

Survival of *Aphelenchoides besseyi* in Infested Rice Seeds Stored under Controlled Conditions¹

RENATA C. VILARDI TENENTE², MARIA MAGALY V. S. WETZEL²,
EDNA STELLA B. G. COSTA MANSO² & ABI SOARES A. MARQUES²

SUMMARY

Tenente, R. C. V.; M. M. V. S. Wetzel; E. S. B. G. C. Manso & A. S. dos S. Marques, 1994. Survival of *Aphelenchoides besseyi* in infested rice seeds stored under controlled conditions. *Nematol. Brasileira* 18:85-92.

The objective of the experiment was to determine the number of live nematodes of species *Aphelenchoides besseyi*, in rice seeds either fumigated or not, stored at 10 and 18°C, as well as to observe the germination percentage and the vigour of infected seeds. (It was carried out for 30 months in which period the effects of temperature, fumigation on the storage of infested seeds were registered every six months. The number of detected nematodes increased significantly with the storage period was higher at 18 than 10°C.) The temperature affected the number of nematodes and the seed vigour but not the germination. The fumigation had no significant effect. The correlation coefficient showed that the percentage of germination and the seed vigour were directly proportional and both were inversely proportional to the number of live nematodes.

Key words: *Aphelenchoides besseyi*, survival, temperature, germination, vigour.

Recebido para publicação: 4/7/1994.

¹ Presented in the Brazilian Congress of Seeds, Brasília, DF, Brazil, October/1985.

² CENARGEN/EMBRAPA. Cx.P. 2372. 70849-970 Brasília, DF - Brazil.

RESUMO

Tenente, R. C. V.; M. M. V. S. Wetzel; E. S. B. G. C. Manso & A. S. dos S. Marques, 1994. Sobrevivência de *Aphelenchoides besseyi*, em sementes de arroz conservadas sob condições controladas. *Nematol. Brasileira* 18:85-92.

Estudou-se a sobrevivência do nematóide *Aphelenchoides besseyi* em sementes de arroz, fumigadas ou não e armazenadas em câmara de conservação a 10° C e em "freezer" a -18° C. A avaliação do número de nematóides vivos nas sementes foi feita a cada seis meses, durante três anos. Paralelamente, foram realizados testes do poder germinativo e vigor das sementes. O delineamento experimental utilizado foi o inteiramente casualizado, com 20 tratamentos e 4 repetições. Os dados foram analisados pelo teste de Duncan a 1% de probabilidade e, as correlações, calculadas entre o número de nematóides, o poder germinativo e o vigor das sementes. O número de nematóides detectados aumentou sensivelmente com o tempo de armazenagem e foi maior em sementes conservadas a -18° C. O número de nematóides não foi alterado pela fumigação das sementes. Foram estudadas as interações fumigação x temperatura, fumigação x tempo de armazenamento, temperatura x tempo de armazenamento e fumigação x temperatura x tempo de armazenamento. Para o número de nematóides, as correlações estudadas foram significativas. Entretanto, para o poder germinativo, nenhuma interação apresentou significância. Em relação ao vigor, somente a interação fumigação x temperatura não foi significativa. O coeficiente de correlação indica que o poder germinativo e o vigor das sementes são diretamente proporcionais entre si e inversamente proporcionais ao número de nematóides detectados.

Palavras-chaves: *Aphelenchoides besseyi*, sobrevivência, temperatura, germinação, vigor.

INTRODUCTION

Several nematode species are known to be associated with rice, *Oriza sativa* L. (Timm, 1965; Sher, 1968). Investigations on the biology of nematode diseases of rice have been concentrated upon four genera: *Aphelenchoides*, *Ditylenchus*, *Heterodera* and *Hirschmanniella*. The white tip nematode, *Aphelenchoides besseyi*, is distributed throughout the world (Ou, 1985; Fortuner and Williams, 1975) and causes economic losses in grain yield (Yoshii and Yamamoto, 1959; Atkins & Todd, 1959; Huang, 1959). It is a seed-borne nematode, capable of withstanding desiccation and may be found in quiescent state beneath the hulls of rice grains (Huang, 1978). They may remain viable for two to

three years on dry grain, but die in four months on grains left in the field (Yoshii & Yamamoto, 1950; Huang, 1978). They do not survive in soil. Huang and Chiang (1975), and Huang et al. (1972), reported that the viability and survival of *A. besseyi* are reduced at low temperatures. However, Yoshii & Yamamoto (1950) found live nematode in grains stored for three years, but they suggested that this nematodes had few possibilities to survive under winter conditions in Japan.

Regarding germination of seeds, fumigation with methylbromide can be hazardous although it can control the nematode population with varying degrees of efficiency (Franklin & Siddigi, 1972). Tullis (1951) reported that *A. besseyi* was killed in infested seeds fumigated with methylbromide for six hours but the seed germination was seriously affected.

Nematologists have been aware of the fact that *A. besseyi* can survive under controlled temperature, but few work has been done, mainly on stored seeds in short, medium and long time periods of germplasm conservation. Favorable conditions for seed conservation could be optimum for maintaining the parasite alive and infective.

Therefore, the present study was designed to verify the ability of *A. besseyi* to survive on rice seeds stored under controlled conditions of temperature and humidity as related to the germination and vigour of the seeds that had been fumigated or not.

MATERIAL AND METHODS

Seeds of rice (var. IAC 425) infested with *A. besseyi* were used for measuring survival of this nematode after storing at two constant temperatures, 10 and -18°C (temperature degrees that conserve seeds for short and long period, respectively). Half of the seed lot was fumigated with aluminum phosphate before storing in the appropriated room under controlled temperature, in a paper bag.

The initial seed conditions for germination, vigour and number of nematodes were determinated and the results are presented in Table 1.

Numbers of *A. besseyi* in the stored seeds were estimated during a 30-month storage period, at 6-month intervals. At collecting time, germination and vigour were also determinated in growth chamber (100% relative humidity) and chamber of accelerated aging respectively.

Table 1 - Initial evaluation of *A. besseyi* infested rice seeds in relation to percentage germination, percentage vigour and nematode numbers before storing at different temperature regimes.

Covariates	Fumigated	Not fumigated
Germination (%)	75.50	82.00
Vigour (%)	46.00	33.50
Nematode number (in 100 seeds)	1.83	9.86

Average of 4 replications.

For extraction of nematodes from seeds, the hulls were removed manually by tweezers and transferred to a modified Baermann funnel (Goodey, 1963) for 24 and 48 hours. Emerged nematodes were then counted, for each treatment.

The experiment comprised 20 treatments with four replications of 50 rice seeds each.

Analysis of variance concerning *A. besseyi* survival on rice seeds, was done with time of observation, storage temperature, and seed treatment (fumigation) as covariates. When necessary, data were transformed (arcsin) prior to the analysis, for germination percentage and vigour of nematode-infested rice seeds (Hoel, 1961).

RESULTS AND DISCUSSION

Significant differences ($P < 0.01$) in survival of *A. besseyi* were seen among the five observation times.

The analysis also showed that numbers of nematode extracted from seeds were not significantly different between seeds with or without fumigation. However, differences were found in interactions between fumigation x storage period and between fumigation x temperature. In a similar way, significant differences were observed in storage period x temperature x fumigation.

Franklin & Siddiqi (1972) found a decreased number of nematodes after fumigation. Similar results were observed by Tullis (1951) when compared to those found by Franklin & Siddiqi (1972).

The germination percentage, was only affected by storage period. Tullis (1951) found that fumigation with methylbromide affected the germination of seeds.

In relation to the seed vigour, significant differences were found when compared to the period of storage with the other interactions, except the interaction between fumigation x temperature (Table 2).

Table 2 - Analysis of variance of nematode number, germination and vigour of rice seeds infested with *Aphelenchoides besseyi*, as affected by storage period, fumigation and temperature regimes.

Treatment	Nematodes in 50 seeds	%	
		Germination	Vigour
L	0.0001	0.0001	0.0001
F	0.0152NS	0.8629NS	0.0818NS
T	0.0001	0.3906NS	0.0063
LxF	0.0001	0.8323NS	0.0001
LxT	0.0016*	0.5414NS	0.0001
FxT	0.0001	0.8064NS	0.3227NS
LxFxT	0.0001	0.2076NS	0.0001
C.V.	17.5437	7.9688	14.3503

L= Storage period; F = fumigation; T = storage temperature.

(*) = Significant at 1%.

Analysis of variance of the studied parameters, within storage period showed a significant increase of the number of nematode in seeds. At 6 and 12-month intervals, the number of nematodes was lower and there were no significant differences between them.

The correlation coefficient between the data showed that seed germination is directly proportional to seed vigour and inversely proportional to recovered nematode numbers. Therefore, between these two first parameters a positive correlation, and negative one for the nematode number and both of them were established (Table 5).

Thus, it is believed that the following survival features of *A. besseyi*, contribute to reinforce its significance as an important plant parasite:

- a) The nematode was not affected by fumigation;
- b) The nematode showed ability to survive under low temperature, and
- c) The nematode population varied, showing increase or decrease during different storage periods.

After 18 months of storage, the number of nematodes increased significantly, as well as after 24 and 30 months of storage.

The highest number was found at the 30th month, which did not show significant difference from the 24th month.

Seed vigour decrease significantly during the observation periods when compared to the initial percentage.

The highest vigour was verified in the 12th month and the lowest one in the 30th month, which were different from the other periods (Table 3).

Analysis of the temperature effect in the studied parameters, showed that from stored seeds at -18°C , it was possible to recover more nematodes than from seeds stored at 10°C . There were significant differences between temperatures.

These results do not confirm the results reported by Huang and Chiang (1975) and Huang et al. (1972), when they observed the *A. besseyi* survival decreases under low temperature. Yoshii and Yamamoto (1950) reported that *A. besseyi* has few possibilities to survive during winter, but our results showed that on infested rice seeds, stored under -18°C , the nematodes remained in good conditions.

This work suggests that *A. besseyi* can survive on infested seeds under low temperatures although the percentage of vigour and germination of these seeds decreased.

Temperature regimes did not show any significant effect on seed germination whereas the vigour was higher at -18°C , showing significant difference at 10°C (Table 4).

Table 3 - Effect of storage period on number of *Aphelenchoides besseyi*, germination percentage and vigour percentage of rice seeds.

Time (month)	Nematode number*	Time (month)	Germination (%)*	Time (month)	Vigour (%)*
30°	11.07a	12°	81.28a	12°	82.66a
24°	10.82a	18°	78.76ab	6°	83.28b
18°	7.35b	6°	76.09b	24°	53.45c
6°	4.93c	30°	74.99b	18°	46.21c
12°	4.32c	24°	67.54c	30°	28.19d

Mean of 4 replications

* Numbers followed by the same letters are not significantly different at the 0.01 level (Duncan's MRT).

Table 4 - Effect of storage temperature on the number of *Aphelenchoides besseyi*, germination and vigour of rice seeds (50/replications).

Storage temperature*	Nematode number	Germination*	Vigour*
-18°	8.86a	76.58a	60.47a
10°	6.15b	75.19a	52.80b

Mean of 4 replications

* Numbers followed by the same letters in columns are not significantly different at the 0.01 level (Duncan's MRT).

Table 5 - Correlation among *Aphelenchoides besseyi* number, germination and vigour of rice seeds stored under controlled temperature.

	Storage temperature (°C)	Seed vigour	Nematode number (50 seeds/replication)
Seed germination	-18	0.06255	-0.18294
	10	0.5815	0.1043
Seed vigour	-18	-	-0.07794
	10	-	0.4920

ACKNOWLEDGEMENTS

We wish to thank Dr. E. C. da Silva for the statistics analysis.

REFERENCES

- ANTONIOU, M. & A. A. F. EVANS, 1987. Diapause in *Meloidogyne naasi* eggs. I. The effect of constant temperature incubation on subsequent hatch. *Nematologica* 33(1): 186-198.
- ATKINS, J. G. & E. H. TODD, 1959. White tip disease of rice. III. *Phytopathology*, 49(4): 189-191.
- FORTUNER, R. & K. J. O. WILLIAMS, 1975. Review of the literature on *Aphelenchoides besseyi*. Christie, 1942, the nematode causing "white tip" disease in rice. *Helmithol. Abstr. B*, 44:1-40.
- FRANKLIN, M. T. & M. R. SIDDIQI, 1972. *Aphelenchoides besseyi*. C. I. H. Descriptions of Plant-parasitic Nematodes, Set 1(4):33pp.
- GOODEY, J. B., 1963. Laboratory methods for work with plant and soil nematodes. 4th ed. Ministry Agric., Fish and Food, Tech. BULL. 2, H. M. Stationery Office, London, 72pp.
- HOEL, P. G., 1961. *Estatística Elementar*. 1. ed. Fundo de Cultura S. A., Rio de Janeiro, R.J., 312pp.
- HUANG, C. S., 1978. O nematóide da ponta branca do arroz, *Aphelenchoides besseyi*, um patógeno transmitido pelas sementes. III Reunião da Sociedade Brasileira de Nematologia. Coleção Mossoroense, Vol. LXII, 5-18.
- HUANG, C. S. & Y. C. CHIANG, 1975. The influence of temperature on the ability of *Aphelenchoides besseyi* to survive dehydration. *Nematologica*, 21:351-375.
- HUANG, C. S.; S. P. HUANG & L. H. LIH, 1972. The effect of temperature on development and generation periods of *Aphelenchoides besseyi*. *Nematologica*, 18:432-438.
- HUANG, Y. P., 1959. White tip disease of rice in Taiwan. *Plant Protection Bulletin*, Taiwan, 1(4):1-4.
- OU, S. H., 1985. Diseases. Commonwealth Mycological Institute, U. K., 2. ed., 370pp.
- SHER, S. A., 1968. Revision of the genus *Hirschmanniella*: *Tylenchoidea* Luc & Goodey, 1963. *Nematoda*. *Nematologica*, 14:243-275.
- TIMM, R. W., 1965. In: A preliminary survey of the plant parasitic nematodes of "Thailand and Philippines" South East Asian Treaty Organization, Bangkok, 18pp.
- TULLIS, E. C., 1951. Control of seed-borne nematode of rice by fumigation with methylbromide. Progress Report, Texas Agricultural Experiment Station, nº 1413, 4pp.
- WATSON, T. R. & B. R. LOWNSBERRY, 1970. Factors influencing the hatching of *Meloidogyne naasi*, and a comparison with M. hapla. *Phytopatology*, 60:457-460.
- YOSHII, H. & S. YAMAMOTO, 1950. A rice nematode disease, Senchú Shingaré byô. I. Symptoms and pathogenic nematode. *Journal of the Faculty of Agriculture*, 9(3): 209-222.