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Full Length Research Paper

Host-status and host-sensitivity of *Cucumis africanus* and *Cucumis myriocarpus* to M*eloidogyne incognita* race 2 under greenhouse conditions

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Four economically important genera in the Cucurbitaceae family, namely *Citrullus*, *Cucumis*, *Cucurbita* and *Langenaria*, have no resistance to *Meloidogyne* species, which cause extensive crop losses. Certain wild, but economically unimportant genera in this family have the potential for use as seedling rootstocks in vegetable husbandry. Preliminary studies suggested that wild watermelon (*Cucumis africanus)* and wild cucumber (*Cucumis myriocarpus)* were compatible with selected nematode-susceptible watermelon *(Citrullus lanatus*) cultivars. Therefore, the host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* to *Meloidogyne incognita* race 2 were investigated to determine if they had the potential to serve as seedling rootstocks in suppression of nematodes in watermelon husbandry. The eight nematode levels were arranged in a randomised complete block design with 5 replicates. Fifty-six days after inoculation, the reproductive factors of *M. incognita* race 2 on both plant species were less than one, whereas the two *Cucumis* species did not suffer any yield loss in response to nematode infection. Consequently, *C. africanus* and *C. myriocarpus* are resistant to *M. incognita* race 2 and could possibly serve as a seedling rootstock to *C. lanatus* cultivars in the management of population densities of this nematode race.

Key words: Cucurbitacin, indigenous plants, plant-parasitic nematodes, nematicides, quadratic relationship.

INTRODUCTION

The Cucurbitaceae family contains 115 genera that include four economically important genera, *namely, Citrullus*, *Cucumis*, *Cucurbita* and *Langenaria* (Pitrat et al., 1999). Yield losses due to plant-parasitic nematodes in the four genera run into millions of US dollars in various parts of the world with tropical climate and sand (Davis, 2005). In watermelon (*Citrullus lanatus)* cultivars and cantaloupe (*Cucumis melo*) resistance to the root-knot nematode (*Meloidogyne* spp.) has not been identified (Davis, 2005; Fassuliotis, 1967; Thomason and McKinney, 1959), suggesting the need for evaluating compatible seedling rootstocks within the family. Certain wild species in the family, for instance, the "fig-leafed" gourd (*Cucurbita ficifolia)* and the African-horned cucumber (C*ucumis metuliferus)* contain some resistance to *Meloidogyne incognita acrita* (Fassuliotis, 1970). Attempts to introgress nematode-resistant genomes from the wild species to the economically important species had not been successful (Fassuliotis, 1977).

The genus *Cucumis* has the centre of diversity in South Africa (Kristkova et al., 2003), where wild species are widely used for local medicines and food. The wild watermelon (*Cucumis africanus*) and the wild cucumber (*Cucumis myriocarpus*) are renowned for containing the bitterest substance known to man, which had been identified as cucurbitacin (Van Wyk et al., 2002). In *C. africanus* all parts of the plant contain this chemical compound, whereas in *C. myriocarpus* the material is in roots and fruits (Van Wyk et al., 2002). Extracts of ground *C. africanus* and *C. myriocarpus* fruits had been successfully used for the suppression of *M. incognita* race 2 in vegetable husbandry (Mashela, 2002; Mashela and Mphosi, 2001), with the efficacy of the material being

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comparable to that of synthetic nematicides, *viz*. aldicarb and fenamiphos (Mashela et al., 2008). The active ingredients were identified as Cucurbitacin A and B in *C. myriocarpus* and *C. africanus*, respectively (Van Wyk et al., 2002) which is the only cucurbitacin that is soluble in water (Chen et al., 2005). Suppression of plant-parasitic nematodes *in vivo* by these plant species which would confer them the potential status of being used as seedling rootstocks for *Citrullus* and other economic *Cucumis* species not documented. The objective of this study was to investigate the host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* to *M. incognita* race 2 in order to establish whether they could serve as seedling rootstocks for the management of nematode numbers.

MATERIALS AND METHODS

Study area and period

The study took place in the greenhouse at the Plant Protection Skills Centre, University of Limpopo, in the Limpopo Province of South Africa (23°53'10"S, 29°44'15"E). Ambient day/night temperatures averaged 28/21°C, with maximum temperatures controlled using thermostatically-activated fans. The two trials were conducted in summer (2008) and repeated in autumn (2009).

Growth condition and inoculum

Thirty-cm-diameter plastic pots, filled with 10 L steam-pasteurised sand and Hygromix (3:1 v/v), were placed on greenhouse benches at 0.5 m inter-row and 0.6 m intra-row spacing. Two days before transplanting 3 g 2:3:2 (22), 2 g 2:1:2 (43) and half-strength Hoagland solution (Hoagland and Arnon, 1950), were mixed into the topsoil in each pot. When required, *M. incognita* race 2 inoculum was prepared by extracting eggs and juveniles from roots of greenhouse-grown nematode-susceptible tomato (*Lycorpersicon esculentum)* cv. Floradade plants in 10% NaOCl (Hussey and Barker, 1973).

Preparation of material, experimental design and irrigation

Fruits of *C. africanus* and *C. myriocarpus* were collected from the local field, cut into pieces, seeds removed and shade-dried for 14 days. Seeds were separately wrapped in hand-sewn cotton handkerchief bags and submerged in running tap-water for 8 h to leach out the germination-inhibiting chemicals prior to planting in seedling trays containing Hygromix growing medium (Mafeo and Mashela, 2009). Uniform three-week-old, nematode-free *Cucumis* seedlings were transplanted to the pots for separate studies one day after irrigating the growing medium to field capacity. A day after transplanting, pots were each infested by dispensing approximate numbers of *M. incognita* eggs and juveniles using a 20-ml plastic syringe by placing into 5-cm-deep holes on the cardinal points of the stem of the plants per replication. The zero untreated control plants received filtrate (25-µm-mesh sieve) of nematode suspension to establish any microbes associated with *M. incognita* in their rhizosphere. The treatments, *viz*. 0, 500, 750, 1, 000, 1,250, 1, 500, 1,750 and 2000 eggs and juveniles, were arranged in a randomised complete block design with 5 replicates. Two sets of Hadeco moisture meter were inserted to 20-cm depths in randomly selected pots of each treatment to monitor soil moisture tension. Plants were irrigated with 1000 ml tap-water as soon as 50% of the moisture meters have readings just below 2 units.

Data collection

At harvest, 56 days after inoculation, plant length was measured from the crown to the tip of the longest runner, shoots cut at the soil level and stem diameters measured 5 cm above the severed ends using a digital vernier calibre. Shoots were oven-dried for 72 hours at 70°C and weighed. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density per total roots per plant. Root galls, when necessary, were assessed using the North Carolina Differential Scale of 1 = no galls, 2 = 1 - 10 galls, 3 = 11 - 100 galls and 4 = > 100 galls (Taylor and Sasser, 1978). All collected roots were separately weighed. Nematodes were extracted from 5 g roots per plant by maceration and blending for 30 seconds in 1% $NaOCl_2$ (Hussey and Barker, 1973) and passed through top-down nested 150, 45 and 25-µm mesh sieves. Contents of the 25-µm mesh sieve were poured into 100-ml plastic containers for counting under a stereomicroscope.

Soil per pot was thoroughly mixed and a 250 ml soil sample was collected. Nematodes were extracted from soil samples using the modified sugar-floatation and centrifugation method (Coolen and D'Herde, 1972). The soil sample was washed through a 45-µm-aperture sieve into a bucket, which was then filled with water and mixed in a swill. After the swill had stopped, the aliquot was poured through a 25-µm sieve, with the contents being washed into 100-ml plastic centrifuge tubes. A teaspoon of kaolin was then added in each tube and contents centrifuged at 1 750 RPM for five minutes. The kaolin solution was then decanted with nematodes having settled at the bottom of the tubes with soil particles. A 469 g sugar/L tap-water was poured into the centrifuge tubes and stirred once prior to centrifuging for one minute at 1 750 RPM. The aliquot was then decanted onto 25-µm sieve; sugar was rinsed off the nematodes, which were then collected from the 25-µm sieve into 100-ml plastic containers for counting under a stereomicroscope. During counting, which was completed in less than 10 days, samples were stored at 5°C. Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 10 L soil per pot. Reproductive factors (RFs), described as final population/initial population numbers, were computed.

Data analysis

Prior to analysis of variance (ANOVA), nematode data were transformed through $\log_{10}$ (x + 1) to homogenise the variances (Gomez and Gomez, 1984). Data were subjected to ANOVA through the SAS software (SAS Institute, Inc., Cary, NC., U.S.A.) to determine the effects of initial population densities (Pi) of nematodes on the RFs and the yield components. Mean separation for significant ($P \leq 0.05$) treatments was achieved through the Duncan's multiple-range test. Lines of the best fit were determined for RFs over the $\log_{10}$ transformed Pi values. Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

RESULTS

The interaction between seasons for each *Cucumis* species was not significantly different and data were pooled and subjected to statistical analysis. Generally, a higher number of nematode juveniles occurred inside of

Table 1. Responses of final population densities (Pf) and the reproductive factors (RF) of *M. incognita* race 2 to five levels of initial population densities (Pi) on *C. africanus* and *C. myriocarpus* under greenhouse conditions.

Treatment (Pi)	*C. africanus*				*C. myriocarpus*			
	Total soil	Total roots	Pf	RF	Total soil	Total roots	Pf	RF
500	41	164	205	0.41	7	224	231	0.46
750	42	208	285	0.38	7	280	293	0.39
1 000	42	251	293	0.29	7	336	343	0.34
1250	37	358	388	0.31	6	313	325	0.26
1 500	32	465	497	0.33	4	290	294	0.20
1750	40	334	368	0.21	6	317	333	0.19
2 000	48	203	251	0.11	7	344	351	0.18
$LSD_{0.05}$				0.074				0.15

Data pooled over two seasons (n = 10).

Table 2. Responses of dry shoot weight, fresh root weight, plant length and stem diameter to five levels of initial population densities (Pi) of *M. incognita* race 2 on *C. africanus* and *C. myriocarpus* under greenhouse conditions.

Treatment	*C. africanus*				*C. myriocarpus*			
	Dry shoot mass (g)	Dry root weight (g)	Plant length (m)	Stem diameter (mm)	Dry shoot mass (g)	Dry root mass (g)	Plant length (m)	Stem diameter (mm)
0	189.32	4.83	10.09	4.57	61.60	3.55	14.85	8.87
500	189.95	4.74	10.14	4.66	53.23	3.26	15.10	8.35
750	187.60	4.4	10.40	4.86	51.44	3.35	15.10	8.22
1 000	185.24	4.06	10.65	5.06	49.64	3.44	15.10	8.09
1250	192.49	4.15	10.53	5.02	50.52	3.54	15.34	8.14
1 500	199.74	4.24	10.41	4.98	51.39	3.63	15.58	8.18
1750	188.46	4.24	10.44	4.97	53.51	3.42	14.60	8.48
2 000	177.18	4.24	10.47	4.95	55.63	3.21	136.17	8.77

Data pooled over two seasons (n = 10).

roots of both *Cucumis* species. In both species, the RFs of *M. incognita* race 2 were less than unity (Table 1). Also, in both species, the RFs and the Pi had negative quadratic relationships. In *C. africanus*, the inoculums level contributed 86% to the total treatment variation (TTV) in the reproductive factors, whereas in *C. myriocarpus*, this source contributed 99% to the TTV in the RFs (Figure 1). In both plant species, the treatment had no effect on dry shoot weight, dry root weight, plant length and stem diameter (Table 2).

DISCUSSION

The higher number of nematode juveniles in the roots of *Cucumis* species than in the soil confirms the view that nematodes locate and penetrate roots of nematode-susceptible and nematode-resistant plants equally (Acedo et al., 1984; Huang, 1986; Ibraham et al., 1980; Raja and Dasgupta, 1986; Steele and Savitsky, 1981; Weischer, 1982). However, there are cases where penetration was prevented (Caswell et al., 1991; McSorley and Gallaher, 1991; Ploeg, 1999; Roberts, 1993; Siddiqui and Alam, 1988; Veech, 1981). The observation reduced RF values at all Pi levels, suggested that nematode-resistant between the two *Cucumis* species and *M. incognita* race 2 was post-infectional.

In plant-parasitic nematodes, nematode-resistance is described using two concepts: (1) host-status and (2) host-sensitivity (Seinhorst, 1965). Host-status is described using the RF, which is a measure of the reproductive potential of a nematode on a given host (Windham and Williams, 1988). All RFs below unity suggest that the nematode failed to reproduce on a given host, whereas values above one indicate that the nematode reproduced. In this study, all RFs of *M. incognita* race 2 on *C. africanus* and *C. myriocarpus* were below unity. Interpretation of the RF values requires the rates (Seinhorst, 1967). In support of this view, in their study of the citrus nematode (*Tylenchulus semipenetrans*) below

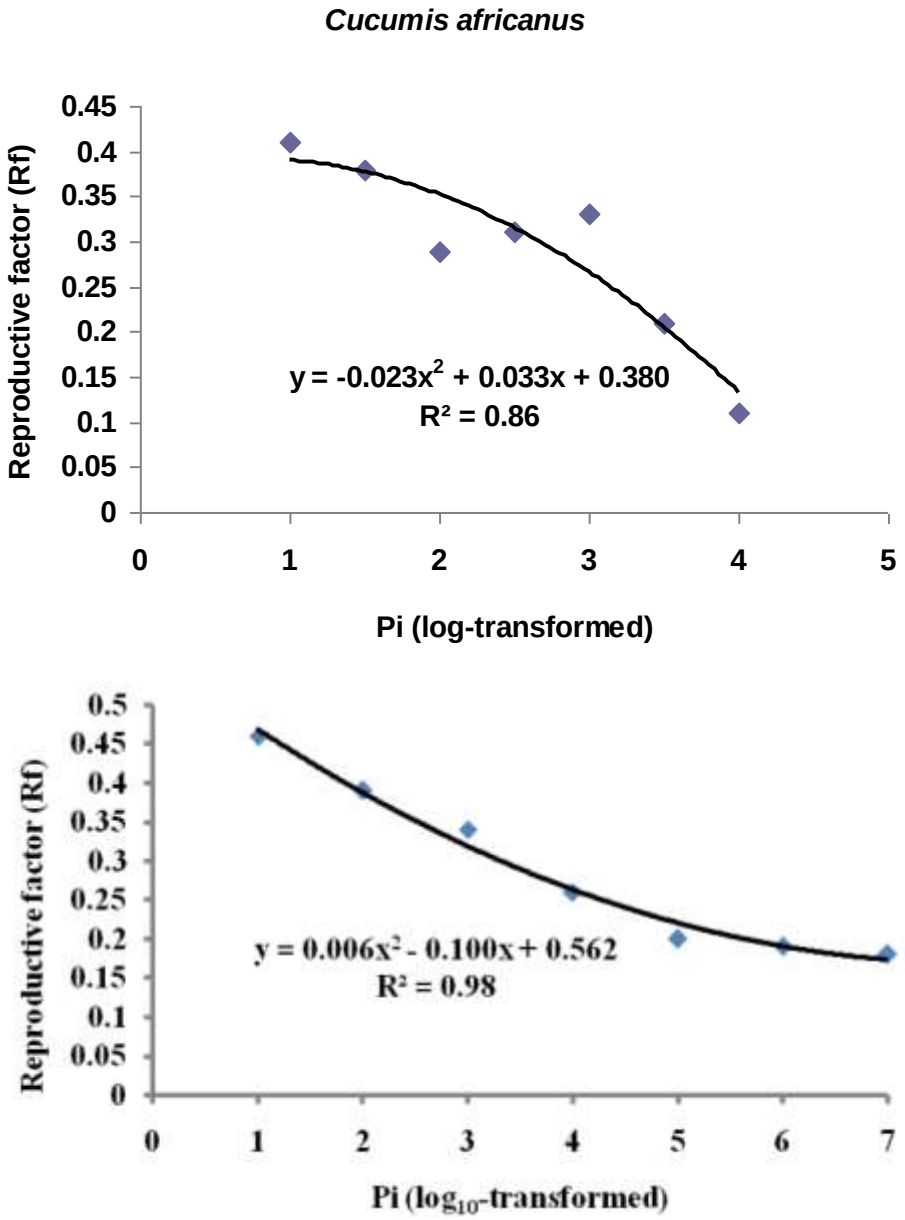

Figure 1. Relationship between reproductive factors of *M. incognita* race 2 and initial nematode level (Pi) on roots of *C. africanus and C. myriocarpus* under greenhouse conditions.

appreciation of the impact equilibrium point (E), nematode reproductive rate. Beyond E all RF values are unity since competition for infection sites is intense, with the consequent of reduced reproductive race in South Africa, Kwaye et al. (2008) used Pi that was far above E on differential hosts that were hosts and non-hosts with the resultant RF values being all below unity. In other words, the Pi used in determining the host-status must invariably be less than E. Duncan and McSorley (1987) argued that for most plants where the RF is greater than unity at Pi values below E, the plants are hosts regardless of whether populations decrease at time - related final populations. This argument suggested that even at inoculums levels of Pi lower than E, with increasing infection time, E may be attained, resulting into a situation where the RFs are below unity, with the subsequent inaccurate inference that the plant was a non-host.

In this study, the E points of *M. incognita* race 2 for both *C. africanus* and *C. myriocarpus* are not documented. Consequently, a series of Pi levels were used and the fact that the RFs were below unity at all inoculum's levels, one can safely infer that this was due to the incompatibility between the test nematode race and the test species. Also, to guide against the impact of time-

related final populations, which may lead to the misinterpretation of RF values due to the cyclic nature of Pf values on various crops (Ferris, 1985), the experiments were harvested 56 days after inoculation. This duration allowed for approximately three reproductive cycles for this nematode species (Sikora and Fernandez, 2005). The negative quadratic relationship between RF and Pi observed in this study support the view of the reproductive cycles in plant-parasitic nematodes. Host-sensitivity describes the responses of hosts to nematode infection (Seinhorst, 1967). When the RF is greater than unity and the plant suffers yield loss, the plant is described as a susceptible host, whereas a host that allows nematodes to reproduce but does not incur yield loss is referred to as a tolerant host. However, if RF is less than unity and there is no yield loss, the test plant is said to be resistant (Seinhorst, 1967). Using the Seinhorst model, both *C. africanus* and *C. myriocarpus* were resistant to *M. incognita* race 2 since the RF values were less than unity and the two species did not suffer any yield losses in response to nematode infection.

Conclusion

Results of this study suggested that *C. africanus* and *C. myriocarpus* were resistant to *M. incognita* race 2. Consequently, these plant species have the potential for use as seedling rootstocks for genera with comparable rootstocks such as *Citrullus* species in the management of this nematode race.

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