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*Full Length Research Paper*

# Using relative penetration and maleness indices in *Meloidogyne incognita* to establish resistance type in *Cucumis myriocarpus*

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**Resistance in plant-parasitic nematodes is broadly classified as pre-infectional or post-infectional. Pre-breeding establishment of resistance type in plant-parasitic nematodes is essential where germplasm is to be introgressed into the rootstock breeding lines since only post-infectional resistance is introgressible. A study was conducted to determine whether resistance in wild cucumber (*Cucumis myriocarpus*) rootstocks to the southern root-knot nematode (*Meloidogyne incognita*) was pre- or post-infectional. Seven treatments: 0, 200, 600, 1000, 1400, 1800 and 2200 eggs and juveniles, were arranged in a randomised complete block design with 10 replicates. At harvest, 56 days after inoculation, the relative penetration index [RPI = ($Pf_{in\ root}/Pf_{in\ soil}$) – 1] and the relative maleness index [RMI = ($Pf_{total\ males}/Pf_{total\ females}$) – 1] were each greater than one, with the reproductive factors of *M. incognita* also being less than one at all levels, without any yield loss. The RPI and RMI suggested that resistance in *C. myriocarpus* to *M. incognita* was post-infectional. Thus, the resistance germplasm in this plant could be introgressed into wild watermelons in the development of nematode-resistant rootstock lines for the management of *M. incognita* race 2 in highly nematode-susceptible watermelon cultivars.**

**Key words:** *Citrullus lanatus*, nematode resistance, reproductive potential, watermelon.

## INTRODUCTION

The southern root-knot nematode (*Meloidogyne incognita*), which is a sedentary endo-parasitic nematode, causes yield losses in watermelon (*Citrullus lanatus*) that run into millions of US dollars (Davis, 2005; Koenning et al., 1999). Commercially grown watermelon cultivars lack resistance to *M. incognita* (Thies, 1996). Wild cucumber (*Cucumis myriocarpus*) seedlings are highly resistant to *M. incognita* race 2 (Pofu et al., 2010). Evidence suggests that *C. myriocarpus* seedling rootstocks are highly compatible to scions of *C. lanatus* cultivars 'Congo' and 'Charleston Gray' (Pofu et al., 2010).

Resistance mechanisms against plant-parasitic nematodes can be described as being either pre- or post-infectional. Sorghum (*Sorghum bicolor*), cowpea (*Vigna unguiculata*), marigold (*Tagetes species*), castor bean (*Ricinus communis*), velvet bean (*Mucuna pruriens*) and sunn hemp (*Crotalaria juncea*) released chemicals into the rhizosphere that avoid infection by nematode juveniles prior to infection (McSorley and Gallaher, 1991; Roberts, 1993). In contrast, rootstocks such as *Poncirus trifoliata* and *Swingle citrumelo* (Citrus paradisi × *P. trifoliate)*, allow nematode juveniles of the citrus nematode (*Tylenchulus semipenetrans*) to penetrate the root system and prevent damage by having cells around the nematode undergoing hypersensitivity.

The first step in determining whether resistance germplasm in plant-parasitic nematodes is introgressible is to establish whether resistance is pre- or post-infectional. Generally, only post-infectional resistance is introgressible. The objective of this study was to use relative penetration and maleness indices to determine whether resistance of *C. myriocarpus* to *M. incognita* race 2 was pre- or post-infectional.

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**Abbreviations: RPI,** Relative penetration index; **RMI,** relative maleness index; **RFs,** reproductive factors.

## MATERIALS AND METHODS

### Study location and growth conditions

This study was initiated in the greenhouse at the Plant Protection Skills Centre, University of Limpopo, in Limpopo Province, South Africa (23°53'10"S, 29°44'15"E). Minimum and maximum ambient temperatures were 19 - 20 and 25 - 27°C, respectively, with the maxima controlled using thermostatically-activated fans. A 14:10 LD photoperiod was maintained for the duration of the study, which was initiated in September 2009 and repeated in December 2009.

### Raising seedlings, experimental design and inoculation

Fruits of *C. myriocarpus* were locally collected from the field, seeds were removed and auto-allelochemicals were leached out in running water (Mafeo and Mashela, 2009). Seedlings were raised in seedling trays containing Hygromix (Hygrotech, South Africa). 20 cm diameter plastic pots (3000 ml) were filled with 2700 ml steam-pasteurised sand and Hygromix (3:1 v/v) and placed on green-house benches at 0.25 m inter-row and 0.25 m intra-row spacings. Two days before transplanting, each pot was fertilised using 0.9 g 2:3:2 (22), 0.6 g 2:1:2 (43) and half-strength Hoagland solution (Hoagland and Arnon, 1950) and irrigated to full capacity.

When required, *M. incognita* race 2 inoculum was prepared by extracting eggs and juveniles from roots of greenhouse-grown nematode-susceptible Kenaf (*Hibiscus cannabinus*) plants in 10% NaOCl. Seven treatments: 0, 200, 600, 1000, 1400, 1800 and 2200 eggs and juveniles, were arranged in a randomised complete block design with 10 replicates.

Uniform three-week-old, nematode-free seedlings were transplanted to the pots one day after irrigating to field capacity. A day after transplanting, pots were each infested by dispensing approximate numbers of *M. incognita* 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. Untreated control plants received filtrate (25-µm-mesh sieve) of inoculum suspension to establish in their rhizosphere any microbes associated with *M. incognita* race 2. Plants were irrigated with 500 ml tap-water every other day and aphids were sprayed with 0.75 ml/l water of aphicide (Dimethoate 400 g/l) when necessary.

### Data collection

At harvest, 56 days after inoculation, stolon lengths were measured from the crowns to the tips, cut at the soil level and stem diameters were measured at 5 cm above the severed ends using a digital vernier calibre. Roots were removed from pots, immersed in water to remove soil particles, blotted dry and weighed. When necessary, rating of galls per root system was based on the scale of 0 to 5, where 0 = absence of galls, 1 = 1 to 2 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls, and 5 being greater than 100 galls (Taylor and Sasser, 1978). Nematodes were extracted from 5 g roots using maceration-blending and sugar-flotation methods (Hussey and Barker, 1973). Aliquots were passed through nested 60 and 38 µm mesh sieves, with contents of the 38-µm sieve poured into 100-ml plastic containers for counting under a stereo microscope. Shoots and the remaining roots were oven-dried at 70°C for 72 h and weighed. Fresh roots from untreated controls were weighed and dried to allow for the estimation of the dry weight of roots in infected treatments.

Soil per pot was mixed, a 250-ml soil sample was collected and nematodes extracted using the modified sugar-flotation and centrifugation method (Coolen and D'Herde, 1972). Briefly, the soil sample was washed through a 60 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 38-µm mesh sieve, with the contents being washed into 100-ml plastic centrifuge tubes. A teaspoon of kaolin was then added and contents centrifuged at 1800 rpm for five minutes (Jenkins, 1964). Kaolin solution was decanted with nematodes having settled at the bottom of the tubes with soil particles. Approximately 469 g sugar/l water was poured into the centrifuge tubes and stirred prior to centrifuging for one minute. The aliquot was then decanted onto 38-µm sieve and sugar was rinsed off the nematodes. Juveniles were then collected from the 38-µm sieve into 100-ml plastic containers for counting under a stereomicroscope. During counting, samples were stored at 5°C for less than 10 days. Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 2700 ml soil per pot, to allow for the computation of the reproductive factors (RFs).

### Data analysis

Prior to analysis of variance (ANOVA) through the SAS software, nematode data were transformed through $\log_2(x+1)$ to homogenise the variances (Gomez and Gomez, 1984), with mean separation for significant ($P \leq 0.05$) treatments being determined through the Waller-Duncan multiple range test (SAS Institute, 1985). Lines of the best fit were determined between the RFs and $\log_2$ – transformed P*i*. Relative penetration and relative maleness indices were computed using the relationships $RPI = (Pf_{in\ root}/Pf_{in\ soil})-1$ and $RMI = (Pf_{total\ males}/Pf_{total\ females})-1$, respectively. Unless otherwise stated, the treatment effects were significant at the probability level of 5%.

## RESULTS

The RPI and the RMI were each higher than unity in both experiments (Table 1). The reproductive factors (RFs) at all levels of inoculation were less than one and when fitted against the transformed-P*i* values, negative quadratic relationships occurred (Figure 1). Treatments contributed 94% to the total treatment variation in the RFs.

Root systems remained mainly fibrous, with almost 98% roots being gall-free, whereas visible galls were small and undeveloped. Nematode treatments at all levels had no significant effect on dry shoot weight, stolon length, dry root weight and stem diameter in both experiments (Table 2).

## DISCUSSION

The high RPI values suggested that a large number of juveniles entered the roots of *C. myriocarpus*. Generally, in *Meloidogyne* species, upon egress, the infective second stage juveniles (J2s) migrate from the roots into the soil and invade the new roots from outside (Ferraz and Brown, 2002). The high number of J2s inside roots suggested the failure of reproduction by this nematode in *C. myriocarpus* and the subsequent migration into the soil. In *Meloidogyne* spp., the J2s neither grow nor develop to subsequent stages when feeding is not allowed (Ferraz and Brown, 2002).

Results of this study confirm those of *Rotylenchulus*

**Table 1.** Relative penetration index (RPI) and relative maleness index (RMI) of second stage juveniles of *M. incognita* race 2 into roots of *C. myriocarpus* from the inoculum in the soil.

| Experiment | Relative penetration index | | Relative maleness index | |
|---|---|---|---|---|
| | Source | Number of nematode | Gender | Number of nematode |
| 1 | Total soil | 72 | Female | 6 |
| | Total root | 188 | Male | 14 |
| | RPI[y] | 1.61 | RMI[z] | 1.30 |
| 2 | Total soil | 110 | Female | 8 |
| | Total root | 311 | Male | 22 |
| | RPI | 1.83 | RMI | 1.75 |

[y]RPI = ($Pf_{in\ root}/Pf_{in\ soil}$)–1; [z]RMI = ($Pf_{total\ males}/Pf_{total\ females}$)–1

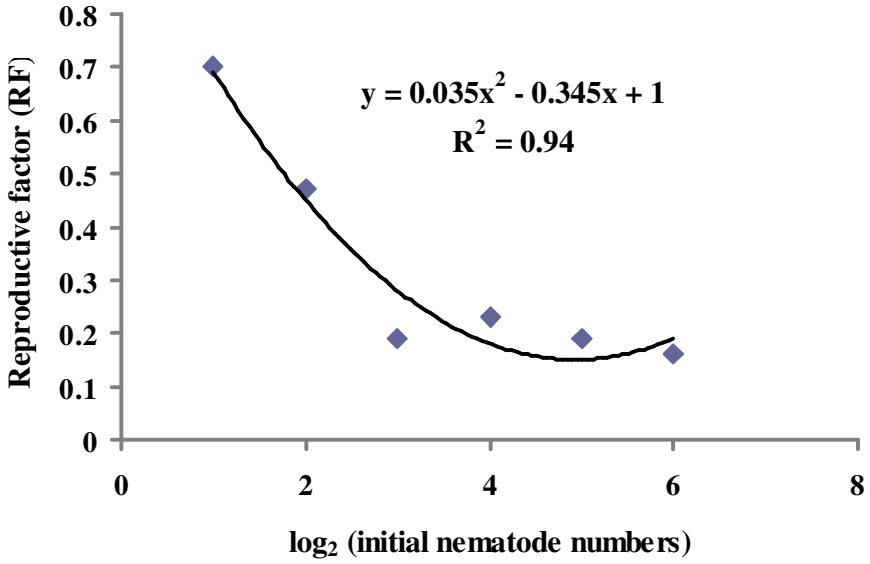


**Figure 1.** Relationship between reproductive factors of *M. incognita* race 2 and initial nematode level (Pi) in roots of *C. myriocarpus*.

**Table 2.** Response of dry shoot mass (DSM), stolon length (SL), dry root mass (DRM) and stem diameter (SD) of *C. myriocarpus* to initial population levels (Pi) of inoculation with *M. incognita* race 2.

| Pi | Experiment 1 | | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | DSM (g) | SL (cm) | DRM (g) | SD (mm) | DSM (g) | SL (cm) | DRM (g) | SD (mm) |
| 0 | 9.57 | 108.17 | 2.01 | 5.40 | 53.95 | 826.00 | 2.38 | 4.00 |
| 200 | 10.59 | 116.25 | 1.82 | 5.79 | 55.42 | 836.33 | 2.41 | 4.38 |
| 600 | 11.15 | 132.00 | 1.89 | 5.06 | 59.14 | 794.00 | 2.81 | 4.70 |
| 1000 | 11.15 | 128.33 | 2.00 | 5.39 | 59.54 | 756.17 | 2.39 | 4.07 |
| 1400 | 9.79 | 136.00 | 2.17 | 5.18 | 54.11 | 692.50 | 2.11 | 4.55 |
| 1800 | 10.11 | 118.33 | 2.28 | 5.33 | 53.65 | 709.17 | 2.22 | 4.50 |
| 2200 | 9.84 | 146.67 | 2.71 | 5.36 | 52.46 | 729.67 | 2.28 | 4.47 |
| $LSD_{0.05}$ | 3.11 | 35.23 | 0.60 | 0.73 | 12.61 | 279.80 | 0.84 | 0.69 |

*reniformis* in *Tagetes patula* and *Meloidogyne* juveniles in *Tagetes erecta* (Caswell and Robert, 1991; Ploeg and Marris, 1999), *Pratylenchus* spp. in *Targetes species* (Siddiqui and Alam, 1988; Veech, 1981), *M. incognita acrita* in some wild races of "fig-leafed" gourd (*Cucurbita ficifolius)* and African horned cucumber (*Cucumis*

*metuliferus)* plants (Fassuliotis, 1970), which were all post-infectional. In post-infectional nematode-resistance, resistance against nematodes is expressed after penetration into roots (Kaplan and Keen, 1980).

Sex conversion when J2s cannot establish a feeding site is common in *Meloidogyne* species. Fassuliotis (1970) observed sex shifts when *M. incognita acrita* J2s were unsuccessful in establishing feeding sites in roots of *Cucurbita ficifolius* and *C. metuliferus.* Results of this study confirmed those of Fassuliotis (1970) where necrotic tissues on roots due to hypersensitivity were not noticeable. Generally, in hypersensitivity reactions, cells around the nematode wither and isolate the nematode from advancing into healthy cells.

In plant-parasitic nematodes, reproductive factor (RF) values of less than one, along with failure of the test nematode to reduce yield components, fit the description of nematode resistance (Ferris et al., 1993) and confirm results observed previously (Pofu et al., 2010). The quadratic relationship between RFs and Pi suggested that the nematode RF, regardless of the host-status, is density dependent (Ferris and Wilson, 1987).

In conclusion, non-host status of *C. myriocarpus* to *M. incognita* race 2 is primarily related to three factors viz: (1) failure of J2s to establish a feeding site, (2) inhibition of J2s to develop beyond this stage and (3) conversion of J2s to males. Since the RPI and the RMI were each greater than unity, resistance to *M. incognita* race 2 in *C. myriocarpus* seedlings is essentially post-infectional and therefore, introgressible.

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