

## Reproduction of *Meloidogyne incognita* and *M. graminis* on Several Grain Sorghum Hybrids

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**Abstract:** A total of 27 grain sorghum hybrids were evaluated in a series of greenhouse experiments to determine their susceptibility to *Meloidogyne incognita* race 3 and *M. graminis*. Each hybrid was inoculated with 2,000 nematode eggs/pot. Reproduction by *M. incognita* was numerically greater than *M. graminis* on 93% of the hybrids tested, indicating that grain sorghum is a better host for *M. incognita* than *M. graminis*. A wide variation in host suitability was observed on these hybrids in a second experiment as reproduction by *M. incognita* ranged from 395 to 3,818 eggs/g of root. Only two hybrids, Terral RV9782 and RV9823, consistently supported <20% reproduction by *M. incognita* compared to the most susceptible hybrid, Golden Acres 5556. Reproduction of four isolates of *M. incognita* was evaluated on six selected hybrids in a third greenhouse experiment. Hybrid susceptibility was similar to that observed in the previous experiment for all isolates. A difference in isolate aggressiveness was observed between two of the four isolates across all hybrids. In fields where damaging populations of *M. incognita* are present, most grain sorghum hybrids will likely maintain or increase the nematode population for the subsequent crop.

**Key words:** grain sorghum, *Meloidogyne graminis*, *M. incognita*, *Sorghum bicolor*, southern root-knot nematode, reproduction, resistance.

The southern root-knot nematode (*M. incognita*) is one of the most important plant-parasitic nematodes affecting cotton (*Gossypium hirsutum*) and soybean (*Glycine max*) production in the United States (Wrather and Koenning, 2006; Starr et al., 2007; Koenning and Wrather, 2010; Koenning, 2015). During the 2014 cotton cropping season, damage from root-knot nematodes was estimated to cause an average yield loss of 3.1% for a total yield loss of 494,000 bales across the United States Cotton Belt (Lawrence et al., 2015). During the 2014 cropping season, an estimated yield loss in soybean to root-knot nematodes was 1.1% for a total yield loss of 12 million bushels across the southern soybean-producing states in the United States (Allen et al., 2015). Generally, management strategies for root-knot nematodes rely on an integrated approach that includes host plant resistance, nematicides, and crop rotation.

The best choices in a crop rotation sequence include crops that are poor or nonhosts to *M. incognita*, and provide adequate income to the grower. Peanut (*Arachis hypogea*) is a nonhost to *M. incognita* and a good rotational crop in areas suitable for peanut production. Grain sorghum (*Sorghum bicolor*) is often selected as a rotational crop to manage *M. incognita* (Rodriguez-Kabana et al., 1990; Rodriguez-Kabana et al., 1991). Grain sorghum has been reported to be resistant to *M. incognita* race 3, *M. arenaria* race 1 and 2, and *M. javanica* (Fortnum and Currin, 1988). In contrast, Birchfield (1983) reported that eight of nine grain sorghum entries evaluated in a greenhouse trial were highly susceptible to *M. incognita*. Similarly, in another greenhouse study, three grain sorghum were reported to be a more suitable host for *M. incognita* race 3 than

*M. arenaria* race 1 (Ibrahim et al., 1993). Given these conflicting reports on the host status of grain sorghum to *M. incognita*, there is need to expand this investigation to include several commercially available red and white grain sorghum hybrids and evaluate the host response to more than one isolate of *M. incognita* collected from different cropping systems.

*Meloidogyne graminis* is one of the more common species of root-knot nematode associated with turfgrass in the United States and its host range has been expanded to include several grain crops, including grain sorghum (Dickerson, 1966; McClure et al., 2012). In one study, *M. graminis* was reported to complete its life cycle on one grain sorghum hybrid, 'RS610', but with no information on the degree of susceptibility. Given the limited information on susceptibility of grain sorghum to *M. graminis*, additional studies are needed to confirm and expand the host suitability of grain sorghum to *M. graminis*.

The objectives of this study were to compare the reproduction of *M. graminis* to *M. incognita* on several grain sorghum hybrids, to determine the susceptibility of several commercially available grain sorghum hybrids to *M. incognita*, and to evaluate the aggressiveness of four isolates of *M. incognita* on selected grain sorghum hybrids.

### MATERIALS AND METHODS

**Nematode cultures and inoculum:** The four isolates of *M. incognita* race 3 used in this study were collected from fields previously cropped in cotton, *G. hirsutum* (Biscoe, Leachville, and 98-1 isolates), or soybean, *G. max* (Kerr isolate), and maintained in the greenhouse on tomato, *Solanum lycopersicum*, 'Rutgers'. All isolates were collected in Arkansas, except 98-1, which was collected from Texas. Nematode species identification was confirmed using Mi-F/Mi-R PCR primers (Adam et al., 2007) and all reproduced on cotton cultivar Deltapine

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DP 0912 B2RF. The isolate of *M. graminis* used in this study was collected from bermudagrass, *Cynodon dactylon*, in Arkansas and cultured in the greenhouse on bermudagrass or zoysiagrass, *Zoysia japonica*, 'Palisades'. Species identification was confirmed by amplifying a DNA product using C2F3/1108 PCR primers that was digested using *SspI* restriction enzyme to produce bands that were similar to that of *M. graminis* type A and B (McClure et al., 2012). This isolate did not reproduce on tomato and males were often observed in culture. Eggs collected from cultures with 0.6% NaOCl (Hussey and Barker, 1973) were used as inoculum.

**Grain sorghum hybrids:** Twenty-seven grain sorghum hybrids were used in this study (Table 1). These hybrids were selected because they were marked for grain sorghum production in Arkansas (Bond et al., 2013). All hybrids were marketed as red, feed grain hybrids with the exception of four white, food-grade hybrids from Richardson Seed.

**Greenhouse experiments:** In the first experiment, due to space limitations, host suitability of these 27 hybrids to *M. incognita* (Leachville isolate) and *M. graminis* was randomly divided into five groups and each group was evaluated separately in five tests. In a second greenhouse experiment, the susceptibility of the 27 grain sorghum hybrids to *M. incognita* (Leachville isolate) was evaluated. In a third greenhouse experiment, the aggressiveness of four isolates of *M. incognita* was evaluated on six grain sorghum hybrids that ranged in susceptibility to root-knot nematode from highly resistant to susceptible. In all experiments, germinated seedlings, 1 wk after sowing, were transplanted into 496-cm<sup>3</sup> poly grow bags (Sunleaves Garden Products, Bloomington, IN) containing pasteurized sand and peat (12:1 v/v) soil mix. Seedlings were inoculated at the second-true leaf stage, 3 to 5 d after transplanting, with ~2,000 eggs (2 eggs/cm<sup>3</sup> soil) distributed among three 2-cm-deep holes per seedling. Each experiment was arranged in a randomized complete block design. In the first experiment, there were four replicates per hybrid per nematode species and each test was conducted once. This was because of the consistent difference in reproduction between nematode species, per objective one. In the second experiment, there were four replicates per hybrid and the experiment was conducted twice. A corn (*Zea mays*) hybrid 'Pioneer 31G71' and cotton cultivar DP 0912 B2RF were used in this experiment as comparative hosts for nematode reproduction. In the third experiment, there were four replicates per hybrid and isolate combination and the experiment was conducted twice. All experiments were maintained in the greenhouse where ambient temperatures ranged from 26°C to 33°C. Fresh shoot weight was collected in experiments one and two at harvest. Roots were collected 7 wk after inoculation, washed of soil, blotted dry with a paper towel, and weighed. Eggs were extracted from a 5-g subsample, collected from the upper root

TABLE 1. Grain sorghum hybrids (*Sorghum bicolor*) tested for host suitability against *Meloidogyne incognita* and *M. graminis*.

Company, location	Hybrid	Experiment no.
B-H Genetics, Ganado, TX	BH 3822	1, 2
	BH 5566	1, 2
Crop Production Services, Portageville, MO	Dyna-Gro 765B	1, 2, 3
	Dyna-Gro GX13662	1, 2, 3
	Dyna-Gro M75GB39	1, 2
	Dyna-Gro M77GB52	1, 2
DuPont Pioneer, Delaware, OH	Pioneer 83P17	1, 2
	Pioneer 83P99	1, 2
	Pioneer 84G62	1, 2
	Pioneer 84P80	1, 2
Gayland Ward Seed, Herford, TX	Gayland Ward	1, 2
	GW 9480	
Golden Acres Genetics, Buchanan Dam, TX	Golden Acres 5556	1, 2, 3
	Golden Acres 5613	1, 2
Richardson Seeds, Lubbock, TX <sup>a</sup>	Richardson XP10413	1, 2
	Richardson XP4053	1, 2
	Richardson XP50113	1, 2
	Richardson XP68653	1, 2
Terral Seed, Lake Providence, LA	Terral RV9562	1,2,3
	Terral RV9782	1, 2, 3
	Terral RV9823	1, 2, 3
	Terral RV9883	1, 2
	Terral RV9794	1, 2
	Terral RV9803	1, 2
	Terral RV9924	1, 2
Triumph Seed, Ralls, TX	Terral RV9973	1, 2
	Triumph TR481	1, 2
	Triumph TRX15401	1, 2

<sup>a</sup> All grain sorghum entries from Richardson Seeds were white, food-grade hybrids.

system with 1.2% NaOCl, and counted using a dissecting microscope.

**Statistical analysis:** Nematode reproduction data (eggs per g root) were square root ( $x + 0.5$ ) transformed to normalize for statistical analysis. Reverse transformed data are reported. Data from experiment one were analyzed by general linear model procedure between species of *Meloidogyne* for each hybrid using SPSS 19.0 (SPSS Inc., Chicago, IL). Nematode reproduction data from experiments two and three were analyzed according to general linear mixed models with experiment repetitions and treatment replications modeled as random variables. Mean separation ( $P = 0.05$ ) was established by Tukey's honest significant difference test.

## RESULTS

Nematode reproduction was observed for both species of *Meloidogyne* on all grain sorghum hybrids. Reproduction by *M. incognita* was greater ( $P \leq 0.05$ ) on 69.5% of the red grain sorghum hybrids than *M. graminis* (Fig. 1). Numerically, more eggs were produced by *M. graminis* on two red grain sorghum hybrids, Pioneer 84P80 and Terral RV9823, than *M. incognita*. Of the four white, food-grade grain sorghum hybrids, reproduction by *M. incognita* was greater than *M. graminis*.

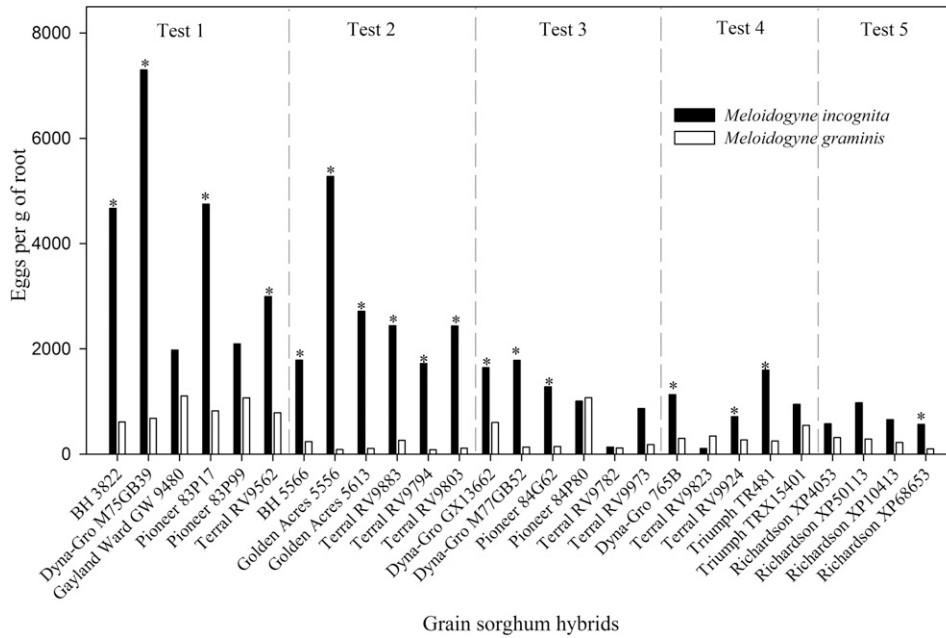


FIG. 1. Reproduction of *Meloidogyne incognita* and *M. graminis* on 27 grain sorghum hybrids. Within each test, \* over bars indicates significant differences at  $P \leq 0.05$  between nematode species per hybrid.

Both nematode species reduced shoot biomass with a negative correlation between shoot weight and reproduction by *M. incognita* ( $r = -0.31, P = 0.0001$ ) and *M. graminis* ( $r = -0.19, P = 0.04$ ). Small galls were rarely observed on hybrids inoculated with either species of *Meloidogyne*; however, more frequently observed were females with egg masses protruding from root systems.

There was no ( $P > 0.05$ ) experiment by hybrid interaction in the second experiment. However, a wide

variation in host suitability was observed as reproduction by *M. incognita* ranged from 395 to 3,818 eggs/g of root across all hybrids (Fig. 2). Of the red grain sorghum hybrids, fewer ( $P \leq 0.05$ ) *M. incognita* eggs were recovered from Terral RV9782, RV9823, RV9562, and RV9803 compared to the most susceptible hybrid, Golden Acres 5556. However, only Terral RV9782 and RV9823 consistently supported  $<10\%$  and  $<20\%$  reproduction by *M. incognita* compared to the

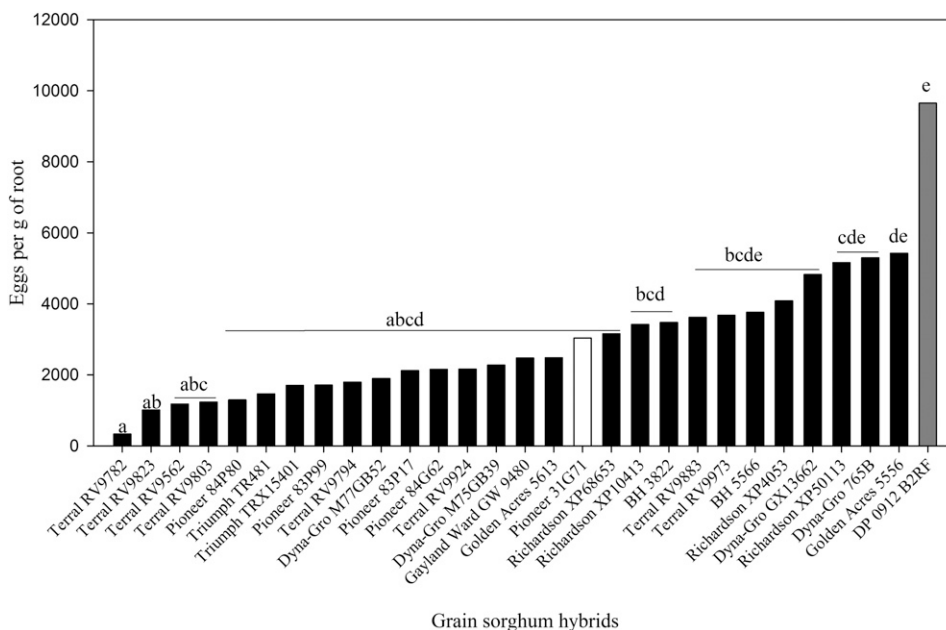


FIG. 2. Reproduction of *Meloidogyne incognita* on 27 grain sorghum hybrids. A cotton cultivar Deltapine DP 0912 B2RF (gray bar) and corn hybrid 'Pioneer 31G71' (white bar) were included for comparison. Different letters over bars indicate significant differences at  $\alpha = 0.05$  according to Tukey's honest significant difference test.

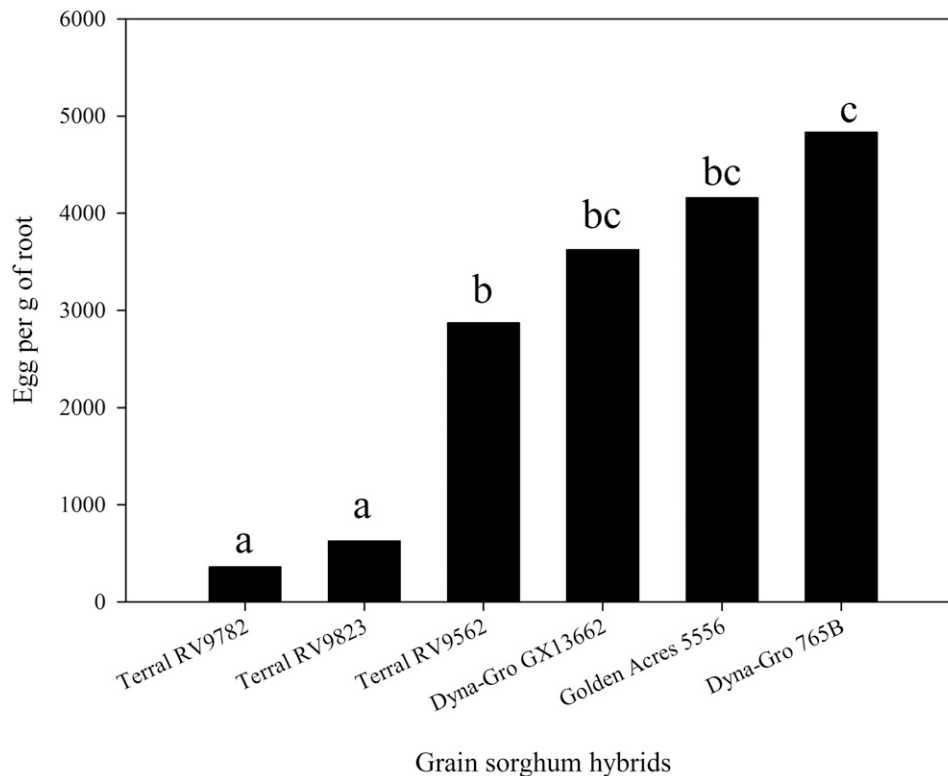


FIG. 3. Reproduction of *Meloidogyne incognita* on six selected grain sorghum hybrids averaged across four nematode isolates. Different letters over bars indicate significant differences at  $\alpha = 0.05$  according to Tukey's honest significant difference test.

most susceptible hybrid, respectively. Nematode reproduction was lower ( $P \leq 0.05$ ) on 74% of the red and 50% of the white, food-grade hybrids compared to that of the cotton cultivar DP 0912 B2RF, while nematode reproduction on all hybrids was similar to that of the corn hybrid 'Pioneer 31G71'. Shoot weight averaged 48.3 g across all hybrids and there was a negative correlation between shoot weight and reproduction by *M. incognita* ( $r = -0.19$ ,  $P = 0.04$ ).

There were no ( $P > 0.05$ ) effects of any interaction combination for experiments, hybrids, or nematode isolates in the third experiment, thus only the main effects of hybrids and isolates are reported. Fewer ( $P \leq 0.05$ ) *M. incognita* eggs were collected from Terral RV9782 and RV9823 compared to Terral RV9562, Dyna-Gro GX13662, Dyna-Gro 765B, and Golden Acres 5556 (Fig. 3). Similar to the second experiment, Terral RV9782 consistently supported <10% nematode reproduction to that of the most susceptible hybrids, Dyna-Gro 765B or Golden Acres 5556, whereas Terral RV9823 supported <20% reproduction to that of these susceptible hybrids. Thirty percent fewer ( $P \leq 0.05$ ) eggs were recovered from hybrids inoculated with the Kerr isolate compared to the Leachville isolate (Fig. 4). The Kerr isolate was collected from a soybean field, whereas the Leachville isolate was collected from a cotton field; however, no statistical difference was observed between the Kerr isolate and other isolates collected from cotton fields (Biscoe and 98-1).

#### DISCUSSION

None of the grain sorghum hybrids were immune to *M. graminis* or *M. incognita*; however, the majority of these hybrids were a relatively poor host to *M. graminis* compared to *M. incognita*. Reproduction by *M. incognita* was at least numerically greater than *M. graminis* on 93% of these hybrids. It is not uncommon to observe differences in nematode reproduction between species of *Meloidogyne* on grain crops, as grain sorghum and corn hybrids were reported to be a more suitable host for *M. incognita* (race 3) than *M. arenaria* (race 2 or 4) or *M. javanica* (Baldwin and Barker, 1970; Windham and Williams, 1987; Ibrahim et al., 1993; Davis and Timper, 2000). In contrast, more eggs were produced by *M. marylandi* than *M. incognita* on one of two grain sorghum hybrids in a host range study (Faske and Starr, 2009). These data confirm that *M. graminis* does reproduce on grain sorghum (Dickerson, 1966; MacGowan, 1984), but generally not to the same magnitude as that of *M. incognita*, which indicate that grain sorghum is a more suitable host for *M. incognita* than *M. graminis*.

There was a wide range in host suitability among these hybrids to *M. incognita*, which is similar to that reported on corn (Windham and Williams, 1987; Davis and Timper, 2000; Todd et al., 2016). Based on nematode reproduction as a percentage of the most susceptible hybrid (Starr and Mercer, 2009), Terral RV9782 was highly resistant (<10%), Terral RV9823 was resistant

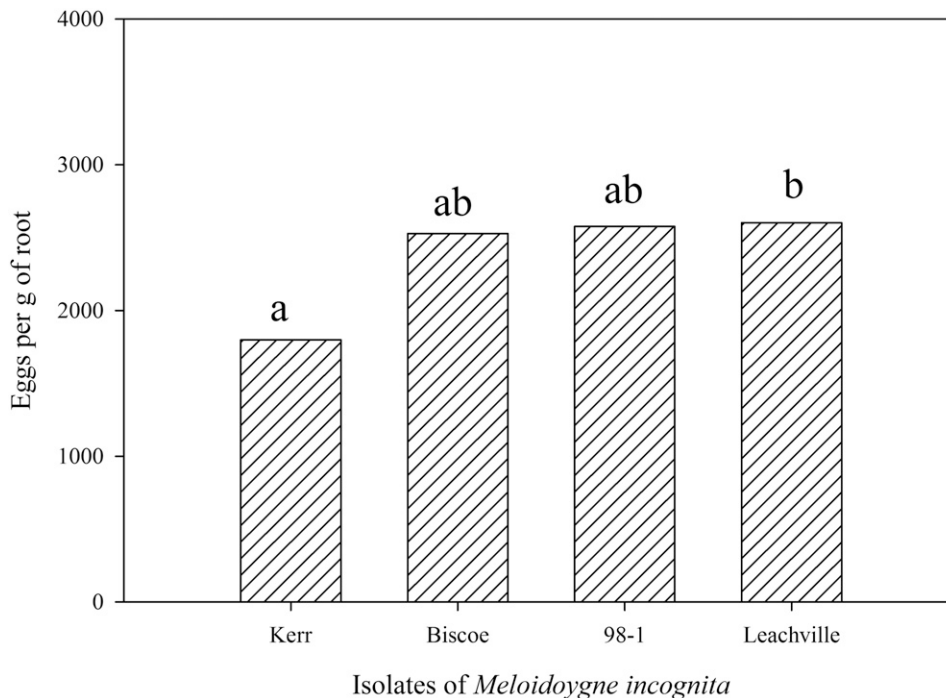


FIG. 4. Reproduction of four isolates of *Meloidogyne incognita* averaged across six grain sorghum hybrids. Different letters over bars indicate significant differences at  $\alpha = 0.05$  according to Tukey's honest significant difference test.

(11%–25%), and those hybrids that supported numerically less reproduction than the corn hybrid were moderately resistant (26%–50%). The remaining 40% of the hybrids, including the white, food-grade hybrids that supported a greater numeric level of reproduction to that of the corn hybrid were susceptible (>50%). These observations suggest that most of these hybrids would maintain or increase *M. incognita* for the subsequent crop at a level similar to that of corn (Roberts, 1992; McSorley and Gallaher, 1993; Kirkpatrick et al., 2014). No hybrids, including corn, were as susceptible as the cotton cultivar, which is comparable to that reported by Orr and Morey (1974). These observations support previous reports that grain sorghum hybrids vary in their susceptibility to *M. incognita* (Birchfield, 1983), which would be an important consideration when utilizing grain sorghum as a rotational crop to manage *M. incognita*.

Of the hybrids tested, Terral RV9782 and RV9823 were considered highly resistant and resistant, respectively, thus either would be a better selection as a rotational crop than the more susceptible hybrids tested. These observations were consistent in two experiments and across all four isolates of *M. incognita* race 3. Two isolates differed in aggressiveness across the six selected hybrids; however, this difference was inconsistent among the other isolates. Differences in isolate aggressiveness are common and have been reported in *M. incognita* (Baldwin and Barker, 1970; Davis and Timper, 2000; Zhou et al., 2000; Anwar and McKenry, 2007). Collectively, these observations suggest that these two resistant hybrids have broad

applicability as a rotational crop and in general, grain sorghum hybrids will likely be a more effective rotational crop with some populations of *M. incognita* than others.

In this study, grain sorghum was a more suitable host for *M. incognita* than *M. graminis*, with significant variation in hybrid susceptibility to *M. incognita*. Thus, in fields where damaging populations of *M. incognita* are present, grain sorghum hybrids, except for Terral RV9782 and RV9823, will probably maintain or increase the population of *M. incognita* for the subsequent crop.

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