EFFECTIVENESS OF ROOT-KNOT NEMATODE (*MELOIDOGYNE* SPECIES) RESISTANT TOMATO (*SOLANUM LYCOPERSICUM* L.) AND PEPPER (*CAPSICUM* SPECIES) CULTIVARS IN GHANA

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ABSTRACT

Kwara, B. K., C. K. Kwoseh, and J. L. Starr. 2014. Effectiveness of root-knot nematode (*Meloidogyne* species) resistant tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum* species) cultivars in Ghana. Nematropica 44:130-136.

Nematode-resistant cultivars are useful, economical, and effective means of managing nematodes. Three root-knot nematode resistant tomato cultivars, Small Fry, Jetsetter, and Celebrity, and three root-knot nematode resistant pepper cultivars, Carolina Cayenne, Carolina Wonder, and Charleston Belle, were evaluated for their reaction to *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* in two field experiments and a soil bioassay. The local cultivars, Power (tomato) and Ohene Sateaa (pepper), were used as susceptible checks. The bioassay used soils from the tomato-growing areas of Tono, Vea, and Pwalugu in the Upper East Region of Ghana. All resistant tomato and pepper cultivars supported low reproduction by *Meloidogyne* spp. and exhibited low root gall rating scores for both the field and bioassay. The soil temperature during the study period in the field ranged from 24 to 32ºC, temperatures that were not likely high enough for a sufficient duration to affect the temperature-sensitive nature of the resistance in tomato. Based on these results, all of the resistant tomato and pepper cultivars that were studied could be useful in managing root-knot nematodes in major tomato and pepper growing areas in Ghana.

Key words: bioassay, fruit quality, reproduction factor, soil temperature.

RESUMEN

Kwara, B. K., C. K. Kwoseh, and J. L. Starr. 2014. Efectividad de cultivares de tomate (*Solanum lycopersicum* L.) y pimiento (especies de *Capsicum*) resistentes a nematodos formadores de agallas en las raíces (especies de *Meloidogyne*) en Ghana. Nematropica 44:130-136.

Los cultivares resistentes a nematodos constituyen una útil, asequible y efectiva medida en el manejo de nematodos. La reacción de tres cultivares de tomate resistentes a nematodos, Small Fry, Jetsetter, y Celebrity, y tres cultivares resistentes de pimiento, Carolina Cayenne, Carolina Wonder, y Charleston Belle frente a *Meloidogyne incognita*, *M. javanica*, y *M. arenaria* fue evaluada en dos experimentos en campo y un bioensayo con suelo. El bioensayo utilizó suelos recolectados en áreas de cultivo de tomate en Tono, Vea, and Pwalugu en la región alta del este de Ghana. Los cultivares locales de tomate Power y de pimiento Ohene Sateaa se usaron como controles susceptibles. Todos los cultivares resistentes de tomate y pimiento sostuvieron una baja reproducción de *Meloidogyne* spp. y mostraron índices bajos de agallas tanto en los experimentos de campo como en el bioensayo. La temperatura del suelo durante el periodo de estudio en campo varió de 24 a 32ºC, temperaturas que no fueron lo suficientemente altas, ni duraron lo suficiente como para afectar la resistencia sensible a la temperatura del tomate. Basado en estos resultados, todos los cultivares resistentes de tomate y pimiento que fueron estudiados, podrían ser útiles en el manejo de *Meloidogyne* spp. en las principales áreas productoras de tomate y pimiento en Ghana.

Palabras clave: bioensayo, calidad de fruta, factor de reproducción, temperatura del suelo.

INTRODUCTION

Vegetable production, especially tomato and pepper, is a key component in the economic stability of most farmers in Ghana. Both crops are well adapted to all the agro-ecological zones of the country, namely; the forest-savannah transition, the savannah, and forest zones. In Ghana, tomato is the most important vegetable crop in the Northern, Upper East, and Volta Regions. It is also an important cash crop in the outskirts of urban areas in the forest zone, in the greater Accra area, Akumadan, Wenchi, and Mankesim (Obeng-Ofori *et al*., 2007). Tomato and pepper are used for the preparation of soups, stew, and sauces. According to Wolff (1999), vegetables account for 9.6% of total food expenditure and 4.9% of total expenditure in Ghana, and tomato alone makes up to 38% of the vegetable expenditure.

Root-knot nematode attack is one of the factors responsible for frequent crop failure in tomato and pepper. Hemeng (1981) reported yield loss of 73% to 100% in tomato in northern Ghana. The main root-knot nematodes in Ghana are: *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood. It is well established that root-knot nematodes pre-dispose plants to other pathogenic infections (Manzanilla-Lopez and Starr, 2009). Diseases caused by fungi and plant-parasitic nematodes are especially important in tomato and pepper production due to limited options for effective management. Use of genetic resistance is often the best approach to disease management because resistance presents no potential hazard to human or environmental health, and can be useful both for protection of yield potential and management of pathogen densities (Starr *et al.*, 2002). Genetic resistance to key vegetable pathogens, including nematodes, has been introgressed into tomato and pepper but such resources are under-utilized in Ghana. The objective of this study was to determine the reaction of resistant tomato and pepper cultivars to local root-knot nematode populations grown under root-knot nematode pressure.

MATERIALS AND METHODS

Two experiments were conducted in this study at the Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. Three root-knot nematode resistant tomato cultivars not commercially used in Ghana, Small Fry (VFN), Jetsetter (VFN), Celebrity (VFN), and three root-knot nematode resistant pepper cultivars, Carolina Cayenne, Carolina Wonder, and Charleston Belle were used. Power and Ohene Sateaa, which are susceptible and locally grown tomato and pepper cultivars, respectively, were used as checks. Seed of the tomatoes and peppers were obtained from Texas A&M University, College Station, TX, USA, and seed of the local cultivars was obtained from Bentronic, a licensed Agrochemical shop at Kejetia, Kumasi.

Experiment 1: Reaction of root-knot nematode resistant tomato and pepper cultivars in the field

A steam-sterilized top soil and river sand mix (3:1 v/v) was used to germinate tomato and pepper seeds separately in plastic pots. The soil mix (1.8 L) was placed into 2-L plastic pots. One week after seedling germination, 5,000 *Meloidogyne* spp. eggs, collected from infected tomato roots using the NaOCl method (Hussey and Barker, 1973), were inoculated onto the seedlings. Two weeks after inoculation, the tomato and pepper seedlings were transplanted to the field. Prior to planting, the field was slashed with a cutlass, and the surface vegetation was burned. Thirty ridges were constructed by hand using a hoe. The ridges were raised with each measuring 3-m long and 50-cm wide with 1 m between ridges. The seedlings were planted 20-cm apart on the ridges. One seedling was planted per hill, and there were five plants each of tomato and pepper cultivars per ridge serving as a replicate. There were three replications per cultivar, and the experiment was arranged as randomized complete block.

Plants were watered copiously immediately after transplanting. A temperature probe (Easy Log (EL) USB-1, Lascar Electronics, Lascar Electronics Inc., Erie, PA, USA) was inserted into the soil in the field, and daily soil temperature was recorded from 1 wk before the start of the experiment until 1 wk after the end of the experiment. The ridges were mulched with grass 1 wk after transplanting to conserve water and control weeds. Recommended rates of 15:15:15 N-P-K fertilizer were applied 2 wk after transplanting. The plot was cleared of weeds three times by using a hoe to prevent competition with the crops for nutrients, sunlight, and water and to prevent the weeds from harbouring nematodes. The plants were watered as necessary to maintain good growth, and they were also sprayed with cypermethrin (Wynca Sunshine Agricultural Products and Trading Company, Accra, Ghana) and copper hydroxide (DuPont, Wilmington, DE, USA) 4 wk after transplanting to protect the plants against insect attack and foliar diseases.

The plants were dug up 120 d after transplanting, and root systems were rated for galling using the gall index (scale 0-5) reported by Hussey and Boerma (1981), where, 0 = complete and healthy root system, no galling, 1 = trace infection with a very few small galls, 2 = less than 25% roots galled, 3 = 25-50% roots galled, 4 = 51-75% roots galled and 5 = greater than 75% roots galled. *Meloidogyne* spp. eggs were collected from each root system using the NaOCl method and counted. The field experiment was carried out during the wet season and repeated in the dry season. The data were subjected to analysis of variance (ANOVA), and treatment means separated with least

significant difference (LSD) at $P = 0.05$ using GenStat for Windows (2007), Discovery edition 3. All count data were square-root transformed before statistical analysis.

Experiment 2: Tomato and pepper bioassays for assessing root-knot nematode population in the soil

Soil was randomly collected separately from tomato- and pepper-growing communities in Tono, Vea, and Pwalugu in the Upper East Region of Ghana. From each of the three communities, composite soil samples were collected with a soil auger from 40 different locations at depth of 0 to 15 cm from each of 10 farmers' fields in each community. The subsamples for each community were then bulked to constitute the composite samples. The bulked soil was put in polythene bags, labelled, and transported to KNUST for the bioassays. Before the bioassay was conducted, the level of root-knot nematode infestation of the soil samples was determined using the modified Baermann tray method (Whitehead and Hemming, 1965).

The bioassay was conducted in the plant house of the Department of Crop and Soil Sciences, KNUST, Kumasi. Each of the tomato and pepper cultivars was grown separately in 1.8-L soil from Tono, Vea, and Pwalugu. Three seeds each of the tomato cultivars, Small Fry, Jetsetter, Celebrity, Power, and the pepper cultivars, Carolina Cayenne, Carolina Wonder, Charleston Belle, and Ohene Sateaa, were sown in each plastic pot. The pots were arranged on wooden benches in a completely randomized design with three replications of each cultivar. One week after germination, the seedlings were thinned to one plant per pot. The plants were watered as needed. The plants were harvested 8 wk after planting, and the roots were washed carefully with running tap water and blotted dry with tissue paper. Nematode damage was rated using the root galling index described above. *Meloidogyne* spp. eggs were extracted with NaOCl and counted using a stereomicroscope.

Reproduction factors (Rf) on field tomato and pepper cultivars 8 wk after planting were calculated where: Rf = final population (Pf)/Initial population (Pi). Consequently, Rf < 1 = no or very low reproduction occurred and Rf > 1 = a reproducing population.

Molecular identification of Meloidogyne *spp. extracted from field tomato and pepper cultivars and soil bioassay*

Equal volumes of the *Meloidogyne* spp. juvenile-water suspension from each tomato and pepper cultivar obtained from the field and bioassay and 98% ethanol were separately put in Eppendorf tubes and tightly covered to preserve the *Meloidogyne* spp. The nematodes samples were sent to the Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA for *Meloidogyne* spp. identification. Species were confirmed based on esterase and malate dehydrogenase isozyme (MDH) phenotypes and by species specific PCR tests according to Adam *et al*. (2007).

The juveniles were handpicked with the tip of a needle and placed in 15 µg of worm lysis buffer (WLB) on a glass microscope slide and cut into two pieces with a needle under a stereomicroscope. The cut nematode, in 10 µL WLB, was then transferred by a pipette into a 0.5-ml centrifuge tube containing another 10 µL of WLB. The tubes were centrifuged at 13,500 rpm for 2 min, and then placed at -80°C for 15 min. Mineral oil (7 µL) was added to each tube and incubated at 60°C for 1 hr, followed by 90°C for 10 min. The mineral oil was removed by pipette after the aqueous sample was frozen at -20°C. PCR amplification using rDNA primers were carried out in 25 µl of DNA extract, or Taq polymerase (Promega). The reactions using Taq polymerase also included 2.5 µL 10 × buffer, 1.5 µL of 50 mm $MgCl_2$ and 2.5 µL 200 mm of each dNTP and two units of enzyme. Amplification products were separated on agarose gels and visualized with UV light after staining with ethidium bromide.

RESULTS

Experiment 1: Reaction of root-knot nematode resistant tomato and pepper cultivars in the field

Field soil temperature recordings where tomato and pepper cultivars were cultivated were taken daily within a 4-mon period, from August to November. The highest temperature reading was 32°C and the lowest recorded was 24°C (Fig. 1), with temperatures being less than 30°C from the initiation of the test in August until mid October.

The results of the wet and dry season experiments followed a similar trend. Gall scores of the pepper cultivars ranged from 1 to 2 with Ohene Sateaa recording the highest and the lowest by Carolina Cayenne, Carolina Wonder, and Charleston Belle (Table 1). Ohene Sateaa also had the highest number of nematode eggs whereas Carolina Cayenne had the least number of eggs. All the exotic pepper cultivars were resistant to the root-knot nematodes, with no detectible root galling and egg production that was extremely low. However, Ohene Sateaa (control) was susceptible to the root-knot nematodes, exhibiting gall ratings of 2.0, and significant reproduction.

No galling was observed for the root-knot nematode resistant tomato cultivars whereas the susceptible control cultivar had a gall score of 4 (Table 1). Root-knot nematode egg counts ranged from 4.5 to 54.6/5 g root with susceptible Power having the highest count. The least nematode reproduction was observed on Celebrity.

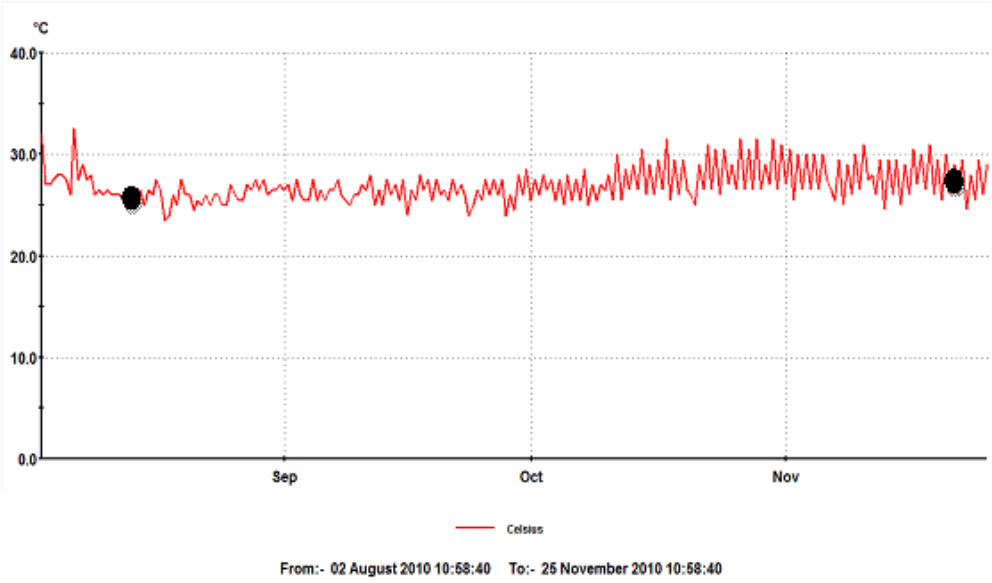

Fig. 1. Daily field soil temperature reading over a 4-mon period in the tomato and pepper field. Dots indicate the beginning and end of the experiment.

Table 1. Root gall ratings, *Meloidogyne* spp. egg counts, and reaction of pepper and tomato cultivars in the field.

Crop and cultivar	Root gall rating[x]	Egg count[y] No./5 g root
Pepper		
Carolina Cayene	1.0	5.1
Carolina Wonder	1.0	6.0
Charleston Belle	1.0	7.1
Ohene Sateaa (Control)	2.0	52.0
LSD (5%)	0.5	1.2
CV (%)	1.6	3.7
Tomato		
Small Fry	1.0	12.3
Jetsetter	1.0	13.3
Celebrity	1.0	4.5
Power (Control)	4.0	54.6
LSD (5%)	0.5	0.3
CV (%)	7.3	0.8

x Rating scale of 1-5 where 0 = no galls on root and 5 = > 75% roots with galls.
y $\sqrt{(x+0.5)}$ transformed before analysis.

Table 2. Root gall score, mean number of *Meloidogyne* eggs, and reproduction factor of pepper and tomato bioassay in Tono, Vea, and Pwalugu soil in the plant house at KNUST.

	Root gall rating[x]			Egg Count[y] No./5 g root			Rf[z]		
Crop and Cultivar	Tono	Vea	Pwalugu	Tono	Vea	Pwalugu	Tono	Vea	Pwalugu
Pepper									
Carolina Cayenne	1.0	1.0	1.0	11.0	9.2	9.2	0.9	0.9	0.8
Chaleston Belle	1.0	1.0	1.0	11.8	8.4	7.4	0.9	0.9	0.8
Ohene Sateaa (Control)	1.0	1.0	1.0	10.4	9.5	5.0	0.9	0.9	0.8
LSD (5%)	2.0	1.0	3.0	33.1	27.4	28.9	1.9	1.7	1.4
CV (%)	0.5	0.5	0.5	2.8	2.0	2.5	0.1	0.1	0.1
Tomato	21.7	26.6	20.4	9.1	7.7	10.6	6.0	4.2	5.4
Small Fry									
Jetsetter	1.0	1.0	1.0	7.8	3.2	5.0	0.8	0.7	0.7
Celebrity	1.0	1.0	1.0	8.6	3.9	6.4	0.8	0.7	0.8
Power (Control)	1.0	1.0	1.0	9.5	9.2	6.7	0.9	0.9	0.8
LSD (5%)	3.0	4.0	2.0	34.5	65.2	26.1	1.9	3.8	13.3
CV (%)	0.5	0.5	0.5	3.6	2.7	1.7	0.2	0.2	0.01
	20.4	17.3	21.7	12.5	7.1	7.9	8.6	5.4	3.9

[x]Rating scale of 1-5 where 0 = no galls on root and 5 = > 75% roots with galls.
[y]$\sqrt{(x+0.5)}$ transformed before analysis.
[z]Reproduction factor = final population (Pf)/Initial population (Pi), where Pi = 375, 300 and 550; for Tono, Vea, and Pwalugu, respectively.

Experiment 2: Tomato and pepper bioassays for assessing root-knot nematode population in soil

The initial root-knot nematode juvenile population density from Vea, Tono, and Pwalugu were 300, 375, and 500/100 cm^3, respectively. No root galls were observed on any of the exotic tomato cultivars while Power, the local susceptible check, had gall scores of 2, 3, and 4 for Pwalugu, Tono, and Vea, respectively (Table 2). The mean number of *Meloidogyne* spp. eggs produced on the tomato cultivars ranged from 3.2 to 65.2/5 g root with the control cultivar recording the highest and the least by Small Fry. Only Power had a reproduction factor greater than 1.0 in soils from Tono, Vea, and Pwalugu, and it is thus considered to be susceptible to the root-knot nematodes.

Root gall score, *Meloidogyne* spp. egg count, and reproduction factors of the exotic pepper cultivars grown on soils collected from Tono, Vea, and Pwalugu were low (Table 2) for the bioassay. Galling scores of 1 were observed for all the exotic pepper cultivars, while Ohene Sateaa, the susceptible local check, had gall scores of 1 to 3. The mean number of *Meloidogyne* spp. eggs of the pepper cultivars ranged from 5.0 to 33.1/5 g root with the control cultivar having the highest and Charleston Belle having the least reproduction. Ohene Sateaa was the only cultivar that had reproduction factors greater than 1.0.

Molecular identification of Meloidogyne *species extracted from field tomato and pepper cultivars and soil bioassays*

The molecular identification of the juveniles extracted from the tomato and pepper cultivars indicated only tropical *Meloidogyne* spp. A few of the *Meloidogyne* spp. populations were *M. incognita*, and most were *M. javanica* with some *M. arenaria* (Table 3).

DISCUSSION

The nematode counts in Power and Ohene Sateaa cultivars, the local tomato and pepper checks, were higher than in the exotic root-knot nematode-resistant tomato cultivars, indicating that the local cultivars were susceptible to the *Meloidogyne* spp. that were present. It has been reported that highly susceptible host plants allow *Meloidogyne* spp. juveniles to enter the roots (Hirunsalee *et al.*, 1995; Karssen and Moens, 2006), reproduce, and produce severe root galling while resistant plants suppressed nematode development and thus, did not allow reproduction. Our study revealed that the resistant tomato and pepper cultivars are effective against the *Meloidogyne* spp. in Tono, Pwalugu, and Vea since the tomato and pepper cultivars did not form galls and *Meloidogyne*

Table 3. Tropical *Meloidogyne* species identified from locations and on tomato and pepper cultivars from the bioassay studies and bulked soil.

Cultivar or Location	Species
Small Fry	*M. javanica* and *M. arenaria*
Jetsetter	*M. javanica* and *M. arenaria*
Celebrity	*M. javanica* and *M. arenaria*
Power (local check)	*M. incognita*, *M. javanica*, *M. arenaria*
Carolina Cayenne	*M. javanica* and *M. arenaria*
Carolina wonder	*M. javanica* and *M. arenaria*
Charleston Belle	*M. javanica* and *M. arenaria*
Ohene Sateaa (local check)	*M. incognita*, *M. javanica*, *M. arenaria*
Tono	*M. incognita*, *M. javanica*, *M. arenaria*
Vea	*M. incognita*, *M. javanica*, *M. arenaria*
Pwalugu	*M. incognita*, *M. javanica*, *M. arenaria*

spp. reproduced very poorly on them. It is likely that the resistant tomato and peppers would yield better than the susceptible local cultivars since the resistant cultivars should have healthy roots, allowing them to absorb water and nutrients more efficiently. Fery and Dukes (1984) reported that marketable yields from resistant cultivars are significantly higher than those from susceptible cultivars grown in nematode-infested soils.

According to Roberts (2002), the Mi gene that confers resistance to *Meloidogyne* spp. in tomato is sensitive to temperature, with almost total loss of expression at or above 28º to 30ºC. However, in our study, soil temperatures of 32ºC were recorded 2½ mon post transplanting when the plants had started fruiting so the high temperature did not seem to adversely affect the resistance of the plants in this study. Nematode identification indicated that the *Meloidogyne* spp. populations were a mixture of species, primarily *M. incognita* and *M. arenaria*. *Meloidogyne incognita* was recorded in only the local checks, Power and Ohene Sateaa, which could indicate that the resistant cultivars were more effective against *M. incognita*.

In summary, the resistant tomato and pepper cultivars were resistant to the root-knot nematodes in the field while the local checks, Power and Ohene Sateaa, were susceptible. There was no root-knot nematode reproduction on the resistant tomato and pepper cultivars from soils collected from Tono, Vea, and Pwalugu. Temperature did not seem to have an effect on the resistant tomato and pepper cultivars evaluated. In light of the above findings, the root-knot nematode resistant tomato and pepper cultivars could be grown successfully in Ghana. However, further evaluation is recommended.

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