RESEARCH NOTE/NOTA DE INVESTIGACIÓN

HOST SUITABILITY OF CARINATA (*BRASSICA CARINATA*) FOR *ROTYLENCHULUS RENIFORMIS*

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ABSTRACT

R. Sandoval-Ruiz, Z. J. Grabau. Host Suitability of carinata (*Brassica carinata*) for *Rotylenchulus reniformis*. Nematropica 53:6-15.

Reniform nematode (*Rotylenchulus reniformis*, RN) is one of the most important yield-robbing pathogens in cotton (*Gossypium hirsutum*). In the southeast United States, carinata (*Brassica carinata*) is an emerging winter crop, but its host status for RN has not been studied. This information is foundational for the establishment of carinata as a crop and for RN management in the region. Therefore, the objective of this research was to determine the relative host suitability of carinata to RN when compared to poor hosts of RN—oat (*Avena sativa*), canola (*B. napus*), and peanut (*Arachis hypogaea*)—or good hosts of RN—hairy vetch (*Vicia villosa*) and cotton. This objective was investigated in a repeated greenhouse experiment with the first trial conducted in 2020 and the second in 2021. In both trials, carinata had reproduction factor values (final population density/initial population density) of less than one, and the abundance of RN per gram of roots and per root system was less for carinata than good hosts of RN. For other crops, results were consistent with the RN host status previously defined. Based on these results, carinata is a poor RN host, and therefore, it may be a useful management option for RN.

Key words: *Arachis hypogaea*, *Avena sativa*, *Brassica carinata*, *B. napus*, canola, carinata, cotton, *Gossypium hirsutum*, hairy vetch, host, management, oats, peanut, reniform nematode, *Rotylenchulus reniformis*, *Vicia villosa*

RESUMEN

R. Sandoval-Ruiz, Z. J. Grabau. Idoneidad de carinata (*Brassica carinata*) como hospedante para *Rotylenchulus reniformis*. Nematropica 53:6-15.

El nematodo reniforme (*Rotylenchulus reniformis*, RN) es uno de los patógenos más importantes que roban rendimiento en algodón (*Gossypium hirsutum*). En el sureste de los Estados Unidos, la carinata (*Brassica carinata*) es un cultivo de invierno emergente, pero no se ha estudiado su condición como hospedante de RN. Esta información es fundamental para el establecimiento de carinata como cultivo y para el manejo de RN en la región. Por lo tanto, el objetivo de esta investigación fue determinar la idoneidad relativa de los hospedantes de carinata para RN en comparación con hospedantes pobres de RN: avena (*Avena sativa*), canola (*B. napus*) y maní (*Arachis hypogaea*) o buenos hospedantes de RN: arveja peluda (*Vicia villosa*) y algodón. Este objetivo se investigó en un experimento repetido de invernadero con el primer ensayo realizado en 2020 y el segundo en 2021. En ambos ensayos, carinata tuvo valores del factor de reproducción de menos de uno, y la abundancia de RN por gramo de raíces, por el total de raíz fue menor para carinata que para los buenos hospedantes de RN. Para los otros cultivos, los resultados fueron consistentes con el estado de hospedante RN previamente definido. Con base en estos resultados, carinata es un hospedante pobre de RN y, por lo tanto, puede ser una opción de manejo útil para RN.

Palabras chave: *Arachis hypogaea*, *Avena sativa*, *Brassica carinata*, *B. napus*, canola, carinata, algodón *Gossypium hirsutum*, arveja peluda, anfitrión, manejo, avena, maní, nematodo reniforme, *Rotylenchulus reniformis*, *Vicia villosa*

Rotylenchulus reniformis Linford and Oliveira (reniform nematode, RN) is a sedentary semi-endoparasitic nematode (Robinson, 2007) identified as one of the major yield-reducing pathogens in cotton (*Gossypium hirsutum* L.). However, it has a broad host range (Dyer *et al.*, 2020) rendering it difficult to manage. In cotton, the symptoms of RN infection include smaller plants, evidence of nutrient deficiency such as chlorotic leaves or yellowing tissue between the veins, poor crop stand, stunted root system, and reduction in the size and number of cotton bolls (Khanal *et al.*, 2018b; Koebernick *et al.*, 2021).

The United States is the most important exporter of cotton and one of the main cotton-producing countries in the world with 11.7 million ha planted in 2021, with a crop value of more than $4 million (USD) (OECD-FAO, 2020; NASS-USDA, 2021a, 2021b). Nematodes, including RN, were the main cause of cotton yield losses in the United States in 2020 (Lawrence *et al.*, 2021). Therefore, RN management strategies are urgently needed. Nevertheless, RN management options are currently limited and rely primarily on the use of synthetic nematicides and crop rotation (Grabau, 2016; Grabau *et al.*, 2021). Commercially viable resistant cultivars have become available recently, but are not extensively adopted (Koebernick *et al.*, 2021). Because RN is dependent on a host crop for its pathogenicity and reproduction (Singh, 1976; Kularathna *et al.*, 2019), and with the increasing focus on environmentally sustainable agricultural strategies, cultural practices such as crop rotation are important for development of RN integrated management practices. Studying the host status of crops for RN is fundamental to establishing crop rotation recommendations.

Brassicas have been widely used as rotation crops to manage nematodes (Dutta *et al.*, 2019; Abd-Elgawad, 2021). Nevertheless, they vary in their host status and thus in their value as rotational crops for RN management. For instance, *Brassica napus* (canola) is defined as a non-host crop for RN (Jones *et al.*, 2006), whereas *B. nigra*, *B. rapa*, and *B. campestris* are known as poor hosts (Khan and Khan, 1973; Dyer *et al.*, 2020). In contrast, *B. juncea* (Waisen *et al.,* 2019), *B. oleracea* var. *botrytis* and *B. oleracea* var. *capitata* are good RN hosts, and RN impairs the growth of the latter two crops (Khan and Khan, 1973). In the Southeast United States, *B. carinata* A. Braun (carinata) is an emerging crop in cotton-producing areas, but its host status for RN has not been defined. Carinata susceptibility to nematodes varies by genus and species. For instance, *Meloidogyne arenaria* and *M. incognita* reproduced on carinata at low and moderate levels respectively, while *M. javanica* and *Pratylenchus neglectus* had a high levels of reproduction on carinata (Potter, *et al.*, 1999; Castillo and Liébanas, 2004).

Carinata is a winter oilseed crop used to produce jet biofuel, an alternative to non-renewable fossil fuels that can help mitigate CO_2 emissions (Cardone *et al.*, 2002). Additionally, it can be processed to obtain various value-added co-products (Schulmeister *et al.*, 2019; George *et al.*, 2021). In the Southeast, winter production of carinata represents a prospect for producers to contribute to local energy requirements. Carinata can be integrated into existing cropping systems—grown between summer crops such as cotton and peanut—providing an opportunity to farm more than 1.4 million ha of land that is typically fallow in winter (Seepaul *et al.*, 2021). Currently, carinata production is not extensive in the Southeast but there are growing commercial and research efforts to establish the carinata industry (Kumar *et al.*, 2020; Seepaul *et al.*, 2021). Because RN is widespread and a serious pest in current cash crops, understanding the host status of carinata for RN is required to establish carinata as part of a double-crop system.

Studying the host suitability of carinata towards RN—determined by population density and reproduction rate on a crop (Trivedi and Barker, 1986)—is critical to determine if RN will negatively impact carinata production. In nematology, the reproduction factor (RF) is used to indicate the suitability of a crop as a nematode host (Sasser *et al.*, 1984). Plants classified as susceptible have an RF>1, while poor or non-host plants have an RF≤1 (Seinhorst, 1967). It is also a foundational step in determining its value for RN management preceding the production of cotton or other crops susceptible to RN. Therefore, the objective of this research was to determine the relative host suitability of carinata to RN when compared to crops with defined RN host status.

Crops were challenged with RN in a repeated greenhouse pot experiment conducted in a polycarbonate greenhouse at the University of Florida Entomology and Nematology Department in Gainesville, FL. This experiment was a completely randomized design with one factor (crop), replicated six times, and performed twice (Trial 1 and Trial 2). Crops evaluated were carinata 'Avanza 641', canola 'Canterra 1918', cotton 'Deltapine 1646B2XF', hairy vetch 'Au merit', peanut 'Georgia 06G', and oat. Carinata 'Avanza 641' was selected because it was the major carinata variety available when this research was conducted.

The RN population used in this study was a pure culture originally obtained from a naturally infested cotton field in Tift County, GA. The culture was maintained on cotton. The RN inoculum was prepared based on Hussey and Barker (1973) with modified sodium hypochlorite (NaOCl) concentration according to Walters and Barker (1993). Cotton roots were cut into 2- to 4-cm pieces and placed in a 250-ml glass flask. Roots were covered with a 0.25% solution of NaOCl (Walters and Barker, 1993) and were shaken at 150 rpm using a VWR standard analog shaker 3500 STD (VWR International, PA. USA) for 1.5 min. The solution and roots were poured into 200-mesh nested over 500-mesh sieves and washed with tap water for 30 sec to remove the bleach. Reniform nematode egg and vermiform stages retained on the 500-mesh sieve were collected in tap water for inoculum and quantified using a Zeiss (NY, USA) PrimoVert™ inverted microscope at 200X magnification. Nematodes were inoculated into pots on the same day RN inoculum was extracted.

The soil used was a Chipley-Foxworth-Albany complex (91% sand, 6.8% silt, and 2.4% clay with 1.7% organic matter), collected from the University of Florida North Florida Research and Education Center-Suwannee Valley, Live Oak, FL. It was autoclaved at 121°C for 90 min. in an Amsco Lab (Ohio, USA) 250 LV autoclave. Clay pots (15-cm diam. as measured at top of pot) were filled with autoclaved soil. Crops were planted on 2 October 2020 and 11 January 2021, respectively, for the first and second trials. Plants were initially seeded at double the recommended density for field production, but two weeks after planting, pots were thinned to the recommended density as adjusted to 15-cm-diam. pots (area = 176.7 cm^2). The recommended plant densities were: 61 to 108 carinata plants/m^2 (Seepaul *et al.*, 2019), 100 canola seeds/m^2 (Harker *et al.*, 2015), 240 to 360 oat plants/m^2 (Forsberg and Reeves, 1995), 50 hairy vetch plants/m^2 (Bamford and Entz, 2016), 2-3 cotton plants/30 cm of row in 91-cm-wide rows (Wright *et al.* 2022), and 3 peanut plants/30 cm of row (Wright *et al.*, 2021). Therefore, the final plant densities were six oat, two carinata, two canola, one peanut, one hairy vetch, and one cotton plant per clay pot. Plants had from two to three true leaves (Hartman and Sasser, 1985) when they were inoculated with RN at 29 and 31 days after planting (DAP), respectively, in Trials 1 and 2. The inoculum consisted of 3,000 RN (mixture of eggs, juveniles, and adults), suspended in 5 ml water, and distributed in three 2.5-cm deep holes around the radical system.

Plants were maintained, after planting, for 94 and 96 days, in Trials 1 and 2, respectively. The temperature was measured using a HOBO MX TidbiT 400® (Onset Computer Corporation. MA, USA). The average temperature for the first trial was 20.87°C (maximum=35.94°C, minimum=5.99°C) and for the second trial was 20.19°C (maximum=42.01°C, minimum=8.90°C). Plants were watered daily by hand, and no supplemental light or fertilizer was used.

At harvest, shoots were clipped from roots at the soil line. Roots were separated from soil and carefully washed with tap water. The fresh weight of both shoots and roots was recorded. Plant growth was compared by crop to show the inherent growth characteristics of each crop. This information could help to establish management strategies.

The soil from each pot was screened through a metal mesh with 0.25 cm^2 holes to homogenize the sample and 100 cm^3 of soil was used for nematode extraction by the sucrose centrifugation method (Jenkins, 1964). Nematodes were extracted from 10 g of roots using the same method that was previously described for the inoculum preparation.

If any of the crops did not have 10 g of roots at harvest, the nematodes were extracted from the entire root system. Next, the abundance of RN (eggs and vermiform life stages) from the root and soil extractions were counted separately using a Zeiss inverted microscope. From these counts, RN soil abundance, RN abundance per gram of fresh root, RN abundance per root system (calculated based on nematodes per gram of root and total root system weight), total RN per pot (including soil and root RN abundances), and RN reproduction factor (total RN per pot final population/inoculated initial population) were calculated.

Statistical analysis was performed in R Studio (RStudio Team, 2021). Trials were analyzed separately because of trial-by-crop interactions (ANOVA, $P \leq 0.05$). Parameters for each trial were analyzed by one-way ANOVA. Before analysis, homogeneity of variances and normality of the residuals were checked for each model using DD-plots and normal probability plots, respectively. Nematode and plant variables were ln(x+1) transformed if needed to meet model assumptions of homogeneity of variances and normality of residuals. For each analysis, when the main effect of a crop in ANOVA was significant ($P \leq 0.05$), a Tukey's test was performed to separate treatment means (α=0.05). Untransformed means are presented.

In both trials, RN reproduction factor (RF) was generally greater in known hosts (cotton and hairy vetch) compared to carinata or known poor hosts of RN (canola, peanut, and oat) (Fig. 1). In Trial 1, the RF of carinata and oat were significantly less than the RF of cotton and hairy vetch, while RF for canola and peanut was less than cotton only. In Trial 2, the crops defined as good hosts, cotton and hairy vetch, had significantly greater RF than carinata, canola, or peanut. In Trial 2, RF was lower for oat than cotton, but it was not significantly different from hairy vetch. The RF on carinata and oat was less than 1 in both trials, classifying them as poor hosts of RN. In contrast, cotton, and hairy vetch had values greater than 1, therefore they are defined as good hosts of RN (Fig. 1). The host status of canola and peanut changed depending on the trial, with RF values near 1 in Trial 1, but less than 1 in Trial 2 (Fig. 1).

The abundance of RN per gram of roots and per root system, in either trial, was significantly greater in cotton and hairy vetch than in carinata, oat, and peanut (Fig. 2). Reniform nematode root abundances were also typically less for canola than cotton or hairy vetch, except that the RN/root system for canola was not significantly different from cotton in Trial 1. Additionally, the abundance of RN per gram of roots was significantly higher in cotton than hairy vetch in either trial (Fig. 2A and B), but RN abundances per root system were not significantly different between cotton and hairy

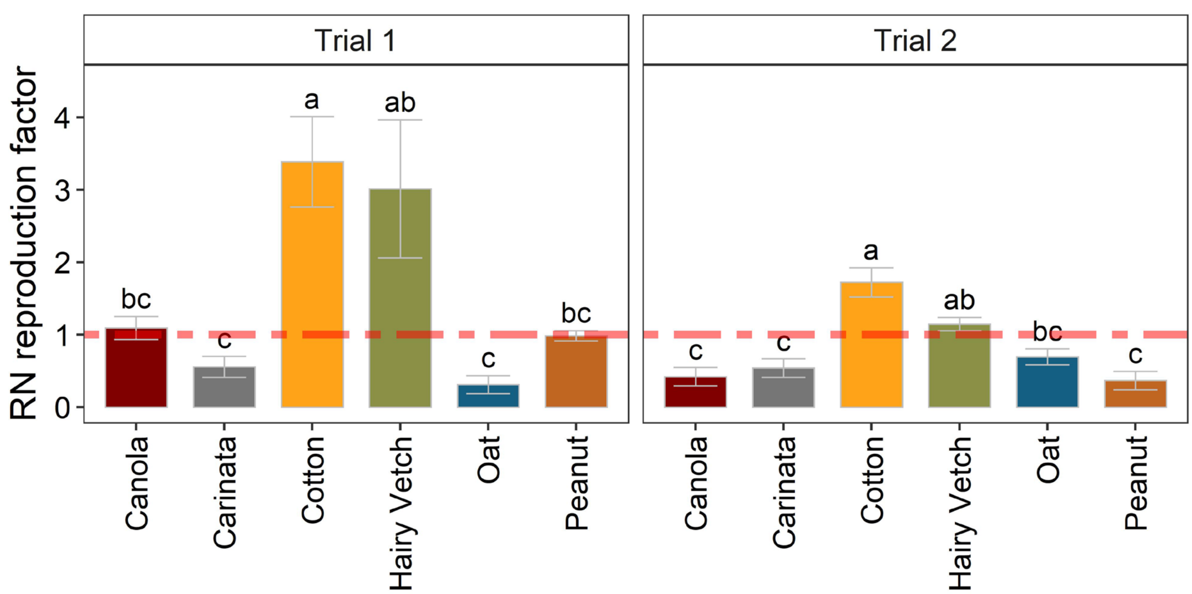

Figure 1. Reniform nematode (RN) reproduction factor (total RN per pot final population/inoculated initial population) by host plant. Letters imply significant differences among means, based on Tukey's HSD, P <0.05.

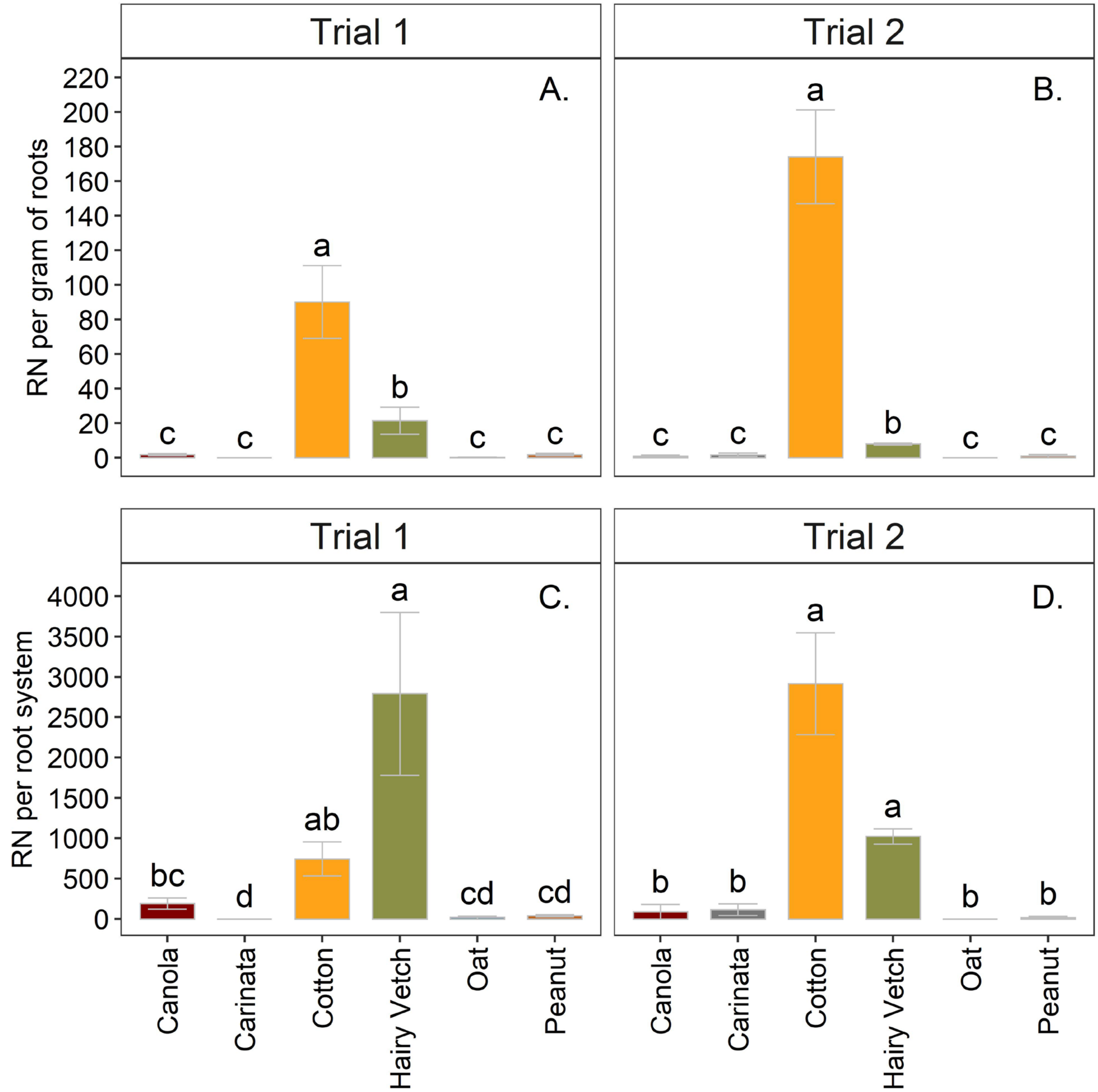

Figure 2. Reniform nematode (RN) abundance per gram of root, in Trial 1 (A) and Trial 2 (B) as well as RN per root system in Trial 1 (C) and Trial 2 (D) as affected by host plant. Letters imply significant differences among means, based Tukey's HSD, P <0.05.

vetch (Fig. 2C and D). One nematode was found in the oat root in Trial 1 while no nematodes were extracted from oat in Trial 2 or from the carinata roots in the Trial 1 (Fig. 2). The abundance of nematodes, in both trials, was also low in canola and peanut (Fig. 2).

In Trial 1, RN soil abundances were significantly higher in cotton and hairy vetch than in carinata and oat, but intermediate for canola and peanut (Fig. 3). In Trial 2, hairy vetch had significantly higher abundances of RN than canola, carinata, oat and peanut whereas cotton had intermediate abundances (Fig. 3). In each trial, fresh shoot weight (FSW) was significantly affected by crop. In Trial 1, FSW was greatest in canola, carinata, and oat, followed by hairy vetch and least in cotton and peanut (Table 1). In the second trial, carinata, canola and oat again had the greatest FSW, followed by hairy vetch, while cotton and peanut had the least FSW (Table 1). In

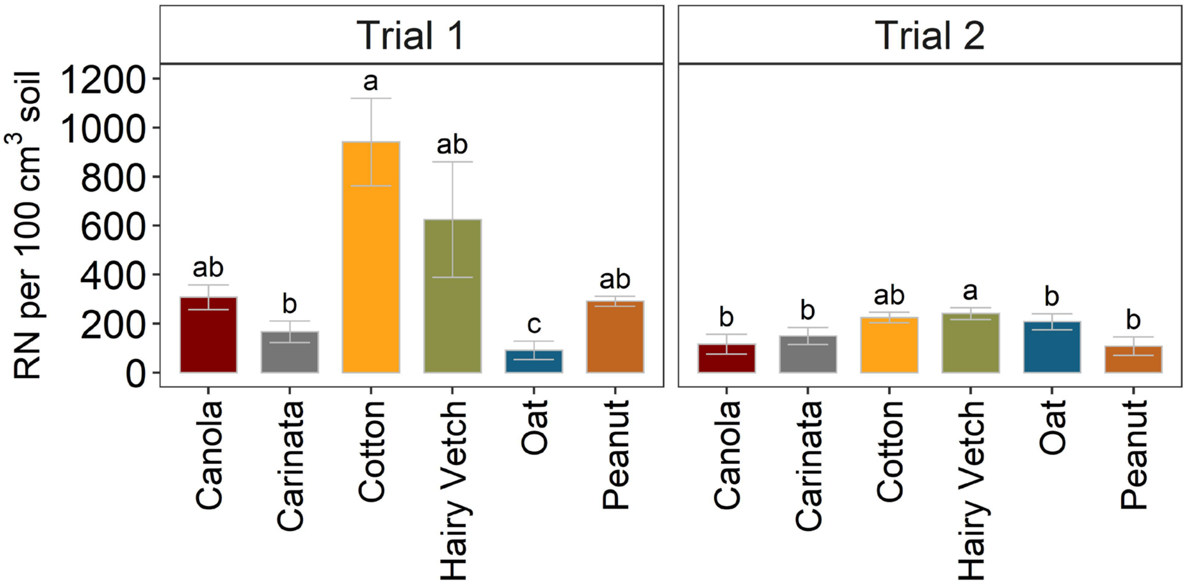

Figure 3. Reniform nematode (RN) soil abundances as affected by host plant. Letters imply different means, based on Tukey's HSD, P <0.05.

each trial, fresh root weight (FRW) was significantly affected by crop (Table 1). In both trials, FRW tended to be greatest for oats and hairy vetch, least for cotton and peanut, and intermediate for carinata and canola.

The results from this study suggest that carinata 'Avanza 641', is a poor RN host. Because RN is a major pest in the Southeast, this result is critical for guiding growers in establishing carinata in this region. Since carinata is a poor host of RN, this nematode is not expected to adversely affect carinata yield. This is an important consideration for the successful production of carinata in the Southeast. In addition, rotating a poor host winter crop can help reduce RN inoculum pressure before planting susceptible summer cash crops, such as cotton or susceptible soybean (Robinson, 2007). Therefore, carinata has potential to be used as a rotational crop during winter to help manage RN. This could increase the value proposition of producing carinata. While this greenhouse study is foundational, more field research would be needed to confirm that these conclusions can be broadly applied to field production.

In this study, only one carinata cultivar was assessed, which is similar to methods in previous initial host differential tests (McSorley, 1999; Acharya *et al.*, 2020). Reniform nematode cultivar dependency, however, can vary by crop and cultivar. For instance, in soybean, RN reproduction has been cultivar-related (Robbins *et al.*, 2002). In contrast, in corn, no variation in RN host status by cultivar was reported (Holguin *et al.*, 2015). Consequently, it is unclear if RN host status is likely to vary by carinata cultivar. From a practical standpoint, the primary cultivar available at the establishment of this research was used in this study. As an emerging crop, the diversity of carinata cultivars commercially available in the region is limited and under development. Therefore, more research will be needed to determine the carinata cultivar host status towards RN as the cultivar or cultivars that will be supplied for commercial production are finalized. Nevertheless, this is the first time, to our knowledge, that the host suitability of carinata for RN has been assessed. Previous experiments have evaluated the status of carinata as a root-knot and lesion nematodes (Castillo and Liébanas, 2004; Potter *et al.*, 1999), but not as a RN host. In addition to testing more cultivars, field assessments of carinata are needed. Field assessments would help confirm that carinata is a poor host of RN, as well as determine if RN does not adversely affect carinata yield.

Carinata is a potential winter cash crop alternative to the current Southeast winter cover crops evaluated in this study (oat and hairy vetch). In this study, carinata helped manage RN, similar to oat, but hairy vetch is detrimental for RN

Table 1. Plant fresh shoot weight and plant fresh root weight by crop in greenhouse trials.

	Plant weight (g) per pot [y]			
	Fresh shoot		Fresh roots	
Crop	Trial 1	Trial 2	Trial 1	Trial 2
P-value[z]	**	**	**	**
Canola	138±10 a	49±4 a	126±11 ab	89±7 b
Carinata	137±10 a	69±4 a	101±12 b	75±6 b
Cotton	23 ±5 c	17±2 c	6±1 d	16±1 d
Hairy Vetch	71±3 b	28±4 b	129±16 ab	147±23 a
Oats	119±7 a	50±2 a	190±20 a	115±8 ab
Peanut	29 ±3 c	25±4 c	35±6 c	39±4 c

[y]Mean ± standard error.
[z] Letters within the same plant parameter and column imply significant differences between crops by trial ($P \leq 0.05$) based on ANOVA with post hoc Tukey HSD (α=0.05).

management, as previously established in other studies, because it increases RN populations. Therefore, carinata enhances the diversity of winter crops that farmers in the area can use to manage RN. Further field research would help to confirm the commercial value of carinata to manage RN in the Southeast, relative to current winter crops.

Overall, the results from this study are consistent with previous reports in terms of the host status of the crops analyzed. In this study, RN reproduction was generally substantial for cotton and hairy vetch, which have been previously described as good hosts for RN (Jones *et al.*, 2006, Robinson, 2007). These two crops are detrimental for RN management. In this research, canola, peanut, and oat commonly sustained lower RN reproduction than hairy vetch or cotton, consistent with their formerly established status as poor hosts for RN (Robinson, 2007; Asmus *et al.*, 2008). However, based on the RF, canola, oat, and peanut were borderline hosts in one of the two trials in this study although they have been reported as poor hosts in prior studies (Jones *et al.*, 2006; Schumacher *et al.*, 2020). These slight differences in RN reproduction in this research relative to previous studies could be related to greenhouse conditions and temperature. Nematodes are susceptible to environmental changes, and, as has been reported, nematode reproduction is influenced by soil temperature in addition to host status (Timper *et al.*, 2006; Yan *et al.*, 2017). For instance, temperatures above 36°C, as observed in Trial 2 in this research, inhibited RN reproduction (Rebois, 1973), this may be one reason RN reproduction was numerically lower in Trial 2 than Trial 1.

Nematological host tests have been normally developed under greenhouse conditions (Taylor and Sasser, 1978; McSorley, 1999; Robbins *et al.*, 2002; Jones *et al.*, 2006), however, the controlled environment can favor RN population increase (Timper *et al.*, 2006) compared to field conditions (Molin and Stetina, 2016). Therefore, a crop that is usually defined as a poor- or non-host crop for RN in field experiments may be classified as a host in greenhouse experiments. For example, in a previous study, hairy vetch was defined as a RN host under greenhouse (RF=3.7), but in field experiments, the RN population did not increase (Jones *et al.*, 2006). Another possible explanation for the slight variation in RN reproduction in this study relative to previous studies is the variation in the aggressiveness of the population used compared to other studies. Previous research reported the presence of virulence phenotypes in RN populations supported by changes in reproduction and pathogenicity according to the RN population (Khanal *et al.*, 2018a). The susceptibility of the crop cultivars used in this research compared to cultivars previously tested could also affect RN reproduction slightly. For example, it is known that soybean cultivars can show different suitability toward RN (Kularathna *et al.*, 2019). Further studies of pathogenicity are suggested to define the virulence diversity of Florida RN populations.

To summarize, this research established carinata ‘Avanza 641’ as a poor RN host that could be useful as a rotational crop for RN management. Since this research was done in greenhouse conditions, further assessment is needed to verify RN management with carinata in the field. Additional research is suggested to determine the host status of additional carinata cultivars for RN, particularly as improved cultivars are released for commercial production.

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