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Can industrial cleaning procedures make *Urochloa ruziziensis* seeds nematode free?

Rita de Cássia Lima Mazzuchelli, Ceci Castilho Custódio, Nelson Barbosa Machado Neto, Fábio Fernando de Araújo*

Agricultural Sciences College at UNOESTE, Presidente Prudente, SP, CEP 19067175, Brazil

***Corresponding author: fabio@unoeste.br**

Abstract

Seeds for agriculture should be pathogens free e.g. from plant-parasitic nematodes that if present in seed lots may be transferred to other clean areas as inoculum to infest other host plants. The aim of this work was to identify and quantify active forms of nematodes during the cleaning procedures of *Urochloa ruziziensis* seeds and also to evaluate its efficiency in turning the seeds nematodes free. Three seed lots with distinct origins were used. They were sampled four times in different parts of the process such as the reception and during the cleaning procedures consisting of the following treatments: unclean seeds, after using air screen cleaner, after using gravity separator (clean seeds) and the dust particles (seed covers and empty seeds). Identification and quantification of nematodes were done in each of the samples. The results showed that there is reduction in active number of *Aphelenchoides* and *Ditylenchus* nemaodes after cleaning procedures of seeds. However, this reduction does not meet the standards to be disseminated as pathogen free seeds for commercialization. The active *Meloidogyne* form was found only in one seed lot, but the cleaning procedures was effective to eliminate the parasites from the clean commercial seed. Others control forms need to be introduced into the system to turn pasture seed free of nematodes.

Keywords: *Brachiaria*, pasture, *Aphelenchoides*, *Ditylenchus*, *Meloidogyne*.

Introduction

The seed production of tropical pastures increased in the past decade to attend the world's demand, contributing to cattle food security, which guarantees their nutrition, principally in tropical zones. The tropical pastures are considered as a healthy and low-cost diet for cattles (Rudel et al., 2015). On the other hand, the use of *Urochloa ruziziensis*, as a component in the integrated crop-livestock and direct sowing systems is being increasing because it produces a large amount of dry mass (27 t ha^{-1} in average), and could be easily eradicated by herbicides. It has a decumbent growth and covers the soil surface with abundant leaves, culms and thin roots, which is an effective contribution to the physic restoration of the soil structure (Marochi et al., 2005).

However, the pasture seed production can be threatened by the presence of phytopathogens and also by the absence of sanity standards for seed commercialization. In this context, seeds maybe a disseminating system for many pathogens and pests, which can affect the main culture in an integrated crop-livestock system, from plant establishment to harvesting (Marchi et al., 2007).

Nematodes are hard to control and can easily hide in seed lots. This can have direct impacts on productivity, forage growth; seed production and natural pasture recover (Favoreto et al., 2010). The best way of control is prevention form introduction into new clean areas, as there is no effective way to eradicate nematodes. Only its populations can be decreased (Coltro-Roncato et al., 2015).

The phytonematodes cause a major obstacle to seed pasture exports, as seed importers may impose phytosanitary restrictions on seeds (Marchi et al., 2007). For pasture seeds, the harvest might be directly from plants, avoiding the soil sweeping process (Mallmann et al., 2013; Alves et al., 2017). Favoreto et al. (2010) pointed out that control of nematodes in pasture seeds can prevent dissemination, especially considering integrated crop-livestock system and the loss of pasture yield to livestock. Seed infection by nematodes may occur by the contact of the seed with the soil. Most *Urochloa* seeds are harvested from sweeping from the ground, where the fallen seeds could be collected from the surface and mechanically removed with soil particles which, in turn, may contain nematodes (Coelho et al., 2006).

In Brazil, *Aphelenchoides* species, especially *A. besseyi*, and some *Ditylenchus* are found in forage seeds (Favoreto et al., 2006). The *Aphelenchoides* and *Ditylenchus* are also pathogens of sugarcane crop, arising major concern since forage areas are being replaced by sugarcane (Tokeshi and Rago, 2005).

Favoreto et al. (2006) found that cleaning procedures in *U. brizantha* seed removed more *Ditylenchus* species than *Aphelenchoides*, since *Ditylenchus* are found in larger dust particles, which are easily removed during the process. *A. besseyi* is the causal agent nematode of rice "white tip" and can survive up to 19 years inside the seeds (Bedendo and Prabhu, 2005). The *A. besseyi* dissemination is mainly occurred by infested seeds; therefore, identification and elimination methods of these nematodes is necessary (Bueno et al., 2002).

Meloidogyne incognita is considered as very harmful nematode to plants and found in more than 2000 plant species inducing the lateral root knot gall. The gall causes a reduction in plant development, even in very low populations (Asmus and Inomoto, 2007).

The *Meloidogyne* genus was less frequently associated with pasture seeds but they are not less harmful if not removed. It can be disseminated by seeds to other areas and present a great potential problem, since it has associations with many crops reducing their growth and yields (Zambiasi et al., 2007).

Table 1. Active forms of *Aphelenchoides*, *Ditylenchus* and *Meloidogyne* nematodes in three unclean seeds lots of *Urochloa ruziziensis*.

Lot	*Aphelenchoides* spp	*Ditylenchus* spp	*Meloidogyne* spp
1	1320.8 c	1483.3 b	87.9 a
2	1691.6 b	1620.8 b	-
3	3054.1 a	3645.8 a	-

Means followed by different letter, on column, statistically differ by Tukey's test ($p<0.01$).

Table 2. Active forms of *Aphelenchoides*, *Ditylenchus* and *Meloidogyne* nematodes on *Urochloa ruziziensis* seeds collected in three steps during industrial seed cleaning procedures, from three seed lots.

		Aphelenchoides spp	
Lot	Unclean	After air screen cleaner	Clean seeds
1	1266.6 cA	983.3 cAB	866.6 bB
2	1866.6 bA	1483.3 bB	1016.6 bC
3	3800.0 aA	2866.6 aB	1516.6 aC
Mean	2000.0 a	2088.8 a	1133.3 b
		Ditylenchus spp	
1	1516.6 bA	1166.6 bB	550.0 cC
2	1300.0 bA	1316.6 bA	1133.3 bA
3	4350.0 aA	4583.3 aA	1816.6 aB
Mean	2388.8 a	2355.5 a	1166.6 b
		Meloidogyne spp	
1	183.3 aA	100.0 aB	-
2	-	-	-
3	-	-	-
Mean	61.7 a	34.0 a	-

Means followed by different letter, lower case on column and upper case on rows, statistically differ by Tukey's test ($p<0.01$).

Table 3. Active forms of *Aphelenchoides*, *Ditylenchus* and *Meloidogyne* nematodes on dust particles of *Urochloa ruziziensis* after cleaning procedures in three seed lots.

	Aphelenchoides spp	*Ditylenchus* spp	*Meloidogyne* spp
Lot			
1	2166.6 b	2700.0 b	67.3 a
2	2400.0 b	2733.3 b	-
3	4033.3 a	3833.3 a	-

Means followed by different letter, on column, statistically differ by Tukey's test ($p<0.01$).

The aim of this work was to identify and to quantify active nematodes forms in unclean, after using air screen cleaner, using gravity separator (clean seeds) and in the dust particles (seed covers and empty seeds). We also verified the efficiency of the industrial procedures in lots of *U. ruziziensis* seeds to make the seeds nematodes free.

Results and Discussion

All evaluated lots showed positive to *Aphelenchoides* and *Ditylenchus* presence. However, the highest concentration of these genera was found in lot 3. The lot 1 showed the lowest *Aphelenchoides* and *Ditylenchus* populations. Nevertheless, in this lot *Meloidogyne* presence was detected (Table 1).

The seed industrial cleaning procedures decreased the active forms of *Aphelenchoides* and *Ditylenchus* although it did not eliminate them. The *Aphelenchoides* and *Ditylenchus* infestation did not differ between unclean seeds and after the air screen clean. However, *Meloidogyne* active forms were eliminated during cleaning procedures of lot 1 (Table 2). Monteiro et al. (2014) studying seed nematodes in *Urochloa brizantha*, suggested that the internal localization between the coat and endosperm was the reason that seed nematodes are not eradicated, making control and its evaluation difficult.

Aphelenchoides was reduced in *U. ruziziensis* seeds after cleaning procedures, but some active forms were detected in the clean seed (commercial ones) (Table 2). In relation to *Ditylenchus* active forms, there was significant reduction of seed pathogen after process cleaning in two lots. The lot 3 showed the highest nematodes amount of this genus (Table 2). The *Meloidogyne* active forms were detected only in lot 1 and the amount was lower than detected to others nematodes forms. *Meloidogyne* is characterized for nematodes that lied in the soils and in roots of susceptible plants (Curto et al., 2005). The penetration in seeds may be superficial as it was observed that the cleaning procedures eliminated those nematodes from seeds at the end of the process, indicating that those nematodes was located in seed external layers, turning the processes more effective (Table 2).

The higher concentration of nematode active forms occurred in dust particles (seed covers and empty seeds) (Table 3). Favoreto et al. (2006) observed that nematodes population differed in cleaning procedures steps and dust particles also exhibited the highest nematodes populations.

The *Aphelenchoides* and *Ditylenchus* in analysed lots were associated to *U. ruziziensis* seeds (Marchi et al., 2007). This can be a problem not only to pastures and livestock but also to crops because those are not specific and can be parasites to other crops (Tokeshi and Rago, 2005). The cleaning procedures partially decreased seed parasites showing that commercial seed can still disseminate nematodes to free areas.

An effort to prevent the use of low quality pasture seed *per se* is necessary as the seed are being used not only for cattle farmers, but in integrated crop-livestock system, compromising the livestock sustainability and extending it to crops low yielding (Mallmann et al., 2013). Besides the seed industrial cleaning procedures, associations with other

methods must be implemented to guarantee that pasture seeds are nematode free. In *U. brizantha* seeds, the use of dry heat (40-57°C), prevented the *A. bessey* dissemination into free areas (Tenente et al., 2006). Methods like this, or biological or chemical options, may be studied and adopted as control methods to eliminate nematodes from pasture seeds during seed processing and to protect free areas. Seed nematodes analysis as a routine test must be inserted along with appropriated phytosanitary legislation.

Materials and Methods

Seed material

Three seed lots of *U. ruziziensis* were used and sub samples of each were taken during the cleaning procedures by the following steps: after using air screen cleaner, after gravity separator (clean seeds) and dust particles (seed covers and empty seeds). Three repetitions per seed lot and for each step were collected.

Nematode extraction and analysis

The nematode extraction method followed Coolens and D'Herde (1972) as follows: 20 g sample was crushed in a blender with 300 mL distilled water during 30 seconds. The supernatant was passed by 400 mesh screen and the retained material collected was washed with the aid of a water flux from a washing bottle to an 80 mL Becker. The solution was transferred to 20 mL centrifuge tube with the addition of 1 cm^3 of kaolin. After homogenization, it was centrifuged at 2000 rpm for four minutes. The supernatant was discarded and the tube completed with 45% sucrose solution. The result solution was homogenized and centrifuged at 2000 rpm for one minute. The supernatant was dropped in 400 mesh screen and the retained material collected was washed with the aid of a water flux from a washing bottle to an 80 mL Becker. One mL of sample was transferred to Peters camera to identify the nematode genera and to count the number of each under optical microscope.

Statistical design

The research was designed as a completely random experiment and the treatments distributed as a factorial array (three seed lots with four distinct cleaning steps) with three repetitions each. The data was submitted to ANOVA using the SISVAR software (Ferreira, 2014) and when significant to the Tukey's test ($p<0.01$).

Conclusion

There was reduction of the active number of *Aphelenchoides* spp. and *Ditylenchus* spp. after cleaning procedures of *U. ruziziensis* seeds. However, this did not mean seeds free of parasites for commercialization. *Meloidogyne* spp. active forms were found only in one seed lot, but the cleaning procedures were effective to eliminate the parasites from it. Others control forms need to be introduced into the system to turn pasture seed nematodes free.

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