WHITE TIP DISEASE IN ITALIAN RICE

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Abstract
Aphelenchoides besseyi Christie is a seed-borne plant-parasitic nematode and is the causal organism of the "white tip" disease of rice. It has been reported from most of the rice growing countries and recently it was found in Italian rice field and seed, even if quarantine has been applied to prevent the nematode introduction in European Union. In the last years all seed lots produced in Italy have been tested for detecting *Aphelenchoides besseyi*. Data are reported concerning the population density of nematode in the seed lots officially inspected for certification in the last 6 years. At the same time experimental research started - to implement information coming from other rice growing countries - on distribution, varietal symptom expression and vulnerability, possible survival in the field, yield loss, economic damage threshold and control by chemicals. Moreover a reliable and efficient method for detecting *Aphelenchoides besseyi* in seed was prepared on the basis of those reported in literature. Results obtained confirm information gathered in other countries. Briefly, seed transmission is the principal dispersal way for *Aphelenchoides besseyi* in Italy. Hot water treatment is confirmed to be the most appropriate control method, unfortunately it cannot be applied to large amount of seeds. Data are reported concerning the relation between seed infestation and crop performance in trials where 2 varieties, 2 cultural systems (flooded or dry-seeded crop) and 4 infestation levels were compared: 30 viable nematodes per 100 seeds is the tolerance limit density beneath which there is no significant damage on flooded rice crop.

Introduction
Aphelenchoides besseyi Christie is a plant-parasitic nematode and is the causal organism of the "white tip" disease of rice. In the 1950-60s this disease was deeply studied particularly in Japan and in the U.S.A.
Aphelenchoides besseyi is borne by rice seed, in which it can survive for up to three years in a dehydrated state. In infested fields the average yield loss ranges from 10% to 30% depending on the susceptibility of the variety grown and, obviously, on nematode population density (1).
Even if the number of studies done on crop loss due to the nematode is very limited, an inverse correlation between the number of nematodes in seeds and crop performance was demonstrated (2). Generally population densities/seed number or weight are counted. It was estimated (3) that the density of approximately 30 live nematodes per 100 seeds can be accepted as a tolerance limit correlated to a maximum 5% loss in yield for susceptible varieties. It was also determined an economic damage threshold density of 300 live nematodes/100 seeds (4).
Surveys showed large number of seed lots infected and high incidences of infection by *Aphelenchoides besseyi* within seed lots throughout the main production areas of the world. It was reported from most of the rice growing countries of Africa, North, Central and South America, Asia, Eastern Europe and the Pacific (5, 6). Recently it was reported from Italy, too (7, 8).
Aphelenchoides besseyi is primarily spread through infested seeds; it adapts to seed transmission thanks to its capacity to remain quiescent upon dehydration and reactivate with rehydration. The number and infectivity of nematodes is reduced as seed age increases (9), however good seed storage conditions probably prolong nematode survival.
Aphelenchoides besseyi can also be dispersed from infested seed by irrigation water and can live in rice debris left in the field after harvest which enables carry over of inoculum from season to season (1), but it cannot survive in the soil (10). Moreover, *Aphelenchoides besseyi* has a wide host range (Italian millet, strawberry, soybean) including weed species of rice field (wild rice, *Echinocloa* spp.) (1, 11, 12).
Since the nematode is seed-borne, treatment of seed is the best control method for *Aphelenchoides besseyi*: the hot water treatment is the most effective, even if not eradicant, but unfortunately it cannot be applied to large amount of seeds. Various chemical seed treatments were used which allowed a good control but did not give eradication (11, 13).
As a seed-borne pathogen, *Aphelenchoides besseyi* is a potential target for quarantine, and many countries (including EU) enacted regulations to prevent its introduction. In EU the Council Directive n. 2000/29 states that *Oryza* spp. seeds can be imported in the Community only after they underwent an official health test and resulted free from *Aphelenchoides besseyi* or they were subjected to an effective treatment (hot water or whatever else). Exchanges of rice seeds between EU countries are not subjected to any control.

White tip disease in Italy
In 1996 white tip symptoms were observed for the first time in some paddy fields in Italy (8) and *Aphelenchoides besseyi* was extracted from different parts of plants harvested in the infested crops (14).

Moreover *Aphelenchoides besseyi* has been detected in seed of the cultivars grown in the infested fields. From then onwards, all seed lots produced in Italy have been tested for detecting *Aphelenchoides besseyi* and only the free lots have been certified. Table 1 shows data concerning the population density of nematode in the seed lots officially inspected for certification.

Table 1 Nematode population density in Italian rice seed lots.

N. of nematode/ 100 seeds	Seed lots									
	1997		**1998**		**1999**		**2000**		**2001**	
	n.	%	n.	%	n.	%	n.	%	n.	%
0	845	90.0	953	96.7	936	97.1	932	96.5	943	96.3
1-30	81	8.6	32	3.2	28	2.9	34	3.5	35	3.6
31-300	8	0.9	1	0.1	0	0.0	0	0.0	1	0.1
>300	5	0.5	0	0.0	0	0.0	0	0.0	0	0.0
Total	939	100.0	986	100.0	964	100.0	966	100.0	979	100.0

Legenda - 30 nematode/100 seeds: tolerance limit density.
300 nematode/100 seeds: economic damage threshold density.

Few lots resulted infested and the population density was higher than the economic damage threshold (300 nematode/100 seeds) in five lots only, in 1997. In the following years the density of *Aphelenchoides besseyi* in the infested lots was lower than the tolerance limit (30 nematode/100 seeds).
Testing all seed lots and rejecting certification of the infested ones showed to be an effective procedure to readily lower the nematode population density in following years, so that no crop damage was ever reported except where untested farm seed was used. However it is worth noting that the percentage of lightly infested lots has remained basically the same for the last five years, so that eradication of *Aphelenchoides besseyi* from rice seed seems to be impossible to achieve.
Testing all seed lots gave also interesting information about the varietal infestation and the management of the disease. A wide difference in susceptibility is known to exist between varieties (1). In table 2 data are reported concerning the percentage of infested seed lots of the main Italian varieties, in the period 1997-2001. The traditional Italian varieties – used to cook *risotto* (Arborio, Baldo, Carnaroli, Vialone Nano, Volano) - were the most infested, while *Aphelenchoides besseyi* was rarely recovered in the seed of indica type varieties (Gladio, Thaibonnet).

Table 2 Percentage of infested seed lots of the main Italian rice varieties.

Variety	Grain type	1997	1998	1999	2000	2001
Baldo	Long A	57.1	16.7	12.2	5.4	6.1
Carnaroli	Arborio-type	50.0	5.9	0.0	3.6	14.3
Volano		46.2	8.0	0.0	5.7	2.9
Arborio		12.2	8.9	5.9	4.4	3.3
S.Andrea		4.2	0.0	5.6	5.7	0.0
Vialone nano		3.6	4.2	0.0	17.4	33.3
Roma		0.0	0.0	0.0	3.0	0.0
Balilla	Round grain	0.0	0.0	0.0	0.0	0.0
Selenio		2.9	0.0	1.8	1.3	0.0
Ariete	Long A for	8.1	0.0	0.0	2.4	2.2
Loto	parboiling	3.6	9.4	13.5	5.6	4.3
Nembo		-	0.0	0.0	0.0	1.9
Gladio	Long B	0.0	0.0	0.0	0.0	0.0
Thaibonnet	Indica-type	3.3	0.0	0.8	0.0	2.7

The plants of the old traditional varieties are very tall (culm length of cv Carnaroli is 120 cm) and susceptible to lodging, as a consequence they are often sown in drills and flooded afterwards. This cultural practice helps the transmission of the nematode from seed to seedling, in fact direct sowing of seeds in water rather than sowing followed by flooding is recommended to reduce the nematode population (13). The results of experimental trials (see below) confirm this statement.
Since the presence of *Aphelenchoides besseyi* in Italy was reported, the foundation seed of the main rice varieties

has been treated yearly with hot water (55-61°C for 10-15 min) to control the diffusion of the nematode. The treatment proved to be effective in quickly and significantly reducing the presence of *Aphelenchoides besseyi* in the commercial seed of those varieties. For some varieties foundation seed did not undergo hot water treatment, and several lots of commercial seed of these varieties (i.e. Vialone nano) are still rejected for certification due to nematode infestation.
Since *Aphelenchoides besseyi* was detected in Italy, experimental research has started to implement information coming from other rice growing countries on yield loss, varietal vulnerability, possible survival in the field, and control methods. Moreover symptoms of the disease were studied in the main Italian varieties for possibly detecting the infestation in field [see the poster presented to this Conference by Giudici and Villa (15)]. An efficient and reliable method for detecting *Aphelenchoides besseyi* in seed was also devised (16), comparing and modifying different available methods, since no official method exists.

Detecting method
All methods available for detecting *Aphelenchoides besseyi* are based on the principle of reactivating the quiescent nematodes soaking seeds in water, resulting in their migrating out of the seeds followed by microscopic detection. For quantitative analysis it is recommended the use of five samples of 100 seeds to obtain consistent nematode recovery (2, 5, 17).
For routine testing the technique cannot be too much time-consuming and laborious, so the critical steps are two: the separation of glumes from the kernel to let the nematodes migrate into the water and the extraction period.
Using undehulled seeds, time of extraction must be longer than two days, to cause the migration of nematodes into the water through the openings in the hulls created by imbibition and the starting of the process of germination. Dehulling of rice seeds results in significantly more nematodes detected in the grains even after only one day of extraction, making it important in quantitative analysis (17). Unfortunately manual dehulling is tedious and time-consuming. The use of a small hand milling equipment, very easy to clean, proved to be suitable: in few minutes kernels and hull can be separated and soaked in water for one day (16).
Two extraction methods were compared: the nylon sieve and the Baermann funnel methods (5). In both cases nematodes were collected at 24-hours intervals up to 10 days. Dehulled seeds gave higher nematode counts than undehulled seeds in both extraction methods. The nylon sieve method consistently gave higher nematode counts than the Baermann funnel method for both types of seeds.
Briefly the procedure adopted for detecting *Aphelenchoides besseyi* in the experimental trials in Italy is the following: seeds (5 samples of 100 seeds) are dehulled with a hand milling equipment, very easy to clean; kernels and hull are transferred to a nylon sieve placed in a 50 ml beaker filled with 40 ml distilled water; the apparatus is left undisturbed for 24 hours; the sieve is removed from the beaker and washed with 20 ml distilled water, finally added to the water in the beaker; the water (60 ml) is transferred to a De Griss counting dish and examined under a stereo-binocular stereo-binocular microscope (50 x) (16).

Seed infestation and crop performance
The relation between the number of *Aphelenchoides besseyi* in the seed and the performance of the subsequent crop had been studied for three years comparing the two cultural practices used in Italy: direct sowing in water and sowing followed by flooding. On the basis of preliminary studies and of the degree of seed infestation, two susceptible varieties were used:
- Baldo, a traditional Italian variety (Arborio-type), whose seed was badly infested and whose plants show typical “white tip” symptoms;
- Cripto, a short grain variety, whose plants does not show any leaf symptom even if they are badly infested.

Materials and methods
The same seed was used both in flooded and in dry seeded condition. For all the trials the seed rate was 180 kg/ha and the following 4 infestation levels, expressed as number of nematodes in 100 seeds, were compared: 0 – 30 – 300 – 700/1200 (the maximum infestation level varied with variety and year). Each trial was laid in a complete randomized design with four replications.
In 1999 seed for the four infestation levels came from four different lots and the lot assumed to be healthy was hot water treated at 59-60 °C for 15' to be sure that the infestation level was “0”. On the contrary in 2000 and 2001 seed came from one badly infested lot where the nematode population density corresponded with the maximum infestation level. Part of this seed was hot water treated at 55 °C for 15' to obtain the infestation level “0”. This treated seed was afterwards mixed with the infested one until the desired infestation levels of “30” and “300” were got. For the nematode detection the method previously described was followed.
In flooded condition, plots, whose size was different each year ranging from 28 m^2 to 37 m^2, were separated lengthways by levees and transversely by ondolux to allow individual irrigation and draining and so preventing dissemination of the nematode through water from one plot to another. Seed was hand spread and after a week plots were drained for a short period to enhance rice seedling establishment. Before harvesting with a plot

combine harvester, in each plot 20 plants at random were measured for plant height and panicle length, and 8 sub-plots of 0,25 m^2 were hand harvested in order to measure panicle density, dry matter, 1000-seed weight and number of nematodes per 100 seeds. Grain yield was evaluated on the whole plot. In 2001 ten panicles per plot were also collected to count the number of spikelets/panicle.

In dry seeded condition plots size was different each year varying from 41 m^2 to 69 m^2 and fields were drilled with a automatic drill-machine. At the 3-4th leaf stage of rice plots were separately flooded. A levee surrounded each plot and individual irrigation and draining were provided to prevent movement of nematodes from plot to plot. Before harvesting with a plot combine harvester, in each plot 40 plants at random were measured and 20 sub-plots of 0,25 m^2 were hand harvested in order to get the above mentioned parameters. Also in this case grain yield was evaluated on the whole plot. In 2001 twenty panicles per plot were collected to count the number of spikelets/panicle.

Results

Results are reported in table 3-5.

Table 3 Crop performance of the cv Baldo in flooded and dry-seeded crop using seed with 4 nematode infestation levels.

Treatments	Grain yield (1) t/ha	Yield reduction (2) %	Plant height (3) cm	Panicle length cm	Panicle density (4) no./m^2	Dry matter (4) g/m^2	1000-seed weight g	Spikelets/ panicle no.
				BALDO - FLOODED CROP				
1999								
0	8.41 a	-	92.8 a	18.2 a	297 a	1760 a	41.0 a	-
30	8.31 a	1.2	91.9 a	18.0 a	311 a	1863 a	40.8 a	-
300	8.12 a	3.5	90.7 a	18.1 a	292 a	1749 a	40.4 a	-
1200	6.45 b	23.3	82.9 b	17.5 a	315 a	1741 a	38.2 b	-
2000								
0	7.22 a	-	95.2 a	16.4 a	386 a	1739 a	36.7 a	-
30	7.05 a	2.5	94.9 a	16.6 a	391 a	1749 a	35.4 ab	-
300	6.43 b	10.9	92.4 ab	16.7 a	381 a	1783 a	34.6 bc	-
900	5.38 c	25.6	90.0 b	15.5 b	386 a	1699 a	33.4 c	-
2001								
0	7.70 a	-	91.6 b	18.7 a	326 a	1608 a	41.8 a	104 a
30	7.85 a	0.0	95.1 a	19.1 a	321 a	1687 a	41.3 a	111 a
300	7.11 b	7.6	90.1 b	18.6 a	329 a	1543 a	39.1 b	103 a
700	6.30 c	18.1	85.8 c	17.5 b	316 a	1381 b	38.8 b	99 a
				BALDO - DRY-SEEDED CROP				
2000								
0	7.61 a	-	96.5 a	16.0 a	286 c	1570 a	35.4 a	-
30	5.19 b	31.8	87.5 b	16.1 a	307 b	1510 ab	28.4 b	-
300	4.44 c	41.7	79.3 c	15.2 b	350 a	1462 bc	25.9 c	-
900	4.41 c	42.0	79.2 c	14.7 c	359 a	1408 c	25.4 c	-
2001								
0	8.30 a	-	94.8 a	18.8 a	249 b	1601 a	41.7 a	128 a
30	7.30 b	12.0	89.8 b	18.5 b	237 b	1430 b	38.1 b	133 a
300	5.73 c	31.0	81.7 c	17.0 c	281 a	1363 b	32.3 c	109 b
700	5.70 c	31.3	81.0 c	17.0 c	287 a	1323 b	32.1 c	101 b

(1) Calculated on the whole plot, 14% moisture content.
(2) Comparison with population density = 0.
(3) Measured from soil to neck node.
(4) Mean of eight 0,25 m^2 sub-plot; dry matter calculated on the whole plant without root.
Means followed by the same letter are not significantly different at $P < 0,05$ (Duncan's test).

Generally the higher is the number of nematodes in 100 seeds, the more serious is the yield loss. All the parameters show the same trend of the grain yield, except panicle density. The differences in the panicle density in 1999 are a consequence of the different 1000-seed weight of the seed lots used and of the reduction of germination due to hot water treatment of seed in the other two years. Taking into consideration the panicle density data it seems that the negative effect of the hot water treatment, which takes place only when rice is heavily infested (data in press), is enhanced in dry seeded rice: this aspect might be considered in future specific trials.

Table 4 Crop performance of the cv Cripto in flooded and dry-seeded crop using seed with 4 nematode infestation levels.

Infestation level	Grain yield (1)	Yield reduction (2)	Plant height (3)	Panicle length	Panicle density (4)	Dry matter (4)	1000-seed weight	Spikelets/ panicle
	t/ha	%	cm	cm	no./m^2	g/m^2	g	no.
				CRIPTO - FLOODED CROP				
1999								
0	8.95 a	-	69.2 a	15.1 a	429 a	1713 a	31.4 a	-
30	8.93 a	0.3	68.9 a	15.3 a	400 a	1761 a	31.1 a	-
300	8.59 a	4.0	67.2 a	14.2 b	423 a	1685 a	31.0 a	-
800	7.99 b	10.8	65.4 a	13.8 b	445 a	1693 a	29.7 b	-
2000								
0	7.70 a	-	72.7 a	15.2 a	464 a	1772 a	27.8 a	-
30	7.60 a	1.3	71.7 a	15.1 a	489 a	1725 a	27.9 a	-
300	6.22 b	19.2	71.4 a	15.0 ab	488 a	1781 a	26.8 b	-
900	4.50 c	41.5	70.8 a	14.7 b	485 a	1630 b	25.9 c	-
2001								
0	8.31 a	-	67.3 a	15.7 a	424 a	1544 a	31.3 a	97 a
30	8.47 a	0.0	67.1 a	15.7 a	414 a	1575 a	31.1 a	107 a
300	7.56 b	9.0	66.9 a	15.6 a	430 a	1531 a	29.7 b	101 a
900	5.92 c	28.8	62.6 b	14.2 b	428 a	1403 b	27.6 c	96 a
				CRIPTO - DRY-SEEDED CROP				
1999								
0	9.50 a	-	71.7 a	16.8 a	252 b	1560 a	32.0 a	-
30	8.27 b	13.0	68.9 b	16.0 b	262 b	1428 b	29.9 b	-
300	6.52 c	31.4	63.0 c	14.9 c	266 b	1265 d	26.5 c	-
800	6.24 c	34.4	62.4 c	14.2 d	313 a	1332 c	25.4 d	-
2000								
0	8.21 a	-	75.0 a	15.4 a	366 b	1726 a	26.7 a	-
30	5.69 b	30.7	71.8 b	14.7 b	377 b	1620 ab	24.3 b	-
300	4.90 c	40.3	66.1 c	13.5 c	385 b	1523 b	23.6 b	-
900	4.76 c	42.1	65.8 c	13.5 c	435 a	1581 b	22.5 c	-
2001								
0	8.71 a	-	67.2 a	16.8 a	307 a	1470 a	31.1 a	130 a
30	8.07 b	7.3	67.1 a	16.7 a	308 a	1448 a	31.1 a	122 ab
300	5.37 c	38.4	62.8 b	16.1 b	342 a	1287 b	26.7 b	110 bc
900	4.09 d	53.1	58.6 c	14.3 c	330 a	1158 c	24.9 c	97 c

(1) Calculated on the whole plot, 14% moisture content.
(2) Comparison with population density = 0.
(3) Measured from soil to neck node.
(4) Mean of eight 0,25 m^2 sub-plot; dry matter calculated on the whole plant without root.
Means followed by the same letter are not significantly different at P < 0,05 (Duncan's test).

Comparing the two cultural conditions, it is evident a greater yield loss in drilled rice in all the years and for both the varieties. At the maximum infestation level, the greatest yield loss was reached in dry seeded crop in 2001 and in flooded rice in 2000, by cv Cripto (Table 4): the former amounts to 53.1%, the latter to 41.5%. Nevertheless it must be pointed out that in 2000 the climate was very favourable to *Aphelenchoides besseyi* and that in the other years the yield loss in flooded crop did not exceed 28.8% (Table 4). In dry seeded condition the grain yield does not show significant differences between the maximum infestation level and level "300", except for Cripto in 2001 (Table 4); on the contrary in flooded condition this difference is significant. The difference between the two cultural conditions is more remarkable at the infestation level "30"; in fact, while in drilled rice yield is significantly lower than yield at level "0", in flooded rice the differences are not significant. The highest yield reduction in flooded rice, amounting to 2.5%, was got by Baldo 30 in 2000 (Table 3) when, as mentioned

above, climate was very favourable to the disease. In dry seeded rice the yield reduction at the infestation level "30" ranges from 7.3% of Cripto (Table 4) to 31.8% of Baldo (Table 3), respectively in 2001 and in 2000.
Both plant height and panicle length are more reduced in drilled than in flooded rice. Also the reduction in the 1000-seed weight is greater in dry seeded crop and this is due to a reduction of the spikelet size. The number of spikelets per panicle, recorded only in 2001, is not significantly different in flooded rice crop, but differs significantly in the dry seeded one.
Taking into consideration the detection of nematodes at harvest, the number of nematodes found in 100 seeds is similar or less than the initial infestation level for all the treatments (Table 5). In particular, in flooded rice, at the infestation level "30" the population density ranges from 15 to 74, the latter was detected when the climate was very favourable to *Aphelenchoides besseyi*. The fact that some nematodes were detected also at the infestation level "0" confirms that the hot water treatment is not eradicant.

Table 5 Nematodes detected at harvest from flooded rice crop sowed using seed infested with 4 different nematode infestation levels.

BALDO		CRIPTO	
Infestation level	Nematodes at harvest (1)	Infestation level	Nematodes at harvest (1)
	1999		
0	0	0	0
30	30	30	53
300	142	300	169
1200	615	800	524
	2000		
0	2	0	1
30	42	30	74
300	422	300	395
900	673	900	668
	2001		
0	1	0	1
30	15	30	17
300	335	300	242
700	417	900	613

(1) Number of nematodes in 100 seeds, average of 5 samples each consisting of 100 seeds.

Conclusion

The trials confirm that the damage caused by *Aphelenchoides besseyi* is more serious in drilled rice. As the infestation level increases, the 1000-seed weight, the dry matter, the number of spikelets per panicle, the plant height and the panicle length decrease as well as the grain yield. Also milled rice quality is affected owing to the reduced size and the deformed shape of the grains.
In flooded rice although the above mentioned parameters gradually decrease, the damage is less serious. In particular at the infestation level "30" all the parameters are not significantly different from those at the infestation level "0". Moreover it is interesting to notice that the number of nematodes in 100 seeds detected at harvest is lower than the initial infestation level. Therefore this data suggests that the tolerance limit of 30 nematodes in 100 seeds reported in literature could be valid also in the Italian environment for the flooded condition, which is the traditional and the most used practice. According to the data such a limit seems to be too high for drilled rice.
The results of other studies performed in Italy confirm information obtained in other rice countries.
A wide difference in susceptibility and symptom expression exists between Italian rice varieties, which can be rank in three groups on the basis of the leaf symptom expression [see the poster presented to this Conference by Giudici and Villa (15)]. Most of indica-type varieties grown in Italy could possibly be ranked in a fourth group, their plants showing no symptoms.
Concerning the transmission of the disease, seed is the principal dispersal method for *Aphelenchoides besseyi*, even if flood water can carry over the inoculum from field to field.
Survival of the nematode in the field was never demonstrated: sowing healthy seed in paddy fields where straw was left of badly infested crops grown the previous year (about 1300 nematode/100 seeds), the disease never occurred in three years trials, both in flooded and dry-seeded rice. However nematodes were detected in rice stubble collected in the above mentioned fields (unpublished data) and in weed seeds as wild rice, *Cyperaceae*

and *Echinocloa* spp. (16).
Hot water treatment of seed confirmed to be the best control method for *Aphelenchoides besseyi*, a treatment of 10-15 min at 55-61 °C destroys the nematodes without affecting germination of the seed of the main Italian varieties, except for seed badly infested (data in press). However it is possible to apply this treatment only to small amount of seed, i.e. foundation seed.
Various chemical seed treatments were also tested, soaking the seed in different solutions for 24/48 hours before sowing. They allowed a good control but did not give eradication (unpublished data).
It is worth noting that none of these chemicals is registered in the European Union to treat rice seed and their use would cause negative environmental impacts. Moreover the practice is still common of sowing farm seed which does not undergo health test and can disseminate the disease, so eradication of *Aphelenchoides besseyi* from rice seed is considered impossible to achieve in Italy and EU. On the other hand the restriction imposed by quarantine on rice seed exchange proved to be ineffective to prevent the introduction of *Aphelenchoides besseyi* in EU. Since 1989 doubts have been indeed expressed by J.C. Prot (18) on the benefits produced by quarantine applied for *Aphelenchoides besseyi* at the international level.
For these reasons, a tolerance limit could be fixed for *Aphelenchoides besseyi* in rice seed provided that all seed lots are officially tested prior to certification. A population density threshold of less than 30 live nematodes per 100 seeds could be feasible to prevent any crop damage.
A hot water treatment is recommended for foundation seed and for all seed lots exchanged for scientific research purposes.

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