Techniques to Eradicate *Aphelenchoides besseyi* Christie (1942) from Infested *Brachiaria brizantha* Seeds

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The efficacy of thermal and chemical treatments to eradicate *Aphelenchoides besseyi* from *Brachiaria brizantha* seeds was evaluated. Seeds were exposed to moist heat (40ºC) for 15 and 30 min., followed by moist heat (57ºC) for 10 and 15 min., respectively. In a second method, seeds were treated with dry heat (60ºC) for 6 hr followed by dry heat (90ºC) for 3 or 6 hr. In a third method seeds were soaked in sodium hyplochlorite (2%) plus formaldehyde (0.5 and 1%) for 20 and/or 30 min. Fifteen treatments had four 200-seed-replicates. Nematode eradication, seed germination, seedling vigour and root length were evaluated. All thermal treatments (dry and moist) eliminated the nematodes from seeds, but the chemical treatment only decreased the nematode number, from 927 per plant (control) to as low as 29 nematodes per plant for best treatment. Seed germination and seedling vigour were not affected by the treatments (Moist Heat and Chemical). Dry treatment affected the germination and vigour, showing significant difference among the variations of treatment compared with control. Root length results showed significant difference among treatments compared with their controls, but the dry treatment increased root length, compared with control. Even if the germination rate or growth rate was slightly impaired by the applied treatments, these would allow the exchange of material, between laboratories in different countries, and after seeds are cleaned they can be used to increase seed for reseachers and growers.

Keywords: Seed-borne nematode, *Aphelenchoides besseyi*, eradication, physical control, chemical control, germination, vigour, length size, seed pasture.

Resumo – Tenente, R.C.V.; A.I. M. de Sousa; V.F. Gomes & A.J.G. Rodrigues Junior. 2006. Técnicas de erradicação de *Aphelenchoides besseyi* Christie 1942 de sementes de *Brachiaria brizantha.*

A eficiência de tratamentos físico e químico para erradicar *Aphelenchoides besseyi* de sementes de *Brachiaria brizantha* foi avaliada. As sementes foram expostas ao calor úmido (40ºC) por 15 e 30 min., seguido por tratamento úmido (57ºC) por 10 e 15 min., respectivamente. Em um segundo método, as sementes foram tratadas por calor seco (60ºC) por 6 horas seguido do mesmo tratamento a 90ºC por 3 ou 6 horas. Em um terceiro método, as sementes foram imersas em hipoclorito de sódio (2%) mais formol-aldeído (0,5 e 1%) por 20 e 30 min. Quinze tratamentos continham 200 sementes por repetição, e cada um foi repetido quatro vezes. A erradicação do nematóide, a germinação das sementes, o vigor e o comprimento das raízes das plântulas foram avaliados. Todos os tratamentos térmicos (úmido e seco) eliminaram os nematóides das sementes, mas o tratamento químico somente decresceu o número de nematóides, de 927 (testemunha) para 29 nematóides por planta para o melhor tratamento. A germinação das sementes e o vigor das plântulas não foram afetados nos tratamentos (Úmido e Químico). O tratamento térmico seco mostrou diferença significativa entre as variações de temperatura, comparado ao controle. Os resultados do comprimento das raízes mostraram diferença significativa entre os tratamentos aplicados comparados com seus controles, mas o tratamento térmico seco aumentou o valor do comprimento das radículas, em relação à testemunha. Portanto, mesmo que a germinação e o vigor sejam levemente alterados pelos tratamentos aplicados, isto permitiria o intercâmbio entre laboratórios de diferentes países, após as sementes serem limpas e estas poderiam ser usadas pelos pesquisadores ou fazendeiros interessados.

Palavras-chave: Nematóides de sementes, *Aphelenchoides besseyi*, erradicação, controle térmico, controle químico, germinação, vigor, comprimento de radícula, sementes de forrageira.

Introduction

The plant-parasitic nematode genus *Aphelenchoides* has economically important species that can cause devastating plant damage and crop losses (Fortuner & Orton Williams, 1975; Tenente & Manso, 1987). *A. besseyi* Christie 1942, the agent of white tip disease of rice, can be disseminated by seeds, in which the nematodes exist for long periods in a state of anydrobiosis (Atkins &, Todd 1959; Yoshii & Yamamoto, 1950 and Tenente & Manso, 1987). Fukano (1962) reported that 30 nematodes per 100 seeds are enough to cause yield losses in rice crops. Also, in rice production the loss can range from 4.7 to 54%, depending on many factors, including the rice varieties (Bridge *et al.*, 1990). Although host races of *A. besseyi* are known to exist, the evidence is limited (Noegel & Perry, 1963, Tenente *et al.*, 1999).

Discovery of procedures that can eradicate nematodes from planting material is important, especially for seeds that are imported for use in areas that are not infested by this nematode.

Several reports of the use of pesticides for seed treatment to manage this nematode species indicate a reduction in nematode infestation levels, but not complete eradication of this parasite *Aphelenchoides besseyi*, (Ou, 1985; Tenente *et al.*, 1994; 2004; Garcia *et al.*, 2000). Chemical treatment with paration, malation, dipterex, carbofuran., thiabendazole, benomyl phenitrothion, phosphine, sodium hypochlorite and formaldehyde vary in their efficacy against the nematode (Garcia *et al.*, 2001; Sousa *et al.*, 2003; Tenente *et al.*, 1999; 2004). Variations in temperature and exposure to heat can eliminate nematodes of several species from a variety seeds, without loss of germination and vigour (Baker, 1972; Kaiser, 1983; Gokte & Mathur, 1993; Tenente *et al.*, 1983; 1993; 1994; 1999; 2004; 2005; Santos *et al.*, 2001 & Sousa *et al.*, 2003; 2005).

Brachiaria spp. germplasm is exchanged by many researchers for breeding programs and the risk of new pest introductions is possible. Indeed, Tenente *et al.* (1985; 1994; 1999; 2002a; 2002b; 2005; 2006) reported the presence of many species of *Aphelenchoides*, *Anguina* and *Ditylenchus* associated with different seeds, including several species of *Brachiaria*. Because quarantine programs must provide seeds without any nematode contamination, there is an urgent need to develop treatments that eliminate nematodes from seeds without significant reduction in germination and seedling vigour. In this paper we present the results of studies conducted by EMBRAPA to use thermal and chemical treatments to eradicate juveniles of *A. besseyi* from *Brachiaria brizantha* (A. Rich.) Stapf seeds.

Material and Methods

Brachiaria brizantha seeds, from Mato Grosso State in Brazil, infected with *A. besseyi*, were treated with moist heat (MT), first experiment; dry heat (DT), second experiment; or chemicals (CT) third experiment, in the following treatments: 1) moist heat of 40°C for 15 and/or 30 min. followed by 57°C for 10 or 15 min., respectively; 2) dry heat of 60°C for 3 and/or 6 hr, after decreasing the relative humidity of seeds to 9%, followed by 95°C for 3 and 6 hr; 3) chemical treatment with sodium hypochlorite (2%) plus formaldehyde (methylene dimethyl ether) (0.5 and 1%) for 20 and 30 min., respectively. Moist heat treatments were applied by placing seeds inside small nylon bags, in a 14L circulating water bath, with mechanical agitation, at the desired temperature. Seeds in small nylon bags were treated with dry heat using a programmable circulating dry air sterilising chamber (model

320 SE, FANEN Ltd., São Paulo State, Brazil). The chemical treatment was carried out in the on Nematological Laboratory at Embrapa Genetic Resources and Biotechnology, Brasília, Brazil, soaking the seeds in a mixture solution of sodium hypochlorite and formaldehyde for the exposure time previously mentioned.

Four replicates of each treatment, each consisting of 200 seeds, were used to evaluate eradication efficacy against nematodes and also to determine the effects on the rate of seed germination, seedling vigour, and root length (ISTA, 1976). Untreated *B. brizantha* seeds were used as control for all variables evaluated.

Seed germination was evaluated one week after treatment, in a growth chamber (Percival, Boone Iowa - 50036), at 25°C and 100% RH. The germinated seeds remained in the incubator and the percentage of seeds still alive two weeks after treatments were used as a measurement of seedling vigour. Root length was also measured after 14 days. Later germination was also observed at this time.

Nematodes were extracted from treated and untreated seeds by soaking seeds in distilled water for 16h. after which they were poured into a blender and macerated for 20 seconds (Zuckermann *et al.*, 1990). The suspension was recovered and rinsed on a 500 mesh (0.025mm opening) sieve and placed on a Baermann funnel for 24 to 72 hr. Nematodes passing the filter were counted using a dissecting microscope.

For all experiments, data for percentage germination and vigour were transformed (arcsin square-root) prior to analysis of variance and mean of comparation by Dunnet's Test to compare each type of treatment with its control. Nematode data were transformed [log (x+1)] prior to analysis of variance. Treatment means for counted nematodes and root lengths were separated using Tukey's Honestly Significant Difference ($P=0.05$) (Hoel, 1961).

Results

In Table 1, all of the moist and dry heat treatments appear to have eradicated *A. besseyi* in *B. brizantha* seeds; however, the variations in dry thermal treatment had variable affects on seed growth and vigour (Table 1). The nematode numbers were reduced by all chemical treatments compared to control (CT 1); but no chemical treatments eradicated the nematodes. None of the treatments increased seed germination and DT treatments reduced it and showed significant difference among treatments, compared with control. In contrast to the moist heat and chemical treatments, dry treatments showed significant difference in vigour parameter, compared with control, except for DT 2 for the vigour of *B. brizantha* seeds. Later germination was observed only for the dry thermal treatment (DTT 2), increasing by 10% compared to seed germination for the same treatment.

Discussion

Because of the need to introduce new germplasm for plant breeding, methods to eradicate nematode in seeds are essential to study and development. The results of this study indicate that moist heat may be one of the most effective treatments for this purpose.

None of the treatments used in this study produced consistent effects on seed growth parameters related to their controls. Chemical treatments were markedly inferior to the other treatments in terms of nematode eradication. Similar results using these chemicals to treat rice seeds were reported by Yoshii and Yamamoto (1950); Cralley and French (1952), Gomy and Kogure (1956), Ishiy *et al.* (1975), Martins *et al.* (1976), Tenente and Manso (1994) and Tenente *et al.* (1999). In contrast, the moist heat treatment did not appear to affect germination and vigour of *B. brizantha* seeds in this study, and detrimental effects of all treatments on root length of seeds were generally less than those of control plants. Cralley (1952) and Todd and Atkins (1958) were unable to eradicate seed-borne nematodes using similar temperature ranges, perhaps due to the methodological differences as seeds were not shaken during treatments. Yoshii and Yamamoto (1950) eradicated *A. besseyi* from rice seeds with moist heat at 57°C, without prior treatment, and Tenente *et al.* (1999), at 60°C (moist) and 95°C (dry) with prior treatments at 40° and 60°C, respectively. Dry heat and chemical treatments produced results nearly comparable to those of moist heat for *B. brizantha* seeds. Results of germination rate or growth rate confirmed the nematodes were eliminated and the material can be exchanged among different countries and they can be used to increase seed for the interested researchers and growers.

In conclusion, these results indicate that there is the potential to successfully eliminate the *A. besseyi*- seed-borne nematode, in *B. brizantha* seeds, using moist or dry heat treatments, avoiding dissemination of this nematode into new free areas, as well to eliminate the nematodes from the seeds. As part of a nematode quarantine program,

Table 1. Results of moist heat (MH), first experiment; dry heat (DH), second experiment; and chemical (CT), third experiment of *Brachiaria brizantha* seed germination, in plant vigour, root length and nematode number.

Applied Treatments	Germination		Vigour		Root Length	Nematode number
	Seedling Number	(%)	Seedling Number	(%)	(cm)	
Control - (MH1) – 1st Experiment	**144.75 a**	**72.38**	**145.75 a**	**100**	**6.59 a**	**245.25 a**
40°C/15'- 57°C/10' - (MH2)	147.25 a	73.62	139.25 a	94.62	4.58 b	0 b
40°C/15' – 57°C/15' - (MH3)	154.00 a	76.95	133.75 a	87.15	4.87 b	0 b
40°C/30'- 57°C/10' - (MH4)	153.00 a	76.50	151.00 a	98.65	4.82 b	0 b
40°C/30'- 57°C/15' - (MH5)	156.75 a	78.12	151.25 a	96.57	5.03 ab	0 b
Control - (DT1) – 2nd Experiment	**114.5 ab**	**57.25**	**111.00 b**	**96.95**	**5.74 ab**	**100 a**
60°C/3h. - 95°C/3h. - (DT2)	89.50 b	44.75	99.75 b	100	5.86 b	0 b
60°C/3h. - 95°C/6h. - (DT3)	139.25 a	69.62	129.75 a	93.18	6.91 a	0 b
60°C/6h. - 95°C/3h. - (DT4)	135.25 a	67.62	122.00 a	90.20	5.85 b	0 b
60°C/6h. - 95°C/6h. - (DT5)	135.00 a	67.50	116.50 a	86.30	6.28 a	0 b
Control - (CT1) – 3rd Experiment	**150.00 a**	**75.00**	**145.25 a**	**96.77**	**5.29 a**	**927.4 a**
NaOCl 2% + Formaldehyde 0.5% /30' - (CT2)	146.75 a	73.37	140.50 a	95.82	4.55 b	29.25 c
NaOCl 2% + Formaldehyde 0.5% /20' - (CT3)	154.00 a	77.00	133.75 a	87.15	4.80 b	180.8 b
NaOCl 2% + Formaldehyde 1% /30' - (CT4)	153.00 a	76.50	151.00 a	98.65	4.80 b	43.7 c
NaOCl 2% + Formaldehyde 1% /20' - (CT5)	158.00 a	78.38	151.25 a	96.58	5.00 ab	48.7 c

Average: four (4) replicates, each consisting of 200 seeds.
Numbers followed by the same letter do not differ (P=0.05) according to Tukey's Honestly Significant Difference Test, in each experiment.

EMBRAPA is using these methods combined with careful nematological evaluation of treated seeds to release imported germplasm to universities and private research centers. The treatments are easily accomplished and do not require the use of dangerous or environmentally hazardous chemicals.

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