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Incidence of Aphelenchoides besseyi in Rice in Louisiana and Host Status of the Most Widely Planted Cultivars

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

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ABSTRACT

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. 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 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 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 grains, and three long grain hybrid rice cultivars currently popular in Louisiana. Reproductive values of *A. besseyi* ranged from 11.9 to 2.9 for medium grain cultivar Jupiter and long hybrid XL 753, respectively. Grain weights of Jupiter, CL 111 and XL 753 plants inoculated with *A. besseyi* were significantly reduced below those of non-inoculated controls. There were significant reductions in plant height for all cultivars, except the long grain cultivar CL 152. Plant weights of Jupiter, CL 111, CL 152, XL 745 and XL 753 plants were reduced significantly when inoculated with *A. besseyi*. Germination and seedling growth studies conducted in the laboratory and greenhouse indicated that *A. besseyi* had a negative effect of 27% on percentage of seeds germinating of medium grain Jupiter. However, the nematode had a significant negative impact of 0.64 on average on the rate of germination for all cultivars except the medium grain Caffey.

INTRODUCTION

Rice (*Oryza sativa*) is one of the world's most important cereal crops, serving as a major source of calories for more than half of the world's population (Skamnioti and Gurr, 2009). Most of the world's rice production and consumption come from Asia where population densities are high, with China and India ranking as the top producers and consumers (USDA, NASS 2017). In 2017, the United States produced approximately 10 billion kg of rice on 1,004,430 hectares, averaging 8,638 kg per hectare in yield, and ranking as the fifth highest rice exporter in the world (USDA, NASS 2017).

Louisiana has been growing this crop for over 300 years and is the third largest producer in the U.S. with 13% of total production (Childs, 2016; USDA, NASS 2017). In Louisiana, rice is one of the top five most economically important crops, with 173,205 hectares harvested area and a total value of $298 million (USDA, NASS 2016). Most of the rice produced in Louisiana belongs to the long grain type, which corresponds to 85% of the crop production while the remaining 15% is medium grain (USDA, NASS 2016).

According to the International Rice Research Institute (2004), rice yield losses of 10% are due to nematodes. There are several genera and species of parasitic nematodes that are parasitic on rice. McGawley and Overstreet (1998) listed white tip nematode (*Aphelenchoides besseyi*), rice stem nematode (*Ditylenchus angustus*), the root nematode (*Hirschmanniella* spp.), rice cyst nematodes (*Heterodera oryzae; H. oryzicola; H. elachista; H. sacchari*) and lesion nematodes (*Pratylenchus zeae* and *P. indicus*) as species of economic importance on rice. Plant parasitic nematodes associated with rice can be divided into two groups, according to their parasitic habits: foliar and root parasites. All rice nematodes cause certain mechanical damage and/or some disturbance on the physiological processes involved in plant development, resulting in poor growth and yield loss (Bridge *et al.*, 2005).

Aphelenchoides besseyi, the causal agent of white tip disease is one of the most important nematodes on rice. This pathogen was first reported in Japan by Kakuta (1915) and was first found in the United States in 1935 by Jodon, but damage was attributed to a nutrient deficiency. Since *A. besseyi* is seed-borne and can be disseminated with grain or seed rice, this disease is becoming important on rice due to its quarantine importance (Khan *et al.*, 2012). In addition, yield losses of up to 60% due to *A. besseyi* have been extensively reported from diverse infested regions (Bridge *et al.*, 2005). According to previous research, when this nematode is present in rice fields, yield reductions were as high as 71% in USSR (Tikhonova, 1966), 60% in India (Muthukrishnan *et al.*, 1974) and Japan (Tamura and Kegasawa, 1959) and 54% in the USA (Atkins and Todd, 1959) on susceptible cultivars. Hung (1959) reported yield losses of 29-36% among 10 susceptible cultivars surveyed in Taiwan. The economic damage threshold of this nematode was determined to be 300 live nematodes per 100 seeds (Bridge *et al.*, 2005).

Usually, the common names of plant diseases are based on the induced symptoms. With *A. besseyi*, the most characteristic symptom of infection is the whitening of the young leaf tips, followed by necrosis and shredding of leaves tips (Yoshii and Yamamoto, 1950). In the rice field, the beginning of the elongation is the stage that the white tip symptoms generally appear. Rice plants attacked by this foliar nematode may also produce other symptoms such as stunting, reduction 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 flag-leaf and reduction of seed swelling (Yoshii and Yamamoto, 1950; Todd and Atkins, 1958). According to Liu *et al.* (2008), other symptoms have been reported, such as small grains and erect panicles.

The presence of *A. besseyi* in the seed can reduce viability and retard germination (Tamura and Kegasawa, 1959). When infested rice seed is planted, the nematode becomes active and will move to the rice meristematic areas during tillering. *Aphelenchoides besseyi* feeds ectoparasitically on the buds of the shoot apical stem (Kyndt *et al.*, 2014). As the plant grows, the nematodes move in a film of moisture to the leaves, where they prefer the rice leaf tips, causing the whitening symptom mentioned above (Bridge *et al.*, 2005). Later, the nematode migrates to the emerging panicle, toward the spikelets before anthesis and feed on embryos, ovaries, and stamens (Huang and Huang, 1972). *Aphelenchoides besseyi* is also able to move from leaf to leaf if the proper moisture exists and can severely injury the rice plant (Lambert and Bekal, 2002). Once established within the seed, the nematode enters a state of anhydrobiosis where it can remain for as long as three years (Bridge *et al.*, 2005). Another characteristic of this nematode genus is that individuals can also feed facultatively on fungi (Kyndt *et al.*, 2014).

Since *A. besseyi* is a seed-borne pathogen, the best way to prevent dispersal of this nematode is to disinfest the seed. Prasad and Varaprasad (1992) reported that soaking seed in a 0.2% solution of mancozeb followed by methyl bromide fumigation for 2 h at 30°C totally killed the nematode with no negative effect on seed viability. Another method for controlling white tip disease through seed treatment is immersion in hot water. Yoshii and Yamamoto (1951) obtained some control by soaking nematode-infested rice seed in water at below 20°C for 16-20 hours followed by immersion at 50°C for 5-10 minutes or immersion of dry seeds in water 56-57°C for 10-15 minutes. Injury to the seeds occurred only at 60°C for more than 20 minutes. Different chemical seed treatments have been used as effective control measures for *A. besseyi*. These include malathion and nicotine sulphate (Anonymous, 20and 16). Benomyl was able to significantly reduce populations of white tip nematodes when used as a seed treatment (Gergon and Prot, 1993; Templeton *et al.,* 1971). Tenente and Manso (1994) have also reported thiabendazole as an effective seed treatment. However, they also found that carbofuran and aluminum phosphate fumigation were not as efficient as chemical seed control. McGawley et al. (1984) also found that Phostoxin, which is used to treat stored rice for insects, significantly reduced *A. besseyi* in rice seed.

Aside from chemical seed treatments, other possible management strategies for *A. besseyi* in rice are resistant cultivars and cultural practices. In order to manage the nematode population, farmers can combine two or more management strategies. One of the most common and effective management combinations is host-plant resistance plus nematicides. Examples of this strategy in other crop/nematode combinations include cotton (Schrimsher *et al.*, 2014), potato (Roberts, 1993; Trudgill, 1987), and soybean (Schmitt, 1991). According to Roberts (1993), the use of nematicide and resistant soybean cultivars followed by a nonhost crop reduces nematode population to insignificant levels for susceptible soybean cultivars. Oka (2014) evaluated the nematicidal activity of fluensulfone in vitro against several migratory nematodes. Of these, *A. besseyi* was affected by 8 mg L^{-1}, the highest concentration used. Cho et al. (1987) reported that effective methods for managing *A. besseyi* in rice were seed disinfectant before seeding and carbofuran 3% G on the day before transplanting or the combination of seed disinfectant and water surface application of carbofuran 3% G at early stages of injury. Lee et al. (1972) also found that soaking seed in several insecticides effectively controlled *A. besseyi* prior to planting as well as root dipping in Sumithion 50% EC, Lebaycid 50% EC, and the nematicide Nemagon 80% EC.

Resistance to *A. besseyi* is genetically carried by the Japanese cultivar Asahi (Nishizawa, 1953) and has been described from India (Rao *et al.,* 1986), Brazil (Oliveira, 1989), Russia (Popova *et al.,* 1994), and Italy (Giudici *et al.,* 2003). Only 1% of the 1,003 cultivars tested by

Popova *et al*. (1994) were highly resistance when inoculated with *A. besseyi*. In the past 40 years in the U.S., Fortuna, Nira, Bluebonnet and Rexoro were the major cultivars with resistance that have been used as the precursors for breeding (De Waele, 2002). Atkins and Marchetti (1959) stated that most short and medium grain cultivars were not resistant to white tip nematode, while the long grain ones were reported as resistant. Currently, there are no reports evaluating resistance and susceptibility in current cultivars against white tip nematode in Louisiana.

Another strategy for white tip disease includes cultural practices. Elimination of inoculum source such as infested seeds, debris and weeds is an efficient approach (Ravichandra, 2008). According to Yoshii and Yamammoto (1951), rice sown before the regular planting time could possibly avoid nematode infestation. Cleaning of weeds, stubble and debris from the previous crop (Vuong, 1969) or direct seeding into water (Cralley, 1956) are also cultural practices that reduce infection by *A. besseyi.* Although these practices have their restrictions, they are a good and inexpensive choice for producers (Ou, 1985).

During the past several years, seed of rough rice samples submitted to both Louisiana and Arkansas nematology labs for quarantine purposes have detected numerous infestations by *A. besseyi* (Sullivan *et al.*, 2016). Since the presence of any *A. besseyi* in these samples results in rejection of those rice seed, 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 could go unrecognized (Feng *et al.*, 2013; Jamali *et al.*, 2008; Pei *et al*., 2012). Therefore, the objectives of the present study were (i) to determine the current level of rice seed infestation with *Aphelenchoides besseyi* in Louisiana, (ii) to evaluate the response of common rice cultivars and hybrids to inoculation with *A. besseyi* in a greenhouse environment and (iii) to determine if the nematode impacts germination and early growth of rice cultivars and hybrids.

MATERIALS AND METHODS

Survey of 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 or hybrids for *A. besseyi* incidence. Samples of rice seed were obtained from the 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 was 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 three seconds, three times. The resultant slurry was concentrated by passage through a 500-mesh sieve and placed in a Baermann funnel. After 24 hours, each sample was removed from the funnel and counted at 40x with an inverted microscope.

Culturing of Nematodes

Rice seed infested with *A. besseyi* was obtained from Mer Rouge in Morehouse parish, LA and used as the original source of nematodes. The nematodes were surface-disinfested with 3% H_2O_2 for 10 minutes, 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 infested cultures were incubated for 4 weeks at 25°C in darkness to increase populations (Liu *et al.,* 2008). Nematodes were extracted from fungus cultures, sterilized with 3% H_2O_2 for 10 minutes and washed with distilled water to eliminate the fungus (Liu *et al.,* 2008). These nematodes were used as inoculum in all greenhouses 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 seed of each cultivar were planted to a depth of 2-3 cm in plastic pots (Pricefalls LLC, Las Vegas, NV) with dimensions of 25.5 cm diameter by 23 cm depth, 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 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 seeds became straw yellow color, after 135 days. One hundred ml 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 each trial, weights of dry shoots, roots, panicles and plants (root plus shoot plus panicle), filled and total grain and 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.

Seed Germination and Seedling Growth Evaluation

Seeds of cultivars harvested from the first and second greenhouse studies were used to determine the effect of the nematode on seed germination. Seed from the second study were used for seedling growth experiments. Two hundred twenty seed were collected from each nematode-inoculated and non-inoculated plant representing the nine cultivars and both greenhouse trials for a total of 3,960 seed. Three thousand and six hundred seed were used in in-vitro seed germination tests and 360 from the second greenhouse study were used for evaluation of early seedling growth and vigor. Seed germination was evaluated by placing 10 seed 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 h light/dark cycle (Moldenhauer and Slaton, 2005). Seed was considered as germinated when the radicle reached 2 mm in length. Seed germination rate was calculated according to protocol of Ellis and Roberts (1981). 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 seed of *A. besseyi*-inoculated and non-inoculated plants of the nine cultivars. 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 were required to represent 5 replications of inoculated and non-inoculated seed of the nine 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 and final plant length/height and seedling fresh weights were determined after 15 days (Vibhuti *et al.*, 2015). A similar experiment was conducted in a greenhouse to evaluate seedling vigor of the inoculated and non-inoculated seed in a soil environment. In this 15-day-duration study, plastic cone containers (SC-10 super cell, Stuewe and Sons, Tangent, OR) with dimensions of 3.8 cm diameter by 21 cm long, 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 other studies were established as randomized block designs with five replications and 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

Survey of White tip Nematode Infestation of Rice in Louisiana

White tip nematode was found in 25 samples representing medium, long grain, and long grain hybrid of rice. Nineteen of the infested samples were from hybrids from outside of Louisiana (Table 1). Among these hybrids, the long grain hybrid XL 729 showed the greatest percent infestation at 70% and the highest number of *A. besseyi* at 288 nematodes per 25 g of seed. In contrast, only six seed samples from Louisiana were infested with *A. besseyi* (Table 2). However, 85% of these infested cultivars represent the cultivars most commonly grown in Louisiana. Only 20% of infested rice samples were medium grain type and the other 80% were long grain cultivars.

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

Hybrid[z]	Grain type	Number of samples assayed	Number of samples containing *A. besseyi*	% infested seed	*A. besseyi* per 25 g of seed (range)
XL 729	Long	10	7	70	106 (2-288)
XL 745	Long	22	7	32	27 (2-160)
XL 753	Long	19	3	16	61 (2-160)
XL 760	Long	12	2	17	90 (4-176)

[z] All hybrids were obtained from the Louisiana Seed Testing Laboratory of the Department of Agriculture and Forestry, Baton Rouge.

Rice Growth under Greenhouse Conditions

Reproduction data for *Aphelenchoides besseyi* were combined over the two full season experiments. However, rice growth parameters were analyzed separately since the experiments were conducted in different seasons and years.

Data from both full season trials indicated that all cultivars tested were, in nematological terms, hosts of the nematode since the numbers recovered after 15 weeks were greater than the 500 per plant inoculation level (Table 3). The cultivar Jupiter supported the highest number, 871 per 100 seed, of *A. besseyi* and this was significantly greater than 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. A total of 645 *A. besseyi* per 100 seed were recovered from XL 745 and this was not significantly than the 871 from Jupiter. Reproductive values of the nematodes, ranged from 2.9 for XL 753 to 12.0 for Jupiter. Cultivars representing all grain types supported both the highest and the lowest levels of *A. besseyi*.

Table 2. Incidence of the rice white-tip nematode, *Aphelenchoides besseyi,* in rice samples from Louisiana during 2015-2016.

Cultivar[z]	Grain type	Number of samples assayed	Number of samples containing *A. besseyi*	% infested seed	*A. besseyi* per 25 g of seed (range)
Caffey	Medium	3	0	0	0
Catahoula	Long	4	0	0	0
Cheniere	Long	21	0	0	0
CL 111	Long	32	2	6.3	15 (2-28)
CL 151	Long	17	0	0	0
CL 152	Long	4	1	25	38
CL 153	Long	2	0	0	0
CL 161	Long	1	0	0	0
CL 163	Long	2	0	0	0
CL 271	Medium	7	2	28.5	11 (2-20)
CL 272	Long	2	0	0	0
Cocodrie	Long	3	0	0	0
Della-2	Long	1	0	0	0
Jazzman	Long	2	0	0	0
Jazzman-2	Long	2	0	0	0
Jupiter	Medium	23	0	0	0
Mermentau	Long	23	0	0	0
Pirogue	Long	1	1	100	4
Sabine	Long	1	0	0	0
Toro-2	Long	1	0	0	0
Wells	Long	1	0	0	0

[z] All cultivars were obtained from the Louisiana Seed Testing Laboratory of the Department of Agriculture and Forestry, Baton Rouge.

Greenhouse Experiment 1- Summer to Fall 2016

Although data of Table 3 showed the highest reproduction rate for *A. besseyi* on Jupiter plants, the nematode did not, relative to non-inoculated controls, negatively impact grain, root, shoot, or plant weights or plant height (Table 4). Similar results were observed for other two medium grain cultivars, CL 271 and Caffey.

Among the long grain rice cultivars, CL 111 and CL 152, plant growth parameters 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 weights of 48% and plant weights averaging 18%. The cultivar CL 151 supported moderate nematode reproduction; but, like CL 271, was not significantly affected by the nematode.

Long grain hybrids were damaged significantly by *A. besseyi*. With the nematode-inoculated hybrid XL 745, significant 32% and 24% reductions were observed in shoot and plant weights, respectively. Hybrid XL 729 also had significantly reduced shoot weights of 25% and was 10% shorter than the non-inoculated plants. With the cultivar XL 753, there was a significant higher grain weight for the inoculated plants, 12.9 and 19.6 g for the control and inoculated, respectively. This result did not occur in the second test.

Table 3. Reproduction of *Aphelenchoides besseyi* on nine rice cultivars after 15 weeks in a greenhouse environment[v].

Cultivar[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

[v] There were no significant experiment by treatment interactions and data were combined over two full season trials and are means 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 / total nematodes per plant inoculation level of 500 *A. besseyi*) value.

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 greenhouse environment from summer to fall 2016[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°C - 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*.

Greenhouse Experiment 2- Spring to Summer 2017

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

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

With the long grain hybrids, *A. besseyi* caused significant reductions in both grain and plant weights as well as plant height. The inoculated hybrid XL 753 had significant 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 significant reduction in inoculated plants of 13% and 10%, respectively.

Table 5. Effects of *Aphelenchoides besseyi* on dry weights and plant heights of cultivars of medium and long grain rice and long grain hybrids after 19 weeks in greenhouse environment from spring to summer 2017[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°C - 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*.

Seed Germination and Seedling Growth Evaluation

The germination percentages of infested and non-infested rice seeds from the first greenhouse study were not significantly different for any of the cultivars or grain types (Table 6). In contrast, the germination rate 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 7.5 per seed found in Jupiter and lowest at 1.1 per seed found in CL 111. Only infested Jupiter seeds from the second study, spring to summer, demonstrated that the presence of the nematode significantly reduced the germination percentage. The cultivars CL 271, Jupiter, CL 111, XL 729 and XL 753 however, had lower germination rates than those of controls. There were no significant differences in the numbers of *A. besseyi* extracted from seed with numbers ranging from 2.1 to 14.1 per seed.

In the soil environment of 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 7). With the infested medium grain Caffey, there was a significant reduction in root length of 1.5 cm and root weights of 0.14 g compared with controls. The length of roots of inoculated long grain CL 111 was significantly shorter by 2.3 cm than that of the control. In contrast to Caffey and CL 111, all growth parameters for inoculated long hybrid XL 745 were significantly affected by *A. besseyi*. Root, shoot and plant lengths of the inoculated plants of this long hybrid were significantly 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 significantly by 0.15, 0.15 and 0.30 g, respectively compared with controls.

Table 6. Effects of *Aphelenchoides besseyi* on rice seed germination after 7 days at 30 C [w].

Cultivar/ Grain type[x]	Seed (summer to fall experiment)					Seed (spring to summer experiment)				
	Germ. % [y]		Germ. index		Number of *A. besseyi* per seed	Germ. %		Germ. index		Number 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] Incubator was Thermo Scientific - Model 3740. There were no significant experiment by treatment interactions and data were combined over two 7 day duration 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 significant 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 = Germination index;

determined by $$\sum \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \ldots\ldots + \frac{\text{Number of germinated seeds}}{\text{Day of last count}}.$$

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

Table 7. Growth parameters of rice seedlings after 15 days in a greenhouse environment for plants inoculated and not inoculated with *Aphelenchoides besseyi* [w].

Cultivar/Grain type[x]	Root				Shoot				Plant			
	Length (cm) [y]		Weight (g)		Length (cm)		Weight (g)		Length (cm)		Weight (g)	
	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-LH	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-LH	20.0 a	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-LH	19.5 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 duration 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 significantly 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 8). For the medium grain CL 271, all seedling parameters of the inoculated plants, except root weight, were reduced significantly. There were 4.5 cm, 2.6 cm and 7.2 cm decreases in root, shoot and plant lengths in comparison with those for non-inoculated seedlings, respectively. Shoot and plant weights were also reduced 33% and 30%, respectively. For the cultivar Caffey, *A. besseyi* had a significant negative effect on root weight only, with a 25% reduction compared with the control. For Jupiter, all plant growth parameters except root length were negatively impacted by the nematode. Shoot and plant heights were reduced by 2.0 cm and 3.7 cm, respectively. Weights of roots, shoots and plants were reduced by 37%, 50% and 45%, respectively, compared with control plants. For the long grain cultivar CL 111 there was significant 29% lower plant weight for the inoculated plant than for the control. 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.*

Table 8. Growth parameters of rice seedlings after 15 days in Cyg germination pouches in the laboratory for plant inoculated or not inoculated with *Aphelenchoides besseyi* [w].

Cultivar/Grain type [x]	Root				Shoot				Plant			
	Length (cm) [y]		Weight (g)		Length (cm)		Weight (g)		Length (cm)		Weight (g)	
	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-LH	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-LH	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-LH	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] There were no significant experiment by treatment interactions and data were combined over two 15 day duration 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 significantly 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*.

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 problem in the U.S. (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 during two years (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 in the following year this number increased to 43%.

In the greenhouse inoculation tests reported herein, all cultivars supported sufficient nematode reproduction to be classified as hosts. However, McGawley *et al.* (1984) reported that seven of ten rice cultivars studied were rated as non-hosts, since the estimated numbers of *A. besseyi* were consistently below the inoculum levels. In this study, Jupiter and the long hybrid XL 745 had the greatest reproductive values for population development of the nematodes, 12.0 and 10.6, respectively. 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 in the study by Tulek (2016). In our study, the cultivar with the lowest reproductive value was 2.9 and occurred with the long grain hybrid XL 753.

There are several reports describing yield losses caused by *A. besseyi*. Earlier studies indicate a decrease of 6.6, 17.5 and 49% in seed weight in the U.S. during different years in the

1950`s (Atkins and Todd, 1959), whereas a reduction from 10 to 30% was observed in an experiment conducted in Japan (Yamada and Shiomi, 1950). Tulek and Çobanoglu (2010) reported a loss as high as 58% for the susceptible cultivar Halilbey. In our greenhouse study, *A. besseyi* significantly reduced rice yield by 30% on the medium grain Jupiter, 23% on the long hybrid XL 753 and 15% on the long grain CL 111. According to Fukano (1962), significant plant damage does not occur until nematode levels reach 30 live nematodes per 100 seeds. In our greenhouse studies, the average number of *A. besseyi* per 100 seed across all cultivars was 449. This number is significantly higher than that reported by Fukano. Tulek *et al.* (2014) also observed similar high levels, 409 *A. besseyi* from 100 seeds.

Although all inoculated rice plants used in this research supported reproduction of *A. besseyi*, only cultivars from one medium grain, two long grain, and two hybrids showed significant decreases in plant weights compared with control plants. Respectively, these 3 inoculated cultivars and 2 inoculated hybrids averaged 26% and 24% reductions in plant weights. Similar results were observed by McGawley *et al.* (1984), in which the inoculated cultivar Melrose had a significant reduction of 22% in plant weight. In the present study, there were significant differences in the heights of inoculated and non-inoculated plants for all cultivars except long grain CL 111. Inoculated plants belonging to the medium grain type averaged 9.2 cm shorter than controls whereas inoculated long hybrids and long cultivars averaged 8.8 cm and 5.6 cm shorter, respectively. Liu *et al.* (2008) observed a significant reduction in heights of rice plants with increasing inoculum concentrations from 0 to 800 *A. besseyi* per plant. According to these authors, plant height for the 0, 100, 400 and 800 inoculum levels of the nematode averaged 63.9, 60.5, 57.3 and 50.3 cm respectively.

Feng *et al.* (2013) reported that, in a greenhouse environment, most inoculated plants showed no symptoms of *A. besseyi* inoculation, yet nematodes were recovered from the seeds in high numbers. Pei *et al*. (2012) suggested that there was no correlation between white-tip symptom and *A. besseyi* reproduction. In both of these 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).

Rahim (1998) reported that rice seed infested with *A. besseyi* are capable of germination. Herein, *A. besseyi* reduced the seed germination percentage only once on the inoculated medium grain cultivar Jupiter. The average mean number of *A. besseyi* per seed was 4.0 in the experiment using seed from summer to fall 2016 and 5.4 with seed from spring to summer 2017 experiment. Togashi and Hoshino (2001) and Ma *et al.,* (2000) have counted 2.0 and 4.2 *A. besseyi* per seed in Japan and China, respectively. According to Ferris (1999), emergence from the seed bed of severely infested seedlings was delayed.

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 U.S.? 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 insecticide Phostoxin has nematicidal properties and this chemical is commonly applied in seed storage. As a seed chemical treatment, thiamethoxan and chlorantriprole 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 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) also 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). It is unclear of 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 U.S.A.

SUMMARY

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 cause significant 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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