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Host status and host sensitivity of Kickapoo white tepary bean to *Meloidogyne enterolobii*

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ABSTRACT

Tepary bean (*Phaseolus acutifolius* A. Gray) is a drought tolerant leguminous crop and due to its multi-purpose use, it can easily be incorporated into different production systems. It being an undertilised crop, its production is usually relegated to poor sandy soils that are highly prone to root-knot nematodes (*Meloidogyne* species). Commonly communal producers of tepary beans use retained seeds with no knowledge of the nematode resistance status of the seeds. Hence the objective of the study was to determine whether *Meloidogyne enterolobii* will be able to reproduce on the Kickapoo white tepary bean and cause a reduction in the plant's growth variables. To achieve this objective, Kickapoo white tepary bean seedlings were exposed to 0, 25, 50, 125, 250, 625, 1250 and 3125 *M. enterolobii* eggs and second-stage juveniles (J2) in 2021 and validated in 2022 under shade-net conditions. At 56 days after inoculation, plant and nematode variables were collected and reproductive factor (Rf) was computed. Plant growth variables were not reduced whereas, nematode variables increased with an increase in nematode levels. In both experiments the Rf values were above a unity for all inoculation levels, indicating that nematodes were able to reproduce.. In conclusion, Kickapoo white tepary bean is tolerant to *M. enterolobii*.

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Host status; Kickapoo white tepary bean; *Meloidogyne enterolobii*; root-knot nematodes; host sensitivity

Introduction

Tepary bean (*Phaseolus acutifolius* A. Gray) is known as a drought-tolerant leguminous crop which originates from low rainfall areas in North-western Mexico and Southwestern US (López et al. 2005). The leguminous crop is well known for its low moisture requirements and its inclined adaptation to warm climates (López et al. 2005). Tepary bean has many health benefits, such as rich protein (24%), lowest glycaemic index, saturated fatty acids (67%) and important mineral elements; (Calcium (Ca), Copper (Cu), Magnesium (Mg)) (Bhardwaj and Hamama 2005). This leguminous crop has seeds containing phytochemicals that help alleviate health concerns such as cardiovascular diseases, colon cancer and coronary heart disease (Jiri and Mafongoya 2016). In crop-production systems, tepary bean is compatible with rotation systems because of its multi-purpose use (Jiménez et al. 2017).

Globally, most of the crop-production systems have been destroyed by the existence of aggressive root-knot nematodes (Pofu 2012). *Meloidogyne enterolobii* is a plant-parasitic nematode recently discovered to be highly aggressive in several cropping systems (Collett et al. 2021). The ability of this nematode to infect several plants and overcome the resistance mechanisms of hosts has been one of the major drawbacks to the management of field populations (Silva et al. 2017). Nematodes have generally been controlled using synthetic chemical nematicides. With most synthetic chemicals being withdrawn from the agrochemical market due to their health hazards and negative impact on the environment (Haq et al. 2020; Navarrete et al. 2018), there is a high demand to find alternatives that are both effective and environment-friendly (Onkendi et al. 2014). Currently, sustainable management strategies have been explored on *M. enterolobii* as an alternative solution. Rashidifard et al (2022) evaluated and reported the efficacy of soil microbiomes, associated with various subtropical fruit trees, on the management of a *Meloidogyne enterolobii* population. The use of nematode-resistant genotypes is one of the most environmentally safe and economically viable nematode management

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alternatives (Mashela et al. 2017). However, the use of nematode-resistant genotypes has been limited by several factors such as (i) partial information on the availability of compatible nematode management alternatives, (ii) unavailability of experience based on empirical data stipulating the extent of alternative crops' resistance to nematodes, (iii) inadequate information on the economic potential of nematode-resistant plants (Nzanza et al. 2013). Generally, the use of nematode resistance for managing nematode population densities should follow the identification of existing *Meloidogyne* species and biological races, with the initial plant being resistant to all existing species and biological races (Pofu 2012; Nkosi 2019). The aim of the study is to develop empirically-based information on the host status and host sensitivity of Kickapoo white tepary bean to selected thermophilic *Meloidogyne* species that occur in crop-production regions of South Africa.

Materials and methods

The study location and preparation of materials

The study was conducted at the University of Mpumalanga, Mbombela campus farm (27°26′14.9″S, 30° 59′53.2″E), Nelspruit suited in Mbombela, South Africa under a shade-net house. The site had a mean annual rainfall of less than 867 mm and minimum/maximum average temperatures of 10/38°C. Seeds of tepary bean cv. 'Kickapoo White' were sown in a 200-cell seeding tray with Hygromix (Hygrotech, Pretoria, South Africa) as a growing medium. Two weeks after emergence, seedlings were hardened off for two weeks by the intermediate withdrawal of water and then transplanted into individual 20-cm-diameter plastic pots, filled with approximately 2700 ml steam-pasteurised loam soil and sandy soil at a 3:1 (v/v) ratio. *Meloidogyne enterolobii* eggs and second-stage juveniles (J2) were extracted from the roots of greenhouse-cultured nematode-susceptible tomato (*Solanum lycopersicum* L.) cv. 'Rodade' in 1% NaOCl solution (Hussey and Barker 1973).

Experimental design and cultural practices

Two separate experiments were initiated in October–November of 2021 and validated in January–February of 2022. The treatments 0, 25, 50, 125, 250, 625, 1250 and 3125 eggs and J2s of *M. enterolobii* were laid out in a randomised complete block design, with eight replications. The treatments were applied using a 20 ml plastic syringe by placing into ca. 3 cm deep holes on cardinal points of the plant's stem. Three days succeeding transplanting 5 g of 2:3:2 (22) NPK fertiliser was used to fertilse each plant to support plant growth. Seedlings were inoculated 7 days after transplanting with respective nematode levels. Scouting for insect pests was performed every other day during the lifespan of the study and when observed necessary measures were deployed. The 20 cm pots were spaced at 30 cm intra × 30 cm inter-spacing.

Data collection

At 56 days after inoculation, plant height was measured from the crown to the tip of the flag leaf and the number of branches was recorded. The plants were cut at the soil line, with stem diameter measured at 5 cm above the severed end using a digital Vernier calliper and then oven-dried at 52°C for 72 h for dry matter determination. Root systems were taken out of pots, submerged in water to remove soil particles, and further blotted dry using a laboratory paper towel before being weighed. Root galls were assessed using the North Carolina Differential Rating scale, where 0 = no gall, 1 = 1–2 galls, 2 = 3–10 galls, 3 = 11–30 galls, 4 = 31–100 galls and 5 ≥ 100 galls (Taylor and Sasser 1978). Nematodes were extracted from total root systems per plant using the maceration and blending method in a 1% NaOCl solution (Hussey and Barker 1973). The blended materials were passed through nested 75 and 25 µm opening sieves, with contents from the 25 µm opening sieve collected for further processing using the sugar floatation and centrifugation method to separate debris from nematodes (Jenkins 1964). The soil from each pot was homogenised and a 250 ml soil subsample was collected for J2 extraction using the sugar floatation and centrifugation method (Jenkins 1964). Eggs and J2 from the root system, and J2 from 250 ml soil subsamples were each placed in 100 ml containers, with water added to the mark, stored in the cold room at 5°C and then counted from a 5-ml aliquot at 60× magnification under a stereomicroscope (Zeiss Stemi 2000, Model number: Z4SV240Vac) within 1 week of extraction (Makhado 2019). Total nematodes in the root system and total nematodes in the soil were generated by converting nematode counts from a 5-ml aliquot to total nematodes per root system and soil, respectively. Nematode numbers from the total root system and total soil were added to get the final nematode population densities (Pf). The reproductive factor (RF = Pf/Pi) was computed by dividing the final population density (Pf) by the initial population density (Pi) (Windham and Williams 1987).

Data analysis

Data were subjected to Shapiro–Wilk test to determine the normality of distribution of the data (Shapiro and

Table 1. Partitioning mean sum of squares of root galls (RG), eggs in roots, second-stage juveniles (J2) in roots, second-stage juveniles (J2) in soil, final population (Pf) and reproductive factor (RF) of tepary bean inoculated with *Meloidogyne enterolobii* under shade-net conditions.

	2021							2022						
	Root galls (RG)	Eggs in roots	J2s in roots	J2s in soil	Pf	RF		Root galls (RG)	Eggs in roots	J2s in roots	J2s in soil	Pf	RF	
Source	Df	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Reps	7	0.956	30.13	0.023	2.20	1.045	0.853	0.638	0.43	0.566	0.369	0.6621	1.523		
Treatment	7	0.605	19.07**	0.852	89.2***	119.011	97.16***	144.22	97.77***	150.835	98.25***	40.509	93.20***		
Errors	49	1.612	50.80	0.083	8.69	2.436	1.989	2.645	1.79	2.117	1.38	2.2936	5.28		
Total	63	3.173	100	0.955	100	122.491	100	147.51	100	153.52	100	43.465	100		
	2022													
	Root galls (RG)		Eggs in roots		J2s in roots		J2s in soil		Pf		RF			
Source	Df	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)		
Reps	7	0.021	7.81	0.049	0.370	0.684	0.490	0.0031	0.075	0.944	0.600	0.009	0.402		
Treatment	7	0.036	13.38^{ns}	10.79	81.09***	122.167	87.47***	4.0902	99.29***	149.184	94.84***	2.177	97.14***		
Errors	49	0.213	79.18	2.469	18.542	16.823	12.044	0.0260	0.6311	7.171	4.559	0.054	2.420		
Total	63	0.269	100	13.32	100	139.674	100	4.1193	100	157.299	100	2.241	100		

***Highly significant at $P \leq .01$, **significant at $P \leq .05$, ns: not significant, TTV (%): Total Treatment Variation, MS: Mean sum of squares, Df: Degree of freedom, Exp: experiment.

Table 2. Effects of initial nematode levels (Pi) on eggs in the root, second-stage juveniles (J2) in roots, second-stage juveniles (J2) in soil, final nematode population density (Pf) and reproductive factor (RF) of *Meloidogyne enterolobii* on tepary bean under shade-net conditions.

	2021						2022					
Treatment (Pi)	RG^{y}	Eggs in root	J2 in root	J2 in soil	Pf	RF	RG	Eggs in root	J2 in root	J2 in soil	Pf	RF
0	0.00^{c}	0.00 (0)^{f}	0.00 (0)^{f}	0.00 (0)^{d}	0.00 (0)^{f}	0.00 (0)^{f}	0.00^{b}	0.00 (0)^{e}	0.00 (0)^{g}	0.00(0)^{d}	0.00 (0)^{d}	0.00 (0)^{e}
25	0.14^{bc}	0.29 (8.8)^{e}	2.06 (153.1)^{e}	4.10 (15250)^{c}	4.1 (15412)^{e}	2.71 (616.5)^{a}	0.10^{a}	0.95(8.50)^{d}	1.67 (88)^{f}	4.45 (42650)^{c}	4.46 (42746)^{c}	3.06 (1710)^{a}
50	0.17^{abc}	0.30 (9.8)^{de}	2.50 (325.0)^{d}	4.28 (22000)^{c}	4.3 (22335)^{de}	2.59 (446.7$^{)a}$	0.01^{b}	1.02 (11.89)^{cd}	2.28 (375)^{e}	4.65 (45000)^{bc}	4.65 (45387)^{bc}	3.03 (1130)^{a}
125	0.35^{a}	0.31 (10.8)^{cde}	3.13 (1875.0)^{c}	4.29 (25700)^{c}	4.4 (27586)^{d}	2.25 (219.5)^{b}	0.06^{ab}	1.22 (15.89)^{bc}	2.90 (947)^{d}	4.78 (64000)^{ab}	4.79 (64963)^{bc}	2.56 (459)^{ab}
250	0.36^{a}	0.33 (14.0)^{bcd}	3.30 (2312.5)^{c}	4.54 (36500)^{b}	4.6 (38826)^{c}	2.18 (155.3)^{bc}	0.04^{ab}	1.27 (17.75)^{ab}	3.57 (5625)^{c}	4.97 (90000)^{ab}	4.97 (95643)^{ab}	2.45 (383)^{ab}
625	0.18^{abc}	0.35 (17.8)^{bc}	3.87 (7812.5)^{b}	4.70 (53500)^{ab}	4.8 (61330)^{bc}	1.97 (98.1)^{cd}	0.05^{ab}	1.34 (21.34)^{ab}	3.93 (8828)^{bc}	4.92 (89000)^{ab}	4.97 (97850)^{ab}	2.10 (157)^{b}
1250	0.31^{ab}	0.40 (269.0)^{ab}	4.06 (13281)^{b}	4.67 (48500)^{ab}	4.8 (62050)^{b}	1.78 (70.7)^{de}	0.04^{ab}	1.47 (28.50)^{a}	4.34 (21869)^{ab}	0.77 (86000)^{ab}	4.92 (107897)^{ab}	1.93 (86,38)^{b}
3125	0.19^{abc}	0.36 (20.1)^{a}	4.66 (48828)^{a}	4.82 (66500) ^{a}	5.1 (115348)^{a}	1.57 (36. 9)^{e}	0.03^{ab}	1.48 (29.25)^{a}	4.65 (55016)^{a}	0.78 (123350)^{a}	5.00 (178395)^{a}	1.74 (57.00)^{b}
$LSD_{0.05}$	88.67	14.05	7.56	5.92	5.21	11.49	157.0	20.24	19.83	3.45	8.90	7.17

^{y}Column means followed by the same letter were not different ($P \leq .05$) according to Fisher's Least Significant Different test. Least significant differences at the 5% level ($LSD_{0.05}$). Pf is the final nematode population. RF is the reproductive potential.

Table 3. Partitioning mean sum squares for the effect of *Meloidogyne enterolobii* on stem diameter (STD) in the 2021 experiment, Pod number (PN) and plant height (PH) in the 2022 experiment at 56 days after the initiation of treatments ($n = 64$).

	2021 STD			2022 PN		PH	
Source	Df	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Reps	7	1.775	26.89	22.734	26.95	5550.7	33.95
Treatment	7	1.200	18.18**	16.484	19.54**	2593.7	15.86**
Errors	49	3.600	54.55	45.140	53.50	8207.6	50.19
Total	63	6.600	100	84.359	100	16351.9	100

**Highly significant at $P \leq .05$, TTV (%): Total Treatment Variation.

Table 4. Response of stem diameter (STD) in the 2021 experiment, pod number (PN) and plant height (PH) in the 2022 experiment of tepary bean to *Meloidogyne enterolobii* under shade-net conditions.

	2021	2022	
Treatments	STD	PN	PH
0	$0.018^{bc} \pm 0.017$	$1.375^{cd} \pm 0.010$	$40.875^{abc} \pm 0.001$
25	$0.020^{abc} \pm 0.014$	$1.8750^{abc} \pm 0.003$	$52.625^{a} \pm 0.004$
50	$0.015^{bc} \pm 0.021$	$2.125^{abc} \pm 0.001$	$46.750^{ab} \pm 0.003$
125	$0.028^{a} \pm 0.003$	$2.375^{ab} \pm 0.002$	$50.375^{ab} \pm 0.004$
250	$0.023^{ab} \pm 0.011$	$1.500^{bcd} \pm 0.002$	$38.125^{bc} \pm 0.001$
625	$0.021^{ab} \pm 0.014$	$0.875^{d} \pm 0.002$	$31.750^{c} \pm 0.003$
1250	$0.019^{bc} \pm 0.014$	$1.750^{abcd} \pm 0.011$	$40.125^{abc} \pm 0.001$
3125	$0.013^{c} \pm 0.0001$	$2.500^{a} \pm 0.006$	$43.125^{abc} \pm 0.001$
LSD	44.24	53.42	30.12

[y]Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.

Wilk 1965; Ghasemi and Zahediasl 2012), with the data depicting normal distribution. Data were then subjected to analysis of variance using Statistix 10.0 software. The degrees of freedom and their mean sum of squares were partitioned to determine the contribution of sources of variation in the total treatment variation (TTV) of the variables. Fisher's Least Significant difference test was used to separate means that were significant at the probability level of 5%. Before the analysis of variance, nematode data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez 1984) but untransformed means were reported.

Results and discussion

Generally, host status and sensitivity in plant nematodes are used as an indicator for establishing the degree of nematode resistance in a single cultivar exposed to different levels of inoculation (Mashela and Pofu 2016). According to Seinhorst (1967), host sensitivity refers to the host's response to nematodes, whereas host status denotes the ability of the nematode to reproduce in a plant. The two phenomena are then used to define three concepts: susceptible, tolerant or resistant plant of the relationship between the plant and parasitic nematode. Host status is expressed using Rf, with Rf values less than one indicating that the plant is a non-host, while those greater than one indicate that the plant is a host (Anwar and McKenry 2010). Host sensitivity is expressed by the plants' response to the presence of nematodes, which could be reduced growth, no effect or increased growth relative to the control plants (Seinhorst 1967; Pofu 2012; Nkosi 2019). A resistant plant is one where Rf is equal to or less than one with the plant not experiencing reduced growth, a tolerant plant has Rf greater than one, but the plant's growth is not reduced (Kayani and Mukhtar 2018). A susceptible plant will exhibit the opposite of a resistant one (Kayani and Mukhtar 2018). In the current study, cultivar treatment had a highly significant effect ($P \leq .01$) on root galls, eggs in roots, J2 in roots, J2 in soil, Pf and RF, contributing 19%, 89%, 97%, 98%, 98% and 93%, respectively, in TTV of the respective variables in the 2021 experiment, whereas in the 2022 experiment, treatments had a highly significant effect ($P \leq .01$) on eggs in roots, J2 in roots, J2 in soil, Pf and Rf, contributing 81%, 88%, 99%, 95% and 97% in TTV, respectively (Table 1). Similarly, thermophilic *Meloidogyne species* havea high significant effect in a resistance status study of sweet potato varieties (Osunlola and Fawole 2015; Pofu et al. 2017). In the current study, Rfs above unity for all inoculation levels in both experiments were observed (Table 2). The Rf values being above unity denoted that the test plant is a host (Seinhorst 1967; Fourie et al. 1999; Pofu et al. 2010). When the Rf is greater than unity and the plant doesn't suffer yield loss (Table 4), the plant is described as a tolerant host (Seinhorst 1967; Pofu et al. 2010). Root galls were visible in both experiments (Table 2), generally, the visibility of root galls on the tested plants has been used as an indicator that the penetrating second-stage juveniles (J2) had successfully established a feeding site, suggesting that the plant allowed the nematode to reproduce (Ferraz and Brown 2002). According to Fourie et al. (2015) although the presence of root galls is a good indicator of nematode reproduction and juvenile hatching, in host status studies this indicator is not reliable. In the current

study, *M. enterolobii* levels had a significant effect ($P \leq .01$) on stem diameter in the 2021 experiment, whereas in the 2022 experiment, treatments had a significant effect on pod number and plant height (Table 3). In the 2021 experiment, treatments contributed 19% in the TTV of stem diameter, whereas in the 2022 experiment, the treatments contributed 20 and 16% in the TTV of pod number and plant height, respectively (Table 3). This observation was also reported by Nkosi (2019), who observed tropical *Meloidogyne* species having a significant effect on sweet potato cv. 'Mafutha' (Table 4).

Conclusion and recommendation(s)

Using the Seinhorst model, the RF indicates that *M. enterolobii* was able to reproduce on Kickapoo white tepary bean variety without causing a reduction in the plant's growth variables; therefore, it can be concluded that the Kickapoo white tepary bean is tolerant to the thermophilic *Meloidogyne* species. The tolerance of Kickapoo white tepary bean and its multi-purpose usage makes it ideal for cultivating in areas with poor soils prone to *M. enterolobii*.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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