RAPID SCREENING TECHNIQUE FOR ASSESSING RESISTANCE TO *MELOIDOGYNE* SPP. IN CASSAVA

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Summary. Root-knot nematodes (*Meloidogyne* spp.) are a particularly damaging group of pests on a wide range of crops and the predominant plant-parasitic nematode occurring on cassava (*Manihot esculenta*). Screening for resistance against these nematodes would be prudent to minimize yield loss and the high costs of alternative control measures. However, existing screening methods using pots are laborious and time consuming. This study was initiated with the major objective of establishing a reproducible and rapid screening protocol for cassava against *Meloidogyne* spp. Three experiments were established using four node cuttings of cv. Nase 4 (SS4) planted in vertically hanging 20 cm diameter, sawdust filled polythene tubing. The study evaluated different irrigation methods, plant spacing and position along the tube following inoculation with *Meloidogyne* spp. using different methods and densities. The technique was refined with progression of the study towards determining a suitable protocol. Individual inoculation of cuttings was superior to drenching the sawdust with a nematode suspension. Irrigating the tubes through a plastic pipe installed down the centre resulted in higher and more uniform galling and *Meloidogyne* spp. densities in roots than without pipes. Plant spacing at 30 × 30 cm and 25 × 25 cm resulted in better plant growth despite inoculation with *Meloidogyne* spp. than narrow spacing (20 × 20 cm). Inoculation levels (1000, 500 and 250 *Meloidogyne* spp.) caused no measurable difference to plant growth or nematode variables although 1000 *Meloidogyne* spp. eggs appears the minimum inoculation level necessary to effect a reduction in plant growth components. The use of a tube for screening against *Meloidogyne* spp. is therefore proposed as a space and resource saving technique suitable for cassava stems and perhaps other plants too.

Key words: Root-knot nematodes, *Manihot esculenta*, nematodes, plant-parasite, galling.

Root-knot nematodes (*Meloidogyne* spp.), as a group, are among the most important biotic constraints affecting crop production (Luc *et al*., 2005). On cassava (*Manihot esculenta*) they represent the most important nematodes affecting the crop (Bridge *et al*., 2005). The nematodes attack cassava feeder roots, causing deformities and swellings, or galls, decay and root death disrupting translocation of water and nutrients to the rest of the plant (Gapasin, 1980; Caveness, 1982; Coyne *et al*., 2003). Consequently, a significant reduction in plant height, fresh root weight, storage root number and yield can result from infection (Bridge *et al*., 1991; Coyne, 2004; Coyne *et al*., 2006). Although assessing the specific importance of *Meloidogyne* spp. attack in the field can be complex, due to the diverse conditions under which cassava is grown and the various pest and disease complexes occurring, studies have shown serious losses in farmer's fields (Theberge, 1985; Bridge *et al*., 1991). In Uganda, high levels of galling in fields (Coyne and Namaganda, 1994) was later related to extensive losses (Coyne, 2004), while more controlled studies support the high potential for damage to cassava of *Meloidogyne* spp. (Caveness, 1981; Talwana *et al*., 1996; Crozzoli and Parra, 1999; Coyne and Talwana, 2000; Makumbi-Kidza *et al*., 2000). The release of cassava cultivars into geographic situations where assessment has not been comprehensively conducted has also highlighted the damaging nature of *Meloidogyne* spp. on otherwise high yielding and promising cassava lines (van den Oever, 1995; Coyne *et al*., 2004, 2005). Screening for resistance would help prevent premature and inadvertent release of cultivars. The use of resistance also represents one of the most cost effective and efficient nematode management strategies (Starr *et al*., 2002). However, while various screening methods are in use, they are not necessarily suited to routine use in resource poor conditions, and particularly where nematology expertise is limited. It was, therefore, the objective of the current study to establish a relatively simple and efficient mechanism to rapidly screen cassava for resistance to *Meloidogyne* spp. that is repeatable, and which could theoretically be a generic system for other crops.

MATERIALS AND METHODS

Polythene tubes were used, suspended vertically with the specific objectives to establish an appropriate irrigation and inoculation method, determine the appropriate stem (plant) spacing and position and establish the optimum *Meloidogyne* spp. inoculation level.

The study was conducted in a screen-house at the International Institute of Tropical Agriculture (IITA) research station at Sendusu. Sendusu is in the mid-elevation humid forest zone, 28 km north of Kampala, Ugan-

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da (32°34'E, 0°32'N) at 1150 m asl with a mean annual rainfall of 1300 mm and mean annual minimum and maximum temperatures of 16 and 28 °C, respectively.

Experiment 1. It was conducted in October 2007. Four-node stem cuttings of cv. Nase 4 (SS4), a locally popular cultivar susceptible to *Meloidogyne* spp. (Talwana *et al.*, 1996), were used throughout the study, planted in 20 cm diameter polythene tubing filled with sawdust and hung vertically, suspended from a wooden frame (Fig. 1). The tubes (approx. 1.25 m length each) were arranged in a split-split plot design with four factors: inoculation method, irrigation method, stem spacing and stem position, with three replicates of each treatment combination. A preliminary assessment of the water capacity was undertaken to establish the volume of water required to fully soak the whole tube of sawdust. The inoculation method had two levels: drench inoculation and individual stem inoculation. The *Meloidogyne* spp. inoculum used for a drench inoculation was equivalent to the sum total inoculum used per stem, corresponding to that particular treatment/tube.

The irrigation method had two levels: presence of an irrigation pipe (2.5 cm inner diameter) placed centrally through the sawdust and no irrigation pipe. The base of the irrigation pipe was sealed and the pipe drilled randomly with holes, increasing in number for each of the three designated sections towards the top, to enable a relatively equal distribution of water within the sawdust. Stem spacing and position had three levels each: spacing was assessed at 20 cm × 20 cm, 25 cm × 25 cm and 30 cm × 30 cm and position was assigned to the lower, middle and upper positions. Triangular shaped planting along the length of the polythene tubes was used to maximise space with rows of plants offset from each other by a vertical distances of 10 cm, 12.5 cm and 15 cm for the respective spacing of 20 cm × 20 cm, 25 cm × 25 cm and 30 cm × 30 cm. Each position was represented by between four and six plants, depending on the spacing: 20 cm × 20 cm = 6 plants per position; 25 cm × 25 cm = 5 plants; 30 cm × 30 cm = 4 plants. The tubes were irrigated at planting and then on a weekly basis at a rate of 5 litres per tube, after prior assessment showed this was sufficient to maintain the sawdust in a moist condition.

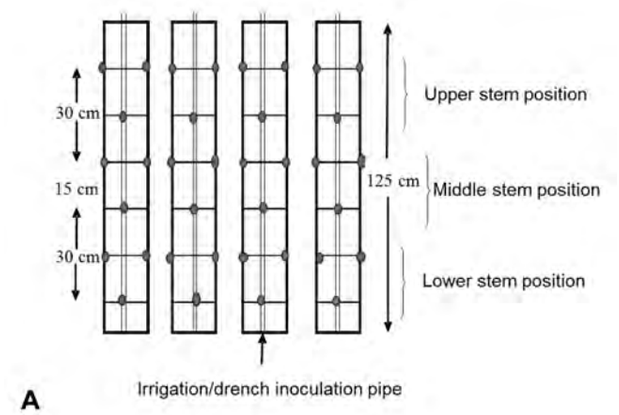

Fig. 1. A. Diagrammatic representation of cassava tube screening set up at 30 cm × 30 cm spacing between plants (the thick dots), B. Photograph of the tubes at 1 month after planting.

Three weeks after planting, *Meloidogyne* spp. juveniles/eggs (Talwana *et al.*, 1996) were used to inoculate the sawdust tubes. For drench inoculation, the inoculum was applied in 1000 ml borehole tap water (used throughout) to ensure thorough distribution. For individual stem inoculation, each stem received 1000 juveniles/eggs applied to the base of the stem (root zone) in 10 ml water using a pipette. The sawdust tubes were irrigated one day prior to inoculation to permit at least one week without irrigation to encourage nematode establishment. The plants were maintained for a further two months. No fertilizer was applied during this time. At harvest, plant height, root fresh weight, tuber fresh weight and galling index data were collected. Galling damage was recorded using a scale of 1-5 according to Gapasin (1980), where 1 = unaffected and 5 = severe galling damage (75-100% of roots severely affected). Nematode densities were determined from a 1 g root sub-sample obtained from each plant after coarsely chopping all roots and nematodes were extracted using a modified Baermann technique (Coyne *et al.*, 2007). Vermiform *Meloidogyne* spp. juveniles and eggs were counted. Based on the results of Experiment 1, modifications were made to the technique before repeating as experiment 2.

Experiment 2. Experiment 2 was conducted in immediate succession to Experiment 1 to confirm initial results and compare inoculation levels. Spacing of the stems was maintained at 30 cm × 30 cm, inoculation was conducted through the perforated centrally placed pipes or through drenching sawdust from the surface. The experiment was arranged according to a randomized block design with four replicates and three inoculation levels of 1000, 500 and 250 *Meloidogyne* spp. eggs applied in water suspension at three weeks after planti-

ng as above. Sawdust tubes were again irrigated one day prior to inoculation and maintained for two months after inoculation. Data collected at harvest included plant height, root fresh weight, galling index, *Meloidogyne* spp. densities as above and egg mass density per g root. The experiment was repeated in July 2009 but using per cutting inoculation at inoculation levels of 1000, 500, 250 and 0 *Meloidogyne* spp. eggs/juveniles.

Experiment 3. This was conducted in March 2008 and repeated in July 2009 in order to confirm and verify the effect of the stem positions (Lower, Middle and Upper) on *Meloidogyne* spp. distribution and growth of the cassava stems. The experiments were conducted in the same manner as Experiment 2 using the equivalent of 1000 *Meloidogyne* spp. juveniles/eggs per stem, and drench inoculation.

Statistical analysis. Nematode population data were square root (x + 0.5) transformed for all experiments prior to analysis of variance to ensure homogeneous variance among treatments (Gomez and Gomez, 1984). Differences between means were compared using Tukey's studentized range test and t-test with SAS (SAS Institute, 1999).

RESULTS

Experiment 1. Analysis of variance indicated that inoculation method significantly ($P \leq 0.05$) influenced root fresh weight, galling index and *Meloidogyne* spp. density (Table I). Irrigation method had a significant ($P \leq 0.05$) influence on plant height, galling index, *Meloidogyne* spp. density and tuber weight. Spacing influenced ($P \leq 0.05$) only plant height and root fresh weight. Stem position affected ($P \leq 0.05$) plant height only. Notable interactions of inoculation method × irrigation method and inoculation method × stem position significantly influenced *Meloidogyne* spp. density and galling index respectively.

Individual stem inoculation resulted in a greater ($P \leq 0.05$) degree of galling and *Meloidogyne* spp. density than drench inoculation (Table II). Drench inoculation resulted in better plant growth ($P \leq 0.05$) than individual stem inoculation. Use of the irrigation pipe resulted in more ($P \leq 0.05$) severe galling and greater ($P \leq 0.05$) *Meloidogyne* spp. densities than without the pipe. Plant height and tuber weight were greater without the pipe. Stem spacing had no influence on final *Meloidogyne* spp. density but the galling index was greater ($P \leq 0.05$) at the widest spacing. Plant growth was also better in the middle position for the wider spaced plants than at narrow spacing. No significant differences were observed for galling index or *Meloidogyne* spp. density between stem position. Growth components were relatively greater, however, in the upper third as opposed to middle and lower positions. Individual stem inoculation resulted in a greater ($P \leq 0.05$) galling index and *Meloidogyne* spp. density than for the drench inoculation, with or without the irrigation pipe (Table III). Under the various inoculation combinations, plant spacing had no significant influence on galling index or *Meloidogyne* spp. populations, although values were relatively greater for individual inoculation. Plant height and root fresh weight increased ($P \leq 0.05$) with plant spacing and were greater under drench inoculation. Galling index and *Meloidogyne* spp. density were greater ($P \leq 0.05$) for the uppermost position than either lower or middle positions under drench inoculation. With the exception of plant height, which was greater ($P \leq 0.05$) in the upper position, all measured variables were similar among the positions for the individual stem inoculation.

Experiment 2. Plant height and root fresh weight were not significantly influenced by inoculation levels using drench. Formation of small tubers was observed on some plant stems, and was significantly ($P \leq 0.05$) influenced by inoculation level. *Meloidogyne* spp. density

Table I. Analysis of variance for the effect of irrigation method, inoculation method, stem spacing and position on growth of cassava and nematode damage and density under tube screening.

Source of variation	d.f.	F Values[1]				
		Plant height	Root fresh weight	Galling index[2]	*Meloidogyne* spp. juvenile density[3]	Tuber fresh weight
Replication	2	5.79**	16.57***	2.75 ns	24.32***	7.05**
Inoculation method (A)	1	0.36 ns	5.71*	146.0***	262.85***	1.02 ns
Irrigation method (B)	1	42.59***	1.49 ns	18.39***	8.68**	45.86***
Sett spacing (C)	2	5.93**	13.76***	2.1 ns	0.18 ns	2.83 ns
Sett position (D)	2	7.04**	0.47 ns	2.00 ns	0.14 ns	3.09 ns
A × B	1	0.83 ns	0.21 ns	1.3 ns	8.05**	0.59 ns
A × D	2	1.98 ns	0.81 ns	4.15*	1.45 ns	0.12 ns
A × C	2	6.62**	0.80 ns	1.29 ns	2.18 ns	4.91**
B × D	2	2.1 ns	0.91 ns	0.70 ns	0.75 ns	2.67 ns

[1]ns, *, ** and *** represent not significant (P>0.05), significant at $P \leq 0.05$, 0.01 and 0.001, respectively.
[2]Rating scale: 1 = unaffected and 5 = severe galling damage.
[3]Square root transformed values used for analysis.

Table II. Effect of various treatment options on plant growth components and nematode variables in vertical polythene tubes[1].

Treatment	Plant growth components			Nematode variables	
	Plant height (cm)	Root fresh weight (g)	Tuber weight (g)	*Meloidogyne*[2] (g^{-1} root)	Galling index[3]
Inoculation method:					
Drench	9.6 a	5.0 a	1.9 a	5.3 a	1.8 a
Per sett	9.3 a	4.5 b	1.6 a	184.3 b	2.6 b
Irrigation method					
Irrigation tube	7.53 a	4.9 a	1.0 a	114.8 a	2.3 a
No tube	11.4 b	4.6 a	2.4 b	71.6 b	2.1 b
Spacing					
20 cm × 20 cm	8.3 a	4.0 a	1.9 a	85.2 a	2.2 ab
25 cm × 25 cm	9.8 b	5.2 b	1.8 ab	97.7 a	2.1 a
30 cm × 30 cm	11.0 b	5.4 b	1.4 b	98.3 a	2.3 b
Sett position					
Lower	9.9 a	4.6 a	1.5 a	89.5 a	2.2 a
Middle	10.6 a	4.8 a	2.0 a	100.1 a	2.1 a
Upper	8.0 b	4.9 a	1.6 a	89.2 a	2.3 a

[1]For each treatment group values within a column followed by the same letter are not significantly ($P \leq 0.05$) different.
[2]Square root transformed values used for analysis.
[3]1 = unaffected and 5 = severe galling damage.

Table III. Effects of combining two inoculation methods with different irrigation methods, spacings and stem positions on plant growth components and nematode variables in vertical polythene tubes[1].

Inoculation method	Option	Plant growth components			Nematode variables	
		Plant height (cm)	Root fresh weight (g)	Tuber fresh weight (g)	*Meloidogyne* spp. juveniles per g of roots	Galling index
	Irrigation method					
Drench	No pipe	11.8 a	4.8 a	2.6 a	7.3 a	1.7 a
	Pipe	7.6 a	5.3 a	1.1 a	3.3 a	1.9 a
Per stem	No pipe	11.1 a	4.4 a	2.2 a	137.3 b	2.4 b
	Pipe	7.5 a	4.5 b	0.9 a	233.2 b	2.8 b
	Spacing					
Drench	20 cm × 20 cm	7.3	4.2 a	2.3 a	7.3 a	1.7 a
	25 cm × 25 cm	11.3 b	5.3 ab	2.3 a	5.6 a	1.8 a
	30 cm × 30 cm	11.0 b	5.9 b	1.0 b	1.8 a	1.8 a
Per stem	20 cm × 20 cm	9.3 ab	3.7 a	1.6 a	169.3 a	2.6 a
	25 cm × 25 cm	8.3 a	5.0 b	1.4 a	192.8 a	2.5 a
	30 cm × 30 cm	10.9 b	5.0 b	1.7 a	194.8 a	2.8 a
	Stem position					
Drench	Lower	9.4 ab	5.1 a	1.6 a	0.3 a	1.6 a
	Middle	11.5 a	5.0 a	2.2 a	1.8 a	1.7 a
	Upper	8.1 b	5.0 a	1.8 a	13.3 b	2.0 b
Per stem	Lower	10.5 a	4.1 a	1.4 a	178.7 a	2.7 a
	Middle	9.7 ab	4.5 a	1.9 a	198.3 a	2.6 a
	Upper	7.8 b	4.8 a	1.4 a	175.2 a	2.6 a

[1]For each inoculation method × option combination values within a column followed by the same letter are not significantly ($P \leq 0.05$) different.
[2]Square root transformed values used for analysis.
[3]Rating scale: 1 = unaffected and 5 = severe galling damage.

Table IV. Effect of inoculation level on growth of cassava and nematode density following drench inoculation in vertical polythene tubes[1].

Inoculation level	Plant height (cm)	Root fresh weight (g)	Tuber weight (g)	Egg masses per g of roots	*Meloidogyne* spp. juveniles per g of roots
250	3.5 a	1.6 a	0.0 a	2.4 a	3.6 a
500	4.2 a	2.0 a	0.0 a	1.7 ab	4.2 a
1000	3.9 a	1.8 a	0.2 a	0.7 b	2.3 a

[1]Values within a column followed by the same letter are not significantly ($P \leq 0.05$) different.

was low and not significantly affected by inoculation level but egg mass density was affected ($P \leq 0.01$), with lower egg mass density occurring at the higher inoculation than at the lower level (Table IV). Galling as a result of *Meloidogyne* spp. infection was not detected in any of the treatments.

Following inoculation of each stem, no significant effect of either stem position or inoculation level, or their interaction, was observed on plant height, root fresh weight or tuber weight. The number of egg masses and *Meloidogyne* spp. density were significantly ($P \leq 0.01$) influenced by inoculum level, while the interaction between inoculum level and stem position significantly influenced ($P \leq 0.01$) only galling index. Egg masses were significantly fewer in the lower stem position compared to the upper sett position (Table V) but *Meloidogyne* spp. densities did not differ among the stem positions.

No statistical differences were recorded among the

Table V. Effect of position in the tube on cassava growth and nematode damage and density of cassava following individual stem inoculation in vertical polythene tubes[1].

Stem position	Plant height (cm)	Tuber weight (g)	Root fresh weight (g)	Galling index[2]	Egg masses per g of roots	*Meloidogyne* spp. juveniles per g of roots
Lower	8.4a	0.5a	1.8a	1.1a	0.6b	1.8a
Middle	10.7a	0.9a	1.5a	1.1a	1.5ab	0.9a
Upper	10.0a	0.9a	2.0a	1.1a	1.7a	1.0a

[1]Values within a column followed by the same letter are not significantly ($P \leq 0.05$) different.
[2]Rating scale: 1 = unaffected and 5 = severe galling.

Table VI. Effect of inoculum level on cassava growth and nematode damage and density following individual stem inoculation in vertical polythene tubes[1].

Inoculation level	Plant height (cm)	Tuber weight (g)	Root fresh weight (g)	Galling index[2]	Egg masses per g of roots	*Meloidogyne* spp. juveniles per g of roots
0	10.3a	1.1a	2.1a	1.0a	0.0c	0.0b
250	8.8a	0.4a	1.1a	1.0a	0.9bc	1.5a
500	8.3a	0.9a	1.8a	1.2a	1.6ab	1.7a
1000	11.2a	0.6a	1.9a	1.2a	2.4a	1.8a

[1]Values within a column followed by the same letter are not significantly ($P \leq 0.05$) different.
[2]Rating scale: 1 = unaffected and 5 = severe galling damage.

Table VII. Effect of position on growth of cassava and incidence of nematode damage and density following drench inoculation equivalent to 1000 nematodes per plant[1].

Stem position	Plant height (cm)		Root fresh weight (g)		Tuber weight (g)		Galling index[2]		Egg masses per g of roots		*Meloidogyne* spp. juveniles per g of roots	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Lower	6.0a	8.8a	2.3a	3.1a	2.1a	0.1a	1.3a	1.3a	0.1a	0.6b	11.7a	9.6a
Mid	6.6a	10.6a	1.8a	1.2a	1.5a	0.5a	1.2a	1.3a	0.4a	1.3ab	15.0a	16.1a
Upper	10.3a	8.9a	1.6a	1.6a	0.4a	0.4a	1.2a	1.1a	0.3a	4.0a	10.7a	14.5a

[1]Values within a column followed by the same letter are not significantly ($P \leq 0.05$) different.
[2]Rating scale: 1 = unaffected and 5 = severe galling damage.

different inoculation levels for plant height, tuber weight, root fresh weight and galling index (Table VI). However, although control plants had slightly better growth and no galling, the measured variables did not differ from those in the other inoculation levels.

Experiment 3. Results obtained in 2008 differed significantly from results obtained in 2009. However, for both seasons none of the measured variables was significantly influenced by stem position in the tubes (Table VII).

DISCUSSION

Although some growth components differed between sections of the drenching method, galling and nematode densities were similar when using an irrigation pipe placed centrally down through the sawdust planting medium. Consequently, for speed of application it can be recommended that polythene tubes, held vertically, and drenched with a suspension of nematode eggs and or juveniles through a centrally placed perforated pipe, can efficiently be used to rapidly assess cassava cuttings for resistance against *Meloidogyne* spp. Individual inoculation of cassava stems did invariably result in significantly greater galling indices and *Meloidogyne* spp. populations than a drench inoculation. Although the drench inoculation was a simpler method and less time consuming in terms of applicability, individual stem inoculation resulted in higher nematode recovery. Given that there were no differences between the positions along the polythene tube though, and that results were repeatable, the system appears suitable as a rapid mechanism for screening cassava. Moreover, it should serve a generic purpose for screening other crops, especially where space is limited. For seed-borne plants, however, it is likely that some provision of fertilizer may be necessary to sustain the plants for the period of assessment. Inoculation method × stem position interaction revealed an even distribution of damage symptoms and growth variables along the polythene tubing, both in drench and individual inoculations, especially in the lower and middle positions.

Watering through an irrigation pipe installed along the centre of the screening tubes resulted in greater galling indices and *Meloidogyne* spp. densities than without a pipe. This indicates that, by watering through the pipes, moisture distribution was well balanced within the polythene tubing with minimum flooding, than when watering directly onto the top of the sawdust with no irrigation pipe. No pipe (direct) irrigation therefore appears associated with irregular water distribution in the polythene tubing leading to the uneven growth observed. Drench inoculation using the pipe gave more uniform nematode damage along the tube, thus support the use of the pipe.

Cassava growth was not affected by stem spacing at 30 cm × 30 cm rather than 25 cm × 25 cm, with greater plant height, root fresh weight and tuber weight, despite inoculation with *Meloidogyne* spp., than in the narrower spacing for both of these spacings. Narrow spacing (20 cm × 20 cm), on the other hand, was characterized by lower growth components and relatively great nematode populations and damage, signifying a possibility of competition for nutrients under high nematode infestation (inoculum). Therefore, it may be wise to recommend a wider spacing, say 25 cm × 25 cm, to ensure that the growth of cassava plants being screened against *Meloidogyne* spp. is not compromised by intra-stem competition for nutrients or light. However, to enable optimum identification of nematode resistance, narrower spacing, such as 20 cm × 20 cm, could be more appropriate, where plants under greater stress but least damaged by nematodes would be the most useful to identify.

Using the different inoculation levels (1000, 500 and 250 *Meloidogyne* spp.) revealed no significant differences in growth or recovered nematode density under drench and per stem inoculation. However, *Meloidogyne* spp. densities recovered were generally small (less than five *Meloidogyne* spp. juveniles per g of root) irrespective of the inoculation levels. The micro-environment in the screen-house combined with management aspects and intra-specific competition may have affected abundance patterns of the nematodes (Wallace, 1963). Drench inoculation, resulted in greater egg mass recovery at lower inoculation levels than at the greater inoculation levels in Experiment 2. The reverse was true with individual stem inoculation. Talwana *et al.* (1996) recorded greatest female fertility when 1000 *Meloidogyne* spp. eggs were used per pot and recommended 1000 *Meloidogyne* spp. eggs to be the least population one can inoculate in order to cause a measurable loss in plant height and root fresh weight.

CONCLUSION

Cassava screening for resistance to *Meloidogyne* spp. in vertically supported polythene tubing can indeed provide a rapid and less laborious protocol compared to other methods, such as use of pots. Spacing of plants should ideally be minimized, with a recommendation of 20 cm × 20 cm, while watering should be made through a perforated hose pipe (2.5 cm inner diameter) inserted along the centre of the growth media. Inoculation on an individual cassava stem basis led to a greater degree of damage and thus presents a greater degree of challenge than inoculation by drenching. However, with greater inoculum levels this shortcoming of drenching may be overcome. Moreover, for speed and ease, the drenching, when applied through the irrigation pipe, can be effective and is suitable for rapid screening purposes. The minimum inoculation level should be 1000 *Meloidogyne* spp. eggs at three weeks after planting of cassava stems.

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