HortScience 58(6):704–713. 2023. https://doi.org/10.21273/HORTSCI17141-23

Managing Southern Root-knot Nematode in Kentucky High Tunnels Using Grafted Tomato

Victoria Bajek and Rachel E. Rudolph
Department of Horticulture, University of Kentucky, 1100 S. Limestone, Lexington, KY 40506, USA

Keywords. host suitability, *Meloidogyne incognita*, plant-parasite, soil microorganism, *Solanum lycopersicum*, temperature

***Abstract.* Infection by root-knot nematode (*Meloidogyne* spp.; RKN) leads to root galling and reduces the host plant's ability to take up water and nutrients. Protected cropping systems, such as high tunnels, create conducive environments for RKN through increased soil temperatures and more intensive crop production. In Kentucky, high tunnel production has increased in the past 10 years, with tomato being the most cultivated high tunnel crop. This has contributed to a lack of rotation and increased pressure from RKN. Tomato grafting with RKN-resistant rootstock is a nonchemical management strategy that has shown promise in other regions of the United States. The primary objective of this 2-year, two-site study (Knox and Boyle Counties) was to determine whether using grafted resistant rootstock could be a viable management strategy in high tunnels naturally infested with *Meloidogyne incognita*. The rootstocks included 'Arnold', 'Maxifort', 'Shin Cheong Gang', and 'Estamino'. 'Primo Red' and 'Cherokee Purple' were the scions and nongrafted controls in Knox and Boyle Counties, respectively. In 2020 and 2021 in Knox County, three of the four grafted treatments produced at least 38% higher yield than the nongrafted control. Grafted treatments had at least 44% fewer RKN eggs/g of dry root compared with the nongrafted control in both years. In 2021 and 2022 in Boyle County, tomato yield was at least five times greater in all four of the grafted treatments compared with the nongrafted control. In 2021, the nongrafted control had three times more RKN eggs/g dried root compared with three of the four grafted treatments. In 2022 in Boyle County, the nongrafted control had four times more RKN eggs/g of dried root than all grafted treatments. In both years and locations, 'Arnold' and 'Estamino' treatments had higher yield and lower RKN population densities in soil and roots compared with the nongrafted controls. Utilization of resistant rootstock will help Kentucky growers maintain crop productivity in soils infested with RKN, but should be combined with other management methods for long-term resiliency of the high tunnel system.**

High tunnels are covered structures that provide a protected environment that extends the growing season and allows growers to capture premium prices through improved yield and quality (Carey et al. 2009; Lamont 2009). High tunnels rely on passive heating and cooling, which means temperatures inside the tunnel can increase rapidly on sunny days and cooling the high tunnel is largely done through venting or opening the sidewalls and end walls (Black and Drost 2010). Studies have shown that increased soil temperatures and overall daytime heat in high tunnels increase yield and optimize early season production for specialty crops such as tomatoes (*Solanum lycopersicum* L.) compared with open field production (Frey et al. 2020; Gude et al. 2022; O'Connell et al. 2012). Tomato is one of the most valuable high tunnel crops per square foot (Galinato and Miles 2013), and its production continues to increase along with the interest in high tunnel production (Janke et al. 2017). This can be partially attributed to financial assistance provided by cost share grants such as the Natural Resource Conservation Service (NRCS) Environmental Quality Incentives Program (Belasco et al. 2011; Ernst et al. 2020; Janke et al. 2017; US Department of Agriculture-Natural Resources Conservation Service 2023). The NRCS incentives program has led to more than 7000 high tunnels being constructed in the southern region, with Kentucky being the most active adopter. Kentucky has more than 1500 high tunnels constructed through the NRCS program (Wheby D, NRCS, personal communication), which is equal to more than 260,128 m^2 of production capacity (Ernst et al. 2020). Although high tunnels can be a high-value infrastructure, providing many production benefits, they can also create a multitude of challenges for growers through increased soil temperatures (Kumari et al. 2014; O'Connell et al. 2012; Zhao and Carey 2009), soil fertility degradation from intensive production (Reeve and Drost 2012), increased soil salinity (Rudisill et al. 2015), and lack of crop rotation and sanitation (Bruce et al. 2019).

One of those challenges includes RKN (*Meloidogyne* spp.). RKNs are one of the most destructive plant-parasitic nematodes worldwide with a host range of more than 3000 plant species (Abad et al. 2003). These sedentary, endoparasites pose a significant threat to crop production (Onkendi et al. 2014). Approximately 5% of crop production worldwide is lost to *Meloidogyne* spp. every year (Karajeh 2008). The infective stage of RKN, second-stage juvenile (J2), detects and penetrates suitable host roots with its piercing mouthpart, called a stylet (Ralmi et al. 2016; Williamson 1998). Although adult males migrate out of the root, females become sedentary and produce large egg masses multiple times throughout a single season (Abad et al. 2003; Mitkowski and Abawi 2003). The establishment of the plant-host relationship creates nutrient sinks from vascular cells causing root galling and inhibits water and nutrient uptake. This leads to wilting, chlorosis, and crop yield loss (Ireri et al. 2018; Mitkowski and Abawi 2003; Onkendi et al. 2014).

Many vegetable crops, including tomato, are susceptible hosts for RKN (Ahmad et al. 2021; Ralmi et al. 2016), and RKNs are difficult to manage because of their wide host range (Gill and McSorley 2011). Growers may be unaware of the problem until the plant is pulled out of the soil at the end of the season and the galled roots are visible. Even then, many growers may not realize what they are seeing. It is unknown how much of this yield loss occurs specifically in high tunnels or in tomato production. Although one of the benefits of high tunnel production is increased soil temperatures for season extension and earlier crop production (Frey et al. 2020; Lamont 2009; Zhao and Carey 2009), this factor may also provide a conducive environment for increased RKN reproduction. In addition, through intensive and repeated crop production there is often little opportunity for nonhost crop rotation and sanitation (Bruce et al. 2019).

The three main species of RKN, *Meloidogyne javanica*, *Meloidogyne incognita*, and *Meloidogyne arenaria*, prefer warmer climates between 35°S and 35°N latitudes (Taylor and Sasser 1978). Of these species, *M. incognita*, or southern RKN, is one of the most damaging crop pathogens in the world due to its

---

Received for publication 3 Mar 2023. Accepted for publication 7 Apr 2023.

Published online 22 May 2023.

This research was funded by a Kentucky Department of Agriculture Specialty Crop Block Grant and supported by the US Department of Agriculture National Institute of Food and Agriculture, Hatch project 1021069.

We thank Maya Horvath, Henry Smith, Nicole Gauthier, Erin Haramoto, Martín Polo, Wayne Kirby, and Alexis Sheffield for their assistance with this project. We also thank the grower cooperators for donating the land, labor, and time.

The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement.

R.R. is the corresponding author. E-mail: rachel.rudolph@uky.edu.

This is an open access article distributed under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/).

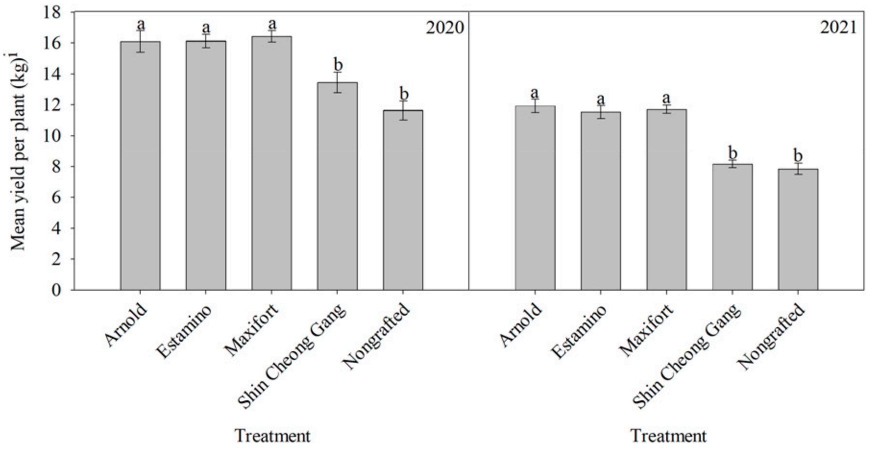

Fig. 1. Marketable tomato fruit yield harvested from grafted and nongrafted 'Primo Red' tomato plants grown in a commercial high tunnel naturally infested with *Meloidogyne incognita* in Knox County, Kentucky, in 2020 and 2021. [i] Values are the means of eight replicates ± standard error. Any two means not followed by the same letter are significantly different at $\alpha \leq 0.05$.

wide host range and presence in many areas (Trudgill and Blok 2001). Soil temperatures, specifically, affect RKN infestation and development (Ploeg and Maris 1999; Roberts et al. 1981; Zacheo et al. 1995). Zacheo et al. (1995) observed that *M. incognita* J2 infection of the tomato seedlings was greatest between 30 °C and 32 °C. At 30.0 °C, the quickest *M. incognita* generation time was observed of 40 d, and the longest was 80 d at 16.2 °C (Ploeg and Maris 1999). The specific activity threshold for *M. incognita* is ∼18 °C indicating that soil migration and root penetration are greatest in environments with soil temperatures above 18 °C (Roberts et al. 1981). Because soil temperatures in high tunnels are higher compared with the open field (Frey et al. 2020; Kumari et al. 2014; Zhao and Carey 2009), this has the potential to create optimal conditions for RKN to reproduce and infect roots and could lead to increased RKN activity earlier in the spring and later in the fall compared with open field production and in more northern regions.

When RKN is identified as an issue, a common method of management is the use of chemicals, called nematicides, applied to the soil. The worldwide nematicide market is ∼\$1 billion, with nearly half of the market used to manage *Meloidogyne* spp. (Haydock et al. 2013). Nematicide costs can amount to ∼\$350 to \$400$\cdot$ha^{-1} (Kratochvil et al. 2004), which would likely be too expensive for small-scale growers. Another complicating factor is that in Kentucky, high tunnels are considered greenhouses (Bessin et al. 2021), which affects which pesticides are permitted for application. There are commercially available nematicides that can be used in a high tunnel, as well as products available for certified organic production (Bessin et al. 2021), but those products are limited. Fumigants, which are commonly used in large-scale commercial farms (Talavera-Rubia et al. 2022), are not permitted for use in Kentucky high tunnels. Soil fumigants are effective at killing nematodes through multiple modes of action, but they require high application rates, can only be applied before planting, and require specialized equipment (Oka 2020). Specialized equipment often means contracting a commercial applicator (Heath 2018). This increases the cost and would likely not be feasible for smaller-scale Kentucky growers, high tunnel or open field. Cost and effectiveness aside, the highly toxic chemical compounds contained in nematicides can also have harmful effects on humans and beneficial insects (Costa et al. 2008; Zasada et al. 2010). Environmental contamination and increased risks on human health have led to the restricted use of several nematicides (Zasada et al. 2010). Ultimately, growers cannot rely solely on chemical products for RKN management.

An alternative management strategy, which can reduce the need for chemical applications, is the use of RKN-resistant crops (Onkendi et al. 2014). Plant resistance to RKN is defined as the ability to restrict growth and development, but there may still be evidence of RKN feeding and reproduction. The resistance gene in tomato is the *Mi* gene, a single dominant gene that has displayed resistance to *M. arenaria*, *M. javanica*, and *M. incognita* (Jacquet et al. 2005). Bailey (1941) was the first to determine that wild tomato (*Solanum peruvianum*) displayed resistance to RKN. Later, Gilbert and McGuire (1956) further determined that resistance resulted from a single dominant gene called *Mi*. Today, the gene is used in breeding programs for commercially available tomato cultivars to inhibit RKN infection at an early stage (Jacquet et al. 2005). Resistant cultivars are often used as rootstocks, the root system, rather than bred for fruit production. These resistant rootstocks can be grafted to susceptible tomato cultivars that produce desirable fruit by using a silicone tube to secure the union of the scion, the upper fruiting body, to the rootstock (Kubota et al. 2008). The main objectives of using grafted rootstocks are to increase resistance to soilborne diseases and RKN, increase fruit yield and quality, and better adapt crops to harsh environments (Kubota et al. 2008). Tomato grafting can result in increased marketable yield, fruit weight, and crop vigor, as assessed by improved stem diameter, leaf area, and above- and below-ground biomass, compared with nongrafted plants (Frey et al. 2020).

Table 1. Tomato plant dry biomass production in a commercial high tunnel naturally infested with *Meloidogyne incognita* in Knox County, Kentucky, in 2020 and 2021.

	Plant biomass (kg)	
Rootstock[i]	2020	2021
Arnold	0.78 ± 0.02 ab[ii]	0.55 ± 0.03 ab
Estamino	0.83 ± 0.02 a	0.58 ± 0.03 a
Maxifort	0.83 ± 0.03 a	0.56 ± 0.04 a
Shin Cheong Gang	0.66 ± 0.04 bc	0.42 ± 0.04 bc
Nongrafted	0.59 ± 0.04 c	0.38 ± 0.04 c

[i] Nongrafted is 'Primo Red' tomato and all rootstocks were grafted onto a 'Primo Red' scion.

[ii] Values are the means of eight replicates ± standard error. Any two means within a column not followed by the same letter are significantly different at $\alpha \leq 0.05$. Three plant samples were collected from each replication and combined.

Table 2. Monthly root-knot nematode (RKN; *Meloidogyne incognita*) population densities in soil surrounding grafted and nongrafted tomato plant roots grown in a naturally infested commercial high tunnel in Knox County, Kentucky, in 2020.

	RKN/100 g dry soil					
Rootstock[i]	March	April	May	June	July	August
Arnold	33 ± 19 a[ii]	33 ± 12 a	14 ± 5 a	9 ± 5 b	8 ± 3 bc	7 ± 3 c
Estamino	76 ± 31 a	26 ± 12 a	14 ± 6 a	24 ± 16 b	4 ± 1 c	7 ± 3 c
Maxifort	40 ± 14 a	36 ± 16 a	22 ± 10 a	71 ± 29 b	67 ± 36 b	340 ± 172 b
Shin Cheong Gang	114 ± 41 a	46 ± 24 a	44 ± 21 a	7 ± 2 b	2 ± 1 c	4 ± 2 c
Nongrafted	41 ± 13 a	12 ± 3 a	75 ± 28 a	356 ± 107 a	506 ± 128 a	1,253 ± 281 a

[i] Nongrafted is 'Primo Red' tomato and all rootstocks were grafted onto a 'Primo Red' scion.

[ii] Values are the means of eight replicates ± standard error. Any two means within a column not followed by the same letter are significantly different at $\alpha \leq 0.05$.

Because management options for RKN in high tunnels are limited because of chemical restrictions, rotation schedules, and crop host suitability, using grafted resistant plants could be a viable management strategy; however, the *Mi* gene can be shut off at soil temperatures above 28 °C (Williamson 1998).

Although resistance through the *Mi* gene can be lost at high temperatures, research has shown success with using grafted RKN-resistant tomato rootstock in high tunnels. Soil population densities of RKN J2 can be significantly reduced using grafted resistant rootstock (Frey et al. 2020; Rivard et al. 2010). Fruit yield and quality can also be significantly higher in grafted treatments in comparison with self- and nongrafted (Rivard et al. 2010). 'Maxifort' rootstock, specifically, is regarded as an industry standard rootstock because of its positive effect on fruit yield (Lang et al. 2020). Yet, 'Maxifort' was the most affected rootstock in a study observing transplants inoculated with *M. incognita* of 'Matissimo' tomato (self-grafted or grafted on rootstock 'Arnold' and 'Maxifort'). 'Maxifort' was observed to have four times the amount of galling in comparison with 'Arnold' (Cukrov et al. 2021). However, even when soil temperatures exceed 28 °C, grafted treatments have shown to have significantly higher tomato fruit yield in comparison with nongrafted controls (Lopez-Perez et al. 2006). The use of rootstocks can result in competitive yield and plant vigor, which is especially important if growers are dealing with RKN infestations; however, there are limited rootstocks commercially available, which suggests that grafting still has potential to grow, especially for RKN management (Barrett et al. 2012; Martínez-Andújar et al. 2020).

Our study evaluated grafted tomato rootstocks containing the *Mi* gene as a viable strategy for *M. incognita* management in naturally infested high tunnels in Kentucky. There were three primary objectives: 1) to determine the efficacy of these rootstocks at managing RKN egg and soil population densities in naturally infested high tunnels; 2) to determine the yield benefits of these same tomato rootstock cultivars in comparison with nongrafted, nonresistant cultivars; and 3) to ascertain the compatibility of resistant rootstocks with the Kentucky high tunnel environment.

Materials and Methods

Sites. A 2-year study was conducted in two high tunnels in Kentucky. The first location was on a commercial farm in Knox County, Kentucky (lat. 36°52′8.5368″N, long. 83°53′32.9244″W, 366 m elevation). The experiment was established in a 9 m × 29 m high tunnel with Latham silt loam/Shelocta channery silt loam soil that was previously planted with 'Primo Red' tomato. The second location was on a commercial farm in Boyle County, Kentucky (lat. 37.680906°N, long. 84.973611°W, 305 m elevation). The high tunnel (9 m × 29 m) with Lowell silty clay loam/shale substratum was previously cropped with 'Cherokee Purple' tomato.

Experimental design. Experimental treatments were arranged as a randomized complete block design with eight replications in Knox County and seven in Boyle County. Treatments included four rootstocks: Arnold (Seedway, Hall, NY), Estamino (Johnny's Selected Seeds, Winslow, ME), Shin Cheong Gang (Johnny's Selected Seeds), and Maxifort (Seedway) with a nongrafted cultivar as the control. 'Arnold', 'Estamino', and 'Maxifort' are reported to have moderate resistance to *M. incognita* (Cukrov et al. 2021; Rivard et al. 2010; Testen et al. 2021) Similar to the other rootstocks, 'Shin Cheong Gang' is also an F1 hybrid and has been reported to have resistance to *Meloidogyne* spp. (Cornell University 2023). 'Primo Red' (Seedway) has no resistance to RKN (Bost 2013; Cornell University 2023) and was the scion grafted onto each rootstock and the nongrafted control in Knox County 'Cherokee Purple' (Johnny's Selected Seeds) was the scion and nongrafted control in Boyle County and has been

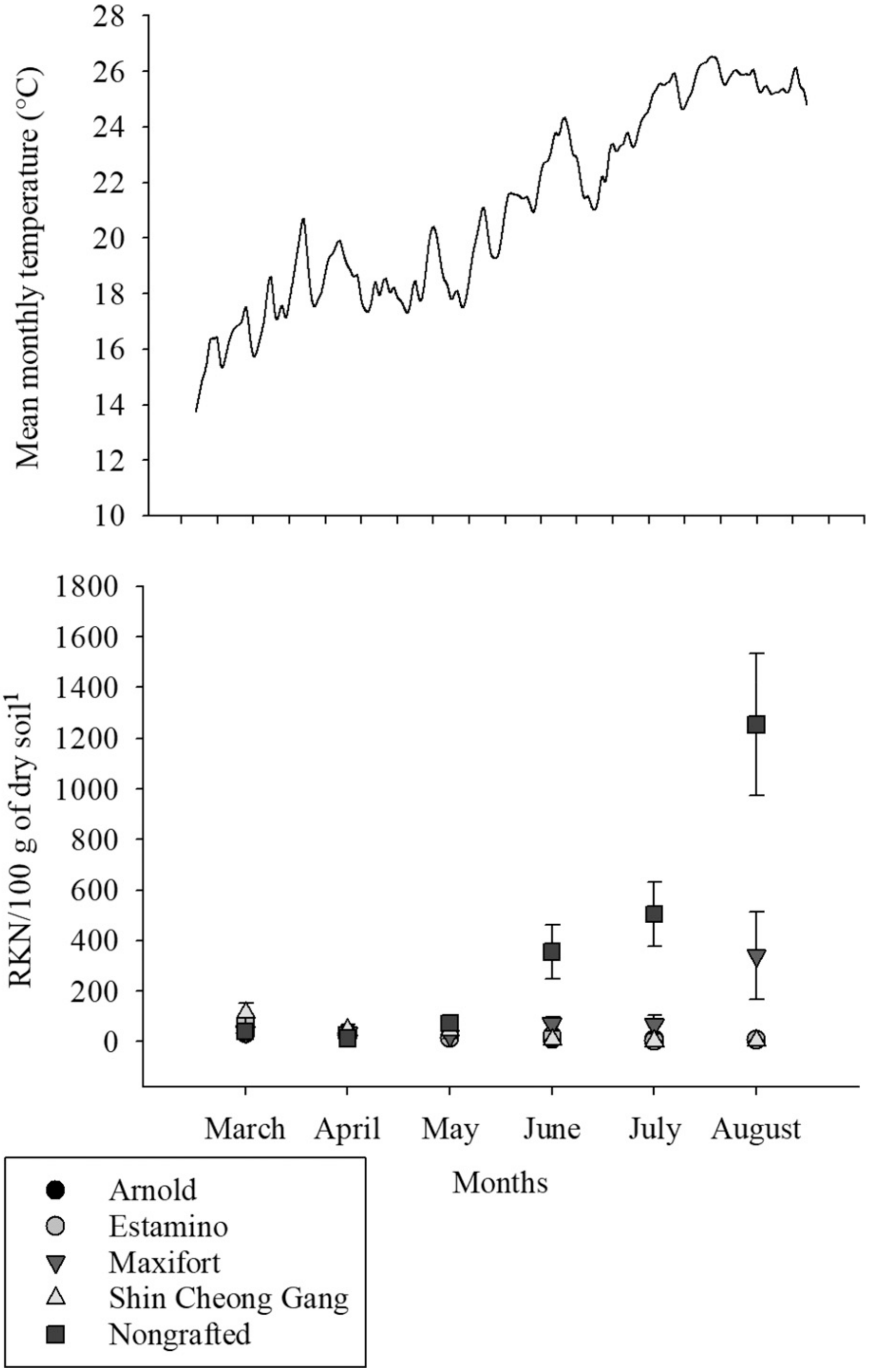

Fig. 2. Root-knot nematode densities (RKN; *Meloidogyne incognita*) population densities in soil surrounding grafted and nongrafted 'Primo Red' tomato plant roots grown in a naturally infested commercial high tunnel in Knox County, Kentucky, and average soil temperatures in that same high tunnel at 15 cm soil depth in 2020. ¹ Mean value of J2 population densities collected monthly from soil ± standard error. The results are the means of eight replicates of each rootstock.

Table 3. Root-knot nematode (RKN; *Meloidogyne incognita*) egg populations in tomato roots grown in a naturally infested commercial high tunnel in Knox County, Kentucky, in 2020 and 2021.

	RKN eggs/g dry root	
Rootstock[i]	2020	2021
Arnold	1,028 ± 255 c[ii]	2,098 ± 1,204 b
Estamino	1,688 ± 820 bc	1,937 ± 728 b
Maxifort	11,009 ± 4,264 ab	8,239 ± 5,291 b
Shin Cheong Gang	731 ± 165 c	1,647 ± 424 b
Nongrafted	19,622 ± 5,033 a	34,078 ± 6,004 a

[i] Nongrafted is ‘Primo Red’ tomato and all rootstocks were grafted onto a ‘Primo Red’ scion.

[ii] Values are the means of eight replicates ± standard error. Any two means within a column not followed by the same letter are significantly different at $\alpha \leq 0.05$. Three root samples were collected from each replication and combined.

reported to be susceptible to RKN (Rivard et al. 2010). In Knox County, each treatment replicate consisted of one row, which included nine tomato plants planted 0.3 m apart. Rows were laid out perpendicular to the length of the high tunnel with a center walkway and were on 0.9-m centers. In Boyle County, seven rows on 1.1-m centers were parallel to the length of the high tunnel. One treatment replicate consisted of seven plants within one row. Treatment plots were re-randomized at both locations in the second year.

Site management. Conventional practices for management and monitoring were followed by the growers, including plant fertility as recommended by UK Vegetable Extension (Bessin et al. 2021). The growers set up drip irrigation to water plants as needed. Woven plastic weed mat covered the entire soil surface inside the high tunnel at both locations. In 2020, the experiment in Knox County began on 27 Feb when plants were transplanted into the high tunnel and ended on 17 Aug with destructive sampling. In 2021, the Knox County experiment began on 29 Feb and was ended on 29 Jul. All plants for the Knox County experiments were grown and grafted at the University of Kentucky Horticulture Research Farm (UKHRF, Lexington, KY). Three-week-old plants were grafted using the splice method and maintained in a dark healing chamber set to 26 °C for 5 d and then gradually exposed to ambient light and temperature between 22 and 24 °C. Nongrafted ‘Primo Red’ was seeded in 50-cell trays (Landmark) with potting soil (Fort Lite, VT Compost County, Montpelier, VT) and grown with ambient light and temperatures between 22 and 24 °C. They were transplanted after 4 weeks.

The Boyle County experiment began 16 Apr 2021 and was ended 2 Aug. Nongrafted plants were sampled on 28 Jul because of their rapid decline. In 2022, the Boyle County experiment began 11 Feb and ended 15 Aug. All grafted plants for the Boyle County experiments were grafted by Trihishtil (Mills River, NC) using the splice method. Nongrafted plants were grown at the UKHRF. Nongrafted ‘Cherokee Purple’ was seeded and maintained as described previously. All plants were transplanted after 4 weeks.

Data collection. Before establishing the experiment, $\sim$15 soil cores with 2.2-cm diameter were collected at 20 cm soil depth and combined. One subsample was submitted to the Nematode Assay Section of the North Carolina Department of Agriculture and Consumer and Agronomic Services (NCDA) (Raleigh, NC) to confirm the RKN species as *M. incognita.* Another subsample was used to establish the initial *M. incognita* population density of 35 and 107 RKN/100 g of dried soil at Knox and Boyle County, respectively. The third subsample was submitted to the University of Kentucky Regulatory Services to analyze soil chemistry. In Knox County, the results of the soil analysis in year 1 were as follows: 1448 kg·ha^{-1} of P, 1240 kg·ha^{-1} of K, 2975 kg·ha^{-1} of Ca, 314 kg·ha^{-1} of Mg, and 2 kg·ha^{-1} of Zn. The pH was 5.3 and lime was applied to increase the soil pH to 6.5. In year 2 in Knox County, the soil sample of the initial RKN population density was 50 RKN/100 g of dried soil. The soil chemistry was 1737 kg·ha^{-1} of P, 1265 kg·ha^{-1} of K, 4931 kg·ha^{-1} of Ca, 532 kg·ha^{-1} of Mg, and 1 kg·ha^{-1} of Zn. The pH was reported to be 6.8. In Boyle County, the soil chemistry results were as follows: 449 kg·ha^{-1} of P, 723 kg·ha^{-1} of K, 3462 kg·ha^{-1} of Ca, 316 kg·ha^{-1} of Mg, 4 kg·ha^{-1} of Zn, and soil pH of 6.2. The year 2 soil analysis results were as follows: 449 kg·ha^{-1} of P, 531 kg·ha^{-1} of K, 4294 kg·ha^{-1} of Ca, 288 kg·ha^{-1} of Mg, and 6 kg·ha^{-1} of Zn. The pH was 5.9 and lime was applied to increase the soil pH to 6.3. The RKN population density for year 2 was 49 RKN/100 g of dried soil.

Directly after transplanting, three data loggers (HOBO U23 Pro v2 Temperature Data Logger, Onset Computer Corporation, Bourne, MA) were programmed to collect hourly soil temperature and were buried randomly at $\sim$15 cm soil depths within the high tunnel. Tomato yield for each treatment replicate was collected and recorded by the growers at each location, approximately two harvests per week. Fruit quality was assessed by the growers according to their market standards and both growers sell at farmers markets.

Soil samples were collected monthly from each treatment replication for analysis of RKN population densities in both sites. We sampled from the rhizosphere of the tomato plants, $\sim$5 cm away from the stem. We collected $\sim$500 g of soil from each treatment replicate from 0 to 20 cm soil depth (2.2 cm diameter) each month. *M. incognita* J2 were extracted from 100 g of soil for 5 d using a modified Baermann funnel method (Hooper 1986). Another subsample of the same soil was dried in an oven at 60 °C and weighed after 5 d to establish the dry weight. *M. incognita* were identified and counted on an inverted microscope (Leica DMI 1, Wetzlar, Germany) at ×10 magnification. Population densities of *M. incognita* are expressed as the number of RKN/100 g of dried soil.

Aboveground symptoms such as stunting, wilting, chlorosis, and necrosis were observed in tomato plants in both years and sites. Destructive sampling occurred at the end of each season and consisted of collecting three plants from each replicate. Roots from each plant were carefully uprooted from the soil, cut from the plant, and washed. The aboveground plant biomass of the three tomato plants was placed into paper bags and oven dried at 60 °C for 5 d and weighed. From each replicate, $\sim$500 g of soil was collected for RKN analysis.

Meloidogyne incognita eggs were extracted from the roots and counted using the methods described by Hussey and Barker (1973). A sugar centrifugation method (Jenkins 1964) was used for heavily sedimented samples. After extraction, the roots were oven dried

Table 4. Monthly root-knot nematode (RKN; *Meloidogyne incognita*) population densities in soil surrounding grafted and nongrafted ‘Primo Red’ tomato plant roots grown in a naturally infested commercial high tunnel in Knox County, Kentucky, in 2021.

	RKN/100 g dry soil				
Rootstock[i]	March	April	May	June	July
Arnold	52 ± 26 a[ii]	34 ± 17 a	48 ± 32 a	58 ± 47 b	45 ± 38 b
Estamino	7 ± 3 a	8 ± 5 a	1 ± 1 a	1 ± 1 b	49 ± 34 b
Maxifort	112 ± 76 a	17 ± 6 a	38 ± 21 a	92 ± 51 ab	103 ± 48 b
Shin Cheong Gang	61 ± 53 a	31 ± 24 a	28 ± 22 a	13 ± 8 b	6 ± 3 b
Nongrafted	80 ± 40 a	54 ± 32 a	152 ± 82 a	429 ± 248 a	503 ± 180 a

[i] Nongrafted is ‘Primo Red’ tomato and all rootstocks were grafted onto a ‘Primo Red’ scion.

[ii] Values are the means of eight replicates ± standard error. Any two means within a column not followed by the same letter are significantly different at $\alpha \leq 0.05$.

for 5 d at 60 °C and weighed. Eggs were identified as described previously and *M. incognita* population densities were expressed as the number of RKN eggs/g of dried root.

Statistical analysis. The RKN soil (J2) and root (eggs) population densities, marketable tomato yield, aboveground tomato plant biomass, and tomato root biomass were subjected to analysis of variance with Tukey as the post hoc test using Statistical Analysis System (SAS) Version 9.3 statistical software (SAS Institute Inc., Cary, NC). Alpha was set at 0.05 for all data. Data from the different sites and years were treated similarly and subject to the same analyses but were analyzed independently due to different scions between the two sites as well as varying grower management strategies from year to year and site to site. Transformations of RKN data were performed using log (x + 10) when necessary if distributions were not normal. Data were then reanalyzed. Although data may have been transformed, the results are presented using the original, nontransformed means.

Results

Knox County, year 1. In 2020, the marketable fruit yield in Knox County was significantly greater among grafted treatments 'Arnold', 'Estamino', and 'Maxifort' compared with yields of the nongrafted control and 'Shin Cheong Gang' ($P < 0.0001$; Fig. 1). There was no significant difference among the yields of 'Arnold', 'Estamino', and 'Maxifort'. These three grafted treatments produced ~16 kg per plant, which was over 19% more than the yield of 'Shin Cheong Gang' (13.4 kg per plant) and nearly 38% more than the nongrafted control (11.6 kg per plant). Differences in aboveground plant biomass production among treatments were significant ($P = 0.0008$; Table 1). 'Arnold', 'Estamino', and 'Maxifort' treatments produced more biomass compared with the nongrafted control, but there was no significant difference between the biomass produced by 'Shin Cheong Gang' and the nongrafted control.

At the first soil sampling in March, *M. incognita* J2 population densities were low and not significantly different among treatments ($P = 0.35$; Table 2). Beginning in June, J2 soil population densities were significantly greater in the nongrafted control compared with all other treatments. This continued through to the project termination in August. *M. incognita* population densities in the soil surrounding the nongrafted control were 30 times greater at the end of the season compared with the first sampling date (Fig. 2). Average soil temperatures increased from 14 °C in March to 26 °C in August. Soil J2 population densities of 'Arnold' and 'Maxifort' were not significantly different in July; however, in August, RKN population densities surrounding 'Maxifort' plant roots were significantly higher than all other grafted rootstock treatments ($P < 0.0001$).

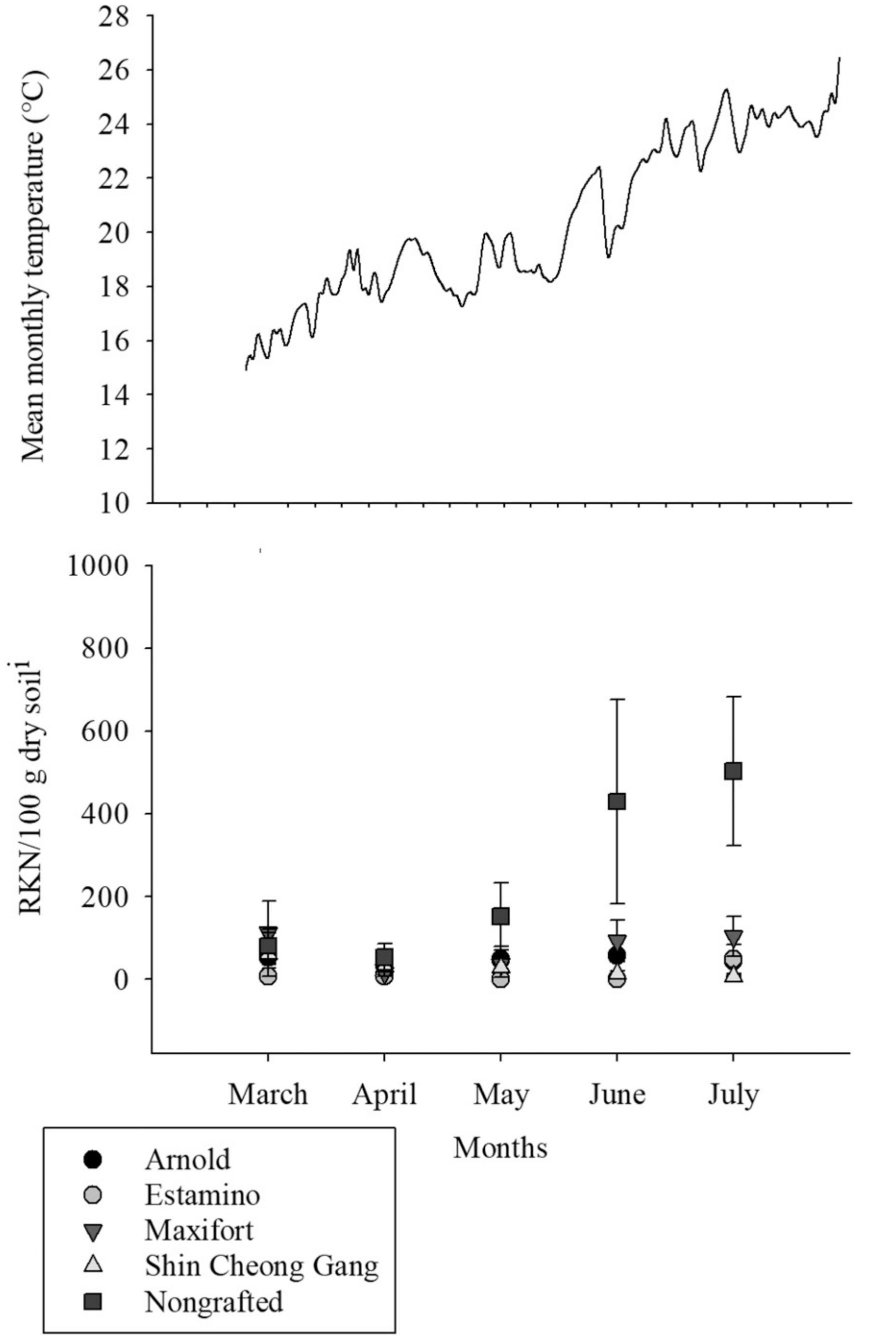

Fig. 3. Root-knot nematode (RKN; *Meloidogyne incognita*) population densities in soil surrounding grafted and nongrafted 'Primo Red' tomato plant roots grown in a naturally infested commercial high tunnel in Knox County, Kentucky and average soil temperatures in that same high tunnel at 15 cm soil depth in 2021. ⁱ Mean value of second-stage juvenile RKN population densities collected monthly from soil ± standard error. The results are the means of eight replicates of each rootstock.

Meloidogyne incognita egg densities per gram of dried root were significantly greater in the nongrafted control compared to 'Arnold', 'Estamino', and 'Shin Cheong Gang' ($P = 0.003$; Table 3). The mean egg density in the nongrafted control was 44% greater than in 'Maxifort' roots, which had the next highest egg population density per gram of root, and 27 times greater than 'Shin Cheong Gang', which had the lowest mean egg population density.

Knox County, year 2. In 2021, 'Arnold', 'Maxifort', and 'Estamino' treatments had significantly higher marketable yield compared with the nongrafted and 'Shin Cheong Gang' rootstocks ($P < 0.0001$; Fig. 1). There were no significant differences among the yield produced by 'Arnold', 'Maxifort', and 'Estamino'. Nongrafted and 'Shin Cheong Gang' also produced similar yields. 'Arnold', 'Estamino', and Maxifort treatments produced an average of ~12 kg per plant, which was ~71% higher than 'Shin Cheong Gang' and the nongrafted control that produced an average of 7 kg per plant (Fig. 1). Aboveground plant biomass produced by the nongrafted control was

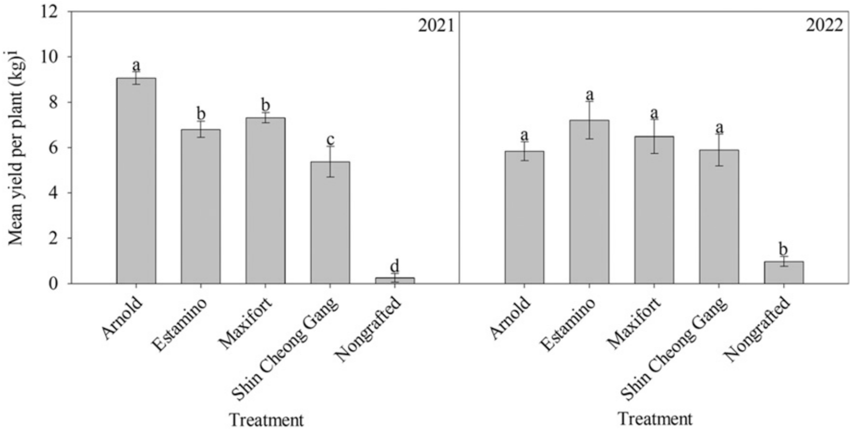

Fig. 4. Marketable tomato fruit yield harvested from grafted and nongrafted 'Cherokee Purple' tomato plants grown in a commercial high tunnel naturally infested with *Meloidogyne incognita* in Boyle County, Kentucky, in 2021 and 2022. [i] Values are the means of seven replicates ± standard error. Any two means not followed by the same letter are significantly different at $\alpha \leq 0.05$.

significantly lower compared with all grafted treatments ($P = 0.0014$; Table 1). There was no significant difference among 'Arnold', 'Estamino', and 'Maxifort' treatments in terms of biomass produced.

The average *M. incognita* J2 soil population densities surrounding the nongrafted plant roots steadily increased from May through July, but were not significantly different among treatments until June (Table 4). In July, the nongrafted J2 soil population densities were significantly greater than all other treatments ($P = 0.0004$; Table 4). The J2 population densities of the nongrafted control were five times higher compared with 'Maxifort', which had the second highest average J2 soil population densities. Average soil temperatures increased from 15 °C in March to 26 °C in July (Fig. 3).

Meloidogyne incognita egg densities per gram of root were significantly greater in the nongrafted control compared with all grafted treatments ($P = 0.002$; Table 3). The mean egg population density was more than four times greater than the population density of the 'Maxifort' treatment, which had the next greatest egg density per gram of root.

Boyle County, year 1. In 2021, all grafted treatments had significantly higher marketable yield compared with the nongrafted control. 'Arnold', which had the highest yield, produced an average of 9 kg per plant, which was 25% higher than 'Maxifort', which had the second highest yield ($P < 0.0001$; Fig. 4). 'Maxifort' and 'Estamino' produced ~7 kg per plant. 'Arnold' was 36 times greater in yield in comparison with the nongrafted rootstock, whereas 'Shin Cheong Gang', the treatment with the second lowest yield, produced a yield that was 21 times greater compared with the nongrafted control. 'Arnold', 'Estamino', and 'Maxifort' treatments produced significantly more aboveground plant biomass compared with the nongrafted control ($P = 0.0004$; Table 5). There was no significant difference among 'Arnold', 'Estamino', and 'Maxifort', or between 'Shin Cheong Gang' and the nongrafted control in terms of biomass produced.

The mean soil population density of RKN surrounding nongrafted plant roots was the highest in July and 16 times greater in comparison to the nongrafted mean population density in May. It was nine times greater than the July mean population density surrounding 'Maxifort' roots, which had the second highest J2 population density that month (Table 6). Nongrafted plants were in visible decline in July. Average soil temperatures increased from 15 °C in May to 27 °C in August (Fig. 5).

'Maxifort' had the largest mean RKN egg densities per gram of root and was significantly greater compared to all other grafted treatments, but was not significantly different from the nongrafted control ($P < 0.0001$; Table 7). The mean egg density of 'Maxifort' was 3, 10, and 17 times more than the egg densities of 'Estamino', 'Arnold', and 'Shin Cheong Gang', respectively.

Boyle County, year 2. In 2022, all grafted treatments had significantly higher marketable fruit yield compared with the nongrafted control. 'Estamino', which had the highest average yield, produced an average of 7 kg per plant, which was approximately seven times more than the nongrafted control that had the lowest yield ($P < 0.0001$; Fig. 4). The average aboveground plant biomass produced was significantly lower in the nongrafted control compared with all other treatments ($P < 0.0001$; Table 5).

Beginning in June, the mean J2 soil population density of the nongrafted control was significantly greater than all treatments except for 'Maxifort' ($P < 0.0001$; Table 8). The density surrounding the nongrafted control was the highest during this month and twice that of 'Maxifort'. Average soil temperatures increased from 12 °C in March to 26 °C in August (Fig. 6).

The mean egg population density per gram of root of the nongrafted control was significantly greater compared with all grafted treatments except 'Maxifort' ($P < 0.0001$; Table 7).

Discussion

High tunnel growers with RKN infestations have limited profitable nonhost options available for rotation. The grower cooperators in our trials had been producing a tomato crop in their high tunnels for several consecu-

Table 5. Tomato plant dry biomass production in a commercial high tunnel naturally infested with *Meloidogyne incognita* in Boyle County, Kentucky, in 2021 and 2022.

	Plant biomass (kg)	
Rootstock[i]	2021	2022
Arnold	0.82 ± 0.06 a[ii]	0.76 ± 0.03 ab
Estamino	0.79 ± 0.09 a	0.83 ± 0.04 a
Maxifort	0.75 ± 0.14 a	0.83 ± 0.08 a
Shin Cheong Gang	0.57 ± 0.03 ab	0.61 ± 0.06 b
Nongrafted	0.26 ± 0.04 b	0.27 ± 0.04 c

[i] Nongrafted is 'Cherokee Purple' tomato and all rootstocks were grafted onto a 'Cherokee Purple' scion.

[ii] Values are the means of seven replicates ± standard error. Any two means within a column not followed by the same letter are significantly different at $\alpha \leq 0.05$. Three plant samples were collected from each replication and combined.

Table 6. Monthly root-knot nematode (RKN; *Meloidogyne incognita*) population densities in soil surrounding grafted and nongrafted 'Cherokee Purple' tomato plant roots grown in a naturally infested commercial high tunnel in Boyle County, Kentucky, in 2021.

	RKN/100 g dry soil			
Rootstock[i]	May	June	July	August
Arnold	435 ± 172 a[ii]	68 ± 23 bc	32 ± 9 c	116 ± 79 b
Estamino	296 ± 67 a	75 ± 23 bc	99 ± 50 c	110 ± 35 b
Maxifort	360 ± 114 a	187 ± 71 b	522 ± 137 b	566 ± 137 a
Shin Cheong Gang	266 ± 135 a	39 ± 13 c	64 ± 22 c	67 ± 37 b
Nongrafted	292 ± 110 a	1,443 ± 563 a	4,689 ± 2,229 a	667 ± 211 a

[i] Nongrafted is 'Cherokee Purple' tomato and all rootstocks were grafted onto a 'Cherokee Purple' scion.

[ii] Values are the means of seven replicates ± standard error. Any two means within a column not followed by the same letter are significantly different at $\alpha \leq 0.05$.

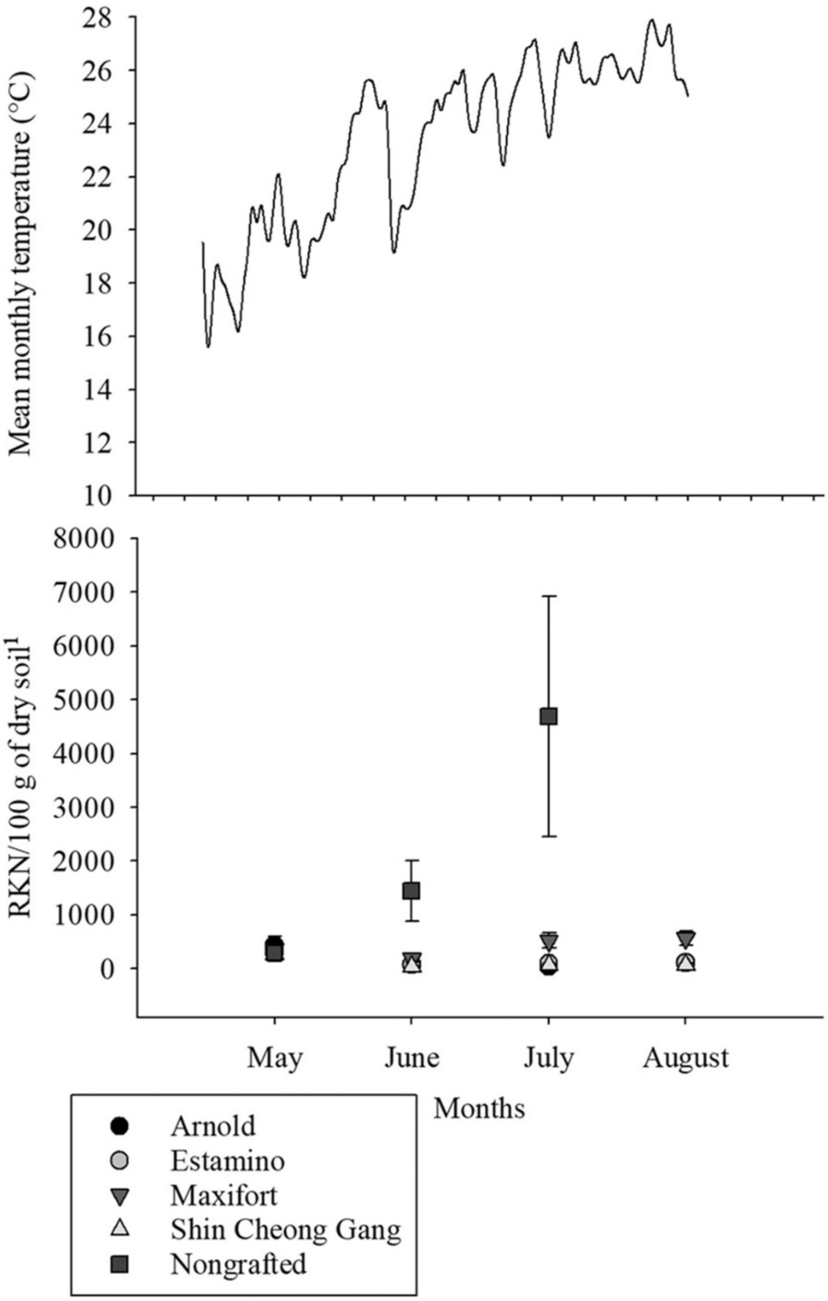

Fig. 5. Root-knot nematode densities (RKN; *Meloidogyne incognita*) population densities in soil surrounding grafted and nongrafted 'Cherokee Purple' tomato plant roots grown in a naturally infested commercial high tunnel in Boyle County, Kentucky, and average soil temperatures in that same high tunnel at 15 cm soil depth in 2021. [i] Mean value of second-stage juvenile RKN population densities collected monthly from soil ± standard error. The results are the means of seven replicates of each treatment.

tive years without rotation before our study. This is a common practice among Kentucky high tunnel growers (Rudolph RE, personal observation). Due to the wide host range of RKN, many profitable vegetable crops are also suitable hosts for RKN (Talavera et al. 2012). The intensive cropping environment as well as lack of weed management can lead to a progression of existing disease or an introduction of new pathogens or pests (Bruce et al. 2019).

Grafting with resistant rootstock is a relatively easy management strategy that can be incorporated into the high tunnel production system with limited disruption to other production or management practices. Previous studies have shown that grafting with RKN-resistant rootstock can result in increased fruit yield (Barrett et al. 2012; Lopez-Perez et al. 2006; Rivard et al. 2010). We observed similar results in our trials, with the nongrafted controls producing significantly lower yield compared with most of the grafted treatments. We observed this at both sites with two different scion cultivars, 'Primo Red' and 'Cherokee Purple'. Another indicator of plant vigor and health is aboveground biomass production (Lang et al. 2020). In our experiments, most grafted treatments produced significantly more aboveground biomass compared with the nongrafted controls. However, for sustainable, long-term crop production, yield and biomass should not be the only factors taken into consideration. Effective management of RKN populations is crucial for the long-term implementation of grafting and use of resistant plant cultivars.

Average *M. incognita* soil population densities surrounding nongrafted plant roots increased over the course of the season in both years and sites ($>$500 J2/100 g dry soil). This was not surprising, as neither of the nongrafted controls are resistant to *M. incognita* (Bost 2013; Rivard et al. 2010). Soil population densities of RKN J2s remained relatively level surrounding grafted plant roots but were never zero. Similar results were observed in Frey et al. (2020) when *M. javanica* soil population densities were tracked over the course of the season. The study observed an increase of $\sim$225 J2/100 cm^3 of soil ($\sim$173 J2/100 g of soil) on nongrafted 'Tribute' and 'Garden Gem' and $\sim$25 J2/100 cm^3 of soil (19 J2/100 g of soil) on the grafted 'Multifort' rootstock. Much like in our study, an increase was observed in RKN soil population densities surrounding nongrafted plants, but population densities remained low surrounding grafted plants. Talavera-Rubia et al. (2022) determined the tolerance limit, the maximum RKN population density of a plant that does not affect growth or yield, of tomatoes to be 91.9 J2/100 cm^3 of soil ($\sim$71 J2/100 g of soil). A treatment strategy should maintain population densities below the tolerance limit of the plant to avoid yield losses and economic impact (Talavera-Rubia et al. 2022). Population densities surrounding the roots of grafted plants remained low and mostly within the tomato tolerance limit, especially in Knox County; however, J2 populations surrounding nongrafted plants were at least five times over the tolerance limit. Nongrafted plants had significantly lower yield, with many plants producing no fruit at all before dying.

Root-knot nematode population densities in all roots were high at the end of each season in both sites. Although significantly lower compared with the nongrafted control, the average RKN root population densities for all grafted treatments were more than 700 eggs/g of root. For sensitive crops, such as lettuce and carrots, one egg per gram of soil can cause sufficient economic loss (Mitkowski and Abawi 2003). One RKN female can produce more than 500 eggs at one time (Mitkowski and Abawi 2003), so any presence of RKN would elicit a recommendation for management action to be taken. Root population densities in both sites and years were well over this density and would suggest that RKN would persist into the next production season and continue to have an economic impact. However, greater egg population densities were observed on nongrafted controls in comparison with grafted treatments with the exception of 'Maxifort' in year 1 in Boyle County. Lopez-Perez et al. (2006) observed egg population densities in

Table 7. Root-knot nematode (RKN; *Meloidogyne incognita*) egg populations in tomato roots grown in a naturally infested commercial high tunnel in Boyle County, Kentucky in 2021 and 2022.

	RKN eggs/g dry root	
Rootstock[i]	2021	2022
Arnold	3,191 ± 1,993 c[ii]	2,452 ± 862 c
Estamino	9,921 ± 2,350 b	3,494 ± 855 b
Maxifort	34,010 ± 3,916 a	7,730 ± 1,423 ab
Shin Cheong Gang	1,944 ± 532 c	783 ± 356 d
Nongrafted	30,815 ± 9,521 ab	29,495 ± 8,053 a

[i] Nongrafted is ‘Cherokee Purple’ tomato and all rootstocks were grafted onto a ‘Cherokee Purple’ scion.

[ii] Values are the means of seven replicates ± standard error. Any two means within a column not followed by the same letter are significantly different at $\alpha \leq 0.05$. Three root samples were collected from each replication and combined.

the millions on RKN-resistant cultivars Beaufort and Blitz. Frey et al. (2020) assessed root galling instead of egg densities and Rivard et al. (2010) examined RKN infestation severity through biweekly destructive sampling to determine the root gall index. Galling is a plant response to RKN infection (Mitkowski and Abawi 2003), but may not provide a clear indication of RKN reproduction (Holbrook et al. 1983). In addition, gall ratings can be subjective depending on each person conducting the ratings. Although determining gall indices can be useful when comparing one treatment with another within an experiment, quantifying egg population densities in roots is more reliable to determine actual RKN reproduction (Holbrook et al. 1983).

In our experiments, average soil temperatures increased from 16 to 26 °C. As a result, the 18 °C activity threshold for *M. incognita* (Roberts et al. 1981) was maintained for most of the season, which allowed for soil migration and root penetration of *M. incognita* J2s. This would contribute to RKN soil population densities being maintained near resistant rootstock treatments. As soil temperatures increased to more optimal temperatures for RKN reproduction, closer to 30.0 °C (Ammati et al. 1986; Ploeg and Maris 1999; Zacheo et al. 1995), so did RKN soil population densities surrounding nongrafted roots. However, an increase in soil population densities was observed surrounding the roots of ‘Maxifort’, particularly in Boyle County in both years. The same was noted by Rivard et al. (2010), who observed that RKN population densities were lower with ‘Maxifort’ in comparison with susceptible ‘German Johnson’ tomato, but not comparable to the “resistant” ‘Big Power’ rootstock. As a result, ‘Maxifort’ was labeled “partially resistant” in that study. In this study, ‘Maxifort’ displayed an average soil population density as high as 810 RKN/100 g dry soil in the middle of summer, which is considered very high and crop damage would be expected, according to the NCDA. However, aboveground biomass and yield were maintained in both sites and years in our study. Another factor to determine resistance or tolerance during high infestations is RKN egg population densities in roots during optimal conditions. ‘Beaufort’ rootstock, initially labeled as RKN-resistant in Lopez-Perez et al. (2006), displayed galling and increased RKN egg populations in roots at the peak soil temperature of 24 °C. The authors concluded that ‘Beaufort’ had become “tolerant” to *Meloidogyne* spp. due to the increased RKN activity on the rootstock. Ultimately, the same could be said for ‘Maxifort’, as it had one of the highest yields across all sites and years, but also high RKN root population densities. As a result, we consider ‘Maxifort’ to be tolerant, rather than resistant, to *M. incognita*.

Higher soil temperatures can lead to increased RKN reproduction (Ploeg and Maris 1999) as well as turn off the RKN resistance gene in rootstocks (Lopez-Perez et al. 2006; Williamson 1998). Temperatures close to 30 °C can accelerate overall life cycles of RKN and lead to increased crop root penetration rates and symptoms of galling (Ammati et al. 1986; Ploeg and Maris 1999; Zacheo et al. 1995). Although the average monthly soil temperatures in our high tunnels did not exceed 26 °C across all years and sites, on individual days, 28 °C was reached. In 2020 in Knox County, 28 °C was never reached at 15 cm soil depth. In 2021 in Knox County, there were only 4 h when soil temperatures reached 28 °C. In 2021 and 2022 in Boyle County, soil temperatures reached or exceeded 28 °C for 47 and 308 h, respectively. Our temperature measurements were collected at 15 cm soil depth. If 28 °C was reached at 15 cm, it is highly likely that it was exceeded at more shallow soil depths. The differences in temperatures across the years and sites is likely due to grower management of their high tunnels, such as opening and closing sidewalls and end walls. Although increased temperatures can be viewed as a benefit in high tunnels, the overall management of temperatures is crucial for successful crop production. High soil temperatures likely encouraged RKN soil population densities in late summer in our trials and may have caused a lack of resistance to RKN in the grafted treatments.

When evaluating rootstocks to be used as a management strategy for RKN, there should be decreases in RKN root and soil populations as well as benefits in plant yield and development. For example, ‘Arnold’ had the highest or second highest yield as well as the second lowest RKN egg density. In comparison, ‘Shin Cheong Gang’ across both years and sites had the lowest egg count, but also the low yield and plant biomass. A reduction in RKN eggs would likely lead to an overall reduction of RKN infestation over time. However, many growers would not want to use ‘Shin Cheong Gang’ because it produced significantly lower yield compared with other grafted treatments. A truly viable management strategy for RKN must demonstrate consistency and benefits to the grower.

Overall, grafting favorable scion to resistant rootstock can potentially result in yield increases and suppression of RKN population densities. In our study, grafting was an effective management strategy because RKN population densities were significantly lower in three of the four rootstocks evaluated in comparison with the nongrafted, susceptible control. However, we still observed RKN feeding and reproduction on these rootstocks, which was also observed in previous studies (Cukrov et al. 2021; Lopez-Perez et al. 2006; Rivard et al. 2010). Complete eradication of RKN is not possible, but using grafting with other management strategies, such as soil solarization and rotation with nonhost crops may be a viable and sustainable option for high tunnel growers. Grafting as a management strategy is promising in tomato production with RKN, as it allows for the opportunity to move

Table 8. Monthly root-knot nematode (RKN; *Meloidogyne incognita*) population densities in soil surrounding grafted and nongrafted ‘Cherokee Purple’ tomato plant roots grown in a naturally infested commercial high tunnel in Boyle County, Kentucky, in 2022.

	RKN/100 g dry soil					
Rootstock[i]	March	April	May	June	July	August
Arnold	430 ± 113 a[ii]	292 ± 102 a	242 ± 94 a	185 ± 49 b	75 ± 23 b	77 ± 40 b
Estamino	100 ± 38 a	102 ± 77 a	180 ± 101 a	74 ± 25 c	134 ± 49 b	77 ± 32 b
Maxifort	302 ± 77 a	266 ± 109 a	810 ± 364 a	685 ± 104 a	204 ± 48 ab	171 ± 40 ab
Shin Cheong Gang	143 ± 52 a	140 ± 76 a	436 ± 237 a	57 ± 8 c	120 ± 49 b	75 ± 23 b
Nongrafted	97 ± 31 a	58 ± 18 a	799 ± 247 a	1,380 ± 151 a	481 ± 131 a	285 ± 60 a

[i] Nongrafted is ‘Cherokee Purple’ tomato and all rootstocks were grafted onto ‘Cherokee Purple’ scion.

[ii] Values are means of seven replicates ± standard error. Any two means within a column not followed by the same letter are significantly different at $\alpha \leq 0.05$.

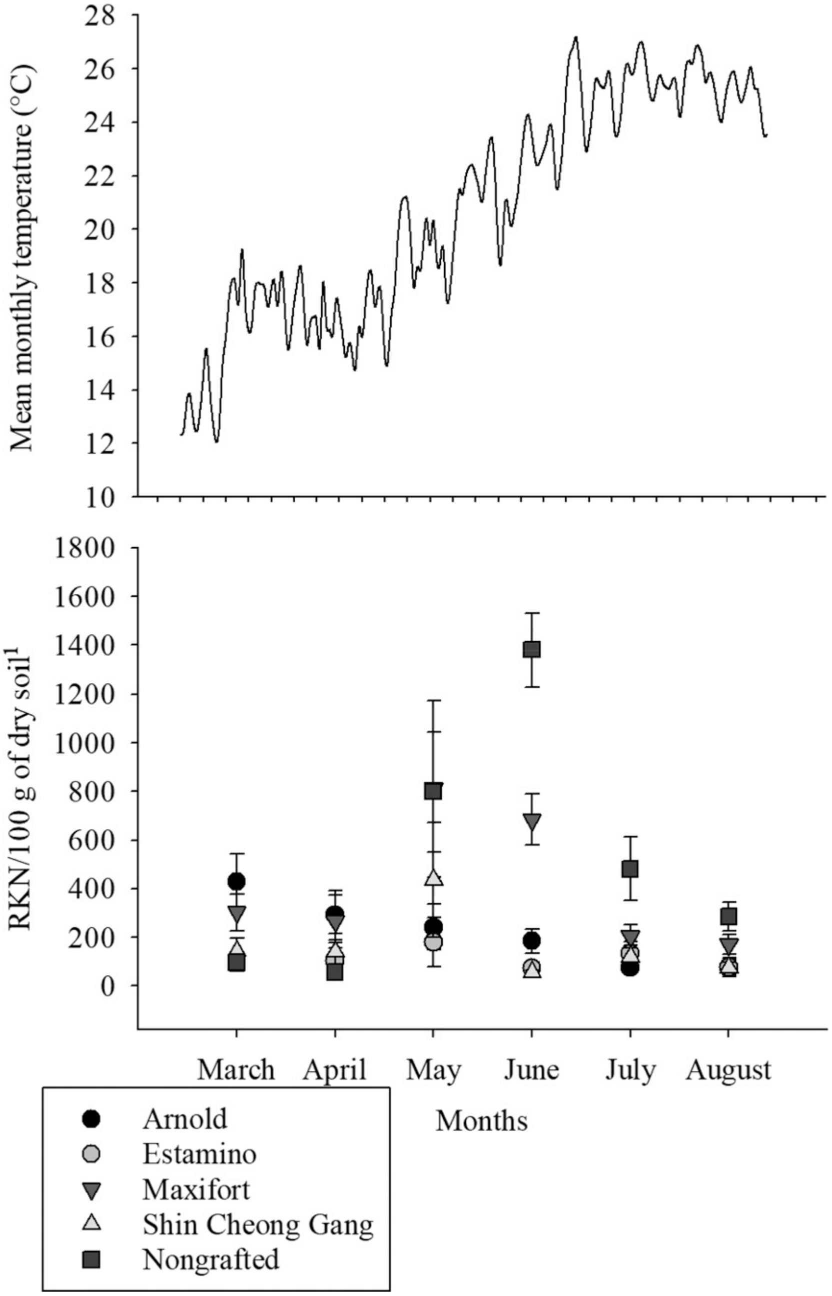

Fig. 6. Root-knot nematode (RKN; *Meloidogyne incognita*) population densities in soil surrounding grafted and nongrafted 'Cherokee Purple' tomato plant roots grown in a naturally infested commercial high tunnel in Boyle County, Kentucky, and average soil temperatures in that same high tunnel at 15 cm soil depth in 2022. [i] Mean value of J2 population densities collected monthly from soil ± standard error. The results are the means of seven replicates of each treatment. Treatment plots were sampled if >50% decline was observed in plants (nongrafted replications were sampled beginning in July).

toward a sustainable, chemical-free option. Grower management of their high tunnel systems must be considered, as well as differences in rootstocks. The compatibility and success of a resistant rootstock is system dependent.

Conclusion

The intensive cropping environment of high tunnel production can lead to increases in population densities of RKN. We see benefits in grafting with resistant rootstock, as it can help growers manage RKN and maintain yield, but more rootstocks should be studied to widen the market of resistant rootstock. Growers will also still need to use other management strategies in combination with resistant rootstocks to decrease population densities as well as manage high tunnel temperatures to the best of their abilities. Grafting resistant rootstock can be used to complement other integrated pest management approaches in high tunnel tomato production systems with RKN infestations.

References Cited

Abad P, Favery B, Rosso MN, Castagnone-Sereno P. 2003. Root-knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. Mol Plant Pathol. 4:217–224. https://doi.org/10.1046/j.1364-3703.2003.00170.x.

Ahmad G, Khan A, Khan AA, Ali A, Mohhamad HI. 2021. Biological control: A novel strategy for the control of the plant parasitic nematodes. Antonie van Leeuwenhoek. 144(7):885–912. https://doi.org/10.1007/s10482-021-01577-9.

Ammati M, Thomason IJ, McKinney HE. 1986. Retention of resistance to *Meloidogyne incognita* in *Lycopersicon* genotypes at high soil temperature. J Nematol. 18:491–495. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2618591/.

Bailey DM. 1941. The seedling test method for root-knot nematode resistance. Proc Am Soc Hortic Sci. 38:573–575.

Barrett CE, Zhao X, Hodges AW. 2012. Cost benefit analysis of using grafted transplants for root-knot nematode management in organic heirloom tomato production. HortTechnology 22:252–257. https://doi-org.ezproxy.uky.edu/10.21273/HORTTECH.22.2.252.

Belasco EJ, Chen C, Ponnaluru SS, Galinato SP, Marsh TL. 2011. An assessment of the interaction between high tunnels and crop insurance for specialty crop producers. Agric Resour Econ Rev. 42(2):403–418. https://doi.org/10.1017/S1068280500004445.

Bessin R, Gauthier N, Fealko E, Rudolph R, Wright S. 2021. Vegetable production guide for commercial growers 2022–23. Univ Kentucky Coop Ext Serv Bul. ID-36. http://www2.ca.uky.edu/agc/pubs/id/id36/id36.pdf.

Black B, Drost D. 2010. Temperature management in high tunnels. Utah State Univ. Coop. Ext. https://digitalcommons.usu.edu/psc_facpub/744.

Bost S. 2013. Tomato wilt problems. Univ TN Ext. SP370-C. https://trace.tennessee.edu/cgi/viewcontent.cgi?article=1030&context=utk_agexgard.

Bruce AB, Maynard ET, Farmer JR. 2019. Farmers' perspectives on challenges and opportunities associated with using high tunnels for specialty crops. HortTechnology. 29(3):290–299. https://doi.org/10.21273/HORTTECH04258-18.

Carey EE, Jett L, Lamont WJ, Nennich TT, Orzolek MD, Williams KA. 2009. Horticultural crop production in high tunnels in the United States: A snapshot. HortTechnology. 19(1):37–43. https://doi.org/10.21273/HORTSCI.19.1.37.

Cukrov M, Podrug I, Urlić B, Mandušić M, Žanić K, Radpudić E, Slatnar A, Dumičić G. 2021. Effectiveness of grafting and biostimulants application as a strategy for enhancing tomato nematode resistance. Acta Hortic. 1320:283–290. https://doi.org/10.17660/ActaHortic.2021.1320.37.

Costa LG, Giordano G, Guizzetti M, Vitalone A. 2008. Neurotoxicity of pesticides: A brief review. Front Biosci-Landmark. 13(4):1240–1249. https://doi.org/10.2741/2758.

Cornell University. 2023. Disease-resistant tomato varieties. https://www.vegetables.cornell.edu/pest-management/disease-factsheets/disease-resistant-vegetable-varieties/disease-resistant-tomato-varieties/. [accessed 27 Feb 2023].

Ernst M, Woods T, Butler A, Wolff B, Jacobsen K. 2020. High tunnel production and marketing survey: Data summary. Univ Kentucky Coop Ext Serv Bul. CCD-SP-17. https://www.uky.edu/ccd/sites/www.uky.edu.ccd/files/HTsurvey.pdf.

Frey CJ, Zhao X, Brecht JK, Huff DM, Black ZE. 2020. High tunnel and grafting effects on organic tomato plant disease severity and root-knot nematode infestation in a subtropical climate with sandy soils. HortScience. 55(1): 46–54. https://doi.org/10.21273/HORTSCI14166-19.

Galinato SP, Miles CA. 2013. Economic profitability of growing lettuce and tomato in western Washington under high tunnel and open-field production systems. HortTechnology. 23(4): 453–461. https://doi.org/10.21273/horttech.23.4.453.

Gilbert JC, McGuire DC. 1956. Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial type tomatoes. Proc Am Soc Hortic Sci. 68:437–442.

Gill HK, McSorley R. 2011. Cover crops for managing root-knot nematodes. Univ Florida Coop Ext Serv. https://doi.org/10.32473/edis-in892-2011.

Gude KM, Pliakoni ED, Cunningham B, Ayub K, Kang Q, Rajashekar CB, Rivard CL. 2022. High tunnel coverings alter crop productivity and microclimate of tomato and lettuce. HortScience. 57(2):265–272. https://doi.org/10.21273/HORTSCI16208-21.

Heath J. 2018. Fumigation for the commercial/noncommercial pesticide applicator: Revision of the 3rd Edition, 2011 (MS Thesis). Univ of Nebraska-Lincoln, Lincoln, NE, USA.

Holbrook CC, Knauft DA, Dickson DW. 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria.* Plant Dis. 67(9):957–958. https://doi.org/10.1094/PD-67-957.

Hooper DJ. 1986. Handling, fixing, staining and mounting nematodes, p 58–80. In: Southey JF (ed). Laboratory methods for work with plant and soil nematodes. HM Stationery Office, London, United Kingdom.

Hussey RS, Barker KR. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. Plant Dis Rep. 57(12):1025–1028.

Ireri DF, Murungi LK, Ngeno DC. 2018. Farmer knowledge of bacterial wilt and root knot nematodes and practices to control the pathogens in high tunnel tomato production in the tropics. Int J Veg Sci. 25(3):213–225. https://doi.org/10.1080/19315260.2018.1499690.

Janke RR, Altamimi ME, Khan M. 2017. The use of high tunnels to produce fruit and vegetable crops in North America. Agro Sci. 8(7): 692–715. https://doi.org/10.4236/as.2017.87052.

Jacquet M, Bongiovanni M, Martinez M, Verschave P, Wajnberg E, Castagnone-Sereno P. 2005. Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene. Plant Pathol. 54(2):93–99. https://doi.org/10.1111/j.1365-3059.2005.01143.x.

Jenkins WRB. 1964. A rapid centrifugal flotation method for separating nematodes from soil. Plant Dis Rep. 48(9):692–695.

Karajeh M. 2008. Interaction of root-knot nematode (*Meloidogyne javanica*) and tomato as affected by hydrogen peroxide. J Plant Prot Res. 48(2):181–187. https://doi.org/10.2478/v10045-008-0021-x.

Kratochvil RJ, Sardanelli S, Everts KE. 2004. Gallagher evaluation of crop rotation and other cultural practices for management of root-knot and lesion nematodes. Agron J. 96(5): 1419–1428. https://doi.org/10.2134/agronj2004.1419.

Kubota C, McClure MA, Kokalis-Burelle N, Bausher MG, Rosskopf EN. 2008. Vegetable grafting: History, use, and current technology status in North America. HortScience. 43(6): 1664–1669. https://doi.org/10.21273/hortsci.43.6.1664.

Kumari P, Ojiha RK, Abhivyakti AW, Rajesh RP. 2014. Microclimatic alteration through protective cultivation and its effect on tomato yield. J Agrometerol. 16(2):172–177. https://doi.org/10.54386/jam.v16i2.1508.

Lamont WJ. 2009. Overview of the use of high tunnels worldwide. HortTechnology. 19(1): 25–29. https://doi.org/10.21273/HORTSCI.19.1.25.

Lang KM, Nair A, Moore KJ. 2020. The impact of eight hybrid tomato rootstocks on 'BHN 589' scion yield, fruit quality, and plant growth traits in a Midwest high tunnel production system. HortScience. 55(6):1–9. https://doi.org/10.21273/HORTSCI14713-20.

Lopez-Perez JA, Le Strange M, Kaloshian I, Ploeg AT. 2006. Differential response of *Mi* gene-resistant tomato rootstocks to root-knot nematodes *(Meloidogyne incognita*). Crop Prot. 25(4): 382–388. https://doi.org/10.1016/j.cropro.2005.07.001.

Martínez-Andújar C, Albacete A, Pérez-Alfocea F. 2020. Rootstocks for increasing yield stability and sustainability in vegetable crops. Acta Hortic. 1273:449–470. https://doi.org/10.17660/ActaHortic.2020.1273.58.

Mitkowski NA, Abawi GS. 2003. Root-knot nematodes. Plant Health Instructor. https://doi.org/10.1094/PHI-I-2003-0917-01.

O'Connell S, Rivard C, Peet MM, Harlow C, Louws F. 2012. High tunnel and field production of organic heirloom tomatoes: Yield, fruit quality, disease, and microclimate. HortScience. 47(9):1283–1290. https://doi.org/10.21273/HORTSCI.47.9.1283.

Oka Y. 2020. From old-generation to next generation nematicides. Agron. 10(9):1387–1403. https://doi.org/10.3390/agronomy10091387.

Onkendi EM, Kariuki GM, Marais M, Moleleki LN. 2014. The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: A review. Plant Pathol. 63(4):727–737. https://doi.org/10.1111/ppa.12202.

Haydock PPJ, Woods SR, Grove IG, Hare MC. 2013. Chemical control of nematodes, p 459-477. In: Perry RN, Moens M. Plant nematology, 2nd ed. CAB International, Oxfordshire, United Kingdom. https://doi.org/10.1079/9781845930561.0392.

Ploeg AT, Maris PC. 1999. Effects of temperature on the duration of the life cycle of a *Meloidogyne incognita* population. Nematol: Int J Fundamental Appl Nematol Res. 1(4):389–393. https://doi.org/10.1163/156854199508388.

Ralmi NHAA, Khandaker MM, Mat N. 2016. Occurrence and control of root knot nematode in crops: A review. Aust J Crop Sci. 10(12): 1649–1654. https://doi.org/10.21475/ajcs.2016.10.12.p7444.

Reeve J, Drost D. 2012. Yields and soil quality under transitional organic high tunnel tomatoes. HortScience. 47(1):38–44. https://doi.org/10.21273/hortsci.47.1.38.

Rivard CL, O'Connell S, Peet MM, Louws FJ. 2010. Grafting tomato with interspecific rootstock to manage diseases caused by *Sclerotium rolfsii* and southern root-knot nematode. Plant Dis. 94(8):1015–1021. https://doi.org/10.1094/PDIS-94-8-1015.

Rudisill MA, Bordelon BP, Turco RF, Hoagland LA. 2015. Sustaining soil quality in intensively managed high tunnel vegetable production systems: A role for green manures and chicken litter. HortScience. 50(3):461–468. https://doi.org/10.21273/hortsci.50.3.461.

Roberts PA, Van Gundy SD, McKinney HE. 1981. Effects of soil temperature and planting date of wheat on *Meloidogyne incognita* reproduction, soil populations, and grain yield. J Nematol. 13(3):338–345.

Talavera M, Sayadi S, Chirosa-Ríos M, Salmerón T, Flor-Peregrín E, Verdejo-Lucas S. 2012. Perception of the impact of root-knot nematode-induced diseases in horticultural protected crops of south-eastern Spain. Nematol: Int J Fundamental Appl Nematol Res. 14(5): 517–527. https://doi.org/10.1163/156854112X635850.

Talavera-Rubia M, Vela-Delgado MD, Verdejo-Lucas S. 2022. A cost-benefit analysis of soil disinfestation methods against root-knot nematodes in Mediterranean intensive horticulture. Plants. 11(20):2774–2785. https://doi.org/10.3390/plants11202774.

Taylor AL, Sasser JN. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University Graphics, Raleigh, North Carolina, USA.

Testen AL, Martínez MB, Madrid AJ, Deblais L, Taylor CG, Paul PA, Miller SA. 2021. On-farm evaluations of anaerobic soil disinfestation and grafting for management of a widespread soilborne disease complex in protected culture tomato production. Phytopathology. 111(6):954–965. https://doi.org/10.1094/PHYTO-07-20-0288-R.

Trudgill DL, Blok VC. 2001. Apomictic, polyphagous root-knot nematodes: Exceptionally successful and damaging biotrophic root pathogens. Annu Rev Phytopathol. 39:53–77. https://doi.org/10.1146/annurev.phyto.39.1.53.

US Department of Agriculture-Natural Resources Conservation Service. 2023. Environmental quality incentives program. https://www.nrcs.usda.gov/programs-initiatives/eqip-environmental-quality-incentives. [accessed 7 Apr 2023].

Williamson VM. 1998. Root-knot nematode resistance genes in tomato and their potential for future use. Annu Rev Phytopathol. 36(1):277–293. https://doi.org/10.1146/annurev.phyto.36.1.277.

Zacheo G, Bleve-Zacheo T, Pacoda D, Orlando C, Durbin RD. 1995. The association between heat-induced susceptibility of tomato to *Meloidogyne incognita* and peroxidase activity. Physiol Mol Plant Pathol. 46(6): 491–507. https://doi.org/10.1006/pmpp.1995.1037.

Zasada IA, Halbrendt JM, Kokalis-Burelle N, LaMondia J, McKenry MV, Noling JW. 2010. Managing nematodes without methyl bromide. Annu Rev Phytopathol. 48(1):311–328. https://doi.org/10.1146/annurev-phyto-073009-114425.

Zhao X, Carey EE. 2009. Summer production of lettuce, and microclimate in high tunnel and open field plots in Kansas. HortTechnology. 19(1):113–119. https://doi.org/10.21273/HORTSCI.19.1.113.