RESEARCH/ INVESTIGACIÓN

INCIDENCE OF *APHELENCHOIDES BESSEYI* IN RICE IN LOUISIANA AND HOST STATUS OF THE MOST WIDELY PLANTED CULTIVARS

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ABSTRACT

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Aphelenchoides besseyi, the causal agent of white tip disease of rice, has been considered a minor pest of rice during the past 50 years in the United States. Recently, this nematode has been found in a number of quarantine samples in Louisiana and Arkansas. The objectives of this research were to determine incidence of this nematode in commercial seed sold to producers in Louisiana and to determine the host status of major rice cultivars currently produced in the state. During 2015-2016, a total of 216 seed samples, representing 3 medium grain, 18 long grain, and 4 long grain hybrid rice cultivars, were examined for *A. besseyi.* The nematode was detected in 12% of the samples, and the highest incidence occurred on long grain hybrids with 30% of the 63 samples infested. Nineteen-week-duration greenhouse studies were conducted to evaluate reproduction of the nematode and pathogenicity to three medium, three long grain, and three long grain hybrid rice cultivars currently popular in Louisiana. Reproductive values of the nematode ranged from 11.9 to 2.9 for medium grain cultivar Jupiter and long grain hybrid XL 753, respectively. Grain weights of Jupiter, CL 111, and XL 753 rice plants inoculated with *A. besseyi* were reduced below those of non-inoculated controls ($P<0.01$). The nematode reduced plant height of all cultivars ($P<0.01$). Plant weights of Jupiter, CL 111, CL 152, XL 745, and XL 753 were reduced when inoculated with *A. besseyi*. Germination and seedling growth studies conducted in the laboratory and greenhouse indicated that *A. besseyi* reduced medium grain Jupiter seed germination by 27% with a decrease in the germination index on 6 of the 9 cultivars evaluated.

Key words: Aphelenchoides besseyi, host suitability, *Oryza sativa,* quarantine, white-tip disease

RESUMEN

Godoy, F. M. C., C. Overstreet, E. C. McGawley, C. A. Hollier, M. T. Kularathna, C. Khanal, and B. McInnes. 2019. La incidencia de *Aphelenchoides besseyi* en el arroz en Louisiana y el estado de hospedaje de los cultivares más ampliamente plantados. Nematropica 49:107-123.

Aphelenchoides besseyi, el agente causal de la enfermedad de la punta blanca del arroz, se ha considerado una plaga menor del arroz durante los últimos 50 años en los Estados Unidos. Recientemente, este nematodo se ha encontrado en varias muestras de cuarentena en Louisiana y Arkansas. Los objetivos de esta investigación fueron determinar la incidencia de este nematodo en la semilla comercial vendida a productores en Louisiana y determinar el estado de hospedante de los principales cultivares de arroz que se producen actualmente en el estado. Durante 2015-2016, se examinaron un total de 216 muestras de semillas que representan 3 cultivares de grano medio, 18 de grano largo y 4 de arroz de grano largo híbrido para *A. besseyi*. El nematodo se detectó en el 12% de las muestras y la mayor incidencia se produjo en los híbridos de grano largo con el 30% de las 63 muestras infestadas. Se realizaron estudios de invernadero de diecinueve semanas de duración para evaluar la reproducción del nematodo y la patogenicidad a tres cultivares de arroz híbrido de grano medio, tres de grano largo y tres de grano largo, actualmente populares en Louisiana. Los valores reproductivos del nematodo variaron de 11.9 a 2.9 para el cultivar de grano medio Jupiter y el híbrido de grano largo XL 753, respectivamente. Los pesos de grano de las plantas de arroz Jupiter, CL 111 y XL 753 inoculadas con *A. besseyi* se redujeron por debajo de los controles no inoculados (P <0.01). El nematodo redujo la altura de la planta de todos los cultivares (P <0.01). Los pesos de las plantas de Jupiter, CL 111, CL 152, XL 745 y XL 753 se redujeron cuando se inocularon con *A. besseyi*. Los estudios de germinación y crecimiento de plántulas realizados en el laboratorio e invernadero indicaron que *A. besseyi* redujo la germinación de semillas de Júpiter de grano medio en un 27%, con una disminución en el índice de germinación en 6 de los 9 cultivares evaluados.

Palabras clave: *Aphelenchoides besseyi*, cuarentena, enfermedad de la punta blanca, idoneidad del huésped, *Oryza sativa*

INTRODUCTION

Rice (*Oryza sativa*) is one of the world's most important cereal crops and is a staple food for more than half of the world's population (Skamnioti and Gurr, 2009). In 2017, more than 10 billion kg of rice were produced on approximately 1 million ha in the USA (USDA-NASS, 2017). Louisiana contributes 13% of the total rice produced in the USA and ranks third among the USA rice-producing states (Childs, 2016; USDA-NASS, 2017). Eighty-five percent of the rice produced in Louisiana is of the long grain type, and the remaining 15% is medium grain rice (USDA, NASS, 2016).

An estimated 10% loss has been attributed to nematodes in rice around the world (Sasser and Freckman, 1987). McGawley and Overstreet (1998) listed white tip nematode, *Aphelenchoides besseyi* Christie, as one of species of economic importance on rice. This pathogen was first reported in Japan by Kakuta (1915) and was first found in the United States in 1934 by Jodon at the Rice Research Station at Crowley, LA. For many years white tip was a significant panicle and leaf disease of rice in Arkansas, Louisiana, and Texas but losses were reduced by widespread use of resistant cultivars (Todd and Atkins, 1958). Yield reductions due to this nematode have been reported to be as much as 71% in USSR (Tikhonova, 1966), 60% in India (Muthukrishnan *et al*., 1974) and Japan (Tamura and Kegasawa, 1959b), and 54% in the USA (Atkins and Todd, 1959) on susceptible cultivars. The economic damage threshold of this nematode was determined to be 300 nematodes per 100 seeds (Bridge *et al.,* 2005).

The most characteristic symptom induced by *A. besseyi* is the whitening of the young leaf tips, followed by necrosis and shredding of leaves (Yoshii and Yamamoto, 1950). Rice plants attacked by *A. besseyi* may also produce symptoms such as reductions in height and presence of small panicles with fewer spikelets and filled grain (Ou, 1985). Other common symptoms described in severe infections are twisting of the flag-leaf, reduction of seed filling, small and unfilled grains, and erect panicles (Yoshii and Yamamoto, 1950; Todd and Atkins, 1958; Liu *et al.,* 2008).

Aphelenchoides besseyi can be disseminated with grain or seed rice, becoming one of the most important pathogens on rice due to its quarantine importance (Khan *et al.,* 2012). The presence of *A.*

besseyi in the seed can reduce viability and retard germination (Tamura and Kegasawa, 1959a). Once established within the seed, the nematode enters a state of anhydrobiosis where it can remain for as long as 3 years (Bridge *et al.,* 2005).

Since *A. besseyi* is a seed-borne nematode, the best approach to prevent dispersal is to disinfest the seed. Soaking the seed in water with a pesticide can efficiently eliminate *A. besseyi* (Hoshino and Togashi, 2000). Although seed immersion in hot water 56-57°C for 10-15 min is an effective way to kill the nematode*,* it is not practical for large producers (Feng *et al.,* 2013). Different chemical seed treatments have been utilized as effective control measures for *A. besseyi*. Benomyl (benzimidazole) was able to significantly reduce populations of *A. besseyi* when used as a seed treatment (Templeton *et al.,* 1971; Gergon and Prot, 1993). Tenente and Manso (1994) have also reported thiabendazole as an effective seed treatment against this nematode. McGawley *et al.* (1984) reported that the insecticide Phostoxin (aluminum phosphide) used to treat stored rice grain had nematicidal properties as well.

Aside from chemical seed treatments, another viable management strategy for *A. besseyi* in rice is the use of resistant cultivars. Resistance to *A. besseyi* was first found in the Japanese rice cultivar Asahi (Nishizawa, 1953) and has been noted in rice from several other countries (Rao *et al.,* 1986; Oliveira, 1989; Popova *et al.*, 1994; Giudici *et al.,* 2003). In the past 40 years in the USA, Fortuna, Nira, Bluebonnet, and Rexoro rice cultivars have been used as the parents for resistance breeding (De Waele, 2002). Atkins and Marchetti (1959) stated that most short and medium grain cultivars were not resistant to white tip nematode, whereas long grain cultivars were reported as resistant. There are no reports evaluating resistance or susceptibility in current cultivars against white tip nematode in Louisiana.

During the past several years, seed of rough rice samples submitted to both Louisiana and Arkansas nematology laboratories for phytosanitary purposes have detected numerous infestations of *A. besseyi* (Sullivan *et al.*, 2016). Since the presence of any *A. besseyi* in these samples results in rejection of those rice seeds, exporters have been experiencing costly delays in shipping. Also, a number of rice cultivars are known to show no visible symptoms of damage from *A. besseyi* and infestations can go unrecognized (Jamali *et al.*, 2008; Pei *et al*., 2012; Feng *et al.*, 2013). Therefore, the objectives of the present study were to: (i) determine the current level of rice seed infestation with *A. besseyi* in Louisiana, (ii) evaluate the response of common rice cultivars and hybrids to inoculation with *A. besseyi* in a greenhouse environment, and (iii) determine if the nematode impacts germination and early growth of rice cultivars and hybrids.

MATERIALS AND METHODS

White tip nematode infestation of rice in Louisiana

This research was conducted during the 2015-2016 growing season to evaluate rice seed across common cultivars and hybrids for incidence of *A. besseyi*. Samples of rice seed were obtained from the Louisiana State Seed Testing Laboratory of the Department of Agriculture and Forestry. These samples represented certified seed that was produced for commercial sale in Louisiana. Long grain hybrids that were produced in Texas for sale to producers in Louisiana were also included. A total of 216 samples representing 25 different cultivars were examined. All rice seed were processed by a modified Baermann funnel technique in order to estimate occurrence and population levels of *A. besseyi* (Hooper, 1990). A 25-g subsample of rice seed was placed into a blender containing 100 mL of water and agitated for 3 secs, three times. The resultant slurry was concentrated by passage through a 25 µm-pore sieve and placed on a Baermann funnel. After 24 hr, each sample was removed from the funnel, and nematodes were counted at 40x with an inverted microscope.

Culturing of nematodes

Rice seed infested with *A. besseyi* was obtained from Mer Rouge, LA, and used as the original source of nematodes. The nematodes were surface-disinfested with 3% H_2O_2 for 10 min., washed with distilled water, and added to actively growing cultures of *Botrytis cinerea*. The fungus was originally isolated from symptomatic strawberry fruit and grown on potato dextrose agar (PDA) at 25°C in darkness (Liu *et al.,* 2008; Yoshida *et al*., 2009). When the mycelium reached one third of the petri dish area at approximately 6 d, 500 mixed-life stages of *A. besseyi* in 1 ml of sterile water were added using a sterile pipette (McGawley *et al.,* 1985). After inoculation, the nematode infested cultures were incubated for 4 wk at 25°C in darkness to increase populations (Liu *et al.,* 2008). Nematodes were extracted from cultures of *B. cinerea* by washing with a 3% H_2O_2 solution into a beaker and allowing the slurry to sit for 10 min. The slurry was then poured through a 38 µm-pore sieve and washed with distilled water to eliminate the fungus (Liu *et al.,* 2008). These nematodes were used as inoculum in all greenhouse experiments.

Rice growth under greenhouse conditions

Rice cultivars employed included three medium grain (CL 271, Caffey, and Jupiter), three long grain (CL 111, CL 151, and CL 152), and three long grain hybrids (XL 729, XL 745, and XL 753). These cultivars are currently the most widely planted in Louisiana. Seven seeds of each cultivar were planted to a depth of 2-3 cm in 25.5 cm-d by 23 cm-deep plastic pots (Pricefalls LLC, Las Vegas, NV) containing 3.0 kg of steam-sterilized soil (61% sand, 21% clay and 18% silt). After germination, plants were thinned to 1 per pot. After 30 days, a 1-ml suspension containing 500 nematodes of *A. besseyi*, a mixture of females, males, and juveniles, was placed between the leaf sheath and culm of the rice plant with a hypodermic syringe. Control plants received an equivalent aqueous suspension without nematodes (McGawley *et al.,* 1984; Togashi and Hoshino, 2003). To avoid spread of the nematode from inoculated to non-inoculated plants, the experiment was arranged as a 9 x 2 x 6 randomized split-plot design representing the nine cultivars x two inoculum levels of *A. besseyi* with six replications. Pots were submerged in water to simulate paddy conditions until 100% of the paddy seedlings became straw yellow color after 135 days. One hundred milliliters of water-soluble Miracle-Gro (Scotts Company LLC, Marysville, OH) fertilizer (24-8-16) was applied every 14 days according to label rates. At the conclusion of the 19 wk-duration trial, weights of dry shoots, roots, panicles and plants (root plus shoot plus panicle), filled and total grain, 100-seed weights, plant height (distance from the soil surface to the base of the tallest panicle), and nematode numbers per 100 seed were determined. A 10-g subsample of rice seed from each plant was processed for *A. besseyi* as described above. This experiment was conducted from summer to fall 2016 (June-October) and repeated in early spring to summer 2017 (March-July).

Seed germination and seedling growth evaluation

Seeds of cultivars harvested from the first and second greenhouse trials were used to evaluate the effect of the nematode on seed germination. Seeds from the second trial were used for seedling growth evaluation. Two hundred twenty seeds were collected from each nematode-inoculated and non-inoculated plant representing the 9 cultivars and both greenhouse trials for a total of 3,960 seed. Three thousand and six hundred seed were used for the *in-vitro* seed germination tests, and 360 from the second greenhouse trial were used for evaluation of early seedling growth and vigor. Seed germination was evaluated by placing 10 seeds from nematode-inoculated plants into each of five 9 cm-diam. plastic petri dishes lined with a double layer of Whatman No. 1 filter paper and moistened with 5 ml of sterile water (Vibhuti *et al.,* 2015). For comparison, five dishes of seed from non-inoculated plants of each cultivar were also established. Plates were maintained in a Thermo Scientific Model 3740 incubator (Thermo Forma, Marietta, OH) at 30°C with a 12 hr light/dark cycle (Moldenhauer and Slaton*,* 2005). Seed was considered to be germinated when the radicle reached 2 mm in length. Seed germination index was calculated according to protocol described by Wang *et al.* (2010). Nematodes were extracted from infested seeds after germination by soaking in distilled water for 15 hr (Zuckermann *et al.*, 1990).

Sterile Cyg germination pouches (Mega International, West St. Paul, MN) with dimensions of 16.5 cm by 18 cm were employed to evaluate vigor of seedlings from plants inoculated and non-inoculated with *A. besseyi.* Ninety seed representing inoculated and non-inoculated plants of each of the nine cultivars were employed for this test. A single seed was placed into the center of the planting-ridge of the germination paper contained in each clear, polyethylene pouch. A total of 90 pouches represented 5 replications of inoculated and non-inoculated seed of the 9 cultivars. Pouches were watered daily with distilled, pH neutral water and maintained under fluorescent lighting and ambient laboratory temperatures of 25-30°C. Root, shoot, final plant length/height, and seedling fresh weights were determined after 15 days (Vibhuti *et al.,* 2015). A similar experiment was conducted in the greenhouse to evaluate seedling vigor from the inoculated and non-inoculated seed in a soil environment. In this 15-day study, 3.8 cm-diam. by 21 cm-long plastic cone containers (SC-10 super cell, Stuewe and Sons, Tangent, OR) containing 175 g of steamed soil were planted with seed from inoculated and non-inoculated plants of the 9 cultivars as described above for growth pouches.

Analysis of data

Except for the greenhouse experiments, all studies were established as randomized block designs with five replications and each experiment was repeated once. Data obtained from all studies were analyzed using Statistix 9 (Analytical Software, Tallahassee, FL) program and examined by Fischer's LSD test at the 0.05 level.

RESULTS

White tip nematode infestation of rice in Louisiana

White tip nematode was found in 25 samples representing medium, long grain, and long grain hybrid cultivars of rice (Table 1). Nineteen of the infested samples were from hybrid cultivars outside of Louisiana. Among these hybrids, the long grain hybrid XL 729 showed the greatest percent infestation (70%) and the highest number of *A. besseyi* (288 nematodes/25 g seed) of all of the rice cultivars evaluated. In contrast, only six seed samples representing four cultivars from Louisiana were infested with *A. besseyi*. However, five of the six Louisiana cultivars are those that are widely grown.

Rice growth under greenhouse conditions

There were no significant experiment by treatment interactions for nematodes and data were combined over two full-season trials. There were, however, significant interactions with plant data between the trials, and it is therefore presented separately.

Data from both full-season trials indicated that all cultivars tested were hosts of the nematode since the populations recovered after 15 wk was greater than the inoculation level (Table 2). The cultivar Jupiter supported the greatest number of *A. besseyi*, 871 nematodes/100 seed, compared to the 571, 569, 384, 342, 309, 211, and 141 per 100 seed recovered from CL 111, Caffey, XL 729, CL 152, CL 271, CL 151, and XL 753, respectively (P<0.01). A total of 645 *A. besseyi*/100 seed was recovered from XL 745, which was not significantly greater than the 871 recovered from Jupiter. Reproductive values ranged from 2.9 for XL 753 to 12.0 for Jupiter. Cultivars representing all grain types supported both the highest and the lowest population densities of the nematode.

Greenhouse trial 1- summer to fall 2016

Although the highest reproduction rate for *A. besseyi* was on Jupiter (Table 2), the nematode did not, relative to non-inoculated controls, negatively impact grain, root, shoot, plant weigh, or plant height (Table 3). Similar results were observed for other two medium grain cultivars, CL 271 and Caffey.

Among the long grain rice cultivars, plant growth parameters of CL 111 and CL 152 were negatively affected by *A. besseyi.* Inoculated plants of CL 111 were 11% shorter and had 51 and 29% lower root and plant weights, respectively, than the non-inoculated control. The same trend was observed for inoculated plants of CL 152, which were 8% shorter than controls and had reductions in root weight of 48% and plant weight reductions averaging 18%. The cultivar CL 151 supported moderate nematode reproduction, but, similar to CL 271, was not damaged by *A. besseyi*.

Long grain hybrids were damaged by *A. besseyi*. With the nematode-inoculated hybrid XL 745, 32%, and 24% reductions were observed in shoot and plant weights, respectively, compared to the non-inoculated plants (P<0.01). Hybrid XL 729 also had reduced shoot weights of 25% and

Table 1. Incidence of the rice white-tip nematode, *Aphelenchoides besseyi,* in rice samples from Louisiana and Texas during 2015-2016.

Cultivar[y]	Grain type	Number of samples assayed	Number infested with *A. besseyi*	*A. besseyi* per 25 g of seed (range)
Caffey	Medium	3	0	0
Catahoula	Long	4	0	0
Cheniere	Long	21	0	0
CL 111	Long	32	2	15 (2-28)
CL 151	Long	17	0	0
CL 152	Long	4	1	(38)
CL 153	Long	2	0	0
CL 161	Long	1	0	0
CL 163	Long	2	0	0
CL 271	Medium	7	2	11 (2-20)
CL 272	Long	2	0	0
Cocodrie	Long	3	0	0
Della-2	Long	1	0	0
Jazzman	Long	2	0	0
Jazzman-2	Long	2	0	0
Jupiter	Medium	23	0	0
Mermentau	Long	23	0	0
Pirogue	Long	1	1	(4)
Sabine	Long	1	0	0
Toro-2	Long	1	0	0
Wells	Long	1	0	0
XL 729[z]	Long	10	7	106 (2-288)
XL 745[z]	Long	22	7	27 (2-160)
XL 753[z]	Long	19	3	61 (2-160)
XL 760[z]	Long	12	2	90 (4-176)

[y]All cultivars were obtained from the Louisiana Seed Testing Laboratory of the Department of Agriculture and Forestry, Baton Rouge, LA.
[z]Hybrid rice cultivars grown in Texas and shipped to Louisiana for planting.

were 10% shorter than the non-inoculated plants. With XL 753, higher grain weight was recorded for the inoculated plants, 19.6 vs. 12.9 g, compared to the non-inoculated plants.

Greenhouse trial 2- spring to summer 2017

Unlike results from the first greenhouse trial in which plants were grown from summer to fall, medium grain rice plants grown from spring to summer were damaged by *A. besseyi* (Table 4). With the cultivar Jupiter, *A. besseyi* reduced grain weight by 30%, plant weight by 20%, and plant height by 17% ($P<0.01$). For the other two medium grain types, CL 271 and Caffey, 7% and 12% decreases in plant height were observed, respectively ($P<0.01$). Among the inoculated plants, a decrease in plant height occurred ($P<0.01$). The height of Jupiter plants was on average 5 cm shorter than those of CL 271 and Caffey.

Table 2. Reproduction of *Aphelenchoides besseyi* on nine rice cultivars after 15 weeks in a greenhouse[u].

Cultivar/Hybrid[w]	Grain type[x]	*A. besseyi* per 100 seed[y]	*A. besseyi* per plant	R values[z]
Jupiter	Medium	871 a	5,976 a	12.0
XL 745	Long Hybrid	645 ab	5,275 a	10.6
CL 111	Long	571 bc	4,394 ab	8.8
Caffey	Medium	569 bc	4,055 abc	8.1
XL 729	Long Hybrid	384 cd	3,911 abc	7.8
CL 152	Long	342 cde	2,889 bcd	5.8
CL 271	Medium	309 de	2,032 cd	4.1
CL 151	Long	211 de	1,603 d	3.2
XL 753	Long Hybrid	141 e	1,428 d	2.9

[u]Means are average of 12 replications.
[w]Most widely planted commercial cultivars and hybrids of rice used in Louisiana in 2015.
[x]The most common grain types planted in Louisiana in 2015.
[y]Within columns, means followed by the same letters are not significantly different according to Fisher's LSD test ($P \leq 0.05$).
[z]Nematodes per 10 g of seed were determined and multiplied by the total seed yield from each plant to estimate the Reproductive (R= total nematodes per plant/inoculation level of 500 *A. besseyi*) value.

The nematode also had a negative effect on the growth of the long grain rice plants. With inoculated CL 111 plants, a 15% reduction in yield was observed and with CL 151 inoculated plants were significantly shorter than non-inoculated plants with final heights of 63.5 and 68.7 cm, respectively. Plant measurements collected for inoculated CL 152 were not different from those of the non-inoculated control.

With the long grain hybrids, *A. besseyi* caused reductions in both grain and plant weights as well as plant height. The inoculated hybrid XL 753 had 8.3 g and 20 g lower grain and plant weights, respectively, and on average were 9 cm shorter than non-inoculated plants. Plant height data for XL 729, and XL 745 indicated a reduction in inoculated plants of 13% and 10%, respectively.

Seed germination and seedling growth evaluation

The germination percentages of infested and non-infested rice seeds from the first greenhouse study were not different for any of the cultivars or grain types (Table 5). In contrast, the germination index was significantly reduced by the presence of the nematode on the cultivars CL 271, Jupiter, CL 151, CL 152, XL 745 and XL 753. Numbers of *A. besseyi* per seed at the end of the experiment varied among the cultivars with highest incidence of 8 per seed found in Jupiter and lowest at 1 per seed in CL 111. Only infested Jupiter seeds from the second study, spring to summer, demonstrated that infestation with the nematode significantly reduced the germination rate. CL 271, Jupiter, CL 111, XL 729, and XL 753, however, had lower germination indices than those of controls. No differences in the numbers of *A. besseyi* extracted from seed were detected with nematode populations ranging from 2 to 14 individuals per seed.

In the plastic cone-containers, *A. bessey*i produced a significant negative effect on seedling growth of one cultivar of each of the 3 types of grain (Table 6). With the infested medium grain Caffey, there was a reduction in root length of 1.5 cm and in root weight of 0.14 g compared with the non-inoculated control. The length of roots of inoculated long grain CL 111 was shorter by 2.3 cm compared to the non-inoculated plants. In contrast to Caffey and CL 111, all growth parameters for inoculated long hybrid XL 745 were affected by *A. besseyi*. Root, shoot, and plant lengths of the inoculated plants of this long grain hybrid were reduced by 3.7 cm, 6.4 cm, and 10.1 cm, respectively. Root, shoot, and plant weights of this hybrid cultivar followed the same trend and were reduced by 0.15, 0.15, and 0.30 g, respectively, compared with non-inoculated controls.

Table 3. Effects of *Aphelenchoides besseyi* on dry weights and plant heights of cultivars of medium and long grain rice cultivars and long grain hybrids after 19 weeks in a greenhouse from summer to fall 2016 (June-October)[w].

Cultivar/ Grain type[x]	Grain (g)[y]		Root (g)		Shoot (g)		Plant (g)		Height (cm)	
	C[z]	I	C	I	C	I	C	I	C	I
CL 271-M	15.1 a	11.0 a	15.7 a	11.9 a	16.9 ab	13.3 ab	47.8 a	36.9 a	71.3 a	69.2 a
Caffey-M	13.8 a	11.4 a	21.2 a	17.2 a	17.8 a	12.1 ab	52.4 a	36.5 a	71.4 a	71.4 a
Jupiter-M	13.1 a	11.8 a	14.3 a	14.1 a	15.6 ab	11.3 b	43.0 a	37.4 a	74.1 a	70.0 a
CL 111-L	12.3 bc	11.6 c	16.8 a	8.3 bc	20.6 a	16.1 a	49.7 a	35.5 c	77.0 a	68.9 bc
CL 151-L	16.4 ab	14.0 abc	8.3 bc	5.9 c	19.1 a	18.3 a	43.8 ab	39.0 bc	76.8 a	72.9 ab
CL 152-L	17.8 a	13.0 abc	14.4 ab	7.5 c	18.3 a	18.4 a	47.2 a	38.6 bc	72.9 ab	67.0 c
XL 729-LH	19.5 a	17.6 ab	13.3 b	13.7 b	24.2 a	18.1 bc	56.7 a	47.1 abc	91.0 a	81.6 b
XL 745-LH	16.8 ab	15.0 ab	20.3 a	15.6 ab	21.4 ab	14.6 c	54.5 ab	41.3 c	79.3 bc	74.2 c
XL 753-LH	12.9 b	19.6 a	14.8 ab	15.7 ab	17.9 bc	19.1 b	45.1 bc	51.8 ab	80.4 bc	80.4 bc

[w]Data are means of six replications per treatment. Plant material was dried at 30-35°C for 2 weeks and plant height was measured at harvest.
[x]Most widely planted commercial cultivars and hybrids in Louisiana in 2015. M (Medium grain cultivars), L (Long grain cultivars), and LH (Long grain hybrids) the most common grain types planted in Louisiana in 2015.
[y]Within grain types and control and inoculated treatments, means with common letters are not significantly different according to Fisher's LSD test ($P \leq 0.05$).
[z]C indicates the non-inoculated control and I indicates inoculation with 500 vermiform life stages of *A. besseyi*.

Table 4. Effects of *Aphelenchoides besseyi* on dry weights and plant heights of cultivars of medium and long grain rice cultivars and long grain hybrids after 19 weeks in a greenhouse from spring to summer 2017(March-July)[w].

Cultivar/ Grain type[x]	Grain (g)[y]		Root (g)		Shoot (g)		Plant (g)		Height (cm)	
	C[z]	I	C	I	C	I	C	I	C	I
CL 271-M	22.9 abc	19.7 c	14.3 a	14.0 a	22.2 a	20.6 a	59.5 ab	54.4 ab	72.0 a	66.7 b
Caffey-M	26.2 ab	20.1 bc	16.4 a	15.9 a	23.9 a	19.6 a	66.7 a	55.8 ab	73.0 a	64.6 b
Jupiter-M	28.9 a	20.2 bc	16.4 a	13.4 a	20.4 a	18.6 a	65.9 a	52.4 b	73.4 a	60.7 c
CL 111-L	27.4 a	23.3 b	11.7 b	10.5 b	20.9 ab	18.9 ab	60.2 a	53.3 ab	66.9 ab	62.8 b
CL 151-L	22.3 b	20.0 b	11.8 bc	10.9 b	18.4 ab	16.6 b	52.8 ab	47.8 b	68.7 a	63.5 b
CL 152-L	23.0 b	20.9 b	15.7 a	14.5 a	22.5 a	20.7 ab	61.2 a	56.4 ab	65.5 ab	64.6 ab
XL 729-LH	30.0 bc	27.9 c	16.7 a	14.9 a	23.9 a	23.2 a	68.9 bc	66.6 bc	69.2 b	60.1 c
XL 745-LH	37.5 a	30.9 abc	16.5 a	15.6 a	25.0 a	23.6 a	79.1 ab	70.4 abc	75.1 a	67.5 b
XL 753-LH	35.7 ab	27.4 c	17.2 a	13.9 a	28.5 a	22.3 a	83.3 a	63.2 c	75.6 a	66.6 b

[w]Data are means of six replications per treatment. Plant material was dried at 30-35°C for 2 weeks and plant height was measured at harvest.

[x]Most widely planted commercial cultivars and hybrids in Louisiana in 2015. M (Medium grain cultivars), L (Long grain cultivars), and LH (Long grain hybrids) the most common grain types planted in Louisiana in 2015.

[y]Within grain types and control and inoculated treatments, means with common letters are not significantly different according to Fisher's LSD test ($P \leq 0.05$).

[z]C indicates the non-inoculated control and I indicates inoculation with 500 vermiform life stages of *A. besseyi*.

Table 5. Effects of *Aphelenchoides besseyi* on rice seed germination after 7 days at 30°C [w].

Cultivar/ Grain type[x]	Seed (summer to fall-2016 experiment)					Seed (spring to summer-2017 experiment)				
	Germ. % [y]		Germ. Index[y]		Numbers of *A. besseyi* per seed	Germ. %		Germ. index		Numbers of *A. besseyi* per seed
	C [z]	I	C	I		C	I	C	I	
CL 271-M	90.0 b	90.0 b	4.2 a	2.9 d	4.6 ab	99.0 a	95.0 ab	3.5 a	3.1 b	8.0 a
Caffey-M	96.0 a	98.0 ab	3.7 b	3.7 bc	2.3 ab	95.0 ab	91.0 ab	3.5 a	3.2 ab	3.2 a
Jupiter-M	94.0 ab	96.0 ab	3.9 ab	3.4 c	7.5 a	90.0 b	63.0 c	3.0 b	1.9 c	14.1 a
CL 111-L	99.0 a	99.0 a	4.3 ab	4.2 b	1.1 b	99.0 a	96.0 a	4.2 a	3.5 c	3.9 a
CL 151-L	98.0 a	98.0 a	4.4 a	3.9 c	5.7 ab	98.0 a	96.0 a	3.9 ab	3.7 bc	3.7 a
CL 152-L	99.0 a	98.0 a	4.5 a	3.8 c	3.9 ab	98.0 a	99.0 a	3.9 ab	3.7 bc	3.2 a
XL 729-LH	97.0 a	98.0 a	4.1 a-c	4.0 bc	2.9 ab	90.0 abc	86.0 c	3.8 b	3.4 c	6.9 a
XL 745-LH	99.0 a	99.0 a	4.2 ab	3.9 cd	4.1 ab	97.0 a	96.0 a	4.3 a	4.2 a	2.1 a
XL 753-LH	97.0 a	97.0 a	4.4 a	3.7 d	3.9 ab	93.0 ab	86.6 bc	3.8 b	3.5 c	4.0 a

[w]There were no significant experiment by treatment interactions and data were combined over two 7-day trials and are means of 10 replications.

[x]Most widely planted commercial cultivars and hybrids in Louisiana in 2015. M (Medium grain cultivars), L (Long grain cultivars), and LH (Long grain hybrids) the most common grain types planted in Louisiana in 2015.

[y]Within grain types and control and inoculated treatments, means with common letters are not significantly different according to Fisher's LSD test ($P \leq 0.05$). Numbers of *A. besseyi* per seed were analyzed across all three grain types.

Germ. % = Germination percentage; number of seed where radicle was at least 2 mm in length. Germ. Index = ∑ Number of germinated seeds/Day of first count +……+ Number of germinated seeds/Day of last count.

[z] C indicates seed from non-inoculated control plants and I indicates seed from plants inoculated with *A. besseyi*.

Table 6. Growth parameters of rice seedlings inoculated and not inoculated with Aphelenchoides besseyi after 15 days in a greenhouse[w].

Cultivar/	Root				Shoot				Plant			
	Length (cm) [y]		Weight (g)		Length (cm) [y]		Weight (g)		Length (cm) [y]		Weight (g)	
Grain type[X]	C[Z]	I	C	I	C	I	C	I	C	I	C	I
CL 271-M	19.7 ab	19.1 a-c	0.32 ab	0.27 b	27.7 ab	23.1 bc	0.32 a	0.29 ab	47.4 ab	42.3 bc	0.64 a	0.56 ab
Caffey-M	20.0 a	18.5 bc	0.41 a	0.27 b	29.3 a	26.5 ab	0.34 a	0.29 ab	49.3 a	45.1 ab	0.75 a	0.59 ab
Jupiter-M	19.2 a-c	18.2 c	0.31 ab	0.23 b	18.6 cd	16.8 d	0.30 ab	0.22 b	37.9 cd	35.1 d	0.60 ab	0.45 b
CL 111-L	18.3 a	16.0 b	0.30 ab	0.21 b	27.5 ab	22.6 b	0.38 ab	0.31 b	45.8 a	38.6 b	0.67 ab	0.52 b
CL 151-L	19.9 a	18.6 a	0.32 a	0.29 ab	25.6 ab	23.7 ab	0.43 a	0.41 a	45.4 a	42.3 ab	0.75 a	0.70 ab
CL 152-L	19.2 a	18.0 ab	0.39 a	0.28 ab	28.8 a	24.8 ab	0.44 a	0.37 ab	48.0 a	42.8 ab	0.83 a	0.65 ab
XL 729-H	19.3 a	18.7 a	0.36 ab	0.31 b	21.3 a-c	18.6 c	0.37 bc	0.33 c	40.6 a-c	37.2 c	0.74 bc	0.64 c
XL 745-H	20.0a	16.3 b	0.45 a	0.30 b	25.9 a	19.5 bc	0.52 a	0.37 bc	45.9 a	35.8 c	0.97 a	0.67 c
XL 753-H	19.3 a	18.1 ab	0.43 a	0.36 ab	24.4 ab	20.5 a-c	0.52 a	0.46 ab	43.9 ab	38.6 bc	0.95 ab	0.82 a-c

[w]There were no significant experiment by treatment interactions and data were combined over two 15-day trials and are means of 10 replications. After 2 weeks fresh roots and shoots were weighed and lengths of roots and shoots were determined.

[x]Most widely planted commercial cultivars and hybrids in Louisiana in 2015. M (Medium grain cultivars), L (Long grain cultivars), and LH (Long grain hybrids) the most common grain types planted in Louisiana in 2015.

[y]Within grain types and control and inoculated treatments, means with common letters are not significant different according to Fisher's LSD test ($P \leq 0.05$). Plant length and weight was the sum of root plus shoot lengths and weights.

[z]C indicates seed from non-inoculated control plants and I indicates seed from plants inoculated with *A. besseyi*.

In the sterile soilless Cyg germination pouches, at least one growth parameter for all cultivars was negatively influenced by inoculation with *A. besseyi* (Table 7). For the medium grain CL 271, all seedling parameters of the inoculated plants, except root weight, were reduced. Root, shoot, and plant lengths decreased 4.5, 2.6, and 7.2 cm, respectively, in comparison with those for non-inoculated seedlings. Shoot and plant weights were also reduced 33% and 30%, respectively. For the cultivar Caffey, *A. besseyi* negatively affected root weight only, with a 25% reduction compared with the non-inoculated seedlings. For Jupiter, all plant growth parameters except root length were negatively impacted by the nematode. Shoot and plant heights were reduced by 2.0 and 3.7 cm, respectively. Weights of roots, shoots, and plants were reduced by 37%, 50%, and 45%, respectively, compared with non-inoculated plants. For the long grain cultivar CL 111, plant weight was 29% lower for the inoculated plants. The nematode caused a 50% reduction in shoot weight of the cultivar CL 151. Inoculated plants of CL 152 showed a reduction in root and plant lengths of 3.9 cm and 5.0 cm, respectively, compared with the control. There were also 40%, 50%, and 43% reductions in root, shoot, and plant weights of inoculated CL 152 compared with control plants. The long grain hybrid XL 729 had a significant 33% reduction in only shoot weight relative to the control. Inoculated long hybrid XL 745 showed reductions of 3.0 and 4.7 cm in root and plant lengths, respectively, compared with non-inoculated plants. Significant decreases of 33%, 50%, and 44% were observed for root, shoot, and plant weights, respectively, when compared with controls. Shoot and plant weights of XL 753 were both reduced by 33% by *A. besseyi.*

DISCUSSION

Even though the white tip nematode is established in most rice-growing areas across the world, it has been reported as only a minor pathogen in the USA (Tulek *et al.,* 2014). In the past 5 years, white tip nematode has been found in high numbers in quarantine samples for shipment overseas from the Arkansas Nematode Diagnostic Service and the Louisiana Nematode Advisory Service (Sullivan *et al.,* 2016). Although the incidence of *A. besseyi* in this survey was only 12%, this percentage was 7% higher compared to a prior assay in Louisiana reported by McGawley *et al.* in 1984. Tulek and Çobanoglu (2010) in a survey conducted in rice growing areas of Turkey between 2007 and 2008 reported higher numbers of seeds infested with *A. besseyi* than the present study. According to these authors, 16.3% of the samples were infested with *A. besseyi* in 2007 and increased to 43% in 2008.

In our greenhouse tests, all cultivars supported sufficient nematode reproduction to be classified as hosts. However, McGawley *et al.* (1984) reported that 7 of 10 rice cultivars studied were non-hosts, since the estimated numbers of *A. besseyi* were consistently below the inoculum levels. In our study, the medium grain Jupiter and the long hybrid XL 745 had the greatest reproductive values for the population of nematodes. Tulek *et al.* (2014) indicated that under field conditions, the rice cultivar Halilbey was the most susceptible to *A. besseyi* showing a reproductive value of 8.4. Moreover, the cultivar Asahi had the lowest multiplication rate (2.0) compared to other three cultivars that supported nematode reproduction (Tulek, 2016).

Yield losses caused by *A. besseyi* vary among cultivars and geographic areas. Earlier studies indicated a decrease of 6.6 to 49% in seed weight in the USA during the 1950s (Atkins and Todd, 1959), whereas a reduction from 10 to 30% was observed in Japan (Yamada and Shiomi, 1950). Tulek and Çobanoglu (2010) reported a loss as high as 58% for the susceptible cultivar Halilbey 60 years later. According to Fukano (1962), significant plant damage does not occur until nematode populations reach 30 nematodes/100 seeds. The average number of *A. besseyi* per 100 seed across all cultivars in our study was 449, considerably higher than the threshold of Fukano. Tulek *et al.* (2014) also observed similar high populations of this nematode across rice cultivars. Yield losses in our study were limited to only one cultivar in each of the medium, long, and hybrid cultivars. By contrast, Tulek *et al*. (2015) reported that yield losses ranged from 7.8-52% on 39 cultivars. Tulek (2016) also reported that cultivars considered resistant supporting only low reproduction of *A. besseyi* still sustained yield losses from 8-19%.

Table 7. Growth parameters of rice seedlings inoculated or not inoculated with *Aphelenchoides besseyi* after 15 days in Cyg germination pouches in the laboratory[w].

	Root				Shoot				Plant			
Cultivar/	Length (cm) [y]		Weight (g)		Length (cm)		Weight (g)		Length (cm)		Weight (g)	
Grain type[x]	C[z]	I	C	I	C	I	C	I	C	I	C	I
CL 271-M	14.4 a	9.9 b	0.07 ab	0.06 bc	7.3 a	4.7 cd	0.03 a	0.02 bc	21.7 a	14.6 c	0.10 ab	0.07 d
Caffey-M	13.4 a	13.6 a	0.08 a	0.06 bc	5.8 ab	7.0 ab	0.02 bc	0.02 ab	19.2 ab	20.6 a	0.09 a-c	0.08 bc
Jupiter-M	13.8 a	12.0 ab	0.08 a	0.05 c	5.7 bc	3.7 d	0.02 ab	0.01 c	19.5 a	15.7 bc	0.11 a	0.06 d
CL 111-L	10.0 ab	9.7 ab	0.05 ab	0.04 bc	9.6 a	8.7 ab	0.02 a	0.02 ab	19.6 a	18.4 a	0.07 a	0.05 bc
CL 151-L	13.0 a	10.6 ab	0.05 ab	0.06 a	7.3 a-c	7.4 a-c	0.02 a	0.01 bc	20.3 a	18.0 ab	0.07 ab	0.07 a
CL 152-L	11.6 a	7.7 b	0.05 ab	0.03 c	6.3 bc	5.3 c	0.02 ab	0.01 c	17.9 a	13.0 b	0.07 a	0.04 c
XL 729-H	12.3 ab	10.1 b	0.05 ab	0.05 ab	7.4 a	7.0 a	0.03 a	0.02 bc	19.7 ab	17.1 b	0.08 ab	0.07 bc
XL 745-H	13.4 a	10.4 b	0.06 a	0.04 b	8.4 a	6.7 a	0.02 ab	0.01 c	21.8 a	17.1 b	0.09 ab	0.05 c
XL 753-H	11.4 ab	10.5 b	0.06 a	0.05 ab	7.4 a	7.8 a	0.03 a	0.02 c	18.8 ab	18.3 ab	0.09 a	0.06 c

[w]Data were combined over two 15-day trials and are means of 10 replications. After 2 wk fresh roots and shoots were weighed and lengths of roots and shoots were determined.

[x]Most widely planted commercial cultivars and hybrids in Louisiana in 2015. M (Medium grain cultivars), L (Long grain cultivars), and LH (Long grain hybrids) the most common grain types planted in Louisiana in 2015.

[y]Within grain types and control and inoculated treatments, means with common letters are not significant different according to Fisher's LSD test ($P \leq 0.05$). Plant lengths and weights were, respectively, the sums of root plus shoot lengths and weights.

[z]C indicates seed from non-inoculated control plants and I indicates seed from plants inoculated with *A. besseyi*.

Only a few of the rice cultivars in this study showed significant decreases in plant weights compared to the control plants. These reductions were similar to those observed by McGawley *et al.* (1984), in which the inoculated cultivar Melrose had a significant reduction of 22% in plant weights. In the present study, there were significant differences in the heights of inoculated and non-inoculated plants for all cultivars. Liu *et al.* (2008) observed a significant reduction in heights of rice plants with increasing inoculum levels from 0 to 800 *A. besseyi* per plant. However, plant height reduction by the nematode was not a good indicator of potential yield loss in our study and may be related to tolerance. This tolerance to the nematode is likely indirect since the nematode has not been a major problem during the past 50 years.

Feng *et al.* (2013) reported that most inoculated plants showed no symptoms of *A. besseyi* infection in a greenhouse environment, yet nematodes were recovered from the seeds in high numbers. Pei *et al*. (2012) suggested that there was no correlation between white-tip symptoms and *A. besseyi* reproduction. In our greenhouse studies, there were no signs of white-tip symptoms on the flag leaves of any of the inoculated plants. This phenomenon is referred to as "masked symptom" and has been described in many experiments with rice cultivars (Rao and Rao, 1979; Zhu and Wu, 1986; Jamali *et al*., 2008). However, symptoms of nematode damage were observed in the present greenhouse experiment on the medium grain Jupiter, long grain CL 152, and long hybrid XL 753. These symptoms were expressed as small and unfilled grains with a black-brown coloration. Although Jamali and Mousanejad (2011) mentioned that infested rice plants may not exhibit any typical symptoms, similar patterns of symptoms were reported by Liu *et al.* (2008) and McGawley *et al.* (1984). Since symptoms of this nematode may be difficult to recognize on many cultivars of rice, recognition of the problem in a field may be more challenging. Tulek *et al*. (2015) did find a significant positive correlation between yield loss and white tip symptoms.

Rahim (1988) reported that rice seed infested with *A. besseyi* is capable of germination. However, Tamura and Keyasawa (1959a) reported that the viability of rice seed that is infested with *A. besseyi* is lowered and germination delayed. Ferris (1999) indicated that emergence from the seed bed was delayed by this nematode. Our study found that seed germination was reduced only once with the medium grain cultivar Jupiter. This would suggest that germination is not impacted on the common rice cultivars grown in Louisiana.

Since all the cultivars tested in our research support substantial numbers of nematodes, the question is why has this nematode not become more of a recognized problem in the USA. The use of insecticides either during seed storage or seed treatment could be impacting the incidence of *A. besseyi*. McGawley *et al.* (1984) reported that Phostoxin has nematicidal properties and this chemical is commonly applied in seed storage (Schowalter, 2007). As a seed chemical treatment, thiamethoxan and chlorantraniliprole are currently the most commonly used insecticides in Louisiana (M. Stout per. comm., 2017). It is unknown as to whether these chemicals have any nematicidal activity against *A. besseyi*. Metalaxyl and fludioxonil are fungicidal seed treatments used in rice in Louisiana. Benomyl has also been reported to be somewhat effective as a seed treatment but must be applied during the infestation stage (Gergon and Prot, 1993). Gergon and Prot (1993) reported that benomyl was not effective when applied at tillering or panicle initiation. Azoxystrobin and propicanazole are two of the most frequently applied fungicides in Louisiana rice areas to manage some of serious diseases associated with this crop (Hollier *et al*., 2017). The role that fungicides or insecticides may have in limiting the spread and development of *A. besseyi* as a major pathogen in rice in the USA is unclear. After this nematode was identified and recognized in the past, cultivars were selected that had strong resistance. This selection could have resulted in lower incidence of this nematode and decreased populations across our production areas. Since the nematode has not been a major problem for so many years, this could explain why many of our newer cultivars don't have the nematode since they have not been exposed to it.

Important conclusions from the research reported herein include: i) most of the rice cultivars produced in Louisiana and released to farmers are relatively free of *A. besseyi*, ii) cultivars that are currently planted in Louisiana are hosts of the nematode and can experience damage and yield loss without displaying symptoms typical of white-tip disease, and iii) *A. besseyi* can cause a marked delay in germination of rice seed and retard seedling growth and establishment.

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