against pathogens using prior colonisation by saprophytes or weak pathogens is the possession principle used to advantage in disease management. Best known is the system developed in England (Rishbeth in Bruehl, 1975). Peniophora gigantea applied to pine stumps protects the stumps against invasion by Fomes annosus. This is the only biological control agent presently registered by the United States Government for use in the United States against a plant pathogen.

Candida species, on the surface of rice leaves caused a remarkable decrease in the number of brown spots induced by Helminthosporium oryzae when innoculated with a mixed suspension of both fungi (Akai and Kuramato, 1968).

Phytoalexins induced by metabolites released by foliar saprophytes, may reduce the germination of pathogen, spores and thus decrease the incidance of disease (Balley, 1971).

A population of Saprobolomyces roseus stimulated to an antagonistic level by exogenous nutrients, reduced infection of wheat leaves by Cochliobolus sativus. Infection by Nigrospora species, inhibits the development of disease induced by Helminthosporium oryzae. The 'lesser' fungi suppress the pathogenic strains as well as by nonpathogenic by virulently pathogenic fungi. Isolates of Trichoderma species were more antagonistic to Rosellinia than to Helicobasidium mompa (Ieki, 1969). Sweet potato sporuts innoculated with a mild strain of the foot rot fungus, Fusarium solani f batatas were protected from subsequent infection by virulent strains of sweet potato wilt fungus, Fusarium oxysporum f batatas (Mc Clure, 1951).

Mycoparasitic fungi are Laetisaria arvalis (Corticum species), a mycoparasite and antagonist of Rhizctonia and Pythium also Sporidesmium sclerotiorum, Gliocladium virens and Coniothyrium minitans and all effectively controlling several of the Sclerotinia diseases. Chaetomium species suppressed Venturia inaqeualis ascospore and conidia production is fallen and growing leaves, respectively. Tubercularia maxima parasitizes the white pine blister rust fungus Cronarticum ribicola, Darluca filum and Verticillum lecanii parasitize several rusts; Ampelomyces quisqualis parasitize several powdery mildews; Tilletiopsis species parasitizes the cucumber powdery mildew fungus Sphaerotheca simplex parasitize two pathogenic species of Alternaria.

Heterobasidion annosum, the cause of root and butt rot of conifers controlled by Peniophora gigantea. Cladosporium herbaceum and Penicillium species controlled by Botrytis cinera.

Hyperparasites or mycoparasites are very common in members of uredinales and erysiphales. In the nature these hyperparasites sometimes control or suppress rust and powdery mildew diseases. Cladosporium species is a parasite and Puccinia conspicua and Darluca species also parasite uredosori of leaf rust of poplars due to Melamspora larici-populina. Hyperparasites play a limited role in reducing epidemics of rusts in plants (Darpaux, 1937). Darluca filum caused a significant reduction in the innoculum potential of race 6AF of oat stem rust, Puccinia graminis f sp. avenae and consequetly lower infection of the host. Cladosporium species decreases the viability of uredospores of Melamspora M. larici-populina and also causes lysis of germinating rust spores. Innoculating half the lamina of Populus nigra variety italica (popular common in Australia) with a mixture of conidia of Cladosporium and viable uredospores of the rust and the uredospores alone. Cladosporium species, also occurs in various powdery mildews as hyperparasites Phyllactinia dalbergiae is reduced by Cladosporium spongiosum.

Gaeumannomyces graminis (Ophiobolus graminis) was reduced by Didymella exitiols. Sclerotia of Sclerotium trifoliorum and S. cepivorum controlled by C. minitans. Sporidesmium sclerotivorum parasitized Sclerotium cepivorum and S. minor and reduced disease development (63-83 %). Phomopsis sclerotioides was controlled by Gliocladium roseum.

Suppressive soils contain bacteria that lyse hyphae inhibit zoospore production and breakdown sporangia. The fungus is not penetrated by the bacteria until lysis has occurred. However, bacteria are attracted to sporangia and cause sporangial breakdown. Since these bacteria multiply and produce considerable bacterial slime on the host, they should be called Nectrotrophic hyperparasites, rather than ontagonistic organisms.

In Western Australia, some soils suppress *Phytophthora cinnamoni* and in these soils a highly susceptible host, *Eucalyptus marginata* will survive and grow even though the pathogen is present (Broadbent and Baker, 1974-a,b; Malajozuker *et al.*, 1977). On the subject of hyperparasites there has been some good news and some bad news. The good news in phytopathology is that hyperparasite or another is recorded in the literature. Many of these hyperparasites have an impact in some microniches, but their effectiveness is limited. The bad news is that hyperparasites have not been effective over the broad range of their hosts.

2. USEFUL ATTRIBUTES

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The following attributes would be useful to increase the probability of hyperparasites providing biological control.

1.) A Capacity for Rapid Dissemination :

Species of Trichoderma, Gliocladium, Fusarium are ideal because they grow rapidly and sporulate profusely under a variety of environmental conditions. The capacity also makes them more visible and they are frequently reported as hyperparasites. If spores can be used, this type of fungus can be quite effective (Wells et al, 1972). A concentration of 600 spores/ml of Fusarium roseum 'Sambucinum' controlled Claviceps purpurea. Several companies have development work underway to provide Trichoderma innoculum for biological control, either as spores or as mycelium on diatomaceous earth granules (Backman and Rodriquez - Kabana, 1975). Trichoderma harzianum was grown in a sand seed medium and broadcast at the rate of 15,000 Kg infected media/ha (Wells et al, 1972).

Unfortunately, many hyperparasites grow very slowly in culture. This makes innoculum production for field applications difficult. Tuberculina, Darluca and Scytalidium uredinicola, 3 slow growing hyperparasites of rust fungi, are difficult to propagate in quantity. Adult insects could facilitate dissemination, since they can locate the target fungus. Because insects, amoebas and nematode require conditions for propagation very different from the fast growing fungi, co-operative research with entomologists, zoologists and nematologists would be needed. However, the speed of hyperparasites spread within the parasite population is an important consideration.

2) Infection of Parasite Throughout its Ecological Range :

Often a hyperparasite that devastates its host in culture or under greenhouse conditions is far less effective under field conditions, because its ecological range is more limited than the hosts. The bacteria that suppress P. cinnamoni are limited to soils with high levels of organic matter whereas P. cinnamoni is not limited by the organic content of soils (Broadbent and Baker, 1974a). Darluca filum prevented telium formation by 99% of the Cronartium steobilinum sori on dwarf leaf oak ground cover. Under these conditions the hyperparasite infected 93% of the sori. In an adjacent less dense stand, the hyperparasite infected only 32% of the rust sori and 26% of the sori produced telia (Kuhlman et al,1978). In the Claviceps purpurea - Fusarium roseum 'Sambucinum' relationship parasite and hyperparasite apparently have similar ecological requirements in California. Sprinkler irrigation facilitated infection by both the parasite and hyperparasite (Mower et al, 1975), whether these ecological requirements would be similar over the broad range of the host and parasite has been explored.

3) Early Attack Before Demage by the Parasite :

The ideal hyperparasite attacks the pathogens infective propagule before it can invade the host. Infection of sclerotia by Trichoderma and Gliocladium eliminates the infective propagule (Wells et al., 1972; Moody and Gindart, 1977). Bacteria in suppressive soils reduce the Puccinia cinnamoni innoculum by hyphal lysis, breakdown of sporaangia and chlymadospores and prevention of zoospore differentiation (Broadbent and Baker, 1974a,b; Malajczuk, et al., 1977). Similarly Darluca filum parasitizes uredospores of Puccinia graminis before they are released from the sorus (Carling et al., 1976). However, infection of the oak host by C. fusiforme is not reduced by simultaneous innoculations of the oak with the parasite and the hyperparasite Darluca filum (Kuhlman et al., 1978).

An exception to this need for an early attack is the Fusarium on Claviceps purpurea because control of products (fungus signs) of the infection not the infection per se was the goal of this biocontrol (Mower et al., 1975). Besides being highly aggressive as a hyperparasite, the Fusarium also broke down ergotamine to psychotropically inert substances. Thus, the most harmful effect of the infection was negated.

4) A Capacity of Kill the Parasite :

Many hyperparasites, however, seem to be associated with the declining stage of the disease cycle. If the hyperparasite is active only during the late stages of the disease cycle, its role may simply be to speed up senescence e.g. Tuberculina maxima. Wicker and Woo (1973) suggested that T. maxima parasitizes the white pine tissue and not the mycelium of C. ribicola. The effect on the parasite would be indirect, since T. maxima would kill the pine cells which in turn would kill the rust. In galls of C. fusiforme pine tissue was similar in T. maxima infected and uninfected tissue (Kuhlman and Miller, 1976), and sporulation by both parasite and hyperparasite occurred for successive season in infected tissue (E.G.Kuhlman, unpublished data), whichever is the host of T. maxima, it is generally agreed that aecial sporulation is reduced but that the rust mycelium persists especially at the gall margin (Powell, 1971b; Vander Kamp, 1970; Wicker and Woo, 1973). Individual plant parasite nematodes may be killed by hyperparasite but entire populations are seldom reduced below the destructive level. Susceptibility of Lovell peach root stock of root knot nematode (Meloïdogyne species) led to its replacement in most areas of California 25 years ago.

Biological control was considered and to be one explanation for the low numbers and an examination indicated a new fungus hyperparasite, Dactyella oviparasitica, was present. The nematode egg masses, the primary substrate for the fungus, were conveniently aggregated on the root surface, enabling the hyperparasite to utilise the entire mass. The fungus also infected adult female nematodes and prevented them from reproducing.

Recently, low numbers of root knot nematodes were found in several old orchards established on Lovell root knot stock (Stirling et al., 1978).

Therefore, the innoculum potential of the nematode remained high on the latter hosts even with the hyperparasite present.

5) Non-Pathogenic to Higher Plant Host :

Ampelomyces quisqualis is a hyperparasite of powdery mildews which reduced powdery mildew infection by Sphaerotheca fuligina and increased fruit production over a non-treated check (Jarvis and Slingsby, 1977). However A. quisqualis also caused on angular leaf spot and long streak lesions on the fruits. The pathogenecity of T. maxima is less economically damaging to the host and can be beneficial if branch galls are prevented from reaching the stem. The failure of T. maxima to advance to the margin of the rust infection suggests this benefit may not be realised.

Mower et al (1975) tested the pathogenecity of Fusarium roseum 'Sambucinum' to wheat and found it to be non-pathogenic. However, Fusarium roseum and F. tricinctum have been shown to be secondary parasites or facultative parasites on snapdragon (Dimock and Baker, 1951). These Fusaria entered the host through rust infections when the plants were held under high moisture conditions. Damage to the host was considerably greater than that due to the rust alone.

Introduced hyperparasites can have long term advantages. Although, a balanced system of hyperparasites, a parasite and host has been discussed, long term persistence of the hyperparasite may be accomplished through the secondary hosts, a saprophytic stage or dormant spore stages Dactyella oviparasitica utilises dead roots and the eggs of several free living and plant parasitic nematodes. Because it can persists when Meloidogyne populations are low. Hyperparasitic bacteria were most abundant in soils rich in organic matter. This finding suggests that the bacteria can be saprophytes or have other hosts that utilise organic matter. A major obstacle to using C. fusiforme is the absence of a mechanism for long term persistence (Kuhlman et al., 1978). Uredial sori are present only briefly and if infection by the hyperparasite occurs, the leaves are shed months before new rust infection occur.

6) A Capacity to Persist :

Unlike most chemical agents, hyperparasites have the potential for long term persistence without harmful residues. The hyperparasite has usually persisted in a balanced relationship with the target parasite is present in the environment, but the hyperparasite limits the ability of the parasite to cause disease. Thus, in suppressive soils P. cinnomoni is present but the long term persistence of the hyperparasitic bacteria renders it non-pathogenic. A dynamic living system once established can continue to protect the crop through susceptible periods. Secondary spread of Fusariium roseum 'Sambucinum' controls Claviceps purpurea infections that the initial spore application misses. In Italy, hypovirulence agents persists in E. parasitica in perennial infections that take on a gall like form because the cambium is not killed (Mittempergha, 1979). Since the virulent forms of E. parasitica have persisted for decade in the United States (Kuhlman, 1979), hypovirulence agents may have a similar capability.

The quest for useful hyperparasites will continue for several reasons. First, they are an attractive means of disease management. Second, the likelihood of insulting the environment is reduced and finally, pathologists will remain alert for changes in disease cycles that are caused by hyperparasites.

Hyperparasites often reveal their presence through changes in the pathogen population in epidemic areas e.g. *Fusarium* on *Claviceps* and *T. harzianum* on *S. rolfsii* were discovered in this way (Mower *et al*., 1975; Wells *et al*., 1972). Hyperparasites of the *Cronarticum* rust have been identified (Kuhlman and Miller, 1976; Kuhlman *et al*., 1976, 1978; Powell 1971 a-c; Wicker and Woo, 1973). Similarly, hyperparasites of dwarf misteloe, *Arceuthobium* species have been found through searches for inexpensive means of controlling this insidious parasite (Muir, 1977). Unfortunately, most of the hyperparasites identified in studies of this kind do not provide significant control of the parasite because the hyperparasites are sufficiently aggressive. Many hyperparasites become effective only after their hosts enter the declining stage of an epidemic or else the signs of the hyperparasite are more obvious than are its effect on the disease.

A recent approach to discovering effective hyperparasites has been through surveys that look for the absence of disease in the presence of host and pathogen and then try to discover the reason for it. The suppressive soils in Australia were discovered in surveys of the incidence and severity of *Phytophthora* root rot in avacado grooves (Broadbent and Baker, 1974-a). The fungal hyperparasites of root knot nematode was also discovered from a disease survey (Stirling *et al*., 1978).

Hypovirulence in E. parasitica was discovered when trees did not die following infection. If plant pathologists make greater use of disease survey to find hyperparasites, we may discover that the fauna is playing an important role too. In areas where C. comandrae is abundant a multitude of mycetophagus animals feed on rust spores and cankers. Insects damaged 41-62 % of the cankers and reduced aeciospore production by 10% (Powell, 1971.c). Giant amoebae can perforate the cell walls and digest the contents of conidia of Cochlibolus sativus and Thieloviopsis basicola (Anderson and Patrick, 1978; Old, 1978). The melanised cell wall of C. sativus had been considered especially persistent to degradation. The environment limits many hyperparasites to a small portion of the parasite population. In soils suppressive to P. cinnamoni, hyperparasite are favoured by high level of organic matter.

Broadbent and Baker (1974-a)suggested that the avacado grower had created a soil environment similar to naturally suppressive rain forest soils by the addition of fowl manure, dolomite and synthetic fertilisers and the expensive use of cover crops. The speed with which these changes can be made and the economics of this type of disease management have been investigated. So the biology of hyperparasitic relationships offers unlimited possibilities for research requires further documentation of its occurrence and potential for success.