



Phytonematodes in Integrated Crop-livestock Systems of Tropical Regions

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ACDA, GOA and JGA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ACDA, LDO and BGT managed the analyses of the study. Authors ACDA, DAF, LDO, BGT, ASCF, MFA, OHSR, JGA, WMP and MR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The integrated crop-livestock system (ICLS) is a model of sustainable cultivation that allows the recovery of degraded pastures and the intensification in pasture and grain production. However, the presence of pathogens in the production fields has hampered the employment of these systems. In order to minimize or eradicate the phytonematodes in the production fields and the seeds used in CLIS, it is necessary to know the nematofauna. Based on this, this work aimed to perform a literature review describing the main forages and agricultural crops used in integrated crop-livestock systems in tropical regions, the major phytonematodes associated with these crops and their control measures. This work was based on a literature review from the Scielo, Scopus

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and Google Scholar databases, with data from 1999 to 2019. The initially used keywords were "tropical weeds"; "Agricultural crops"; and "ICLS" and their respective terms in the Portuguese language. From the initial results, we used the keywords "*Brachiaria* syn. *Urochloa* sp. "; "Phytonematodes"; "Millet"; "Maize"; "*Panicum* sp."; "Soy"; "Sorghum", and "ICLS" and their respective terms in the Portuguese language. Publications that did not meet the criteria of this study (analyzed by titles and abstract) were considered as exclusion criteria, as well as repeated works in the databases. Based on the literature, the cultivation of forages belonging to the genera *Brachiaria* syn. *Urochloa* sp. and *Panicum* sp., along with and soybean, maize, sorghum and millet are predominant in the ICL system of tropical regions. The phytonematodes *Meloidogyne* sp., *Pratylenchus brachyurus*, *Heterodera glycines*, *Rotylenchulus reniformis*, *Aphelenchoides* sp., *Ditylenchus* sp. and *Filenchus* sp. cause greater severity of damage to the main cultures used in ICL. No control method can eradicate the phytonematodes. The most effective control for the studied phytonematodes is through integrated pest and disease management.

Keywords: *Brachiaria* syn. *Urochloa* sp.; phytonematode control; maize; *Panicum* sp.; soybean.

1. INTRODUCTION

The concern with environmental preservation has resulted in restrictions by the current legislation to the opening of new areas for agriculture and exploitation of native wood. Allied to this, there is a growing demand for food due to the increase in the world population, which requires an increase in the agricultural output [1,2].

To aggravate the situation, it is estimated that 20% of world pastures are in the process of degradation [3]. In the tropical region, pasture degradation is highlighted as a reflection of poor management, which reduces the productive potential of cattle. Therefore, there is a need for the development of agricultural and livestock processes and technologies, aiming at the efficient use of inputs [1,2,4].

Sustainable crop models were developed in this regard, such as the integrated crop-livestock system (ICL), allowing the recovery or renewal of degraded pastures, the intensification in pasture production, and grain production. The maximization of food production in response to intensified livestock farming rules out the need to open up new areas so that the demands may be met [2,5,6,7].

However, the presence of pathogens in production fields has hampered the implementation of integrated systems since they may contain soils and crops that are susceptible to these pathogens. In addition, the incidence of pathogens has become a problem of growing importance, and it is believed that phytonematodes can influence the forage and the intercropped agricultural crops from the establishment up to harvest stages [8,9].

Several strategies have been reported in studies in order to minimize the phytonematodes in the production fields and, consequently, in the seeds employed in the agricultural production systems [10,11,12,13]. However, it is necessary to know the nematofauna in the production fields of the main forages and agricultural crops, so that corrective actions may be applied in order to reduce or eradicate the incidence of phytonematodes in the production fields of integrated crop-livestock systems.

Based on the foregoing, a literature review was performed to describe the main forages and agricultural crops used in integrated crop-livestock systems in tropical regions, as well as the main phytonematodes associated with these crops and their possible control measures.

2. METHODOLOGY

This work was based on a literature review from the Scielo, Scopus and Google Scholar databases, with data between 1999 and 2019.

The initially used keywords were "capins tropicais"; "culturas agrícolas"; and "ILP", and their respective English terms "tropical grasses"; "agricultural crops"; and "ICL". At that moment, the purpose was to describe which forages and agricultural crops were the most used in integrated crop-livestock systems in tropical regions.

For the inclusion criteria, publications (papers, books, theses, dissertations and scientific communications) released within 1999 and 2019, in the Portuguese and English languages, which fit the purpose of describing the main forages

and agricultural crops used in tropical regions were employed in the research.

Publications prior to 1999 that did not fit the study aim (analyzed by titles and abstract) were considered as exclusion criteria, as well as works that were repeated in the databases. Based on the results, the second stage of the study was carried out, dealing with the description of the phytonematodes associated with these main crops.

Therefore, the keywords employed were "fitonematoides"; "*Brachiaria* syn. *Urochloa* sp."; "*Panicum* sp."; "soja"; "milho"; "sorgo"; "milheto"; "controle" and "ILP" and their respective English terms "phytonematodes"; "*Brachiaria* syn. *Urochloa* sp."; "*Panicum* sp."; "soybean"; "maize"; "sorghum"; "millet"; "control" and "ICL"; under different combinations.

After reading the titles of the papers and other publications, 97 works that met the initially proposed criteria were selected and read in full.

3. RESULTS AND DISCUSSION

3.1 Main Forages and Agricultural Crops in ICL of Tropical Regions

The widely used forage grasses in the ICL systems of tropical regions belong to the genera *Brachiaria* syn. *Urochloa* and *Panicum*. In the case of *B. syn. Urochloa*, the species *B. syn. Urochloa ruziziensis* and some cultivars within the species *B. syn. Urochloa brizantha* are highlighted, such as Marandu, Xaraés and Piatã [14,15,16,17].

In general, forage grasses of the genus *B. syn. Urochloa* are widely employed in integrated crop-livestock systems in tropical regions, as they may present drought, fire and shade tolerance, pest resistance, along with high levels of productivity, nutritional value, and regrowth capacity. In addition, there is a need for well-drained soils, from average to high fertility [14,15,16,17].

Regarding the forage of the genus *Panicum*, the *Panicum maximum* cultivars Tanzânia and Mombaça are highlighted. These cultivars have tolerance to grasshoppers, which makes them a good alternative when aiming to implant an ICL system. Despite having high demands on soil fertility and requiring deep and well-drained soils, they successfully adapt in tropical regions [18,19,20].

The main agricultural crops are soybean and maize, followed by sorghum and millet, with the main factor behind the great exploitation of these cultures being economical, along with the sowing period, specific agricultural defenses and tolerance to water stress [18,21,22,23,24,25,26].

Soybean has been used in rotation with other grain crops and forage grasses, since it minimizes the use of inputs for being a nitrogen-fixing plant, also favoring the recovery of degraded areas. The intercropping with grasses is difficult, but there are indications that the application of narrow leaf herbicide subdoses helps to reduce the loss of grain yield in broadleaf crops (such as soybean), at the same time allowing the grasses to settle [18,23].

Maize is a widely employed crop as it can be used for human consumption and animal feed by marketing all grain or surplus production. Its management presents a diversity of post-emergence narrow-leaf herbicides that are selective to maize, facilitating the intercropping with grasses in ICL systems. Moreover, it is the most frequently employed alternative in soybean succession in integrated systems of tropical regions, maintaining production levels [22,27,28,29].

Sorghum and millet have an excellent ability to withstand environmental stresses, maintaining yield in water deficit and low soil fertility conditions. In addition, they have shown to be an excellent alternative to be used in succession to summer crops in ILC systems of tropical regions, especially soybean. Besides, these crops are used for soil covering and straw formation in systems of minimum cultivation or no-tillage, in the case of millet [21,24,25,26].

Due to the aforementioned expressiveness of the referred crops in integrated crop-livestock systems, they were selected for the analysis of the nematofauna from the seeds and from the production system.

3.2 Main Phytonematodes Associated with Major Forages and Agricultural Crops in ICL of Tropical Regions

There may be a reduction in the plant stand and development when the association of pathogens with forage seeds occurs (*B. syn. Urochloa brizantha* and *Panicum maximum*), as well as in the persistence of the forages in the area, seed production, dry mass and nutritional value,

adversely affecting the production of products such as milk and meat. It is also noted that the presence of the pathogen in the seeds constitutes a barrier to commercialization [30,31].

The main phytonematodes found in association with seeds of tropical forage grasses are *Aphelenchoides* spp. and *Ditylenchus* spp. [32]. The seeds of *Panicum maximum* may be infected with *Aphelenchoides* spp. and *Ditylenchus* spp. [31]; whereas the seeds of *B. syn. Urochloa brizantha* may be associated with *Aphelenchoides* sp., *Ditylenchus* spp., *Filenchus* sp., *Meloidogyne* sp., *Pratylenchus brachyurus*, *Heterodera glycines* and *Rotylenchulus reniformis* [30,31,33].

Although *Ditylenchus* spp. phytonematodes tend to be associated with seeds and impurities (soil, straw, and so forth.) of commercial lots of *P. maximum*, and *Ditylenchus* spp. and *Filenchus* sp. to seeds and impurities of commercial lots of *B. syn. Urochloa brizantha*, only secondary damages have been verified to the *B. syn. Urochloa brizantha*, *P. maximum*, and the main agricultural crops (intercropped, succession and/or rotation) used in the ICL system in tropical regions [32].

In the main agricultural crops used in the ILC, the phytonematodes that can be propagated via seeds and are the most expressive in terms of affecting productivity are *Meloidogyne* sp., *Pratylenchus* sp., *Heterodera* sp., and *Rotylenchulus* sp. [33,34,35,36].

Regarding the phytonematodes and their damage to agricultural crops, soybean is the most negatively affected cultivation due to the allied farming system, the agricultural operations that provide proper conditions for the development of these pathogens. Thus, certain phytonematodes have become one of the primary phytosanitary problems of the soybean and maize crops, being able to drastically reduce grain yields and even turn farming areas unfeasible [33].

3.3 *Aphelenchoides* sp.

Studies that relate to the pathogenicity of the genus *Aphelenchoides* sp to *B. syn. Urochloa brizantha* are scarce and inconclusive. The phytonematode *Aphelenchoides besseyi*, despite being found in association with *P. maximum*, causes secondary damage to this forage [10,33].

However, studies have related the incidence of *A. besseyi* to the appearance and severity of the Mad Soy II disease (MS-II), which causes green stem and leaf retention in the soybean crop, also being able to attack cotton crops [33,37,38].

This disease is a serious problem in Brazil, affecting soybean and cotton yields, with estimated yield reductions from 60 to 100% due to a high index of abortion of flowers and pods and loss of grain quality, along with the wrinkling and darkening of leaves [19,20,33,38,39].

The presence in the seeds and production fields of pathogenic fungi such as *Fusarium graminearum*, *Fusarium pallidoroseum*, *Alternaria*, *Sclerotinia*, *Phomopsis* spp, *Rhizoctonia*, *Phytium inflatum* and *Phytophthora sojae* is another problem to be considered, since they act as multipliers of *Aphelenchoides* sp. [40].

The literature suggests that the adopted cultural management can help to reduce the incidence of Mad Soy II. The practice of cultural control can be adopted through post-harvest weed control measures, such as the early desiccation at sowing, the use of crop rotation or resistant cultivars, properly considering that *Crotalaria juncea*, *C. spectabilis*, *C. ochroleuca*, maize, millet, sorghum, and rice are not multipliers of these phytonematodes. *B. syn. Urochloa brizantha* and *B. syn. Urochloa ruziziensis*, however, present a low or no final population of *A. besseyi* [41,42,43,44].

3.4 *Meloidogyne* sp.

Phytonematodes of the genus *Meloidogyne* present several hosts, causing damage to numerous crops (coffee, cotton, soybean, maize, etc.), with greater impact and importance on soybean and maize [33,45].

Furthermore, they have a wide geographical distribution, with *M. incognita* and *M. javanica* being the main species for tropical regions. *M. incognita* predominates in areas of coffee and cotton farming, whereas *M. javanica* occurs in a generalized way [33,45].

The damages caused by these phytopathogens are the intense abortion of pods after flowering, chlorotic spots or necrosis between the veins, small and yellowish plants in reeds, besides the early maturation of the plants. In addition, with the occurrence of the "Indian summer"

phenomenon, the damage might be more severe [46,47].

However, in the roots of the plants infected by the pathogen, the main characteristic of this parasitism is the formation of galls or tumors. Galls have their number and size dependent on the susceptibility of the cultivar and on the population density of the pathogen [46,47].

With regard to nematode incidence control methods, studies indicate that the forages *Brachiaria* syn. *Urochloa brizantha* and *P. maximum* may favor phytonutrient suppression by *M. incognita* and *M. javanica* [48,49].

Furthermore, it is verified that the use of genetic resistance associated with biological and chemical control provides an increase in the green mass production of the shoot part of the soybean crop in soils infested with *Meloidogyne* spp. [50]. Biological control with the fungi *Paecilomyces lilacinus* (Thom.) and *P. chlamydosporia* may contribute to the control of *M. incognita* [51,52,53].

With regard to chemicals with nematicidal activity, we highlight avermectin and oxime methylcarbamate, used via seed treatment in soybean. They act by suppressing the phytopathogen in the initial development stage of the crop [54,55,56,57]. Moreover, the use of organic acids such as humic and fulvic acids may control the incidence of *M. incognita* and *M. javanica* in crops such as sugarcane [58].

Based on the chemical treatment of phytonematodes, the use of abamectin via seeds has shown to be promising for *M. incognita* in the cotton crop [59], as well as and via soil in the control of *Meloidogyne javanica* [60].

However, it is worth noting that the aforementioned control methods did not promote the eradication of phytonematodes in the area, but only a reduction in its incidence. Therefore, there may occur failure of the integrated systems with agricultural crops (soybean, maize, etc.) and susceptible forages in areas of *Meloidogyne* sp., as the remaining parasites could reproduce and develop, increasing the population index and causing severe damage to the susceptible crops implanted in the productive system [33].

3.5 *Pratylenchus brachyurus*

Another nematode that causes economic losses in tropical regions, especially in crops such as

soybean, rice, sugarcane, sorghum, cotton, beans, tropical grasses, and especially maize, is *Pratylenchus brachyurus* (root lesion nematode) [34,61].

The damages caused by the root lesion nematode are dependent on the susceptibility of the crop, the population level within the productive system, the environmental condition and the type of soil. In addition, the level of severity and the appearance of some symptoms from the attacks of this nematode are associated with conditions of water stress, compacted and mainly sandy soils, conditions in which the pathogen can cause significant damage to production even in a low population density [33,62].

There was a reduction of 30% in the production of infected soybean in field experiments, and 90% damage in common bean infected with *P. brachyurus*. However, in tropical regions, this severity can compromise up to 50% of the soybean production from infested areas [34,63].

In production fields from the state of Mato Grosso do Sul, Brazil, there are frequency reports of 82, 79 and 87% in the cities of Chapadão do Sul, Costa Rica, and São Gabriel do Oeste, respectively [61,64], causing a loss of 30% in soybean yield [33].

The attacks and the manifestation of symptoms can occur in defined areas, easily visualized in the field. The parasitized plants present reduced growth but remain green, being in a stage of partial or total obscuration during the penetration of cortical and corticoid cells, instead of neutralizing the toxins during the feeding process. In addition, the movement of the parasites through the root is disorganized, aiding in the destruction of cells [33,65].

Furthermore, root lesions caused by the nematode serve as an entry point for bacteria and fungi, primarily the wilt-causing fungi *Fusarium* and *Verticillium*, causing necrosis and rot in the plant [33,66].

With regard to the possibility of *P. brachyurus* suppression, studies have found that *B. syn. Urochloa brizantha* and *B. syn. Urochloa ruziziensis* allowed the population increase of phytonematodes of different origins (okra (Pb20) and cotton roots (Pb24)). However, the Pb24 cotton showed a higher proliferation in areas cultivated with the forages studied in this work,

as well as with soybean and maize cultivars [67,68].

This suggests that this nematode may be severely reduced in integration systems considering succession, rotation or consortium utilizing tropical grasses, soybean, maize and cotton [67,68].

A viable alternative is the use of rotating crops with species that are not hosts and susceptible to the pathogen, such as *Crotalaria spectabilis* and *C. breviflora*, which present *P. brachyurus* reproduction factors of approximately 0.1 and 0.2%, respectively [67,68,69,70,71].

In addition to the use of *Crotalaria spectabilis* and *C. breviflora*, other non-multiplying species for *P. brachyurus* can be used in integrated systems, such as *C. ochroleuca*, *C. juncea*, black oat (*Avena strigosa* Schreb), amaranth (*Amaranthus cruentus*), and the intercropped of *C. spectabilis* and millet (*Pennisetum glaucum*). The result is a reduction in the population density of this phytonematode in the productive systems [70,72,73].

Furthermore, the employment of soybean cultivars with a low *P. brachyurus* reproduction factor can be used as a palliative method in high incidence areas, such as BRS GO Chapadões; Conquista; MG/BR 46; M-SOY 8378; M-SOY 8360RR; M-SOY 8800; UFUS Guar; and UFUS 37 [74,75]. Another palliative alternative is the biological control through the fungus *Pochonia chlamydosporia* [76], and the chemical control with abamectin and imidacloprid with thiodicarb via seed treatment [77].

As previously explained, it should be noted that the aforementioned control methods did not promote the eradication of phytonematodes in the area, but only a reduction in their incidence. This will generate the same complications already discussed.

3.6 *Heterodera glycines*

Another phytonematode that causes economic losses in the soybean crop is the *Heterodera glycines* (soybean cyst nematode). This nematode penetrates the roots of the plant, hinders the absorption of nutrients and water and prevents the development of the plant. Due to this, the plant can manifest small size and chlorosis in the shoot part [33,78].

This nematode penetrates the roots of the soybean plant and impedes the absorption of water and nutrients, which results in reduced size and chlorosis in the shoot part; these are known as yellow dwarfism. However, in regions with more fertile soils and good rainfall distribution, the absence of symptoms in the shoot part of the plant is common. This shows that the definitive diagnosis always requires the observation of the root system [33,78].

Generally, root-related symptoms of infected plants begin 30 to 40 days after soybean sowing. The root system is reduced in this period, exhibiting female nematodes. After the death of this female, the body is transformed into a hard structure of dark brown color, called cyst, that detaches itself from the root and is allocated into the soil [45,46,47].

The dissemination of the cyst occurs primarily due to the transporting of the infected soil; therefore, poorly harvested seeds, implements, and machinery, as well as winds and water can disseminate the nematode into new areas. In properties where the ICL system is employed, it is very important to know the origin of the forage seeds, as it may contain the inoculum of the cyst nematode [33].

In areas with the presence of *H. glycines*, the adoption of joint control measures (integrated management) is a more effective strategy [79, 80]. Biological control is a promising option to be used in conjunction with cultural control (crop rotation), soil management and the use of resistant cultivars [81,82,83,84].

Biological control with the fungus *Pasteuria lilacinus* associated with chemical control, as well as the use of abamectin along with *Pasteuria nishizawae* and *Pasteuria metchnikoff*, in isolated ways, are effective options to control *H. glycines* in the soybean crop [84,85,86]. The control via seeds is the most effective and viable way to apply such treatments [81,82].

With regard to the crop control and the resistance of cultivars, it is seen that millet is resistant to *Heterodera glycines* [87]. Due to their efficiency and economic viability, the use of resistant cultivars is the most adopted control method for *H. glycines* [88].

3.7 *Rotylenchulus reniformis*

There are reports that the phytonematode *Rotylenchulus reniformis* (reniform nematode)

has only caused severe damage to cotton. However, after the 1990s, there were also attacks on soybean crops. It is believed that this phytonematode occurs in high population densities and is present in 29% of soybean-cultivated areas in the state of Mato Grosso do Sul, Brazil [46,47].

The symptoms of nematode attack in soybean plants resemble those caused by problems of soil compaction and mineral deficiency, with the presence of unevenness in the crops, extensive areas of underdeveloped plants characterized by dwarfism, chlorosis, and foliar yellowing. There is no formation of galls; however, in some points of the root, it is possible to observe an outer layer of soil adhered to the egg mass of the nematode [33,89].

Sexually immature reniform females cause attacks and subsequent manifestation of symptoms. These migrate in the soil in search of the roots of the host plant and penetrate them until reaching the region before the pericyclic cells, initiating the parasitism. Upon reaching sexual maturity, the outer portion of the root system acquires a resemblance similar to that of a kidney, giving rise to the name "reniform nematode" [89,90].

The control of *Rotylenchulus reniformis* can be performed with the cultural method and resistance of cultivars, by cultivating sorghum and millet. In addition to the mentioned species, soybean cultivars are being studied with regard to the resistance mechanism to this phytonematode, with promising present results [87,91].

However, long periods of rotation with resistant crops are necessary, since this parasite can survive for up to two years in the soil due to the mechanism of anhydrobiosis [92,93].

In general, for some crops, there is still the recommendation of chemical control for all treated phytonematodes. These control substances are known as nematicides and can be applied via soil or seed. However, their effectiveness and economic viability are still poorly understood [94], varying according to the form of application and the chemical group employed [60].

It is believed that the joint application via seed and soil has a greater residual effect in the control of phytonematodes [60]. Several

nematicides of the carbamate and organophosphorus chemical groups are available for seed treatment in Brazil [64].

3.8 Phytonematodes Eradication

In general, the currently used phytometric control methods are preventive control (to avoid entry and dissemination in the production field), crop control (rotation and/or succession of resistant and host crops), genetic control (resistant cultivars), chemical and biological control [94].

Phytonematode control through different methods is important for reducing the damage caused by the parasites [50]. It is always preferable to prevent the spread of the nematode than to treat an infested area [95], since they are difficult to eradicate from the production field. Furthermore, the occurrence of multiple phytonematode species in one area may be a control problem, since different strategies can be adopted [96].

There are several tested techniques for the eradication of phytonematodes present in forage seeds. In the *P. maximum* seed the treatment of the seeds at 60°C for 10 minutes or at 57°C for 15 minutes eradicated the seed phytonematodes without compromising seed quality [10].

In addition, the use of irradiation has been promising as an effective method for the eradication of seed pathogens. This is due to high control efficiency without toxic residues and no side effects [10].

However, it is emphasized that the above-mentioned technique was only used in laboratory experiments and with a small amounts of seeds. In order for this method to be used in commercial forage seeds, it will be necessary to develop techniques that allow large-scale eradication and with no any side effects on the seeds [97].

4. CONCLUSION

The cultivation of forages belonging to the genus *Brachiaria* syn. *Urochloa* and *Panicum*, as well as soybean, maize, sorghum and millet are predominant in the ICL system of tropical regions.

The phytonematodes *Meloidogyne* sp., *Pratylenchus brachyurus*, *Heterodera glycines*, *Rotylenchulus reniformis*, *Aphelenchoides* sp., *Ditylenchus* sp. and *Filenchus* sp cause greater

severity of damage to the main crops used in ICL of tropical regions.

No control method can eradicate the phytonematodes.

The most effective control of the studied phytonematodes is through integrated pest and disease management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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