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Occurrence of *Aphelenchoides besseyi* in Louisiana Rice Seed and Its Interaction with *Sclerotium oryzae* in Selected Cultivars[1]

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Abstract: *Aphelenchoides besseyi*, the nematode causal agent of white-tip disease of rice, was recovered from 5.5% of 474 seed samples obtained from rice seed warehouses in Louisiana. Laboratory tests in which *A. besseyi*-infested rice seed was treated with Phostoxin®, a compound used for control of insects in stored grain, indicate that it also has nematicidal properties. In 18-week-duration greenhouse tests, populations of *A. besseyi* increased 4–5-fold on the cultivars Saturn and Melrose and 3-fold on Nova '76. Green weights of Nova '76 plants inoculated with *A. besseyi* and *Sclerotium oryzae*, the causal agent of rice stem rot, were significantly reduced below those of plants inoculated with either organism alone or with distilled water. Weights of Melrose plants were reduced significantly by treatments with *A. besseyi* alone and *A. besseyi* plus *S. oryzae*, but not by *S. oryzae* alone. Saturn plant weights were not reduced significantly by either organism alone or by the two in combination.

Key words: *Oryza sativa*, host suitability, nematode–fungus interaction, white-tip disease, chemical control.

White-tip disease of rice (*Oryza sativa* L.) incited by the seed-borne nematode *Aphelenchoides besseyi* Christie was first described by Kakuta (6) in Japan in 1915. Since then, the disease has been found in most of the rice producing areas of the world (2,4). Symptoms, which are usually apparent during and after the booting stage of growth, include tattered white to brownish leaf tips, stunting of plants, reductions in panicle length and in the number of spikelets produced, and small and distorted kernels.

The early work of Atkins and Todd (1) not only indicated that many Louisiana rice cultivars supported reproduction of *A. besseyi* but also demonstrated that under both natural and inoculated field test conditions, yield suppression of 17% in susceptible and 7% in resistant cultivars were possible. During the last several years, white-tip disease symptoms have been observed periodically in fields of commercial rice. In addition to symptoms of white-tip disease, rice plants in some of these fields were also severely infected with the stem rot fungus, *Magnaporthe salvinii* Catt. (*Sclerotium oryzae* Catt.). Both *A. besseyi* and *S. oryzae* were isolated from rotted stems in these fields.

Reported herein are results of 1) a survey conducted to determine the degree of

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A. besseyi infestation in Louisiana rice cultivars; 2) a laboratory test conducted to determine if Phostoxin® (Degesch Co., Frankfurt, West Germany), a chemical commonly used for treatment of grain insects in stored rice, is effective against *A. besseyi*; and 3) greenhouse experiments designed to gauge the response of nine commercial rice cultivars to inoculation with *A. besseyi* alone and in combination with *S. oryzae*.

Materials and Methods

Seed sample survey: The Louisiana State Seed Testing Laboratory collected a total of 798 seed samples from seed warehouses throughout the rice producing area of the state for certification of germination percentage and freedom from weed seeds. A 10-g subsample from each of 474 samples was placed into an Osterizer blender containing 100 ml water and agitated for 45 seconds. The resultant rice grain : rice hull suspension was concentrated by passage through a 75-μm (200-mesh) sieve, placed on a Baermann funnel apparatus, and examined for *A. besseyi* after 24 hours.

Phostoxin test: Two hundred fifty grams of *A. besseyi*-infested Brazos seed obtained from Puerto Rico were placed into each of eight 0.95-liter mason jars. Seed in four of the jars were treated with 10 mg Phostoxin (equivalent to the recommended rate for insect control of six 40-g tablets per ton of grain); jars were then sealed and rotated on a jar mill for 12 hours. At intervals of 10, 20, and 30 days, 10 g of seed were removed from each jar and processed for *A. besseyi* as described above. This test was repeated twice.

Greenhouse experiments: Fifteen-day-old rice seedlings of the cultivars Brazos, Labelle, Lebonnet, Mars, Melrose, Nato, Nova '76, Saturn, and Starbonnet germinated from seed sown in flats of autoclaved planting medium were transplanted singly into the center of 15-cm-d clay pots containing 1.5 kg of steam-sterilized sandy loam soil. The pots were arranged in a four-replication randomized complete block design on greenhouse benches and supplementary light was provided to give a 16-hour photoperiod. When the seedling culms were about 30 cm in length (25–30 days old), they were inoculated with either *A. besseyi*, *S. oryzae*, both organisms, rice grain: rice hull concentrate minus nematodes, or distilled water. Nematode inoculum, 350 individuals in 5 ml water, was collected from infested seed and applied with a hypodermic syringe between the leaf sheath and culm. Sclerotia of *S. oryzae* were obtained by the method of Rush (9) and applied by sprinkling 0.5 g around the base of each plant. At 14 weeks after transplanting, the tillers (culms) were excised just above the soil line and the panicles removed. Panicles and soil were assayed for *A. besseyi* and culms were rated for stem rot infection using the 0 (healthy, no symptoms) to 9 (culm collapsed with sclerotia in the interior cavity, two or more nodes infected) scale described by Hoff et al. (3). Plant weight was measured as fresh weights of tops plus weights of washed, blotted roots. An abstract of this work has been published (7).

Results and Discussion

Of the 798 seed samples collected from nine rice cultivars, 474 were assayed for *A. besseyi*; the incidence in stored rice averaged 5.5% (Table 1). The low rate of recovery of *A. besseyi* from warehoused seed samples was probably related in part to the use of Phostoxin. Results of lab tests conducted with *A. besseyi*-infested grain and Phostoxin suggest this chemical has nematicidal as well as insecticidal properties (Table 2). At each interval between 0 and 30 days, greater ($P = 0.05$) numbers of white-tip nematodes were recovered from untreated, *A. besseyi*-infested seed lots than from seed lots treated with Phostoxin.

Table 1. Incidence of the rice white-tip nematode, *Aphelenchoides besseyi*, in rice samples collected from seed warehouses in Louisiana.

Rice cultivar	No. samples		% infested seed
	Assayed	Containing *A. besseyi*	
Brazos	35	0	0.0
Labelle	50	1	2.0
Lebonnet	52	2	3.8
Mars	7	0	0.0
Melrose	54	10	18.5
Nato	60	1	1.6
Nova '76	6	3	50.0
Saturn	150	7	4.7
Starbonnet	60	2	3.3

TABLE 2. Efficacy of Phostoxin† against the white-tip nematode, *Aphelenchoides besseyi.*

Days after treatment	No. living *A. besseyi*/10 g seed	
	Phostoxin-treated	Control
0	229	229
10	88**	214
20	75**	179
30	40**	184

† Treatment rate = 10 mg/250 g seed.
** Indicates a difference from control which is significant at the 1% level.

In greenhouse inoculation tests, only the cultivars Saturn, Melrose, and Nova '76 supported sufficient nematode reproduction to be classified as hosts. The other seven cultivars were rated as nonhosts because the estimated numbers of *A. besseyi* per plant at 14 weeks after inoculation were consistently below the initial inoculum level (350/plant). Nematodes were recovered only from above ground plant parts. Roots and soil were assayed for *A. besseyi*, but none were recovered. During the 14-week period following inoculation of Saturn and Melrose rice plants with either *A. besseyi* or both *A. besseyi* and *S. oryzae*, nematode population increase averaged 4–5-fold (Table 3). On Nova '76, however, there was a 3-fold increase in nematode numbers on plants inoculated with *A. besseyi* alone and a significantly ($P = 0.01$) greater population increase, 9-fold, on plants inoculated with the nematode and *S. oryzae*. Iyatomi and Nishizawa (5) reported the ability of *A. besseyi* to feed and reproduce on mycelium of *S. oryzae*, and we have observed *A. besseyi* feeding on sclerotia of *S. oryzae* in culture in a manner similar to that reported for *Bursaphelenchus xylophilus*, the pinewood nematode, and the fungus *Gliocladium virens* (8). Research is in progress to determine whether or not *A. besseyi* and *S. oryzae* come physically into contact in/on Nova '76 rice plants.

Although Saturn rice plants supported reproduction of both organisms, the 18-week plant weights were not reduced significantly below those of controls by either organism alone or by the combination of both. On the cultivar Melrose, however, significant weight reductions ($P = 0.05$) were observed both in plants inoculated with only *A. besseyi* and in those inoculated with *A. besseyi* and *S. oryzae*, but not in plants inoculated with only *S. oryzae*. On this cultivar, neither disease severity nor pathogen density was influenced by combined inoculation with both organisms.

Weights of Nova '76 plants inoculated with either organism alone were not significantly different from weights of control plants. However, the plant weight difference from control was highly significant in the *A. besseyi* plus *S. oryzae* treatment, and this was accompanied by both an increase in *S. oryzae* disease index rating, disease severity, and *A. besseyi* population density.

The rice cultivars Saturn, Melrose, and

TABLE 3. Fourteen-week reproduction by and effects of *Aphelenchoides besseyi* and *Sclerotium oryzae* on the rice cultivars Saturn, Melrose, and Nova '76.

Rice cultivar	Treatment	18-wk plant weight (g)	*S. oryzae* (disease index)	*A. besseyi* (no. per plant)
Saturn	Nematode (N)	40.1 a*	0	1,540
	Fungus (F)	39.4 a	1–2	
	N + F	42.0 a	2	1,365
	Control	45.8 a	0	
Melrose	N	27.5 s	0	1,295
	F	30.0 rs	3	
	N + F	16.1 t	2–3	1,190
	Control	38.2 r	0	
Nova '76	N	63.1 x	0	1,085
	F	42.7 x	3	
	N + F	28.3 y	5–6	3,047**
	Control	59.7 x	0	

* Data analyzed by Duncan's new multiple-range test; numbers within columns, *by cultivar*, followed by different letters are significantly different at the 5% level.
** Indicates a difference from control which is significant at the 1% level.

Nova '76 all supported nematode reproduction. However, symptoms of nematode damage, under the greenhouse conditions of this test, were apparent only on Melrose and Nova '76 and were expressed as tattered leaf tips and unfilled grain.

Results of this study suggest that a majority of the rice cultivars presently grown in Louisiana do not support white-tip nematode reproduction. However, recovery of *A. besseyi* from 5% of the seed lots sampled, some of which may have been exposed to the effects of Phostoxin, coupled with the greenhouse data, especially with regard to the cultivars Saturn, Melrose, and Nova '76, indicate that a potential for damage exists. At present we are planning a more intensive field survey and looking more closely into the possibly synergistic association between *A. besseyi* and *S. oryzae.*

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