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Posted Date: 5 March 2024

doi: 10.20944/preprints202403.0258.v1

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Article

Morphometric Adjustment in *Meloidogyne enterolobii* to Botanical Chemicals in Sorghum Cultivars with a Wide Degree of Pre-Infectional Nematode Resistance

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Abstract: Sorghum-nematode interactions were previously purported to produce chemicals that confer pre-infectional nematode resistance to the infective second-stage juveniles (J2) of the root-knot (*Meloidogyne* species) nematodes in the rhizosphere, without any contact to J2 bodies. We tested the null hypothesis in four sorghum cultivars with a wide range of pre-infectional nematode resistance relative to the susceptible tomato cultivar. Post a 56-day exposure of *Meloidogyne* enterolobii, J2 from soil and root samples were subjected to step-wise fixative solutions and then mounted on slides. Length-related and diameter-related morphometrics were measured using an OMAX microscope – equipped with a digital measuring software. The study was conducted over two seasons and the seasonal interactions on morphometrics were not significant. Relative to the tomato cultivar, sorghum treatments generally increased the length-related morphometrics, but decreased the diameter-related variables in both soil J2 and root J2. The null hypothesis was therefore rejected in favour to the assumption that upon exposure of J2 bodies to botanical chemicals in both soil and root samples, with certain morphometrics being adjusted to ameliorate the potential damage of hydrostatic pressure on internal organs of the infective juveniles. In conclusion, in pre-infectional nematode resistance, J2 bridge chemicals in the rhizosphere and enter the root system, where chemicals still have effects on morphometrics of nematodes.

Keywords: adjustment; botanical chemicals; hydrostatic pressure; *Meloidogyne enterolobii*; morphometrics; nematode-sorghum interactions; pseudocoelom

1. Introduction

Prior to the 1980s the influence of the host plant on morphometrics of nematodes was reported on *Ditylenchus destructor* Thorne 1945 [1], *Paratylenchus nanus* Cobb 1923 [2], *Trichodorus christiei* (Allen 1957) Sididqi 1974 [3], *Heterodera glycine Ichinohe* 1952 [4], *H. rostochiensis* Wollenweber 1923 [5], *Aphelenchoides fragariae* (Ritzema Bos 1890) Christite 1932 [6] and *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 and *M. incognita acrita* Chitwood 1949 [7–10]. However, the observed differences were ascribed to the natural existence of different nematode biotypes or to the variability induced by chemicals released by host plants [10,11]. In the mid-1980s, using inocula from a single egg mass, females of *M. incognita* developing on roots of 17 plants from seven genera, exhibited significant variation in morphometrics, except for females on non-host species, where the development to maturity was inhibited [12]. Recently, In vitro studies demonstrated that crude extracts containing cucurbitacins from fruits of wild *Cucumis* species consistently affect morphometrics *Tylenchulus semipenetrans* Cobb 1913 [13], *Scutellonema brachyurus* (Steiner 1938) Andrassy, 1958 [14], *Steinernema feltiae Filipjev* 1934 [15] and *M. incognita* [16].

In nematode-plant interactions, two mechanisms of resistance are broadly classified as pre-and post-infectional nematode mechanism of resistance [17]. In pre-infectional nematode resistance plant

 doi:10.20944/preprints202403.0258.v1

chemicals are released by roots into the rhizosphere, with nematostatic responses that are widely reversible [17]. Plant hosts that release chemicals with nematostatic responses have the ability to mainly repel nematode second-stage juveniles (J2) away from the rhizosphere [18,19]. *Sorghum* species with pre-infectional nematode resistance, primarily release dhurrin ($C_{14}H_{17}NO_7$), a glycoside that breaks down to produce hydrogen cyanide (HCN) – a highly toxic chemical compound to nematodes [19]. Additionally, most sorghum cultivars exude sorgoleone ($C_{22}H_{29}O_4$) – a root exudate that also contains potent nematicidal properties [19]. Sorgoleone is a soil-active hydrophobic compound that also has herbicidal properties that supress growth of a wide range of plant species [20]. Sorgoleone-producing sorghum cultivars have the potential to suppress nematode population densities when sorghums are included in different crop rotation systems [21–24]. Sorghum cultivars have been shown to exhibit a wide range of resistance to *Meloidogyne* species [19], but with a wide range of variabilityIn most sorghum-nematode interactions, the work was limited to *M. incognita and M. javanica* thermophiles, with the focus being on population densities in context of crop rotations [17]. Usually, reduced densities were ascribed to the repulsive nature of the chemicals to the infective second-stage juveniles (J2). In this context, J2 run away from the exuded chemicals, with limited effects of the chemicals on the nematode body. Additionally, information on interactions of sorghum cultivars and *Meloidogyne enterolobii* Yang and Eisenback, 1983 – currently viewed as an emerging threat in various crop industries, is scant.

Meloidogyne enterolobii is the most aggressive among the known thermophilic *Meloidogyne* species, with both a wide distribution and host range, the shortest life cycle of 15-days and the inherent capacity to reproduce on tomato genotypes with the most efficient Mi resistance genes [26–28]. In most instances, J2 are thought to 'run" away from the rhizosphere of plants with pre-infectional nematode resistance, with limited number of J2 ever entering the root system [17]. However, the use of such plants in crop rotations intended to manage population densities of nematodes result in a wide range of inconsistent results [18–24]. In part, the incosistent results could be explained on the wide degree of nematode resistance in *Sorghum* species, cultivars or land races. For example, using the Seinhorst [25] descriptors of nematode resistance, sorghum-Sudan grass hybrid cv. 'PAN 868' and sweet sorghum cv. 'Ndendane-X1' could be clasified as being highly resistant (HR) to *Meloidogyne* species, whereas sorghum cv. 'Avenger' is moderately resistant (MR) and cv. 'Titan' is highly susceptible (HS) [19], thus, according the opportunity to test the "running" away hypothesis. The "runnning" away from chemicals in the rhizosphere suggests that J2 bodies hardly come into contact with the bioactive botanical chemicals, let alone entering the root system, which could be validated by measuring morphometrics of J2 from both soil and root samples. The objective of the current study was to investigate the effects of sorghum cultivars with a wide range of nematode resistance on length-related and diameter-related morphometrics of *M. enterolobii* from both soil and root samples under greenhouse conditions.

2. Results

Effects of seasonal interactions on test morphometrics were significant and therefore the seasonal data for Experiment 1 and Experiment 2 were not pooled [29]. Length-related morphometrics reported included body length, stylet length, anterior to excretory pore, tail length and hyaline length (Table 1). In contrast, four diameter-related morphometrics included head region base, mid-body, anus and excretory pore (Table 2). In some cases the treatment effects were observed either on soil J2 or root J, or on both.

Table 1. Relative impact (R.I.) of sorghum cultivars with pre-infectional nematode resistance to susceptible tomato cv. 'Floradade' on *enterolobii* from soil and root samples at 56 days after inoculation.

				1A: Body length		
		Experiment 1				
Sorghum		Soil		Root		Soil
Cultivar	Degree[y]	BL[z] (µm)	R.I. (%)	BL (µm)	R.I. (%)	BL (µm)
Floradade	HS	382.13[a]	–	399.18	–	426.31[a]
Titan	HS	381.69[a]	–0	389.31	–2	379.31[ab]
Avenger	MR	375.07[a]	–2	384.64	–4	370.48[b]
Ndendane-X1	HR	358.48[b]	–6	374.75	–6	360.61[b]
PAN 868′	HR	397.20[a]	4	399.31	0	363.41[b]
TTV (%)[w]	-	39[**]	-	26[ns]	-	62[**]
				1 B: Stylet length		
Sorghum		Soil		Root		Soil
Cultivar	Degree[y]	SL[z] (µm)	R.I. (%)	SL(µm)	R.I. (%)	SL (µm)
Floradade	HS	14.57	–	13.37[b]	–	14.99[a]
Titan	HS	14.66	1	17.49[a]	31	13.94[ab]
Avenger	MR	13.46	–8	13.38[b]	0	14.74[ab]
Ndendane-X1	HR	13.58	–7	13.78[b]	3	13.67[b]
PAN 868	HR	14.26	–2	13.19[b]	–1	13.87[ab]
TTV (%)	-	32[ns]	-	63[***]	-	54[**]
				1 C: Anterior to excretory pore (AEP)		
Sorghum		Soil		Root		Soil
Cultivar	Degree[y]	AEP[z] (µm)	R.I. (%)	AEP (µm)	R.I. (%)	AEP (µm)
Floradade	HS	73.90[a]	–	79.19	–	69.66[c]
Titan	HS	75.98[a]	3	76.65	–3	73.08[bc]
Avenger	MR	66.94[b]	–9	74.75	–6	73.24[bc]
Ndendane-X1	HR	66.51[b]	–10	74.63	–6	77.14[a]
PAN 868	HR	76.79[a]	3	72.66	–8	74.87[ab]
TTV (%)[w]	-	95[***]	-	45[ns]	-	58[**]
				1 D: Tail length (TL)		
Sorghum		Soil		Root		Soil

Cultivar	Degree[y]	TL[z] (µm)	R.I. (%)	TL (µm)	R.I. (%)	TL (µm)
Floradade	HS	47.04[ab]	–	49.08	–	48.95[c]
Titan	HS	47.61[ab]	1	47.59	–3	46.20[ab]
Avenger	MR	44.54[c]	–5	46.42	–5	46.41[ab]
Ndendane-X1	HR	45.96[bc]	–2	45.48	–7	44.88[b]
PAN 868	HR	48.94[a]	4	48.44	–1	45.13[b]
TTV (%)	-	68[**]	-	42[ns]	-	53[**]
				1 E: Hyaline length (HL)		
Cultivar	Degree[y]	HL[z] (µm)	R.I. (%)	HL (µm)	R.I. (%)	HL (µm)
Floradade	HS	7.67[ab]	–	9.20[b]	–	5.91[c]
Titan	HS	8.09[a]	5	10.66[ab]	16	7.13[bc]
Avenger	MR	5.97[b]	–22	12.47[a]	36	9.82[a]
Ndendane-X1	HR	9.50[a]	24	8.93[b]	–3	9.28[ab]
PAN 868	HR	8.97[a]	17	8.72[b]	–5	10.23[a]
TTV (%)	-	70[**]	-	59[**]	-	71[***]

Table 2. Relative impact (R.I) of sorghum cultivars with a wide range of pre-infectional nematode resistance to tomato cv. 'Floradade' on *enterolobii* from soil and root samples at 56 days after inoculation.

2A: Head region width (HRW)

		Experiment 1				
Sorghum		Soil		Root		Soil
Cultivar	Degree[y]	HRW[z] (µm)	R.I. (%)	HRW (µm)	R.I. (%)	HRW (µm)
Floradade	HS	4.61[a]	–	4.53[ab]	–	7.58[a]
Titan	HS	4.59[a]	–0	4.54[ab]	0	4.45[b]
Avenger	MR	3.93[b]	–14	4.30[b]	–5	4.62[b]
Ndendane-X1	HR	4.61[a]	0	4.40[ab]	–3	4.59[b]

PAN 868	HR	4.41[a]	–4	4.85[a]	7	4.66[b]	
TTV (%)[w]	-	35[**]	-	38[**]	-	90[***]	

2B: Mid-body width (MBW)

Sorghum		Soil		Root		Soil	
Cultivar	Degree[y]	MBD[z] (µm)	R.I. (%)	MBD (µm)	R.I. (%)	MBD (µm)	
Floradade	HS	13.41	–	11.21		12.66	
Titan	HS	13.76	3	13.31	18	14.14	
Avenger	MR	13.29	–1	13.27	20	12.79	
Ndendane-X1	HR	13.88	4	12.47	19	13.58	
PAN 868	HR	14.17	6	13.40	–	14.11	
TTV (%)[w]	-	10[ns]	-	35[ns]	11	46[ns]	

2C: Anal body diameter (ABD)

Sorghum		Soil		Root		Soil	
Cultivar	Degree[y]	ABD[z] (µm)	R.I. (%)	ABD (µm)	R.I. (%)	ABD (µm)	
Floradade	HS	8.81[a]	–	8.31	–	8.30[a]	
Titan	HS	8.55[ab]	–3	8.59	3	7.94[a]	
Avenger	MR	7.95[ab]	–10	8.49	2	6.96[b]	
Ndendane-X1	HR	7.74[b]	–12	8.56	3	8.53[a]	
PAN 868	HR	8.67[a]	–2	8.81	6	8.06[a]	
TTV	-	52[**]	-	14[ns]	-	74[**]	

[x]Degree of nematode resistance depicted as highly susceptible (HS), moderately resistant (MR) and highly resistant HR). [y]Column mean
($P \leq 0.05$) according to Tukey test. [z]Total treatment variation percentage, where [ns] signifies not being significant at $P \leq 0.05$, with [**] a
respectively.

 doi:10.20944/preprints202403.0258.v1

2.1. Length-Related Morphometrics

Variability, as shown by the relative impact and magnitude of TTV values within and across the two seasons, along with the source from which J2 were extracted, was prominent in various length-related morphometrics.

2.1.1. Body Length

Cultivar treatments consistently affected body length of soil J2, contributing 39% and 62% in TTV of the variable in Experiment 1 and Experiment 2, respectively (Table 1A). However, in root J2, the treatment effects were significant only in Experiment 2, contributing 62% in TTV of the variable. Relative to the susceptible tomato standard cv. `Floradade`, cv. 'Ndendane-X1` consistently decreased body length, regardless of the source from which J2 were extracted in both experiments.

2.1.2. Stylet Length

Except for soil J2 in Experiment 1, cultivar treatments consistently affected stylet length, contributing from 54 to 60% in TTV of the variable of root J2 (Table 1B). Relative to the susceptible standard, the cultivar treatments decreased stylet length of soil J2 in both experiments, whereas in root J2 the treatments had a tendency to increasing the stylet length, but such effects were not significant

2.1.3. Anterior to Excretory Pore

Treatments significantly decreased the distance from anterior to excretory pore of soil J2, but did not affect the variable of in root J2 for both experiments (Table 1C). Relative to the susceptible standard, the treatments decreased and increased the variable of soil J2 in Experiment 1 and Experiment 2, respectively. Notably, relative to the standard cultivar, cv. 'Ndendane-X1` with high degree of resistance, decreased and increased the variable in Experiment 1 and Experiment 2 by 10 and 11%, respectively.

2.1.4. Tail Length

Treatments had significant effects on tail length of soil J2, contributing 68 and 53% in TTV of the variable in Experiment 1 and Experiment 2, respectively (Table 1D). Relative to the standard, cv. 'Avenger` reduced the variable in both experiments, whereas cv. `Ndendane` reduced the variable only in Experiment 2. However, in both experiments the treatments did not have significant effects on the variable of root J2.

2.1.5. Hyaline Length

Except for root J2 in Experiment 2, cultivar treatments significantly affected hyaline length of soil and root J2, contributing 70% in TTV of the variable of soil J2, along with 59% and 71% in TTV of the soil J2 variable in Experiment 1 and Experiment 2, respectively (Table 1E). Except for soil J2 where cv. 'Avenger` significantly decreased the hyaline length, the other cultivars had a tendency to increase the variable. However, in Experiment 2, relative to the standard, chemicals from all cultivars significantly increased the variable in soil J2 from 21 to 73%.

2.2. Diameter-Related Morphometrics

Diameter-related morphometric measurements focus on characters that are almost spherical in shape, which for the purpose of the study were limited to collecting data at three body regions: Head region, mid-body region and anal body region.

2.2.1. Head Region Diameter

 doi:10.20944/preprints202403.0258.v1

Regardless of the J2 source, the head region diameter was affected by the test treatments, with TTV values ranging from for cultivars 'Ndendane-X1` and 'Avenger` for root J2, cultivar treatments had a tendency of decreasing the diameter of the head region. (This should have Table 2A)

2.2.2. Mid-Body Diameter

Treatment effects on soil J2 in both experiments, along with root J2 in Experiment 1, were not significant for mid-body diameter, except for root J2 in Experiment 2, where the treatment effects contributed 70% in TTV of the variable (Table 2B). Relative to the standard, all cultivars decreased the mid-body diameter of root J2.

2.2.3. Anal Body Diameter

The treatments had significant effects on the anal body diameter of soil J2 in both experiments, contributing 52 and 74% in TTV of the respective variables (Table 2C). Relative to the susceptible standard, the cultivars had a tendency of decreasing the test variable.

3. Discussion

3.1. Positive and Negative Relative Impact Values

In the current study, significant positive and negative relative impact values, along with those that were not significant, demonstrated that for the test morphometrics, the cultivars released chemicals with concentrations that exhibited density-dependent (DDG) patterns. The latter constitute a major feature depicting the responses of various entities to increasing concentration of botanical chemicals [13,30]. The observed DDG patterns fall within one of three phases, namely, stimulation, neutral and inhibition phases. Significant positive and negative relative impact values suggested that the morphometric characters were exposed to chemical concentration ranges that coincided with the stimulation and the inhibition phases, respectively. In contrast, relative impact values that were not significantly different illustrated that the concentration ranges coincided with the neutral phase in context of DDG patterns or were below the concentration that induces the stimulation, technically referred to as threshold stimulation concentration [31].

3.2. Chemical Concentrations in Context of Degree of Nematode Resistance

In plants with pre-infectional nematode resistance mechanisms, plants release chemicals with nematostatic properties into the rhizosphere [19]. Differences in morphometrics of any individual character as measured in our study showed that the test sorghum genotypes could have been releasing different quantities of sorgolene and/or dhurrin, which are known to depict nematostatic properties. Chiuta [19] reviewed studies demonstrating that the degree of nematode resistance in sorghum genotypes were largely depended upon the concentration of the chemicals released into the rhizosphere. In addition to the concentration, widespread losses to the environment, emanating from microbial degradation and the half-life of the chemical were reported. The losses were viewed as being responsible in the much observed inconsistent results in suppression of nematode population densities when sorghum genotypes were used in context of crop rotation [19].

3.3. Biological Importance of Positive and Negative Relative Impact Values

The DDG patterns for various morphometrics were observed in soil and root J2. Responses of the test morphometrics demonstrated that the concept that in pre-infectional nematode resistance J2 are repelled from the rhizosphere is not entirely correct since morphometrics of soil J2 and root J2, were, with limited exceptions, similarly affected. Previously the Chiuta [19] review demonstrated that Sudangrass with potent degree of nematode resistance exhibited both pre-and post-infectional mechanisms of nematode resistance. However, in the current study, in certain morphometrics, responses to cv. 'Ndanene-X1` with high degree of nematode resistance and cv. `Avenger' with

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moderate resistance, exhibited significantly stronger responses than those in Sudangrass cv. 'PAN 868', which could, in context of DDG patterns, be due to differences in concentration of the chemicals.

Broadly, a nematode is a "tube-in-a tube", with the outer tube being the multi-layered rather rigid cuticle, whereas the internal tube comprises a single-layered intestine [11,15,16,32]. The inner tube is technically embedded in a fluid referred to as the hydrostatic fluid, which induces hydrostatic pressure [16,32]. Since the cuticle is rigid, when nematodes are exposed to solutions that increase the hydrostatic pressure in the pseudocoelom, adjustments of certain organs as illustrated by increases in length-related morphometrics avoid damage to internal organs by the increasing hydrostatic pressure [15,16]. The increase in length of certain morphometrics, supported by the existence of longitudinal muscles in J2 bodies [11,32], could to a certain degree, explain the observed increases in length-related morphometrics in this and other studies where infective stages of nematodes were exposed to increasing concentration of botanical chemicals [13–16].

Generally, the treatments in both soil J2 and root J2 increased the hyaline length, which was not clear to us as to how this was related to the adjustments that are being alluded to. Hyaline layer is a transparent (fin-like) layer on the ventral side of the nematode tail – which could also be species-specific.Apparently, environmental effects on diameter-related morphometrics are automatically inversely proportional to diameter-related morphometrics. As in the case of hyaline length, the adjustment related to the stylet length under the test botanical chemicals of our study, for now, was devoid of any explanation in terms of the alluded adjustments. Generally, the decrease in diameter-related morphometrics in soil J2 and root J2 serves as a confirmation of increases in length-related morphometrics, both supporting the view that in plants with pre-infectional nematode resistance J2 in both soil and roots are morphometrically-affected by the botanical chemicals.

3.4. Differences in Soil J2 and Root J2 Morphometrics

In plants with pre-infectional nematode resistance, the "running" away hypothesis, which was previously not subjected to hypothesis-testing suggested that J2 were hardly allowed to penetrate the root system [19]. However, findings in our study showed that J2 bridged the pre-infectional nematode resistance mechanism and penetrated the root system, where they were subjected to the bioactive chemicals. The latter observation could be interpreted as post-infectional nematode resistance mechanism [17]. The differences observed in the same morphometrics of soil J2 and in root J2 in our study demonstrated that differences existed for a wide range of characters. The observation could be attributed to differences in the concentration of the bioactive chemicals in the two environments, along with the sensitivity of the measured characters to the test chemicals. Chiuta [19] noted that sorgolene in soil environments was short-lived primarily due to biodegradation. Limited information at our disposal on whether the higher concentration of the test chemicals was in soil or in root, compromise our conclusive discussion of the observed differences.

3.5. Seasonal Interaction in J2 Morphometrics

Seasonal interaction in J2 morphometrics were significant and thus, the findings in the two experiments were reported separately instead of pooling the data [29]. The observed differences in morphometric responses during the two seasons, although validation was done during the same period, could possibly ascribed to climatic change during summer 2021 and 2022 [34] demonstrated that for studies initiated in greenhouse during autumn and validated during spring of the same year, reproductive potential of *Meloidogyne* species was highly significant, and attributed the differences to climatic differences during the two seasons. In that study it was recommended that nematode trials be validated the following year, but during the same season in order to avoid seasonal differences. Notwithstanding, when the seasonal interactions are not significant, data could be pooled and reported as being from a one experiment, which improves the precision of estimating the population mean by the sample mean, statistically referred to as inference [29].

4. Materials and Methods

 doi:10.20944/preprints202403.0258.v1

4.1. Description of the Study Site

The study was conducted under greenhouse conditions at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, Limpopo Province, South Africa (23°53′10″S, 29°44′15″E) during summer (November-January – southern hemisphere) 2021 and validated in 2022. The size of the greenhouse was 20 m × 100 m, with the roof covered with a green shade-net that allowed through at least 65% photosynthetically active radiation. Ambient day/night temperatures averaged 28/21°C, with maximum temperatures controlled using thermostatically activated fans on the northern sidewall and the wet wall on the southern sidewall, which also ensured that the relative humidity was retained between 60 and 70%.

4.2. Treatments, Research Design and Procedures

Treatments comprised hybrid sorghum-Sudan grass hybrid cv. 'PAN 868' (HR), sweet sorghum cv. 'Ndendane-X1' (HR), and cv. 'Avenger' (MR) and sorghum cv. 'Titan' (HS), laid out in a randomised complete block design, with nine replications. Blocking was done was done to ameliorate shading from the greenhouse wall in the mornings and wind streams induced by the fans. In each replication, tomato cv. 'Floradade' (HS) was included as a nematode susceptible standard. Seeds were sown into 20-cm diameter plastic pots filled with steam-pasteurised loam, sand and Hygromix-T (Hygrotech, Tshwane, South Africa) at the ratio of 3:1:1 (v/v), respectively, and placed on benches at 10 × 10 cm spacing. Inocula of *M. enterolobii,* cultured on nematode-susceptible tomato cv. 'Floradade', was prepared by extracting eggs and second-stage juveniles (J2) in 1% NaOCI solution [27]. Seven days after seedling emergence, inoculation with ca. 5000 eggs + J2 were placed into 3-cm-deep holes on cardinal points of seedlings using a 20-ml plastic syringe. Seedlings were fertilised once a week after inoculation using 1 g N:P:K [2:3:2 (43)] fertiliser mixture and 2 g 2:3:2 (26) N:P¨K + 0.5% Zn + 5% S + 5% Ca fertiliser mixture. Seedlings were irrigated using 500 ml chlorine-free tapwater/plant when tensiometer readings averaged below 10%.

4.3. Data Collection

At 56 days after inoculation, second-stage juveniles (J2) were extracted from a representative soil subsample of 250 ml and root subsample of 10 g using the modified Baermann funnel [33]. Fresh J2 from soil and root subsamples were separately fixed in stepwise Fixative I, Fixative II and Fixative III solutions [30]. Length-related and diameter-related morphometrics of at least 10 fixed J2 from soil and root samples per treatment were separately measured using an OMAX microscope, which was equipped with a digital measuring software.

4.4. Statistical Analysis

Data were subjected to analysis of variance using Statistix 10.0 software. The degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) [29]. Significant treatment means were separated using the Tukey test and unless otherwise stated, treatment effects were discussed at the probability level of 5%. Seasonal interactions were not significant and thus data in the two experiments were not pooled [29].

5. Conclusions

Responses of morphometrics to botanical chemicals in sorghum cultivars with a wide range of the degree of pre-infectional nematode resistances illustrated that the chemicals have direct effects to nematode bodies in both soil and root. Supported by the results, we reject the hypothesis which suggested that in plants with pre-infectional nematode resistance nematode "run" away from the rhizhospere with limited contact to botanical chemicals. The variation in morphometric responses as affected by the degree of nematode resistance suggested that, although costly, the use of molecular technologies in identification of nematode species should be continued until stable morphometrics that could be of use in nematode taxonomy are empirically established.

 doi:10.20944/preprints202403.0258.v1

Author Contributions: Conceptualization, PWM. and KMP.; methodology, PWM , formal analysis, PWM.; investigation, KMP.; resources,; data curation, PWM; writing—original draft preparation, PWM.; writing—review and editing, KMP; project administration, KMP; funding acquisition, PWM. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research Foundation of South Africa and the Department of Agriculture, Rural Development and Land Reform (Grant number 136124).

Data Availability Statement: All data used in this study are included in the paper and further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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