Past and present of the nematode *Radopholus similis* (Cobb) Thorne with emphasis on *Musa*: a review

Pasado y presente del nematodo *Radopholus similis* (Cobb) Thorne con énfasis en musáceas: una revisión

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ABSTRACT

This review presents a historical background on different aspects of the nematode *Radopholus similis* (Cobb) Thorne, an important pathogen of *Musa*, black pepper and anthurium. It examines the history of its discovery and its name, new approaches to its taxonomy and its genome, and the process of dissemination and pathogenic variability. Finally, it documents the evolution of the control measures in banana, from chemical control to bio-protection and managing the habitat of the pathogen.

Key words: history, taxonomy, pathogenicity, management.

RESUMEN

En la presente reseña, se hace un recorrido histórico sobre diferentes aspectos del nematodo *Radopholus similis* (Cobb) Thorne, importante patógeno de musáceas, de la pimienta negra y del anturio. Se analiza la historia de su descubrimiento y de su nombre, los nuevos planteamientos sobre su taxonomía y su genoma, su proceso de diseminación y su variabilidad patogénica. Finalmente, se documenta la evolución de las medidas de control en banano, desde el control químico hasta la bioprotección y el manejo del hábitat del patógeno.

Palabras clave: historia, taxonomía, patogenicidad, manejo.

Introduction

Radopholus similis (Cobb), with the name "nematodo barrenador" in Spanish and burrowing nematode or "Fiji banana-root nematode" in English, is one of the ten most important phyto-helminths in the tropics (Haegeman *et al.,* 2010). Known in the past by many names (Luc, 1987), it parasitizes more than 250 species of plants (Haegeman *et al.,* 2010), and because of geographic expansion, especially in the second half of the twentieth century, has become a major pathogen in banana (*Musa* sp.), causing the so-called "blackhead banana disease", "banana toppling disease" or "pourriture vermiculaire du bananier." Similarly, it devastated the cultivation of black pepper on an Indonesian island in the early 1930s (MacGowan, 1982; Ramana and Eapen, 2000; Thorne, 1961) and currently has a high position in the ranking of important pathogens of ornamental plants such as *Anthurium*, *Calathea* and *Dracaena* (Uchida *et al*., 2003). Therefore, this review relates the story of the discovery and origin of this pathogen, the ups and downs of its official name, as well as some of its newly discovered biological attributes, and the evolution of management strategies in *Musa*.

Origin and spread of *R. similis*

The discovery, the first morphometric description, and the first names of this nematode were the fruits of research by the American Nathan Cobb August in Australia after the pathological examination of banana roots collected in Fiji in 1891, of the roots of sugar cane of Hawaii, and bananas from Jamaica in 1915, which allowed him to articulate three species that in the end were the same: *Tylenchus similis, T. granulosus and T. biformis* (Cobb, 1915; Luc, 1987). Between 1934 and 1936, Filipjev placed this pathogen in four genera (*Tylenchorhynchus, Bitylenchus, Rotylenchus and Tetylenchus*) (Esser *et al*., 1984; Luc, 1987) until Thorne (1961) proposed the genus *Radopholus* in 1949 - ("Lover of roots") - in order to "accommodate five didelphic species that resemble *Pratylenchus*," keeping *R. similis* as the type species. This new term gave rise to two other genera: *Radopholoides* and *Neoradopholus* (now invalid).

The continued exploration of soil nematodes has helped not only to expand the universe of species of this taxon, but also to speculate about their origin. Luc (1987) developed a list of 25 species of this genus, of which over 60% was discovered in Australia- lending support to the

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widespread hypothesis of an Australian origin (Stirling and Pattison, 2008), which in turn is being replaced by the current thesis of a Southeast Asian connection. In this sense, Quénéhervé (2009) suggested that *R. similis* was probably introduced into that Oceania territory from plants of *Musa* in the 1860's, being rather Melanesian in origin, ie from the Fiji Islands and New Caledonia to Papua New Guinea. Marin *et al*. (1998) were in favor of the Indo-Malayan region as a center of origin of the most important hosts of *R. similis*, and in the opinion of Tan *et al.* (2010), Australian populations of the burrowing nematode were "almost certainly" introduced because they share a common global haplotype, which came from Southeast Asia. The inventory of the genus has continued to grow: ten new species of *Radopholus* discovered in the last fifteen years have been found in Vietnam (3), New Zealand (4), Australia (2) and Indonesia (1), although *R. similis* has not been reported in the first two countries.

However, the current pandemic must have been the result of anthropogenic introduction and transoceanic movement of infected *Musa* corms, to the extent that the burrowing nematode is found in several South American countries, the U.S., Central America, the Caribbean, Southeast Asia, Europe and parts of Africa where it was not present before 1960 (Quénéhervé, 2009). Its entry into the New World may have been begun in 1516 (on La Española island) or better yet by Martinique in the 1830s, but its main dissemination took place after 1960 with the planting of banana cultivars of the Cavendish subgroup (*Musa* AAA), resistant to Panama disease (*Fusarium oxysporum* f.sp. *cubense*) but susceptible to the burrowing nematode (Marin *et al*., 1998). As for Colombia, subsistence *Musa* crops have been registered at least since 1880, seven years later, banana plantations started in the department of Magdalena, and in 1968, the burrowing nematode-tolerant Gros Michel variety led to the planting of Cavendish in Urabá (Augura, 1978), which would presumably coincided with the introduction of this parasite. Now, the nematode is found mainly in these two banana-producing areas in Colombia, and in some non-banana regions, such as Caldas, Quindio, Cauca, Valle del Cauca and the Llanos Orientales, in association with the plantain.

A member of Pratylenchidae?

Based on morphology studies, Filipjev *et al*. (1941) placed the genus *Radopholus* in the family Hoplolaimidae, unlike the popular scheme of Thorne (1961) which placed it in the family Pratylenchidae. Allen and Sher (1967) invalidated this group and moved *Radopholus* to the Tylenchidae family, and created the subfamily Radopholinae, a taxon not validated by Luc (1987) but by Siddiqi (2000) and Golden (1971) that reannexed it to Pratylenchidae - already restored. The closeness of this taxon with the spiral nematode, created in 1941, was hosted by Siddiqi (2000) when he relocated it to the superfamily Hoplolaimoidea with the family Pratylenchidae, and now this hoplolaimoid nexus is supported by molecular analysis (Subbotin *et al.,* 2006; Haegeman *et al*., 2010).

Intraspecific variability

Intraspecific variability of *R. similis* has been studied based on morphometric, physio-biological and molecular markers. Leaving aside the unsubstantiated hypothesis of the alleged relationship between the shape of the tail of the female and the aggressiveness of the pathogen, the morphometric analysis of *R. similis* specimens from three continents, seven countries and five hosts was able to confirm the suspected morphological variation, females being shorter in banana from Costa Rica than from black pepper of Indonesia and males being longer in European crops of *Philodendron* (Elbadri *et al*., 1999). The researchers inferred that the Costa Rican and Cuban populations are more similar to each other than the Panamanian population which deviates by having a longer body and tail and with the excretory pore far from the head. Furthermore, if these results are collated with those of Múnera *et al.* (2010), it follows that the females in plantain from Los Llanos, Orientales and banana in Urabá, Antioquia, very similar in morphometry, are on average shorter than the three aforementioned American populations, drawing attention to the case of Panama females measuring 681.8 μ on average (Elbadri *et al*., 2002) and females of Colombia with a length of 594-599 μ (Múnera *et al*., 2010).

In physiological terms, of parasitism and geographic spread, since 1956, it raised the existence of citrus race in Florida, which is responsible for the disease "spreading decline of citrus", and the banana race, cosmopolite and polyphagous (Esser *et al*., 1984; Gowen and Quénéhervé, 1990). These two types were renamed *R. citrophilus* and *R. similis*, by the cytogenetic differences, and it is now known that there is gene flow between these variants, which share a single karyotype (n = 4), and not two as previously reported (Gowen and Quénéhervé, 1990). Therefore, the original concept of a single valid species returned- *R. similis*, with two pathotypes, races or subspecies, *R. similis similis* and *R. citrophilus similis* (Elbadri *et al.,* 2002; Luc, 1987; Siddiqi, 2000).

This species, with about 3200 genes and a mitochondrial genome with unique features (Jacob *et al*., 2008), is not homogeneous at the molecular level, which seems to suggest that it could be a complex species (Múnera *et al*., 2010). Both Fallas *et al.* (1996) and Elbadri *et al.* (2002) detected molecular variation between isolates of the pathogen that could be divided into two groups. According to Fallas *et al.* (1996), isolates from Costa Rica, Nigeria and Cameroon belong to a different genomic than the isolated group of Guinea, Guadeloupe, Ivory Coast; while the majority group conceived by Elbadri *et al.* (2002) included isolates from Costa Rica, Cuba, Australia and Guinea but not those of Indonesia, Sudan and Belgium that formed another group. Overall, a fixed and invariable relationship between genomic similarity and pathogenic homogeneity or virulence within the same group has not established. On the other hand, Cuban populations of *R. similis* reached a high population density in roots of the banana 'Grand Naine' at 60 d after inoculation, while Brazilian and Costa Rica populations reached equivalent levels at 90 and 120 d, respectively, indicating that the Caribbean population starts to damage banana more quickly (Costa *et al*., 2008). In the Philippines (Herradura, 2009), as in Australia (Cobon, 2003) and Brazil (Costa *et al*., 2008), two isolates of the burrowing nematode were detected with different levels of aggressiveness, and the populations of Honduras and Belize had a lower reproductive rate than that of Costa Rica (Marin *et al.,* 1999).

Biology and disease cycle

This migratory and generally amphimictic endoparasite fulfills its life cycle in 20 to 25 d at 24 to 32°C (Gowen and Quénéhervé, 1990; Haegeman *et al.,* 2010) and has abundant genes for reproduction, development, parasitism and survival (Haegeman *et al*., 2010). Except for the first juvenile stage (L_1) and the male, the other states are infective, especially in the female (Gowen and Quénéhervé, 1990; Quénéhervé, 2009). Rapid penetration in the rhizodeposition area of banana is supported by the four endoglucanases discharged for the degradation of cellulose and endoxylanase responsible for the degradation of xylan hemicellulose (Haegeman *et al*., 2010). The endoglucanases transcripts were found in the esophageal glands (Haegeman *et al*., 2010), which supports the conjecture of Cobb (1915) who referred to the discharged "pathogenic" products as the "three salivary glands." Three endoglucanases genes are not expressed in the male, while in the female there is complete gene expression, but both sexes discharged endoxylanase (Haegeman *et al*., 2010). In any case, the infective states penetrate the deep layers of the root with little effect on the central cylinder, empty the contents of the cortex parenchyma cells that develop into cavities and tunnels, and at the same time, the necrotic tissues acquire a reddish and finally blackish tone (Gowen and Quénéhervé, 1990).

In the absence of bananas, this parasite survives in weeds such as *Caladium bicolor*, *Commelina diffusa*, *Echinochloa colona* and *Phenax sonnerati*, which are efficient reservoirs equal to *Musa* (Quénéhervé *et al.,* 2006) and in the absence of hosts, survives in the soil for more than six months without becoming anhydrobiotic as in *Pratylenchus* (Chabrier *et al*., 2010a; Chabrier *et al*., 2010b), with males surviving longer than females and juveniles (Chabrier *et al*., 2010b). Under these conditions, the male ensures fertilization without competing with the female for food (Chabrier *et al*., 2010b), which survives by reproducing through hermaphroditism and/or perhaps using metabolites supplied by the bacterial endosymbiont *Wolbachia,* common in filarial nematodes that usually infect arthropods but not phyto-helminths (Haegeman *et al*., 2010).

Damage indicators

Quénéhervé (2009) stated three successive levels of damage, all associated with lengthening of the vegetative phase: a) mild and insignificant damage, b) other obvious effects in terms of bunch production of poor quality and reduction of harvest, and c) irreversible damage with reduction of harvest, destruction of plants and shortening the longevity of the crop. To address this issue, we must take into account the injury rate, which bears some relation to the proportion of dead roots and reflects the functionality of the root system and the risks to toppling of the plants (Peng and Moens, 2003). The primary roots taken close to the pseudostem (Bridge and Gowen, 1993) at a depth below 30 cm (Pattison *et al*., 2011) should be considered for evaluation based on a system of five classes (Bridge and Gowen, 1993; Moens *et al*., 2001): 0 = no necrotic roots and undamaged, 1 = less than 25% of roots with necrosis, slight damage, 2 = 26 to 50% of roots with necrosis, moderate damage, 3 = 51 to 75% of roots with necrosis, severe damage 4 = more than 75% of roots with necrosis, very severe damage. In Australia, the rate of disease is determined by another formula, which is derived from the economic threshold ranging from 20.5-30.5% in the subtropics and 9.2 -15.6% in the tropics (Stirling and Pattison, 2008). Additional indicators are the root weight of the bunch and the number of hands (Araya and De Waele, 2004), and not so much the diameter of the pseudostem, the number of functional leaves at flowering time and the number of fingers (Fogain, 2000). The economic threshold is not determinative, which varies between 1,000 and 20,000 nematodes/100 g of roots, depending on environmental conditions (Quénéhervé, 2009).

Ecological aspects

In *R. similis,* a strictly bound organism, the population dynamics is closely related to the phenology of bananas, although temperature and rainfall regime are also regulatory factors. The pathogen does not like root hairs or tertiary roots short-lived (Araya and De Waele, 2005) and instead prefers young tissues, and primary, durable roots, located about 30 cm in the pseudostem, within the first 30 cm of depth which accounts for 90% of root biomass. Its density declines in growth phases and from the end of flowering to harvest in bananas, and is maximized at the time of pre-flowering or after flower emergence which in turn coincides with the period of the majority of emergence or renovation of the radical system (Quénéhervé, 2009). Chávez-Velásquez and Araya-Vargas (2009) hinted at a possible positive correlation between the level of potassium or manganese and the population of *R. similis*, while zinc, according to Pattison (2010), appears to decrease the population. However, its population is always higher in roots than in the soil, conforming to the necrosis progress.

As for abiotic factors, in general, there have been significant negative correlation coefficients between the density of *R. similis* and precipitation (Quénéhervé, 1991), and a marked influence by altitude. It predominates in banana zones located below 400 m and with a temperature of 27°C (Price, 2000) and is not adapted to locations at 1400 m s.a.l. (Fogain, 2001) and/or with temperatures below 19°C (Price, 2000). While in Costa Rica, soil acidity is the main factor limiting the population of *R. similis,* and causes low diversity in the structure of the nematofauna, ie low predatory nematode populations, whereas in Australia, low carbon and excess nitrogen are part of the regulatory factors (Pattison, 2010). This author discussed the need to ascertain the possible suppressing effect of the burrowing nematode on predatory and omnivorous nematodes in slightly acidic soils.

Chemical control

This strategy, almost solely directed toward eliminating vital inoculum (P_i) was dominant for about three decades starting in the 1960s, first with fumigants like DD (Dichloropropane - Dichloropropene), EDB (ethylene dibromide) and DBCP (dibromochloropropane), and then with non-fumigant organophosphates nematicides (fenamiphos, ethoprop, terbufos, isazofos) or carbamates (carbofuran, aldicarb, oxamyl). In Australia, the application of fensulfothion, fenamiphos, oxamyl and ethoprophos increased yield by 44, 29, 29 and 21% respectively (Stirling and Pattison, 2008), there were even reports of increased production in the order of 119% in the first cycle with this class of pesticides (Quénéhervé, 2009). This stimulated intensive use under a scheme of routine quarterly applications of 2-3 g/plant i.a., regardless of many key factors. Stirling and Pattison (2008) suggested that nematicides should be applied in banana in Australia only when the economic threshold, expressed as a disease index, was less than 10%, and showed that soils frequently possess an inherent ability to degrade nematicides and that rotation should be a rule to delay the rate of biodegradation, considering that the benefits were not permanent, but temporary. Other factors to consider are the phenology of the plant, ie the root emission period, the dosage of nematicides correlated with parasite density levels in the roots and the percentage of rootless plants (Quénéhervé, 2009), the climate and soil type, so that this intervention is rational, timely, selective, environmentally "friendly" and with an acceptable economic return.

A "variant" could be chemical control with biopesticides synthesized from bacteria and fungi, this tactic is akin to biocontrol. For example, biological nematicides Deny® and Bionem®, respectively derived from *Bacillus cepacia* and *B. firmus*, had a paralyzing effect on *R. similis*, *Paecilomyces lilacinus* is a proven ovicidal, and *Myrothecium verrucaria*, marketed as DiTera®, as well as a ovicidal, stimulates the production of root biomass and the suppressive reaction of the plant (Twomey *et al.,* 2002), although some of its direct and indirect effects were called into question by Spence and Lewis (2010). On the other hand, a avermectin-product of *Streptomyces avermitilis*-injected into the pseudostem of banana, brings new promising chemical molecules and poses a new tactic to protect seedlings in doses from 100-500 mg/plant, and was more effective than soil application of organophosphate nematicides with reference purposes and had protectant, curative and residual effects, at least for 56 d (Jansson and Rabatin, 1997). Under field conditions, Daneel *et al.* (1999) recorded a population decline of *R. similis* in the roots with the injection of 2 mL of avermectin in the pseudostem or applications of DiTera 50 kg, doses that also increased the bunch weight.

Management

In principle, management could cover two key objectives: the regulation of P_i, and the conservation of soil biodiversity. The protagonistic tactics in banana are planting material sanitation, composting, organic mulches, bioprotection and genetic resistance.

The elimination of injury and thermotherapy of corms are recommended tactics, especially when planted on land that had not been planted with *Musa* before. Kashaija *et al*. (1999) reported that in Uganda just peeled corms could increase banana production 30 to 50% in each of the first three harvest cycles, even increasing the number of production cycles, compared to fields planted with infected seed and bark. As a complement, peeled corms can be immersed in hot water (53-55°C/10 to 20 min), avoiding possible adverse effects (Elsen *et al*., 2004; Gowen and Quénéhervé, 1990). Although this practice is not attractive for large producers, it managed to delay recolonization for many months and yielded collateral benefits in terms of flowering, offspring production, bunch and functional roots and in the vigor of the plant and the weight of the fruit (Elsen *et al*., 2004). Coyne *et al*. (2010) showed that immersion of plant material in boiling water for thirty seconds represents an effective alternative, given the reduction in the rate of necrosis and nematode density in treated corms.

On the other hand, cultural practices for managing phytonematodes populations are being resurrected, something feasible even for large plantations of bananas (Sikora and Schuster, 1999). For example, after evaluating nine types of organic manure, Pattison *et al*. (2011) determined that residues of those vegetables that are high in N and C such as the banana and some grasses are highly recommended to decrease the *R. similis* population, similarly McIntyre *et al*. (1999) observed that the mulch with waste banana or corn mitigated the impact of this parasite. Although each type of organic manure has its own impact on the density of nematodes, this practice has physical, biological and chemical benefits (Tabarant *et al.,* 2011). They decrease soil temperature and often the rate of reproduction of the nematode (Araya and De Waele, 2005), increase soil porosity, absorption capacity and replacement of water, root biomass, increase the concentration of C, K and Mg in the planted crop, especially in poor soils (McIntyre *et al*., 1999) and the biological mineralization of nitrogen (Tabarant *et al.,* 2011). They stimulate antagonists (Pattison *et al*., 2011) and by improving the health status of the roots, the production of offspring and increase the longevity of the cultivation (Coyne *et al*., 2005).

Plant biosecurity with endophytic microorganisms, ie those that are mutualists, saprophytes, commensals or weak pathogens that live in plant tissues (Sikora and Schuster, 1999), is an aspect of managing with a great future, with promising endomycorrhization, use of rhizobacteria and non-pathogenic isolates of *Fusarium oxysporum*. Vos *et al*. (2012) found that colonization of banana plants by the vesicular-arbuscular fungi *Glomus mossae* or *G. intraradices* conferred an induced pre-infection resistance to *R. similis*, given decreased penetration of juveniles, thanks to a soluble root exudate which repel nematode during chemotaxis. In a previous study, Elsen *et al*. (2003) had postulated that this increased tolerance of mycorrhizal plants to phytonematodes attack was due to a higher density of roots, lignification and suberization of cell walls, and increased content of P, Ca, Mg and minor elements, and similarly, the colonization of banana roots by endophytic non-pathogenic isolates of *F. oxysporum* apparently induced the expression of both pre-infection resistance genes (Paparu *et al.,* 2007) and post-infection and systemic resistance (Athman *et al*., 2007). Thus, the expression of these resistance genes has repercussions both on the capacity of penetration of infectious states in the plant and production in females.

Regarding rhizobacteria, Lodewyckx *et al.* (2002) counted more than eighty genera with nematicidal effects, and in India, 80% of endophytic bacteria isolated from the rhizosphere of black pepper is Gram positive, dominated by the genera *Bacillus*, *Arthrobacter*, *Micrococcus* and *Curtobacterium* (Aravind *et al*., 2009). In Cuba, the application of a special isolate of *B. thuringiensis* in banana plantations slowed the nematode population by 87% after treatment, and finally, in tests under controlled conditions, *Corynebacterium paurometabolum* proved to be an effective antagonist (Quénéhervé, 2009). As is known, *B. thuringiensis* produces parasporal crystals and especially the family of Cry 5 that affects at least six phylogenetic groups of nematodes, mainly causing intestinal damage, as in insects (Wei *et al*., 2003). But in general, bacterial biocontrol depends mainly on the production of compounds (antibiotics, cyanide, lytic enzymes) and a stimulation of systemic resistance through the activation of defense genes that encode the production of jasmonate, peroxidases and phytoalexins (Lodewyckx *et al.,* 2002).

On the other hand, production of commercial banana cultivars resistant to *R. similis* has not been very successful despite the fact that there are two traditional sources of resistance such as the AA diploid subgroup Pisang Jari Buaya (PJB) and Yangambi km5 (YK5) (*Musa* AAA Ibota subgroup) (Peng and Moens, 2003) and other diploid and triploid sources from Papua New Guinea, Malaysia and the Philippines (Quénéhervé *et al.,* 2009). In many cases, the resistance depends on phytoanticipinas and phytoalexins.

For example, lignin and ferulic acid esters are preformed in the cell wall of the bark of YK5 and PJB (Wuyts *et al.,* 2007), which strengthens the cover and reduces its digestibility and pathogen penetration; these plants can synthesize also other chemical barriers such as dopamine and proanthocyanidins for the oxidation of phenols (Wuyts *et al.*, 2007). Atkinson (2003) highlighted the promising results obtained with the transformation of banana with two cystatins in hopes of achieving transgenic resistance to burrowing nematode and also emphasized the future use of some lectins capable of misleading nematodes and joining glycoconjugates associated with chemoreceptors.

The future

With the contribution of molecular biology, hopefully the genetic and molecular basis of parasitism of the burrowing nematode will be deciphered in the near future and transgenic resistant banana plants will be developed through the incorporation of transproteins toxic to nematodes from microorganisms or plants with natural resistance to these worms, without undermining the evaluation of the agronomic qualities or biosafety of these putative transgenic plants. It will also be crucial to implement multifaceted management strategies or different modes of action, as suggested by several of the current proposals (Hauser, 2000; Sikora and Schuster, 1999; Mendoza and Sikora, 2009; Chaves *et al.,* 2009; Aravind *et al.,* 2009).

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