TECHNIQUES TO ERADICATE PLANT PARASITIC NEMATODES FROM INFESTED MAIZE, OAT AND RICE SEEDS

R. C. V. Tenente,[1] V. Gonzaga,[1] F. P. Pinheiro,[2] P. Tarchetti,[3] and V. Rodrigues[3]

EMBRAPA/ CENARGEN, Cx. Postal 2372–(70849- 970) Brasilia, DF, Brazil,[1] Research Associates of CNPQ,[2] and EMBRAPA,[3] Brasilia, DF, Brazil.

ABSTRACT

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The efficacy of several physical and chemical techniques to eradicate *Aphelenchoides besseyi, Ditylenchus* sp. and *D. dipsaci* in maize, oat and rice seeds was evaluated. Rice seeds, were exposed to moist heat (40°C) for 15, 30 or 60 min, followed by moist heat (60°C) for 5, 8, or 10 min, respectively. In a second treatment regime, seeds were treated with dry heat (60°C) for 6 hr followed by dry heat (90°C) for 4, 6, 8 or 12 hr. Chemical treatments included soaking seeds in sodium hypochlorite (1% or 2%) + 0.5% formal for 15 or 30 min, or in carbofuran 350 SX at 1× and 2× the recommended dosage for 30 min. To evaluate eradication efficacy, nematodes were recovered from four, 50-seed-replicates for each treatment by placing seeds on Baermann funnels. Four separate replicates were used to determinate the percentage seed germination, root length, and seed vigor. All treatments eradicated both nematodes species from rice seeds. Untreated controls exhibited an average of 97% germination, 96% vigor, and 6.45 cm root length. Sodium hypochlorite-formal treatments reduced root growth compared to controls. Moist heat of 40°C for 30 min, followed by moist heat of 60°C for 8 min resulted in the best seed performance compared to controls (96% germination, 94.5% vigor). For oat seeds, only the moist heat treatment and NaOCl plus formal eradicated *D. dipsaci,* but the latter treatment reduced seed germination. Thermal treatment of maize seeds at 40°C for 30 min, followed by 60°C for 8 min eradicated nematodes and the germination and vigor were similar to those of controls.

Key words: *Aphelenchoides besseyi,* chemical control, *D. dipsaci,* regulatory nematology, sanitation, thermal treatment.

RESUMEN

Tenente, R. C. V., V. Gonzaga, F. P. Pinheiro, P. Tarchetti y V. Rodrigues. 1999. Técnica de erradicación de nematodos parasitadores de plantas de las semillas de maíz, avena y arroz infestadas. Nematrópica 29:17-24.

La eficacia de varias técnicas químicas y físicas en la erradicación de *Aphelenchoides besseyi, Ditylenchus spp.* y *D. dipsaci* de las semillas de maíz, avena y arroz fue evaluada. Semillas de arroz, fueron expuestas a calor humedo a 40°C durante 15, 30 ó 60 minutos, y luego a 60°C por 5, 8 ó 10 minutos respectivamente. En un segundo régimen de tratamiento, las semillas fueron tratadas con calor seco (60°C) durante 6 horas, seguido por 90°C durante 4, 6, 8 y 12 horas. Los tratamientos químicos incluyeron el remojado de las semillas en hipoclorito de sodio (1 ó 2%) + formal 0.5% por 15 ó 30 minutos, o en carbofurano 350 SX a 1× y 2× de la dosis recomendada por 30 minutos. Para evaluar la eficacia de erradicación, se recuperaron nematodos de cuatro replicas de cincuenta semillas por cada tratamiento, poniendo las semillas en embudos Baermann. Cuatro replicas separadas se utilizaron para determinar el porciento de germinación de las semillas, largo de la raíz y vigor. Todos los tratamientos erradicaron ambas especies de nematodos de las semillas de arroz. Los controles no tratados exhibieron un promedio de 97% germinación, 96% vigor, y un largo de raíz de 6.45 cm. Los tratamientos con hipoclorito de sodio-formol redujeron el crecimiento de la raíz en comparación a los controles. El calor humedo a 40°C por 30 minutos, seguido por calor humedo a 60°C por 8 minutos

rindió el mejor comportamiento de las semillas en comparación a los controles (96% germinación, 94.5% vigor). Para las semillas de avena, solamente el tratamiento con calor humedo y NaOCl más formal erradicó *D. dipsaci*, pero el ultimo tratamiento redujo la germinación de las semillas. El tratamiento término de las semillas de maíz a 40°C por 30 min., seguido por 8 min. a 60°C erradicó a los nematodos y la germinación y el vigor mostraron resultados similares al control.

Palabras claves: Aphelenchoides besseyi, D. dipsaci, nematología regulatoría, sanidad, tratamiento término.

INTRODUCTION

The plant parasitic nematode genera *Aphelenchoides* and *Ditylenchus* have important species that can cause devastating plant damage and crop loss (Tenente and Manso, 1987). *A. besseyi* Christie, 1941, agent of white tip disease of rice, can be disseminated in seeds, in which the nematodes exist for long periods in a state of anhydrobiosis (Todd and Atkins, 1959; Yoshii and Yamamoto, 1950). Fukano (1962) reported that 30 nematodes per 100 seeds is the damage threshold for rice. Yield loss data for this nematode have been widely reported, and range from 4.7 to 54% of potential yield, depending on the rice variety, environmental conditions and initial population density in seeds (Bridge *et al.*, 1990). Although host races of *A. besseyi* are thought to exist, the evidence is limited (Noegel and Perry, 1963).

Ditylenchus angustus (Butler, 1913) Filipjev, 1936 causes "ufra" disease of rice which can also result in significant yield loss. Mondal and Miah (1987) reported that 60-70% of low lying areas in Bangladesh are infested with this species of nematode, and the nematode was reported to cause yield losses of 50-100% in both paddy and lowland rice in Vietnam (Cuc and Kinh, 1981). Yield losses of 90% and 30% were reported in West Bengal; in India, respectively (Pal, 1970; Rao *et al.*,1986).

The stem and bulb nematode, *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936, has a very wide host range (eg. broad bean, lentil, onion and garlic, potato, tobacco) and crop loss can be as high as 100% depending on the crop and conditions. Physiological races of the nematode were documented as early as the last century (Ritzema Bos, 1892), and up to 21 races of the nematode have been recognized by various workers (Hesling, 1965; Seinhorst, 1956, 1957).

Several reports of the use of pesticides for seed treatment to manage these nematode species indicate a reduction in nematode infestation levels, but not complete eradication of the pests (Cralley and French, 1952; Gomy and Kogure, 1956; Ishiy *et al.*, 1972; Martins *et al.*, 1976; Silveira, *et al.*, 1983; Tenente and Costa Manso, 1994; Tenente and Marques, 1983; Vuong and Rodrigues, 1972; Yoshii and Yamamoto, 1950). Pesticides tested include DPX-1410, methomyl, methyl tiophanate, carbofuran, parathion, folidol, phosphamidon, thiabendazole, and benomyl in different dosages and periods of exposure. Highest reduction of nematodes numbers have generally been achieved by use of parathion and carbofuran.

Thermal treatments of rice seeds, using hot water and varied temperatures and exposure times, have also been used to manage nematode infection (Cralley, 1949; 1952; Tenente and Costa Manso, 1994; Todd and Atkins, 1958; Yoshii and Yamamoto, 1950). Treatments using high temperatures (56-61°C) eradicated *Aphelenchoides besseyi* from rice seeds, but also decreased germination and seedling vigor of treated seeds.

Because quarantine programs must provide seeds without any nematode contamination, there is still a need to develop

treatments that eradicate nematodes from seeds without significant reduction in germination or seedling vigor. In this paper, we report results of studies conducted by EMBRAPA to use thermal and chemical treatments to eradicate juveniles of *A. besseyi* and *D. angustus* on imported rice seeds, and *D. dipsaci* on imported oat and maize seeds.

MATERIAL AND METHODS

Nematode disinfestation of rice seeds. Rice seeds infested with *A. besseyi* and *D. angustus*, imported from Philippines, were treated with moist heat, dry heat, or chemicals in the following treatment regimes: 1) moist heat of 40°C for 15, 30, or 60 min, followed by moist heat of 60°C for 5, 8, or 10 min, respectively; 2) dry heat of 60°C at 7% relative humidity (RH) for 6 hr followed by dry heat of 90°C for 4, 6, 8, or 12 hr; 3) chemical treatment with sodium hypochlorite (1% or 2%) plus 0.5% formal (methylene dimethyl ether) for 15 or 30 min, or with carbofuran 350 SX (35% a.i.) at 34 ml/L or 64 ml/L for 30 min. Moist heat treatments were applied by adding seeds to beakers of water, with mechanical agitation, in a 14 L circulating water bath at the desired temperature. Seeds in beakers were treated with dry heat using a programmable circulating dry air sterilizing chamber (model 320 SE, FANEN Ltd.).

Four replicates of each treatment, each consisting of 50 seeds, were used to evaluate eradication efficacy on infested rice seeds. An additional four replicates were used to determinate the effects of treatments on percent seed germination, seedling vigor, and root length (ISTA, 1976). Untreated rice seeds were used as controls for all variables evaluated. Rice seed germination was evaluated following 7 days in a growth chamber, at 25°C and 100% RH. Germinated seeds remained in the incubator, and the percentage of the seeds which germinated on day 7 that were still alive on day 14 was used as a measurement of seedling vigor. Root lengths were also measured after 14 days.

Nematodes were extracted from treated and untreated seeds by soaking seeds in distilled water for 15 hr 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 (25 (m opening) sieve and placed on a Baermann funnel for 24 to 72 hr. Nematodes passing the filter were counted using a dissecting microscope.

Nematode disinfestation of oat seeds. Ditylenchus dipsaci-infected oat seeds, imported from U.S.A., were treated with some of the successful treatment regimes used in the previous experiment. Seeds were dried at 22°C and 15% RH for 7 days to reduce the water content from 15% to 5%, after which they were treated with dry heat of 60°C for 6 hr, followed by dry heat of 95°C during 6 hr. A moist heat treatment regime consisted of pre-heating seeds in water at 40°C for 30 min, followed to 60°C for 8 min. In a third treatment, seeds were agitated in a solution of sodium hypochlorite (1%) + formal (0.5%) for 30 min. Eight replicates (50 seeds each) of each treatment were used to evaluate nematode survival in infested oat seeds, and 8 additional replicates were used to evaluate effects of treatments on seeds by the methods described previously.

Nematode disinfestation of maize seeds. Maize seeds infected by *D. dipsacy* were treated with the following regimes: 1) moist heat of 40°C for 30 min followed by either 60°C for 10 min, or 60°C for15 min; 2) dry heat of 60°C for 6 hours followed by either 95°C for 6 hr, or 95°C for 5 hr (after drying maize seeds to 7% moisture content); 3) sodium hypochlorite (1%) + formal (0.5%) for 30 min or 40 min.

Untreated seeds served as controls. Treatment efficacy was evaluated as described previously using 4 replicates of 50 seeds each.

Data analyses. For all experiments, data for percentage germination and vigor were transformed (arcsin square-root) prior to analysis of variance and mean separation by Dunnet's Test to compare all treatments with the control. Root lengths and transformed (log [X + 1]) nematode data were also subjected to analysis of variance and mean separation using Tukey's Honestly Significant Difference Test ($P = 0.05$).

RESULTS

All treatments except the low rate of carbofuran appear to have eradicated *A. besseyi* and *D. angustus* in rice seeds; however, the treatments had variable affects on the different seed growth parameters (Table 1). Compared to untreated seeds, moist heat and chemical treatments had little if any affect on seed germination, whereas dry heat generally depressed germination. In contrast to both moist and dry heat, chemical treatments greatly reduced the length of roots from germinating rice seed. Moist heat treatments also had the least detrimental effect of all treatments on seedling vigor.

The single moist heat treatment and the chemical treatment involving NaOCl and formal were the only treatments that appear to have eradicated *D. dipsaci* from oat seeds (Table 2). All treatments reduced the length of roots substantially. Unlike the results for rice, both chemical treatments reduced the germination rate of oat seeds substantially, but did not depress the length of roots of surviving seeds more than the moist heat treatment. The dry thermal treatment depressed all root parameters of oats in a manner similar to those for rice.

Growth of maize seeds was severely impaired by dry heat treatments (Table 3). Moreover, while moist heat and chemical treatments did not appreciably impair the germination or vigor of maize seeds, they both reduced the lengths of the roots substantially. All treatments appear to have eradicated the *D. dipsaci* in the seeds.

DISCUSSION

The most commonly encountered nematodes detected in these imported seeds were in the genus *Ditylenchus*. The large number of races that exist among *D. dipsaci* make it particularly important from a regulatory standpoint (Seinhorst, 1956; 1957; Hesling, 1965; Metlitsky, 1972). Contamination of imported plant material by *D. angustus* is of similar concern because there is no occurrence of this species in the Americas (Tenente and Manso, 1987). Because of the need to introduce new germplasm for crop improvement, methods to detect and eradicate nematode pests in propagative material is crucial. The results of this study indicate that use of 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 relative to the other treatments. Chemical treatments were markedly inferior to the other treatments in terms of root length of rice and maize, and germination and vigor of oats. Similar results using these chemicals to disinfest rice were reported by Yoshii and Yamamoto (1950), Cralley and French (1952), Gomy and Kogure (1956), Ishiy *et al.* (1975), Tenente and Manso (1994), and Martins *et al.* (1976). In contrast, the moist heat treatments did not appear to affect germination of any seed type in this study, and detrimental effects of moist heat on vigor and root length of seeds were gener-

Table 1. Mean percent seed germination, seed vigor, root length, and number of nematodes in rice seeds following treatment with wet or dry heat or chemicals.

Treatment	Germination (%)	Seed vigor (%)	Root length[z] (cm)	Number *A. besseyi*	Number *D. angustus*
Moist Heat					
40°C/15 min + 60°C/10 min	96.0	92.0	6.82 ab	0.0 c	0 b
40°C/30 min + 60°C/8 min	96.0	94.5	7.42 ab	0.0 c	0 b
40°C/60 min + 60°C/5 min	96.0	93.0	5.80 bc	0.0 c	0 b
Dry Heat					
60°C/6 hr + 95°C/4 hr	89.0*	88.5	7.75 ab	0.0 c	0 b
60°C/6 hr + 95°C/6 hr	94.0	93.5	7.08 ab	0.0 c	0 b
60°C/6 hr + 95°C/8 hr	92.5	90.5	7.18 ab	0.0 c	0 b
60°C/6 hr + 95°C/12 hr	87.0*	83.5*	8.82 a	0.0 c	0 b
Chemical					
NaOCl (1%) + formol (0.5%)/15 min	96.6	87.5	1.06 f	0.0 c	0 b
NaOCl (1%) + formol (0.5%)/30 min	94.0	91.5	1.45 ef	0.0 c	0 b
NaOCl (2%) + formol (0.5%)/15 min	96.6	87.5	1.15 ef	0.0 c	0 b
NaOCl (2%) + formol (0.5%)/30 min	96.0	92.0	1.30 ef	0.0 c	0 b
Carbofuran 35% 32 ml/L/30 min	94.5	93.0	3.92 cd	1.3 b	0 b
Carbofuran 35% 64 ml/L/30 min	94.0	89.5	3.18 de	0.0 c	0 b
Untreated Control	96.0	96.0	6.45 b	18 a	2 a

*Treatment differs ($P = 0.05$) from untreated control according to Dunnet's Test to compare all treatments against a control.

[y]Means of 4 replicates, each consisting of 50 seeds.

[z]Treatments followed by the same letter do not differ ($P = 0.05$) according to Tukey's Honestly Significant Difference Test.

ally less than those of other types of treatments. Cralley (1952) and Todd and Atkins (1958) were unable to eradicate seed-borne nematodes using similar temperature ranges, perhaps due to methodological differences (seeds were not shaken during treatment). Yoshii and Yamamoto (1950) eradicated *A. besseyi* from rice seeds with moist heat at 57°C, without any prior treatment. While dry heat produced results nearly comparable to those of moist heat for rice and oats, dry heat severely compromised the performance of maize seeds.

In conclusion, these results indicate good potential to successfully eradicate certain seed-borne nematodes in rice seeds, using moist or dry heat treatments and also in oat and maize seeds using

Table 2. Mean[y] percent seed germination, seed vigor, root length, and number of nematodes in oat seeds following treatment with wet or dry heat or chemicals.

Treatment	Germination (%)	Seed vigor (%)	Root length[z] (cm)	Number *D. Dipsaci*
Moist Heat				
40°C/30 min + 60°C/8 min	92.5	87.3	5.1 c	0.00 c
Dry Heat				
60°C/6 hr + 95°C/6 hr	83.3*	82.0*	6.8 b	1.00 bc
Chemical				
NaOCl (1%) + formol (0.5%)/30 min	75.8*	72.3*	7.5 b	0.00 c
Carbofuran 35% 32 ml/L/30 min	53.3*	60.0*	4.8 c	2.25 b
Untreated Control	95.5	95.0	11.2 a	8.00 a

*Treatment differs (P = 0.05) from untreated control according to Dunnet's Test to compare all treatments against a control.
[y]Means of 8 replicates, each consisting of 50 seeds.
[z]Treatments followed by the same letter do not differ (P= 0.05) according to Tukey's Honestly Significant Difference Test.

Table 3. Mean[y] percent seed germination, seed vigor, root length, and number of nematodes in maize seeds following treatment with wet or dry heat or chemicals.

Treatment	Germination (%)	Seed vigor (%)	Root length[z] (cm)	Number *D. Dipsaci*
Moist Heat				
40°C/30 min. + 60°C/10 min	98.0	99.0	2.54 cd	0 b
40°C/30 min + 60°C/15 min	97.0	97.3	2.59 cd	0 b
57°C/10 min	97.8	97.3	3.47 bc	0 b
Dry Heat				
60°C/6 hr + 95°C/6 hr	6.3*	0.0*	4.25 bc	0 b
60°C/6 hr + 95°C/5 hr	7.5*	0.0*	5.11 b	0 b
Chemical				
NaOCl (1%) + formol (0.5%)/30 min	95.8	92.8*	1.66 d	0 b
NaOCl (1%) + formol (0.5%)/40 min	94.8*	95.8	1.54 d	0 b
Untreated Control	98.0	98.5	14.18 a	2 a

*Treatment differs (P = 0.05) from untreated control according to Dunnet's Test to compare all treatments against a control.
[y]Means of 4 replicates, each consisting of 50 seeds.
[z]Treatments followed by the same letter do not differ (P= 0.05) according to Tukey's Honestly Significant Difference Test.

moist heat. As part of a nematode quarantine program, EMBRAPA is using these methods combined with careful nematological evaluation of treated seeds, to release imported germplasm to university 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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