PERSPECTIVE-PROSPECTO

PHYTONEMATODE PATHOLOGY: FUNGAL MODES OF ACTION. A PERSPECTIVE

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Accepted:
8.III.1985
Aceptado:

ABSTRACT

Morgan-Jones, G., and R. Rodriguez-Kabana. 1985. Phytonematode pathology: fungal modes of action. A perspective. Nematropica 15:107-114.

The likely involvement of opportunistic soil fungi in cyst and root-knot nematode population dynamics is discussed. Such fungi are thought to play an important role in inducing suppressiveness in agricultural soils. Possible modes of fungal activity are broached, particularly the effects of fungi on cysts and eggs. Both enzymatic and exopathic diffusible toxic effects are considered possible, in addition to direct physical disruption brought about by invading hyphae. Factors regulating vulnerability to invasion, and thereby destruction, are reviewed.

Additional key words: biological control, pest management, nematode ecology.

RESUMEN

Morgan-Jones, G. y R. Rodríguez-Kábana. 1985. Patología de los fitonematodos: manera de actuar de los hongos. Prospecto. Nematrópica 15:107-114.

Se considera la posibilidad que la dinámica poblacional de los nematodos enquistadores y de las agallas dependa de las actividades de hongos "oportunistas" del suelo. Se cree que estos hongos tienen un papel importante en el dessarollo del fenómeno de supresión en suelos agrícolas. También se discuten varias y posibles maneras de actuar de los hongos y en particular sus efectos sobre las estructuras reproductivas de los nematodos especialmente sobre quistes y huevos. Se considera que son posibles efectos de los hongos sobre los nematodos causados por las actividades enzimáticas o de caracter exopático y difusible de los mismos asi como también efectos disruptivos debidos al contacto físico de las hifas al invadir los hongos los nematodos. También se discuten factores que gobiernan la vulnerabilidad de los nematodos a la invasión y por ende a la destrucción por los hongos.

Palabras claves adicionales: combate biologico, manejo de plagas, ecologia de nematodos.

INTRODUCTION

Recognition of the widespread occurrence of suppressive soils, where populations of plant-parasitic nematodes are held in check or reduced, has stimulated a renewed search for the mechanisms involved in natural disease control (5,26). As the phenomena which bring about suppression are elucidated, ability to enhance biocontrol of disease-inducing organisms by manipulation of the soil environment should become increasingly possible.

Interactions between fungal antagonists and nematodes in agricultural soils have been known for many years (1,12,13,17,18). Nematode-destroying fungi are common and abundant in various soils and, although their total contribution to soil biology is not yet fully understood, they undoubtedly play a role in maintaining the balance of microbial life (1,17). Fungi capable of destroying nematodes consist of a wide variety of organisms, both free-living and obligately parasitic. They range from the common, trapping, so-called predacious fungi which are principally parasites of vermiform, free-living nematodes to obligate, endoparasitic species and facultative opportunistic fungi which colonize cysts and eggs of phytonematodes.

The need for increased knowledge of interactions between phytonematodes and other soil organisms, of which antagonistic activity forms an integral part, has been felt as biological control potentials are assessed. It is essential to document interactions before successful attempts at suppressing undesirable elements can be made with consistency. This is especially the case where specific microorganisms, considered to have control potential, are introduced into the soil. Likewise, it is important that information be gathered on the peculiarities of organisms used as biocontrol agents including competitive ability, growth requirements, enzymatic capacity, and capability of biosynthesis of toxic or antibiotic metabolites.

The soil must always be recognized as an extremely complex environment where many modifying influences and varying parameters are operating. Some control of phytonematodes has been achieved by the introduction of certain types of organic amendments to soil (9,19,25). The precise mechanism by which this control is achieved is not, however, fully understood. The release of toxic decomposition products or promotion of antagonistic microbial populations, or a combination of both, is thought to possibly bring about this effect. Rodriguez-Kabana *et al.* (25) suggested that reduction in phytonematode numbers following chitin amendment of soil was, at least in part, the result of the presence of fungi having chitinolytic capacity. Although involvement of fungal antagonists in cyst and root-knot nematode population dynamics has not been established unequivocally, much evidence points in that direction. Kerry (14) and Kerry *et al.* (16) have shown that when the activity of fungi known to be parasitic on females of *Heterodera avenae* Wollenw. is controlled by formalin, nematode numbers increase. It is reasonable to assume that if populations of antagonistic organisms, such as fungi, are greatly increased, a biocontrol influence may become apparent. The role and possible modes of actions of fungi which colonize cysts and eggs form the subject of this paper.

Nematodes belonging to the family Heteroderidae Skarbilovich are vulnerable to attack by fungi at sedentary stages of their life cycles either within root tissue or, more frequently, when exposed in the rhizosphere or in the soil. In the genera *Heterodera* Schmidt and *Globodera* Mulvey and Stone, obese females breaking through the root cortex become increasingly susceptible to attack, as do emerging egg masses of the genus *Meloidogyne* Goeldi (7). In the rhizosphere, where fungal growth is stimulated and enhanced by root exudates, the likelihood of fungal invasion is increased. Cysts and eggs released into the soil, where they may remain for a lengthy period of time before larval hatching, are additionally vulnerable.

That fungi colonize the reproductive structures of plant-parasitic nematodes, especially cysts and eggs, has been known for some time. The first record of a parasite of cyst nematodes was made in 1877 by J. Kühn, who discovered *Catenaria auxilliaris* (Kühn) Tribe, a fungus destructive to females of *Heterodera schachtii* Schmidt. Thorne (27) made the seminal statement that "cysts containing dead eggs or larvae usually are filled with fungus mycelia". Tribe (28,29) provided reviews of the involvement of fungi in the pathology of cyst nematodes. Since that time a number of researchers have reported the occurrence of fungi in association with females, cysts, and eggs of cyst and root-knot nematodes (6,7,10,15,20,21). Some of those attacking females, including three zoosporic fungi belonging to the division Mastigomycota, appear to be obligate parasites while the majority implicated in destruction of eggs are opportunistic fungi whose precise activity vis-a-vis parasitism is not altogether clear. An *a priori* assumption that parasitism is involved cannot, however, be made in many instances since it appears that some physiological disorder of eggs is a prerequisite to invasion. These opportunistic fungi, while taxonomically diverse, have common biological ability to penetrate and colonize nematode eggs. They are relatively restricted in number in any given situation compared with the total soil mycoflora, indicating some measure of specialization and possession of peculiarities enabling them to exploit an unique ecological niche. Most of the fungi belong to the sub-division Dueteromycotina, a few to the Ascomycotina. In surveys conducted by ourselves and others in various geographical locations, much the same sort of fungi occur in association with cysts and eggs, again indicating specialization (6,7,10,11,20,21, 24,28).

MODES OF ACTION

The relationship between fungi and nematode hosts is likely multidimensional and variable. The main types of activity likely to deleteriously affect the reproductive phases of the nematode life cycle are enzymatic disruption of structural elements, and physiological and metabolic disturbances brought about by biosynthesis and transfer of diffusible toxic substances by the fungi (22,23). Additional effects might involve indirect modification of the environment through the presence of fungal biomass; for example, by depletion of oxygen supply available to juveniles. In any particular situation the fungal mode of action leading to a demonstrable detrimental effect on the nematode will depend on such factors as stage of nematode development (especially in the case of cysts and eggs), amount of proximal fungal mycelium, and ability of individual fungi to institute change exogenously by production of translocatable metabolites, either enzymatic or toxic, or to colonize and thereby disrupt endogenously directly. The nature of a sedentary female, a cyst, or an egg as a host varies considerably according to its state of development. The cyst as a protective entity is only partly successful in shielding the eggs since fungal hyphae can readily enter through the natural openings remaining from its life as a female. Once inside a cyst, fungal biomass may increase due to the available nutrients present from decomposition of the internal organs of the female following egg differentiation.

Whether within a cyst or in an extruded mass enveloped by a gelatinous sac as in *Meloidogyne,* eggs are frequently exposed to, and are often in relatively close contact with, fungal hyphae. Although an egg is probably the most resistant stage in a nematode's life cycle to natural environmental stress, some fungi appear to be able to overcome the high degree of protection afforded the developing larvae by the shell. Eggs at varying stages of development have different degrees of vulnerability (Morgan-Jones, unpublished data). At an early stage, when the egg is filled with undifferentiated granular material and a central nucleus is apparent, it is in its most vulnerable condition. The egg shell, at this stage of development, appears to be more easily penetrated by fungal hyphae, presumably because its various layers are not fully elaborated. A fungal hypha coming into contact with a very young egg is able to forcibly buckle the shell and to eventually penetrate by a relatively wide, irregular rupture (22, Morgan-Jones, unpublished data). As the egg matures and cleavage of its contents begins, hyphae cannot easily pierce the egg shell and do so only at a minute locus in the form of a penetration peg (23). Our *in vitro* experiments have clearly indicated that young eggs are more readily colonized than older ones, particularly those containing a fully-developed larva. It is not known if enzymatic weakening of the egg shell preceeds hyphal penetration but this is more likely the case in mature eggs.

The ability of eggs to withstand adverse environmental effects depends, in large part, on the impermeability of their shells. Bird and McClure (3) in their studies on the structure, composition, and permeability of the tylenchid egg shell, report permeability to be related to the lipid layer which is protected from physical and chemical stresses by a narrow vitelline layer and a broad chitin layer external to it. In young eggs at the amorphous granular stage of development, it is not known if the highest degree of shell impermeability has been achieved. In relation to fungal antagonism this is critical. Should diffusible toxic metabolites reach such eggs and inward seepage occur, some physiological disordering could be expected, even leading to abortion of embryonic development. The same effect could be achieved, irrespective of stage of egg maturity, should the permeability of the shell be affected by chemical means, specifically the enzymatic hydrolysis of key elements in its composition. In this regard it is perhaps important to note that many of the fungi that have been demonstrated to be consistently associated with the pathology of phytonematodes have in common the characteristic of being chitinolytic (8). It seems possible that a combination of enzymatic activity and inward movement of toxic substances could be involved in bringing about a diseased condition. Tribe (28) and Morgan-Jones and Rodriguez-Kabana (20), have noted many cysts to contain substantial numbers of lysed, shrivelled, coagulated, or decayed eggs. Frequently no fungal hyphae could be observed within such eggs. In cysts where eggs are found in this condition fungal mycelium is often present, strongly suggesting the operation of an exopathic effect where eggs are damaged without any physical disruption of the shell and penetration by hyphae.

Evidence exists of biotypic variability in capacity to destroy eggs, either by hyphal colonization or through a toxic predisposing effect, among some fungal species. Experiments have shown that some isolates of *Fusarium oxysporum* Schlecht. obtained from cysts of *H. glycines,* for example, can effectively parasitize eggs of *Meloidogyne arenaria* (Neal) Chitwood *in vitro* while others derived from the same source fail to do so (Morgan-Jones and Rodriguez-Kabana, unpublished data). Given that a cyst is frequently occupied by only one fungus (21) the possibility that some biotypes may render a protective function should not be overlooked. That would occur if their preoccupation of the ecological niche prevented entry by other, perhaps destructive biotypes or by destructive separate species.

Our *in vitro* studies have shown the presence of some fungi to have a stimulatory effect on larval hatching, others to have an inhibitory effect. Hatching in at least some of the Heteroderidae is known to occur in response to stimuli provided by exudations from roots of host plants. Some nematodes, such as *Globodera rostochiensis* (Wollenw.) Stone, respond to a narrow range of hatching factors (4) while others, such as *Heterodera schachtii,* hatch in response to a wide range of hatching agents (2). Nematode egg hatching factors are known to induce the larvae to secrete enzymes which weaken the shell, thereby easing the process of emergence. Such enzymes also undoubtedly alter the permeability of the shell since the lipid layer, being proximal to the larvae, is the first affected. Pre-emergence larval movement may also emulsify this layer, again altering permeability. Inward seepage of toxic metabolites may, in fact, induce increased pre-emergence larval movement, thereby indirectly bringing about greater permeability. In the case of hatching regulation, the nature of fungal metabolites to which a larva is exposed will determine the outcome. Should a strongly toxic metabolite enter the egg shell, death would soon follow, resulting in no hatching. Should a metabolite be non-toxic but serve as a hatching agent, enhanced larval emergence, perhaps prematurely, would ensue. Once a larva emerges from an egg, especially if this were to occur prematurely, it is potentially vulnerable to incapacitation by fungal metabolites should a mycobiomass be present proximally. All told, a number of biochemical interactions between fungi and nematode eggs seem possible without direct parasitism involving invasion of the eggs by fungal hyphae. The total effect probably involves disruption of embryonic development and the demise of the larva resulting in inability of the nematode to reproduce.

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Received for publication:
24.IX.1984
Recibido para publicar: