RESEARCH/INVESTIGACIÓN

REPRODUCTION OF *MELOIDOGYNE INCOGNITA* ON HYBRID RICE AND SURVEY OF PLANT-PARASITIC NEMATODES IN FURROW-IRRIGATED RICE IN ARKANSAS

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ABSTRACT

Kelly, J., N. R. Bateman, and T. R. Faske. 2022. Reproduction of *Meloidogyne incognita* on hybrid rice and survey of plant-parasitic nematodes in furrow-irrigated rice in Arkansas. Nematropica 52:94-102.

Furrow-irrigated hybrid rice production has increased in Arkansas and the mid-southern U.S. The susceptibility of hybrid rice to *Meloidogyne incognita*, and the incidence and population density of plant-parasitic nematodes in furrow-irrigated rice is unknown. Reproduction of *M. incognita* on hybrid rice was evaluated in three greenhouse pot experiments, and a nematode survey was based on soil samples collected after harvest from 16 furrow-irrigated rice fields. All rice hybrids were susceptible to *M. incognita* race 3 but were less susceptible than soybean. The susceptibility of four rice hybrids was consistent across three isolates of *M. incognita*, and the reproduction of the isolates was similar across hybrids. A 57% reduction in *M. incognita* reproduction was observed on subsurface-irrigated rice, which is similar to furrow-irrigated rice, compared to overhead-irrigated rice. Spiral nematode (*Helicotylenchus*) and lesion nematode (*Pratylenchus*) were detected in 94 and 69% of furrow-irrigated fields sampled, respectively, and a low density of root-knot nematode (*Meloidogyne*) was detected in two fields. Hybrid rice grown in a furrow-irrigated system can maintain a population of *M. incognita* and other plant-parasitic nematodes for the subsequent crop.

Key words: *Meloidogyne incognita*, *Oryza sativa*, resistance, rice, southern root-knot nematode

RESUMEN

Kelly, J., N. R. Bateman y T. R. Faske. 2022. Reproducción de *Meloidogyne incognita* en arroz híbrido y estudio de nematodos fitoparásitos en arroz regado por surcos en Arkansas. Nematrópica 52:94-102.

La producción de arroz híbrido irrigado por surcos ha aumentado en Arkansas y el sur medio de los EE. UU. Se desconoce la susceptibilidad del arroz híbrido a *Meloidogyne incognita*, y la incidencia y densidad de población de los nematodos fitoparásitos en el arroz irrigado por surcos. Se evaluó la reproducción de *M. incognita* en arroz híbrido en tres experimentos en macetas de invernadero y se basó un estudio de nematodos en muestras de suelo recolectadas después de la cosecha de dieciséis campos de arroz regados por surcos. Todos los híbridos de arroz fueron susceptibles a *M. incognita* raza 3 pero fueron menos susceptibles que la soja. La susceptibilidad de cuatro híbridos de arroz fue consistente en tres aislados de *M. incognita* y la reproducción de los aislados fue similar en todos los híbridos. Se observó una reducción del 57 % en la reproducción de *M. incognita* en el arroz con riego subterráneo, que es similar al arroz con riego por surcos, en comparación con el arroz con riego por aspersión. Se detectaron nematodos espirales (*Helicotylenchus*) y nematodos lesionados (*Pratylenchus*) en el 94 y 69% de los campos regados por surcos muestreados, respectivamente, y se detectó una baja densidad de nematodos agalladores en dos campos. El arroz híbrido cultivado en un sistema de riego por surcos puede mantener una población de *M. incognita* y otros nematodos parásitos de plantas para el cultivo posterior.

Palabras clave: Meloidogyne incognita, *Oryza sativa*, resistencia, arroz, nematodo agallador del sur

INTRODUCTION

Rice (*Oryza sativa* L.) is a major agronomic crop in Arkansas with an annual average production of 0.6 million ha (Hardke, 2021a). Historically, all rice production was flood-irrigated, but recently, furrow-irrigated rice production has increased from 2.7% in 2016 to 16.9% in 2020 (Hardke and Chlapecka, 2020). The increased use is due to lower expenses than flood-irrigated rice and availability of blast-resistant (foliar fungal disease caused by *Pyricularia oryzae* Cavara) hybrids which facilitates crop rotation. Each year, approximately two-thirds of Arkansas rice production is in rotation with soybean [*Glycine max* (L.) Merr.] (Hardke, 2021b). The incidence and population density of soybean nematodes in furrow-irrigated rice and thus the need for nematode management for the subsequent soybean crop is unknown.

Meloidogyne incognita (Kofoid and White), Chitwood, the southern root-knot nematode, is an important and yield-limiting pathogen of soybean. In research plots, grain yield losses of more than 75% have been reported in Arkansas (Emerson and Faske, 2021). Root-knot nematodes were among the top three most destructive diseases of soybean from 2015-2019 in the southern U.S., with a total estimated grain yield loss of 56 million bushels (Bradley *et al.*, 2021). Management of *M. incognita* consists of utilizing resistant cultivars, nematicides, and crop rotation (Kirkpatrick *et al.*, 2014; Faske *et al.*, 2021). Rice (flood-irrigated) is suggested as a rotational crop in Arkansas (Kirkpatrick *et al.*, 2014), but that was before the increase in furrow-irrigated rice production.

Flood-irrigated rice is a good management option because soils remain flooded for at least three months during the cropping season. The duration of flooded soils and warm temperatures are needed to significantly reduce *M. incognita* densities (Rhoades, 1982). In a field study, approximately 2 months of flood-irrigated rice significantly reduced *M. incognita* densities and infection in the subsequent crop compared to upland (non-irrigated) rice (Thames and Stoner, 1953). Unlike flood-irrigated rice, furrow-irrigated rice fields are not completely flooded, especially the beds in the upper half and furrow in the upper third of the field. These aerobic conditions may allow root-knot nematode and other plant-parasitic nematodes to reproduce on furrow-irrigated hybrid rice; however, nematode incidence and population density following furrow-irrigated rice production has yet to be investigated.

A few studies have investigated the susceptibility of upland rice cultivars to *M. incognita*. In Egypt, upland rice was reported as resistance to *M. incognita* race 3, whereas in Brazil, rice was susceptible (Taylor and Sasser, 1978; Ibrahim *et al.*, 1983; Silva *et al.*, 2004; Silva *et al.*, 2011). Currently, no study has investigated the susceptibility of rice hybrids or conventional cultivars grown and selected for production in Arkansas or the mid-southern U.S. to *M. incognita* race 3. *Meloidogyne incognita* is the most common root-knot nematode species in the state (Ye *et al.*, 2019) and given the long history of cotton production, race 3 is the most common race (B. Robbins and T. Kirkpatrick, University of Arkansas, personal communication). Given the limited research in furrow-irrigated rice production on nematode management, the objectives of this study were to: (i) determine the host suitability of eight rice hybrids to *M. incognita*; (ii) evaluate the reproduction of three *M. incognita* isolates on four rice hybrids; (iii) determine the impact of subsurface-irrigation on *M. incognita* reproduction of hybrid rice; and (iv) assess the incidence and population density of plant-parasitic nematodes in furrow-irrigated rice.

MATERIALS AND METHODS

Nematode cultures and inoculum

Three isolates of *M. incognita* race 3 used in this study were collected from Arkansas fields previously cropped in cotton, *Gossypium hirsutum*, (Leachville isolate) or soybean, (Kerr and Black Oak isolates) and maintained in the greenhouse on tomato, *Solanum lycopersicum*, 'Rutgers'. Nematode species identification was confirmed using Mi-F/Mi-R PCR primers (Adam *et al.*, 2007) and all reproduced on cotton 'Deltapine (DP) 0912 B2RF' (Bayer Crop Science, St. Louis, MO). Eggs collected from 8- to 10-wk-old *M. incognita* cultures with 0.5% NaOCl (Hussey and Barker, 1973) were used as inoculum.

Rice hybrids

Eight rice hybrids were used in this study (Table 1). These hybrids are recommended for use by the University of Arkansas System, Cooperative Extension Service in furrow-irrigated rice production (Hardke and Chlapecka, 2020). Two conventional cultivars, 'Diamond' and 'CL 153' are popular and sometimes grown in furrow-irrigated production and serve as a comparison of rice suitability to *M. incognita*.

Greenhouse experiments

Three greenhouse experiments were conducted to evaluate the reproduction of *M. incognita* on hybrid rice (Table 1). In the first experiment, the susceptibility of eight rice hybrids to *M. incognita* (Leachville isolate) was evaluated. In a second greenhouse experiment, the reproduction of the three isolates of *M. incognita* was evaluated on four rice hybrids that varied numerically in susceptibility to *M. incognita* in the first experiment. In a third experiment, the reproduction potential of *M. incognita* (Kerr isolate) was evaluated in two irrigation systems, overhead vs. subsurface, on a susceptible rice hybrid, RT 3201. The subsurface irrigation system was designed to simulate partially flooded rice, which is commonly observed in furrow-irrigated rice. In experiment one and two, two seeds were sown in 656 cm^3 cone-tainers (Model D40L; Stuewe and Sons, Inc., Tangent, OR) containing pasteurized sand (100% sand; < 1 mm diam). Seedlings were thinned to one seedling per pot four days after planting (DAP). Each seedling received 2 ml water that contained ~2,000 eggs (3 eggs/cm^3 soil) at the first-leaf stage [(7 days after planting (DAP)]. Inoculum was distributed among three cavities around the seedlings created by pushing a 4-mm-diam glass rod 2 cm into the root zone. In experiment three, the procedure and materials were the same, except smaller cone-tainers (262 cm^3; Model D16H) were used. Furthermore, each seedling received 2 ml water that contained ~4,000 eggs (15 eggs/cm^3 soil) at the second-leaf stage (14 DAP), and 7 days after inoculation 50% of the cone-tainers were placed in trays that contained reverse osmosis water. The water level was maintained at 60% of the cone-tainer depth (i.e., 10.8 cm flood depth). Treatments were arranged in a randomized complete block design, except for experiment three, which was in a completely randomized design. In the first experiment, there

Table 1. Rice hybrids (*Oryza sativa*) tested for host suitability against *Meloidogyne incognita.*

Company, location	Hybrids/cultivars	Experiment No.
RiceTech Inc., Alvin, TX	RT 3201	1, 2, 3
	RT 7301	1, 2
	RT 7501	1
	RT 7321 FP	1
	RT 7521 FP	1, 2
	RT CLX745	1, 2
	RT Gemini 214 CL	1
	RT XP753	1
Horizon Ag, LLC, Memphis, TN	CL 153^z	1
University of Arkansas System, Fayetteville, AR	Diamond^z	1

^{z}Conventional rice cultivar

were five replicates per hybrid and the experiment was conducted twice. A *M. incognita*-susceptible soybean 'Delta Grow (DG) 4880 GLY', (Delta Grow Seed Co. Inc., England, AR) was used in the experiment as a comparative host for nematode reproduction. In the second experiment, there were five replicates per hybrid and isolate combination, and the experiment was conducted twice. In the third experiment, there were eight replicates per hybrid and watering combination, and the experiment was conducted twice. All experiments were maintained in the greenhouse where ambient temperatures ranged from 26 to 33ºC. Roots were collected 7 wk after inoculation, washed of soil, blotted dry with a paper towel, and weighed. Eggs were extracted from an 8-g subsample, collected from the upper root system (top 15 cm), with 1.0% NaOCl, and counted using a dissecting microscope. Nematode reproduction is reported as egg per gram of root and/or as a nematode RF (reproduction factor = total eggs collected per root system/initial inoculum).

Nematode survey in furrow-irrigated hybrid rice

An assessment of plant-parasitic nematode incidence and density in furrow-irrigated hybrid rice was conducted from 2018 to 2020 in 16 fields across five Arkansas counties (Arkansas, Craighead, Independence, Jackson, and Monroe). Three soil samples were collected from each field at 7 to 10 days after harvest. Soil samples were a composite of 10 core samples taken 15 to 20 cm deep from beds and within 5 to 8 cm of individual rice plants with a 2-cm-diam soil probe. In a preliminary study, a greater population density (>50% more) of plant-parasitic nematodes was detected in the upper third of furrow-irrigated hybrid rice fields compared to the lower third, which is often flooded through most of the cropping season. Therefore, soil samples reported in this study were collected from the upper third of the field. Nematodes were collected from soil using a Baermann funnel (Chapman, 1958). Plant-parasitic nematodes were collected with a 25-µm-pore sieve after 4 hr. Plant-parasitic nematodes were identified by genera and enumerated using an inverted microscope (Auxio Vert.A1, Carl Zeiss Microscopy, Thornwood, NY).

Statistical analysis

Nematode reproduction data (eggs per gram of root and RF) was $\log^{10}$ (x + 1) transformed to normalize for statistical analysis and non-transformed data are reported. The Shapiro-Wilk procedure was used to confirm normality. Data were analyzed according to general linear mixed models with cultivars and/or nematode isolates modeled as a fixed variable, and experiment repetitions and treatment replications modeled as random variables using SPSS 27.0 (International Business Machines Corp., Armonk, NY). Means were separated according to Tukey's Honestly Significant Difference (HSD) procedure at α = 0.05. Pierson's correlation coefficient was used to identify correlations between eggs per gram of root and RF.

RESULTS

Reproduction by *M. incognita* was observed on all rice hybrids and conventional cultivars. There was no (P > 0.05) experiment by cultivar interaction in the first experiment. Little variation in host susceptibility was observed as reproduction by *M. incognita* ranged from 2,500 to 3,775 eggs/g of root across all rice entries. Fewer ($P \leq 0.05$) eggs per gram of root and a lower ($P \leq 0.05$) RF was observed for all rice hybrids and conventional cultivars than for the susceptible soybean 'DG 4880 GLY', except for RT 7521 (Fig. 1). There was a strong positive correlation between eggs per gram of root and RF (r = 0.95; P = 0.001), thus only eggs per gram of root are reported for remaining experiments. Small, spindle-shaped galls were casually observed on rice roots.

There was no (P > 0.05) effect of any interaction combination for experiments, hybrids, or nematode isolates in the second experiment, thus only the main effects of hybrids and isolates are reported. All selected rice hybrids supported a similar (P > 0.05) level of reproduction by *M. incognita* with an average of 2,830 eggs/g of root across hybrids (data not shown). Furthermore, there was no (P > 0.05) difference in reproduction among the three isolates of *M. incognita* with an average of 2,880 eggs/g of root across isolates (data not shown).

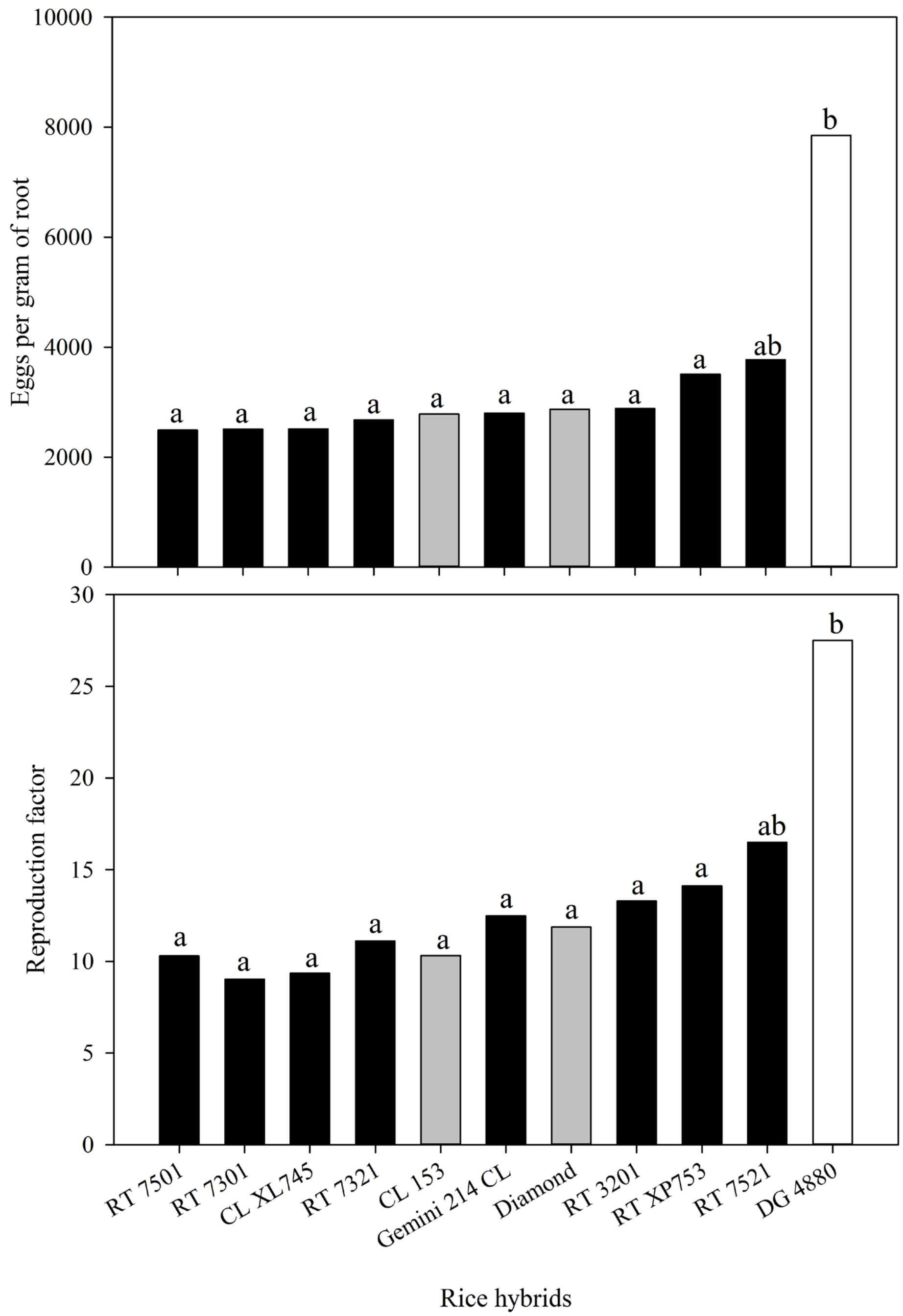

Figure 1. Reproduction of *Meloidogyne incognita* on eight rice hybrids. A soybean cultivar, 'Delta Grow (DG) 4880 GLY' (white bar), and conventional rice cultivars (gray bars) were included for comparison. Different letters over bars indicate significant differences at $\alpha = 0.05$ according to Tukey's HSD procedure. Reproduction factor = Pf/Pi (Pi =2,000 eggs).

There was no ($P > 0.05$) experiment by irrigation interaction in the third experiment. *Meloidogyne incognita* was able to reproduce on partially flooded hybrid rice, albeit fewer ($P \leq 0.05$) eggs were recovered from subsurface-irrigated compared to overhead-irrigated rice (Fig. 2).

A few plant-parasitic nematodes that are common on row crops in Arkansas were observed in furrow-irrigated rice. Of the genera detected,

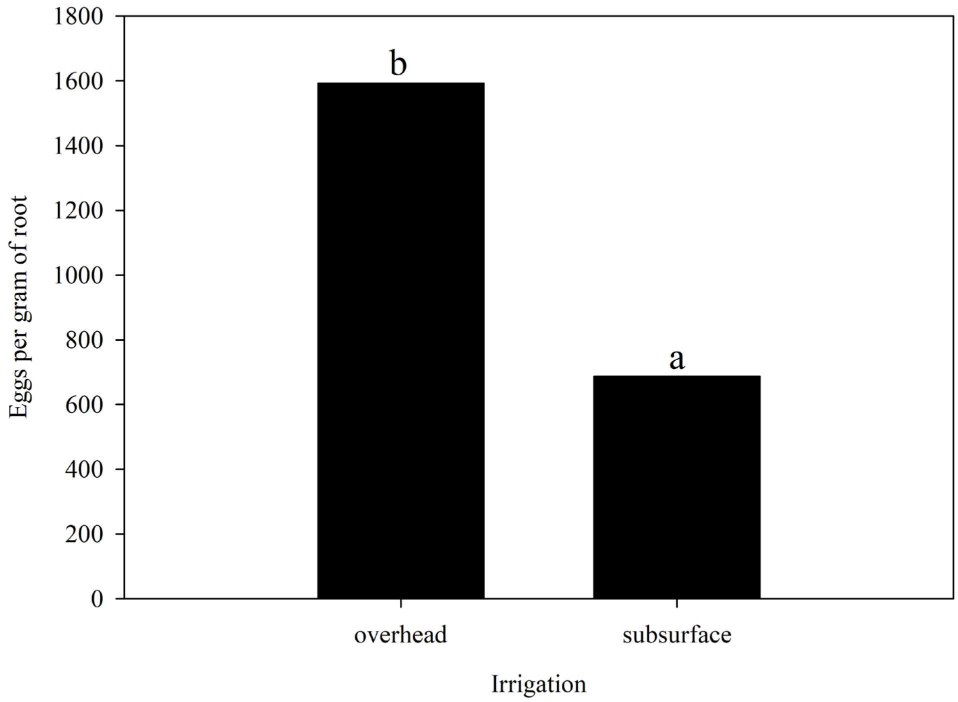

Figure 2. Reproduction of *Meloidogyne incognita* on a rice hybrid, RT 3201, in two different irrigation systems. Different letters over bars indicate significant difference at $P \leq 0.05$ according to analysis of variance procedure.

from most to least frequent included *Helicotylenchus*, *Pratylenchus*, *Tylenchorhynchus*, and *Meloidogyne* (Table 2). Other genera found in at least one field at <10 individuals/100 cm^3 soil consisted of *Hoplolaimus* and *Paratylenchus*. The data indicate that *Meloidogyne* and other plant-parasitic nematodes can be detected in furrow-irrigated rice and provide a reference for nematode densities at the onset of this new cropping system, yet this is not intended to represent a statewide survey. The two samples of *Meloidogyne* were detected in fields with silt loam soils, which is the most common soil texture for rice production in Arkansas (Hardke, 2021b), and is representative of most (n = 13) of the fields sampled in this study. Other soil texture classes consisted of loam (n = 1) and sandy loam (n = 2) soils. All samples contained at least one plant-parasitic nematode genus and various free-living nematodes were casually observed in all soil samples.

DISCUSSION

All rice hybrids were susceptible to *M. incognita* race 3 with no variation in host suitability. The susceptibility of upland rice cultivars to *M. incognita* race 3 has been reported in Brazil (Silva *et al.*, 2004; Silva *et al.*, 2011) and races 1, 2 and 4 in Brazil and West Africa (Plowright *et al.*, 1999; Silva *et al.*, 2004; de Araújo Filho *et al.*, 2010). These data support the susceptibility of rice to *M. incognita* race 3 and expands to include cultivars and hybrids in the U.S. In contrast to the current study, some variation in host suitability was reported in upland rice cultivars against *M. incognita* (Babatola, 1980; Diomande, 1984; Plowright *et al.*, 1999; de Araújo Filho *et al.*, 2010; Silva *et al.*, 2011). The lack of variation in host suitability in the current study may be due to selection of rice for production in flood-irrigated systems, which has historically and currently accounts for the majority of rice production in Arkansas (Hardke, 2021b). Though hybrid rice is susceptible to *M. incognita*, it is a less suitable host compared to cotton (Silva *et al.*, 2011) and soybean.

The susceptibility of rice hybrids was similar among three isolates of *M. incognita* race 3 with no difference in reproduction among isolates. Differences in *Meloidogyne* reproduction among

Table 2. Percent and population density of four plant-parasitic nematode genera sampled after harvest from 16 furrow-irrigated rice fields from 2018 to 2020 in Arkansas.

		Nematodes per 100 cm^3 soil			
Nematode genera	% fields with nematode	1 to 24	25 to 99	100 to 499	≥ 500
Helicotylenchus	93.8	1	2	9	4
Pratylenchus	68.8	6	1	4	0
Meloidogyne	12.5	2	0	0	0
Tylenchorhynchus	62.5	5	3	0	1

races and species collected from rice has been reported in upland rice in Egypt and Brazil (Ibrahim *et al.*, 1983; Silva *et al.*, 2004; Manchado and Araujo Filho, 2014). In the current study, isolates were collected from cotton and soybean, which may also account for some of the lack of variation in *M. incognita* reproduction on hybrid rice. However, the similarity in nematode reproduction among isolates in the current study suggests that *M. incognita* race 3 reproduction is also likely to be similar on hybrid rice grown in a furrow-irrigated production system regardless of cropping history across the state and region.

A low population density of *M. incognita* was detected following furrow-irrigated hybrid rice production. Furthermore, nematode reproduction was reduced by 57% when grown in a partially flooded soil, but not eliminated. Flooded soils can be an effective tool to manage *M. incognita*, but the duration of flooding and soil temperature are important parameters to consider. In a lab study, two months of flooded soils were needed to reduce *M. incognita* densities at soil temperatures greater than 20°C (Rhoades, 1982). In a field study, four months of flooded soils were needed to kill all root-knot nematode second-stage juveniles, but not eggs (Brown, 1933). Furthermore, it was estimated that more than 12 months of flooded soils was needed to kill nematode eggs. Therefore, even in flooded soils, *M. incognita* may not be eliminated. Furthermore, flood-irrigated rice is an option to manage lesion nematode (*Pratylenchus*) in Arkansas (Kirkpatrick *et al.*, 2014), but lesion nematode was frequently detected in furrow-irrigated fields. With the continued use of furrow-irrigated rice, other nematode species previously managed by flooding could become problematic for the subsequent soybean crop.

The data from the current study support the susceptibility of hybrid rice to *M. incognita* race 3 and that root-knot nematode was detected following furrow-irrigated rice production. Because furrow-irrigated rice can maintain a population of *M. incognita* and other plant-parasitic nematodes, additional management practices may be required in the future to manage plant-parasitic nematodes in the subsequent soybean crop.

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