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Evaluation of cacao (*Theobroma cacao* L.) clones for resistance to root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood

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ABSTRACT

Objective: To evaluate cacao clones for reaction to *Meloidogyne incognita,* the most common root-knot nematode species in Nigeria.

Methodology and results: Experiments were carried out in the screen house and in nursery, laid out in a completely randomized design with four replicates. The two factors were *M. incognita* inoculum at two levels (0 and 5000 eggs per seedling) and the twelve cocoa clones (MXC67, T86/2, PA150, LCTEEN, T12/11, T53/5, T101/15, T65/7, ICS1 and AMAZ 15-15). Effect of nematode on plant height, number of leaves, stem girth, fresh root weight, fresh shoot weight, fresh leaf weight and total dry matter were considered. Based on gall index, nematode reproduction factor and growth parameters, it was concluded that clones MXC67, T86/2, PA150, T101/15 and T53/5 were susceptible to the nematode while clones T65/7 and ICS1 were tolerant. A high degree of resistance was exhibited by LCTEEN, T12/11 and AMAZ 15-15. Compared to F_3 Amazon and Amelonado varieties, the two most famous cocoa varieties in Nigeria, four clones (LCTEEN, T65/7, ICSI and AMAZ 15-15) were superior to the check (F_3 Amazon and Amelonado).

Conclusion and application of findings: This study showed that among clones that had been previously screened and certified as being resistant to black pod disease caused by *Phytophthora megakarya*, only three are resistant to root-knot nematode and only two are tolerant (are able to tolerate nematode reproduction). Breeding for disease resistance in cocoa can therefore no longer neglect the effect of nematodes, particularly the root-knot group.

Key words: Cocoa, *Meloidogyne incognita,* nematode, resistance

INTRODUCTION

Theobroma cacao is a member of a the large Malvaceae family which comprises of the former families Sterculiaceae (cacao and kola), Bonbacaceae (baobab, durian and kapok), Malvaceae sensu lato (cotton, hibiscus, and okra), and Tiliaceae (basswood) (Ploetz, 2007). The production of cocoa in Nigeria has witnessed a downward trend since the early 1970s due to numerous factors, e.g. ageing trees, shortcomings in applying recommended agronomic techniques by farmers, and the effects of pests and diseases. The root-knot nematode *Meloidogyne* spp is a

well-known pest of many tropical and sub-tropical plants. *Meloidogyne* species are the most important nematodes of cacao due to their pathogenicity and wide distribution in cocoa producing regions (Campos & Villain, 2005). It is a common pest of cacao in West Africa (Whitehead, 1969; Asare-Nyako & Owusu, 1979; Fademi *et al*., 2006).

Symptoms of *M. incognita* damage on cacao seedlings are dieback, stunting, wilting, chlorosis and reduction in size of the leaves, and galling of the root or complete death of the seedlings (Afolami & Caveness, 1983; Orisajo & Fademi, 2005; Orisajo *et al*., 2007). Although control strategies have been based on the use of chemical nematicides (Afolami, 1993), the chemicals are usually too expensive for resource-poor farmers and their use often adversely affects many soil biological processes. There is need to develop new management tools that are environmentally and toxicologically safe (Gullino *et al*., 2003). The need for alternatives to nematicides has stimulated research focusing on sustainable tactics for management of plant parasitic nematodes (McSorley & Poranzinska, 2001). The objective of this research was to evaluate ten clones of cacao for their reaction to *Meloidogyne incognita*.

MATERIALS AND METHODS

Experiments were carried out both in the green house and nursery at the research farm of the Cocoa Research Institute of Nigeria (CRIN) at Ibadan, Nigeria (latitude 7.26°, longitude 3.54° and 122m above sea level). The annual rainfall ranges between 1200 - 2500 mm, distributed over 5 - 7 months from April to October. The average daily temperature range is 26 - 30°C.

Sandy-loam top soil normally used for raising cacao seedlings was collected in bulk from the CRIN research field and sterilized in an autoclave at 1kg/cm^3 for 15 minutes before distributing into 5 L plastic pots. The factorial experiment was laid out in a Completely Randomized Design with four replicates. The two factors were *M. incognita* inoculum at two levels (0 and 5000 eggs per seedling) and the twelve cocoa clones (T12/11, T65/7, T101/15, T53/5, T86/2, LCTEEN, PA150, AMAZ 15-15, MXC67, ICS1, F_3 Amazon and Amelonado). F_3 Amazon and Amelonado (both susceptible) served as checks.

Two seeds of the appropriate cocoa clone were sown in each of the 192 pots in January 2007; with eight replicate pots for each of the twelve clones. Seedlings were thinned to one per pot at five days after emergence. On the seventh day, the seedlings in four of the eight pots of each cocoa clone were inoculated with 5000 *M. incognita* eggs extracted from a culture of the nematode maintained on *Celosia argentea* L. roots. Nematodes were cultured by inoculating *Celosia* seedlings grown on sterilized soil with eggs and juveniles of *Meloidogyne incognita* identified through perineal pattern examination. The eggs were extracted using the Hussey and Barker (1973) sodium hypochlorite (NaOCl) method. The experiment was terminated 24 weeks after nematode inoculation.

The screening experiment was re-validated in the nursery for all the clones, using four replications per treatment. Pots were arranged on nursery benches in a randomized complete block design with four replications. After sowing, regular visual observations were made on disease symptoms expression. The experiment was terminated 26 weeks after planting. The growth parameters such as plant height, stem girth, and numbers of leaves were recorded.

To assess infection, the roots were carefully rid of soil, washed under a gentle stream of tap water, mopped and galls counted using a hand lens at 3-5 X magnification. Root galling was assessed using the 0-5 gall index (Sasser *et al*., 1984). Nematode eggs were collected from each root system using the sodium hypochlorite method (NaOCl) of Hussey and Barker (1973), and counted. Aliquots of 100 cm^3 soil samples from each pot were assayed for juveniles of *M. incognita* using the modified Baermann technique (Coyne *et al*., 2007).

Data analysis: Analysis of variance (ANOVA) and correlation analysis were carried out on data collected and the means were compared using the Least Significant Difference (LSD) test. Resistance rating was carried out using the quantitative scheme for assigning crop varieties into resistance categories based on crop yield, reproduction factor R, and gall index (GI) (Afolami *et al.,* 2004).

RESULTS

For some clones plant growth as expressed by height, number of leaves and stem girth was significantly (P= 0.05) suppressed by *M. incognita* at the initial population of 5000 nematodes per plant (planted in 5kg soil) while other clones exhibited resistance or tolerance. Inoculated plants of clone PA150 manifested stunted growth with reduced stem girth and total dry matter. Later investigation revealed poor root development with galls when compared to the nematode-free plants having good growth and root development (Figure 1). For clone T101/15 seedlings the presence of the nematode suppressed the growth as expressed by reduced height, leaf number, stem girth and poor root development when compared to the nematode-free plant. The presence of nematodes in stimulated growth of cv. AMAZ 15-15, leading to better growth as expressed by significant height with more leaves, though having similar girth to the uninoculated plants. Root investigation revealed good root development with fine root hairs (Figure 2). This cultivar showed comparatively healthy shoot growth for both inoculated and *Meloidogyne*-free plants.

Inoculated plants of clone LCTEEN expressed good growth with healthy shoot development, having a significantly increased stem girth and total dry matter. Root investigation revealed a healthy root system with fine root hairs. For cv. MXC67, the nematode-free plant showed an incipient growth advantage in terms of leaf area, stem girth and good root development over the inoculated plant that manifested narrow leaves in the 16th week, which later led to leaf drop that gave them an unthrifty appearance. The inoculated plant was drastically affected by nematode infection at latter stages of growth, with a significant reduction in leaf number, stem girth and total dry matter with a reproduction factor of 1.35 and gall index of 3.0.

Cultivar Amelonado and F_3 Amazon began manifesting chlorosis sixteen weeks after inoculation with *M. incognita*. They both manifested stunted growth, and later investigation revealed poor root development with galls in these plants. Nematode infection of cv.T86/2 led to a reaction that stimulated rapid growth but with plant having reduced leaf number, stem girth and poor root development when compared to the nematode-free plant which showed physiological superiority by exhibiting jorquetting (Figure 3) and having good growth. Ten weeks after nematode infection, vein clearing and narrowing of leaves were observed in clone T53/5. The vein clearing was not persistent and root investigation revealed poor root development with galls. Clone ICS1 plants also exhibited better growth in terms of the growth indices in the presence of *M. incognita* infection as expressed by significantly increased height, leaf number and stem girth.

Inoculated plants of clone T65/7 compared favourably to the nematode-free plants in terms of growth parameters. In the presence of the nematode clone T12/11 plants had growth advantage in terms of leaf number, height, stem girth and total dry matter. Root investigation revealed good root development with fine root hair when compared to the nematode-free plants.

Of the ten clones evaluated, nematode inoculation drastically reduced the height of Amelonado, which is a susceptible variety. The highest plant height in the presence of nematode inoculation was recorded in clone T12/11 both in the green house and in the nursery (Figure 4). Considering the shoot weight, the presence of *M. incognita* stimulated good shoot development with clone T12/11 exhibiting the highest shoot weight both experiment and was closely followed by ICS1 (Figure 5). With regard to root development, the nematode presence enhances root development in some of the clones with T12/11 having the highest root weight (Figure 6). Dry matter, which corresponds to yield, was highest in T12/11 and was closely followed by T101/15 in the presence of nematodes (Figure 7).

Data in table 1 show the response and rating of ten clones of cocoa using the modified version of Afolami (2000) quantitative scheme for assigning crop varieties into resistance categories. Clones LCTEEN, T12/11 and AMAZ 15-15 were poor hosts of *M. incognita* and they exhibited resistance to the nematode, which was unable to reproduce in these clones. These clones showed good root development in spite of *M. incognita* infection. Although the galls were well developed, the root hairs were not destroyed. The plants of these clones showed comparatively healthy shoot growth for both inoculated and *Meloidogyne*-free plants.

Clones MXC67, T86/2, PA150, T101/15 and T53/5 were obviously highly susceptible as *M. incognita* successfully established itself in these clones. Mean dry matter of the inoculated plants was significantly lower than that of the uninoculated plants.

Clones T65/7 and ICS1 were rated as tolerant because although the nematode was able to establish itself successfully in the clones, the mean dry matter of

the inoculated plants remained equal to or higher than that of the uninoculated plants, i.e. the clones did not suffer any statistically significant loss due to infection. When visually rated, cultivar F_3 Amazon compared favourably to the best five clones, i.e. LCTEEN, T12/11, AMAZ 15-15, T65.7 and ICS1 (Table 1), but was inferior in root growth to clones T65/7, LCTEEN and T12/11. Clones Amelonado was inferior to all the clones that were evaluated.

Figure 1: Inoculated plant of cocoa cv. PA150 (left) and nematode free plant (right); **Figure 2**: Inoculated plant of T86/2 (left) and nematode free plant (right). The inoculated plant exhibited stimulated growth.

DISCUSSION

Based on a combination of root-gall index, nematode reproduction factor, total dry matter and other growth indices, five of the ten clones tested were rated as susceptible, three were resistant and two tolerant. All the ten clones showed individual variations in growth response when inoculated with *M. incognita* eggs. According to Nwanguma *et al.,* (2005), there are indirect relationships between the various growth indices and the nematodes' population, which are largely due to genetic basis of the plant characters.

The relatively low populations of the nematodes in clones AMAZ 15-15, T12/11 and LCTEEN indicated some degree of resistance, which probably explains the absence of damage on the root system and thus insignificant impact on the growth characters. The superiority of clones AMAZ 15-15, T12/11 and LCTEEN (resistant clones) and T65/7 and ICS1 (tolerant clones) in terms of plant height, leaf number and total dry matter could be attributed to the low level of root nematode infection which resulted in enhanced root development and ability to tap nutrients from the soil maximally. It is also a known fact that the ability to withstand nematode attack, good plant vigour and high yield are all indices of high level of plant tolerance (Ploeg, 2001; Ononuju & Fawole, 1999).

Figure 3: Inoculated plant of T53/5 (left) and nematode free plant.

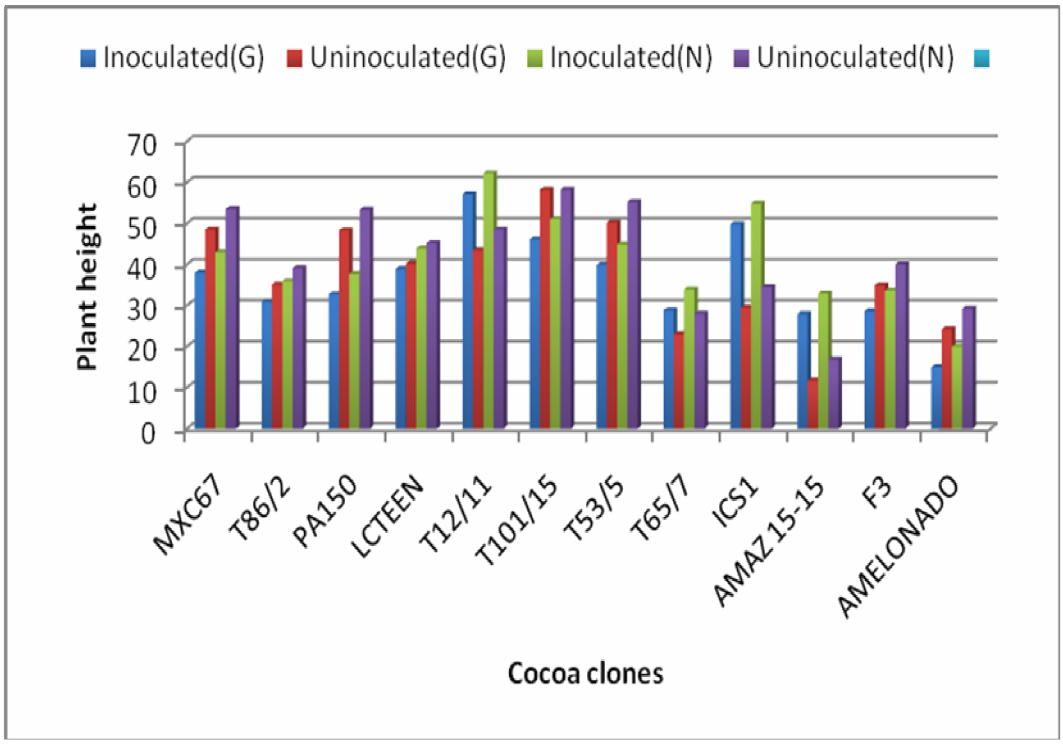

Figure 4: Effect of *M. incognita* on plant height 24 weeks after inoculation both in the greenhouse and in the nursery.

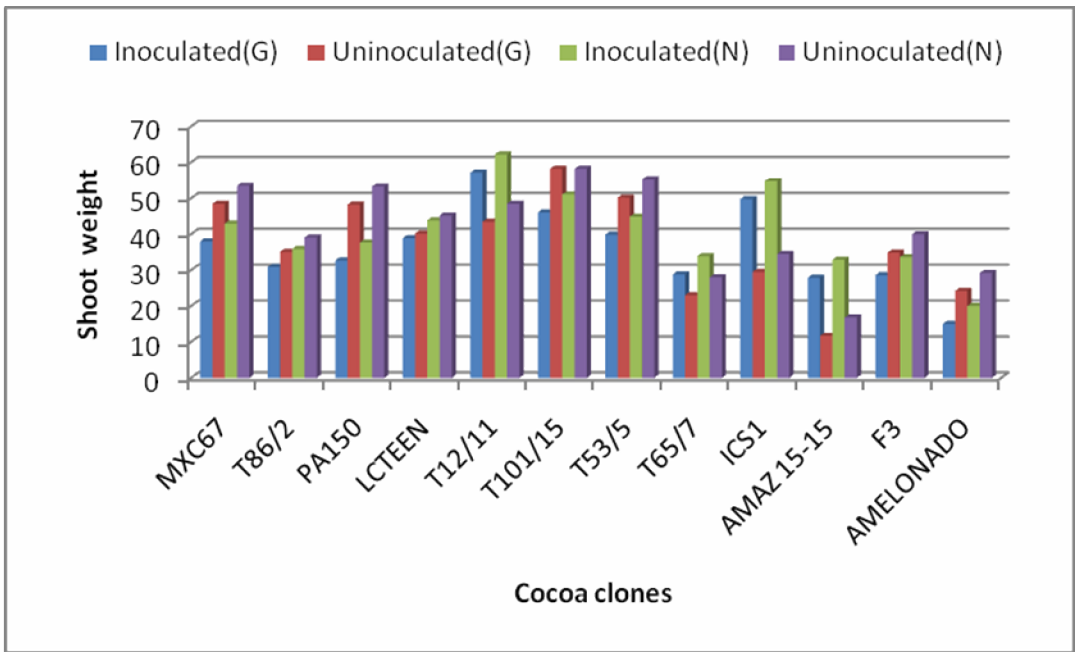

Figure 5: Effect of *M. incognita* on shoot weight 24 weeks after inoculation both in the greenhouse and in the nursery.

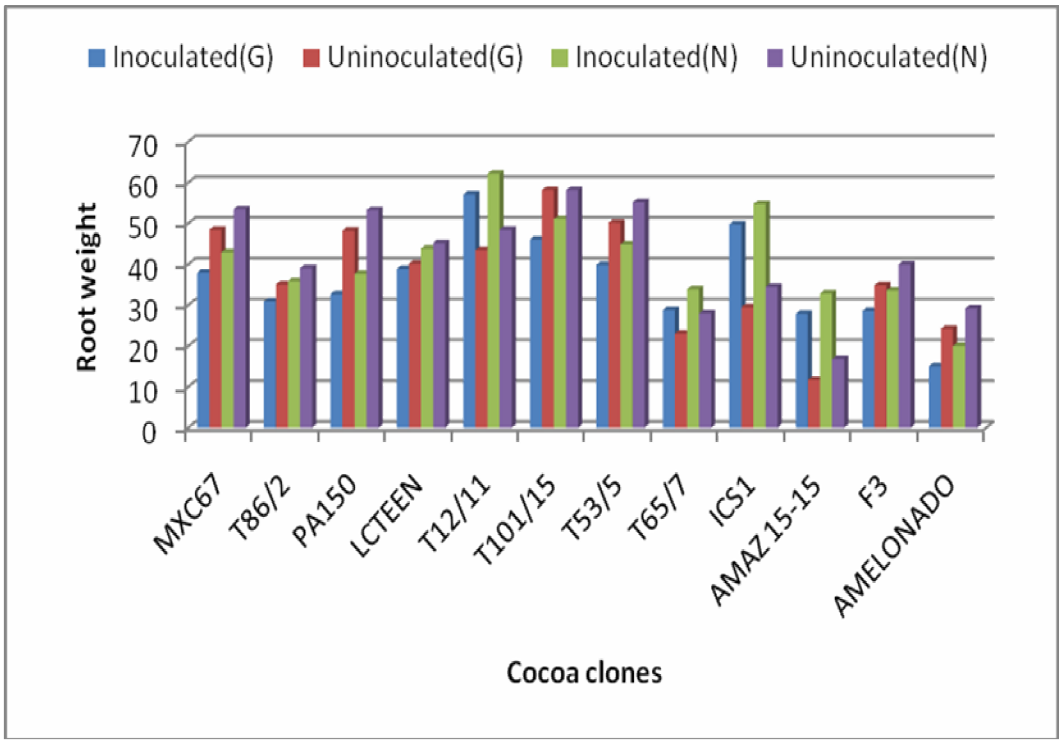

Figure 6: Effect of *M. incognita* on root weight 24 weeks after inoculation both in the greenhouse and in the nursery.

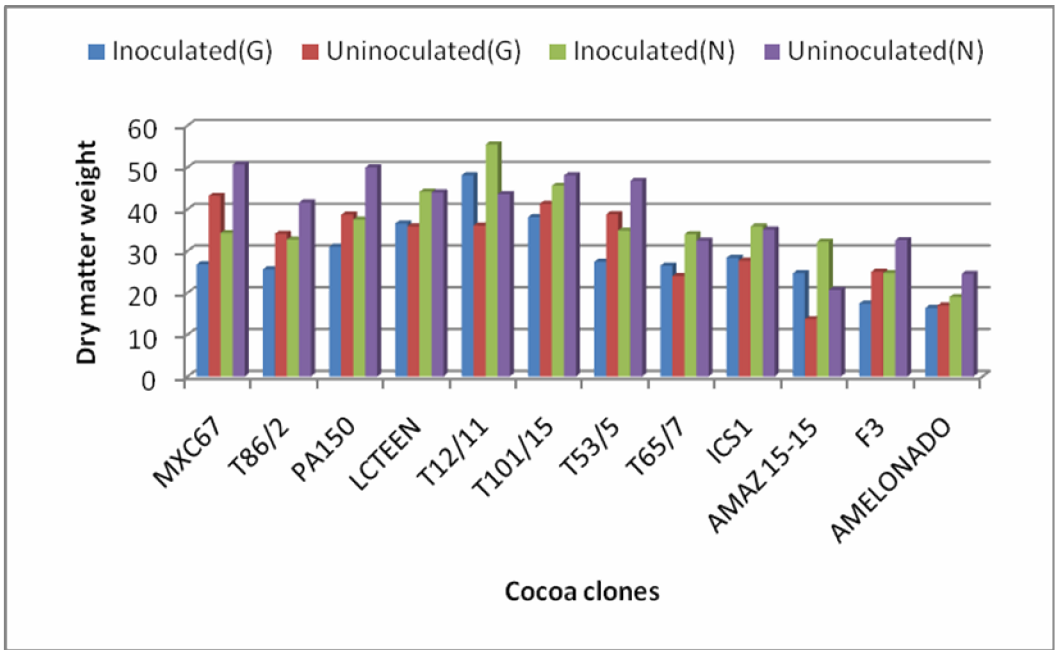

Figure 7: Effect of *M. incognita* on dry matterweight 24 weeks after inoculation both in the greenhouse and in the nursery.

Table 1: Resistance rating of twelve clones of cocoa using the quantitative scheme for assigning crop varieties into resistance categories based on dry matter, reproduction factor and gall index (Afolami, 2000).

	GREENHOUSE EXPERIMENT							NURSERY EXPERIMENT							
Clones	Mean No of Gall	Gall Index (I)	Rep. Factor (R)	Mean Dry Weight of Seedlings A	B	A-B	Resitance Category	Mean No of Gall	Gall Index (I)	Rep. Factor (R)	Mean Dry Weight of Seedlings A	B	A-B	Resitance Category	Remarks
MXC67	27.5	3.0	1.35	28.7	43.2	-14.5*	Susceptible	28.0	3.0	1.36	34.3	50.7	-16.4*	Susceptible	Consistent
T86/2	27.3	3.0	1.53	25.7	34.1	-8.4*	Susceptible	27.3	3.0	1.48	32.7	41.6	-8.9*	Susceptible	Consistent
PA150	27.0	3.0	1.69	31.0	36.7	-5.7*	Susceptible	26.0	3.0	1.72	37.5	50.0	-12.5*	Susceptible	Consistent
LCTEEN	2.0	1.0	0.97	36.6	35.9	0.7NS	Resistant	1.8	1.0	0.90	44.2	44.0	0.2NS	Resistant	Consistent
T12/11	3.0	2.0	0.99	48.0	36.1	11.9*	Resistant	2.8	2.0	0.99	55.3	43.6	11.7*	Resistant	Consistent
T101/11	24.3	3.0	2.24	38.1	41.3	-3.2*	Susceptible	23.8	3.0	2.28	45.5	48.0	-2.4*	Susceptible	Consistent
T53/5	24.8	3.0	1.20	27.4	38.8	-11.4*	Susceptible	24.5	3.0	1.20	34.9	46.3	-11.4*	Susceptible	Consistent
T65/7	28.3	3.0	1.93	26.5	24.0	2.5*	Tolerant	25.5	3.0	1.94	34.0	31.5	4.5*	Tolerant	Consistent
ICS1	28.6	3.0	1.45	28.4	27.6	0.8NS	Tolerant	26.6	3.0	1.46	35.9	35.0	0.9NS	Tolerant	Consistent
AMAZ 15-15	2.5	2.0	1.00	24.7	13.8	10.9*	Resistant	2.3	2.0	1.00	32.2	20.8	11.4*	Resistant	Consistent
F3 AMAZON	15.8	3.0	1.62	17.4	25.0	-7.6*	Susceptible	15.3	3.0	1.04	24.7	32.6	-7.9*	Susceptible	Consistent
AMELONADO	18.5	3.0	2.82	11.4	17.0	-5.6*	Susceptible	18.4	3.0	2.04	19.0	24.5	-5.5*	Susceptible	Consistent

GI = Gall index where 1 = 1 – 2 galls; 2 = 3 – 10 galls; 3 = 11 – 30 gals; 4 = 31 – 100 galls; 5 = > 100 galls (after Taylor and Sasser, 1978).
R = Nematode Reproduction Factor = Final number of juveniles and eggs (P_f)/initial inoculum (P_i)
* = Statistically Significant Difference ($P \leq 0.05$).
NS = Not significant
A = Inoculated
B = Uninoculated

Visual observation showed that the leaves of parasitized plants were smaller and narrower, as reported by Sharma and Maia (1976). In their study, *M. incognita* damaged the seedlings of "Catongo" cultivar of cocoa in the greenhouse in Brazil causing significant reduction in height, stem diameter, dry shoot weight and reduction in number and longevity of leaves. Similar observations were made by Asare-Nyako and Owusu (1979) for seedlings of hybrids T63/967 X Sca 6, Wacri Series IIJ, WACRI Series II D and Amelonado in Ghana.

In Nigeria, Afolami and Ojo (1984) screened ten cocoa hybrids for resistance and they reported that cacao hybrids C77 x 23, C74 x C23, C73 x C75, C72 x C23 and C43 x C74 manifested serious shoot damage and galling. Afolami (1981) also reported that *M. incognita* caused a reduction in leaf size, stem height and root galling of cv. F_3 Amazon and Amelonado seedlings at 16 and 32 weeks after inoculation, respectively.

In our study, the cocoa clones were vulnerable to nematode attack under the enclosed screen house conditions apparently due to heavy inoculum density of the nematodes and some exogenous factors (e.g. high ambient temperature >33.15 ^{0}C) associated with green house studies. This observation is corroborated by the report of Nwanguma *et al*. (2000), Nelson (1985) and Canto-Saenz and Brodie (1984) who noted that temperature gradients, shading-effect, moisture differential and light intensity were some of the factors influencing outcomes of green house experiments. In addition, Canto-Saenz and Brodie (1984) observed that non-efficient hosts of *M. incognita* become progressively efficient as temperature rises while the stress caused by high temperatures increases nematode activity and makes the plant more vulnerable to attack.

The results of this study clearly show that in spite of the resistance of all these clones to *Sahibergella singularis* and black pod disease (Otuonye *et al.,* 2007), only three of the clones are resistant while two are tolerant to *M .incognita* infection. It is therefore evident that breeding for resistance can no longer neglect the effect of nematodes.

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