INFLUENCE OF INOCULUM DENSITY OF ROOT-KNOT NEMATODE [*Meloidogyne incognita* (Kofoid and White) Chitwood] AND PLANT AGE ON YIELD OF AMPALAYA (*Momordica charantia* L.)

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ABSTRACT

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A pot experiment was conducted to determine the most susceptible stage of growth of ampalaya, *Momordica charantia* L. to the root-knot nematode, *Meloidogyne incognita* infestation and the inoculum density of the nematode that could affect the yield.

Gall and egg mass indices and nematode population recovered from the roots and soil were significantly higher in 2- and 4- wk old plants compared with 6- and 8- wk old plants regardless of inoculum density. Likewise, plants inoculated earlier had heavier root weight compared with plants inoculated at later stage but not the fresh weight of vines. Fresh root and vine weight were generally heavier at inoculum density of 20,000 compared with the other inoculum density regardless of age of the plants.

Total fruit yield of 2- and 4-wk old plants inoculated with 1,000 and 5,000 eggs were reduced by 68 to 70% and increased to 81 to 82% when inoculum density was increased to 10,000 and 20,000, respectively. Percent yield reduction was lower on 8-wk old plants regardless of inoculum density compared with 2-, 4- and 6-wk old plants; however, percent yield reduction at this growth stage increased as inoculum density was increased.

Interaction effects of inoculum density versus age of plant on the production of galls and egg masses, fresh vine weight and weight of marketable and non-marketable fruits were significant.

KEYWORDS: Ampalaya. Egg mass index. Gall index. Inoculum density. *Meloidogyne incognita*. *Momordica charantia*. Root-knot nematode. Yield.

INTRODUCTION

The bitter gourd (*Momordica charantia* L.) or ampalaya is a slender, climbing plant with bitter fruits which are used as food including the leaves and tender shoots. In its production, plant diseases constitute one of the natural hazards. Disease outbreaks are often the principal factors that alter production levels and imperil consumer needs. Among the nematode diseases, the root knot disease caused by *Meloidogyne* spp. is very important. They are responsible for 95% of all infestation in cultivated land and cause an average crop loss of 5% worldwide (Taylor and Sasser, 1978). Yield losses of 55% due to nematodes in tomato and pepper were reported in Benin and Malaysia. Chapman (1960) also reported damage by *M. incognita* on leguminous crops like Lucerne (Atlantic variety) and Red Clover (Kenland variety), incurring 2-7% and 77-83% damage, respectively. Likewise, Ducusin and Davide (1972) reported that *Meloidogyne* spp.could reduce yield of tomato to 50% in severe cases. They further observed that inoculum levels of 100, 500, 1,000 and 5,000 larvae per plant gave a corresponding yield loss of 24.1, 29.1, 29.5 and 38.7%, respectively, on tomato. Yield loss of about 83% when tomato plants were inoculated at planting and 40% at flowering were also observed.

Ampalaya, squash and upo which are extensively grown in some localities in Leyte were found to be highly susceptible to root-knot nematodes. However, information on the most susceptible stage of growth of ampalaya to root-knot infestations and the effect of varying inoculum density of the nematode is lacking. This information is very important and necessary in formulating management strategies against the organism. This study, therefore, aims to assess the growth stage of ampalaya that is most susceptible to root-knot infestation and determine the inoculum density of the nematode that will affect its yield.

MATERIALS AND METHODS

Ampalaya seeds (variety Chinensis) were sown in 25-cm diameter clay pots filled with sterilized soil. Thinning was done leaving only one plant per pot 10 days after sowing. Two-meter bamboo poles were set up near each plant in each pot when plants have developed vines. The joints were tied securely with wires to serve as trellis for the ampalaya. The plants were watered whenever necessary. Complete fertilizer was applied at planting and plants were protected from aphids by spraying Seven.

Infested tomato roots previously inoculated with pure culture of *M. incognita* were harvested, washed with tap water and chopped into pieces. Eggs of the nematode were extracted following the procedure of Hussey and Barker (1973). The desired number of eggs as inoculum was counted under the stereomicroscope with the aid of a hand tally counter. The inoculum densities were approximately 1,000, 5,000, 10,000 and 20,000 eggs per pot. The different inoculum densities were inoculated at four different growth stages of the plant (e.g. two, four, six and eight weeks after planting).

Pots were randomly arranged outside the screenhouse with 16 treatment combinations and a control. The experiment was laid out in a factorial design arranged in completely randomized design and was replicated five times. Data gathered included crop yield, fresh vine and root weight, gall and egg mass indices and nematode population in the roots and in the soil.

RESULTS AND DISCUSSION

Root gall and egg mass indices

Root gall index in ampalaya plants inoculated with different inoculum density of *M. incognita* at different growth stages is presented in Table 1. Severe to highly severe root gall indices were observed on 2- and 4-wk old plants inoculated

Table 1. Root gall index as influenced by different inoculum densities of *Meloidogyne incognita* inoculated at different growth stages of the plant.

Inoculum density (Eggs/plants)	Growth stage, wks[1]				Mean[3]
	2	4	6	8	
1,000	4.8a	4.0bc	2.8d	0.2e	2.95
5,000	4.6ab	4.4ab	4.4ab	3.4cd	4.20
10,000	5.0a	4.6ab	4.4ab	3.4cd	4.35
20,000	5.0a	5.0a	4.4ab	3.8bc	4.55
Mean[2]	4.85	4.5	4.0	2.7	

[1]Data within the four columns having a common letter are not significantly different at 5% level based on Duncan's Multiple Range Test (DMRT).

[2/3]The main effect of the growth stages and inoculum densities is meaningless since interaction effect is significant.

with 1,000 eggs and in 6-wk old plants inoculated with 5,000 eggs. However, trace to slight galling on roots were observed on 8-wk and 6-wk old plants inoculated with 1,000 eggs. The results indicated that younger plants were more susceptible to infection and therefore can easily be penetrated by root-knot nematodes, hence, producing more galls even at low inoculum density.

Older plants seem to be more resistant to nematode damage as indicated by lower gall index compared to younger plants even at high inoculum density. A similar result was obtained by Castillo et al. (1972) who revealed that galls in *Arachis* spp., P-246 and P-258 were significantly higher when inoculated at the age of 1 1/2 mos than when inoculated at the age of 3 mos. Likewise, Olasiman (1981) observed that sweetpotato var. BNAS #51 inoculated with approximately 20,000 eggs of *M. incognita* at 8 wks after planting had lower gall index compared to plants inoculated earlier.

Egg mass indices of less than 3 were obtained on 8-wk-old plants inoculated with 1,000 eggs and in 6-wk-old plants inoculated with 20,000 eggs (Table 2). Statistical analysis showed significant differences among treatment combinations suggesting further that egg mass production is highly influenced by inoculum density and the time of inoculation.

Table 2. Egg mass index as influenced by different inoculum densities of *Meloidogyne incognita* inoculated at different growth stages of the plant.

Inoculum density (Eggs/plant)	Growth stage, wks[1]				Mean[3]
	2	4	6	8	
1,000	3.6bc	3.4bc	1.8d	0.0e	2.20
5,000	4.4ab	3.6bc	2.8cd	2.6cd	3.35
10,000	4.0ab	4.0ab	2.6cd	2.2d	3.20
20,000	4.8a	4.6a	4.0ab	2.8cd	4.05
Mean[2]	4.2	3.9	2.8	1.9	

[1]Data within the four columns having a common letter are not significantly different at 5% level based on Duncan's Multiple Range Test (DMRT).

[2/3]The main effect of the growth stages and inoculum densities is meaningless since interaction effect is significant.

The high egg mass indices from plants inoculated with 1,000 eggs at 2 and 4 wks after planting indicate the susceptibility of younger roots to the attack of root-knot nematodes. More larvae have penetrated young roots and female nematodes developed more rapidly consequently producing more egg masses. In contrast fewer larvae have penetrated roots of older plants and this may have resulted in fewer egg masses produced from these plants even at high inoculum density.

Nematode population in roots and soil

Plants having the highest inoculum density of 20,000 eggs per plant obtained the highest nematode population both in the roots and in the soil (Tables 3 and 4). Significant differences were obtained on nematode population in roots receiving an initial inoculum density of 20,000 eggs compared with those plants receiving 1,000, 5,000 and 10,000 initial inoculum density. Likewise population in the soil on treatments having an initial inoculum density of 20,000 eggs 3 was significantly different with treatments having an initial inoculum density of 5,000 and 10,000 eggs.

The age of plants at the time of inoculation greatly affected nematode population in the roots and in the soil. Nematode population decreased significantly

Table 3. Nematode population in roots (1 g/plant) as influenced by different inoculum densities of *Meloidogyne incognita* inoculated at different growth stages of the plant.

Inoculum density density (Eggs/plant)	Growth stage, wks[1]				Mean[3]
	2	4	6	8	
1,000	187.6	107.2	37.0	7.6	84.85c
5,000	265.0	204.8	202.8	39.8	178.10ab
10,000	264.4	165.4	41.2	33.2	126.05bc
20,000	399.8	289.4	136.4	70.0	223.90a
Mean[2]	279.2a	191.7b	104.35c	37.65d	

[1]Interaction effects are not significant at the 5% level.

[2/3]Means having common letter are not significantly different at the 5% level based on Duncan's Multiple Range Test (DMRT).

Table 4. Nematode population in soil (300 cc/pot) as influenced by different inoculum densities of *Meloidogyne incognita* inoculated at different growth stages of the plant.

Inoculum density (Eggs/ plant)	Growth stage, wks[1]				Mean[3]
	2	4	6	8	
1,000	329.8	302.8	234.2	59.2	231.5ab
5,000	355.2	175.6	153.6	74.0	189.6b
10,000	183.2	161.4	93.2	101.6	134.8b
20,000	537.6	367.4	172.4	98.6	299.0a
Mean[2]	356.4a	251.8ab	163.35bc	83.35c	

[1]Interaction effects are not significant at the 5% level.

[2/3]Means having common letter are not significantly different at the 5% level based on Duncan's Multiple Range Test (DMRT).

when plants were inoculated at 6 and 8 wks after planting (Table 3) supporting the findings of Castillo et al. (1972) that recovery of nematodes on peanut was significant in younger plants than in older plants of wild and known species. They concluded that lower nematode recovery in older plants may be due to mechanical exclusion by the mature root system or the greater synthesis of repellent or toxic substances.

Nematodes recovered in the soil were also higher in plants inoculated earlier. More egg masses were produced on younger plants (Table 2) and some may have hatched at the time of soil collection and have contributed to higher nematode recovery in the soil (Table 4).

Fresh vine and root weights

The influence of inoculum density of *M. incognita* inoculated at different growth stages of ampalaya on fresh vine and root weight is presented in Tables 5 and 6. The highest fresh weight of vines was obtained on plants inoculated with 20,000 eggs at 8 wks after planting and this differed significantly with the uninoculated plants and other treatment combinations. The general increase in vine weight of plants inoculated 2 and 4 wks after planting could be attributed to stimulation of top growth due to infection by the nematodes. Stimulation of top growth was reported by Davide and de la Rosa (1974) on Ladu (citrus variety)

Table 5. Fresh vine weight (g) as influenced by different inoculum densities of *Meloidogyne incognita* inoculated at different growth stages of the plant.

Inoculum density (Eggs/ planat)	Growth stage, wks[1]				Mean[3]
	2	4	6	8	
0	35.98	29.94	28.60	31.78	0.32bcd
1,000	28.29bcd	17.56d	29.14bcd	42.20bc	29.47
5,000	34.32bcd	35.24bcd	42.16bc	32.30bcd	36.00
10,000	15.22d	21.52d	36.06bc	34.90bcd	26.92
20,000	27.2cd	44.96bc	47.12b	104.26a	55.88
Mean[2]	28.34	28.84	36.62	49.09	

[1]Data within the four columns having a common letter are not significantly different at 5% level DMRT.

[2/3]The main effect of the growth stages and inoculum densities is meaningless since interaction effect is significant.

Table 6. Root weight (g) as influenced by different inoculum densities of *Meloidogyne incognita* inoculated at different growth stages of the plant.

Inoculum density (Eggs/plant)	Growth stage, wks[1]				Mean[3]
	2	4	6	8	
0	35.98	29.94	28.60	31.78	30.32bcd
1,000	28.29bcd	17.56d	29.14bcd	42.20bc	29.47
5,000	34.32bcd	35.24bcd	42.16bc	32.30bcd	36.00
10,000	15.22d	21.52d	36.06bc	34.90bcd	26.92
20,000	27.2cd	44.96bc	47.12b	104.26a	55.88
Mean[2]	28.34	28.84	36.62	49.09	

[1]Data within the four columns having a common letter are not significantly different at 5% level DMRT.

[2/3]The main effect of the growth stages and inoculum densities is meaningless since interaction effect is significant.

inoculated with 20,000 individuals of *Tylenchulus semipenetrans*. However, at 30,000 individuals there was reduction in top growth of the plants. Likewise, Coursen and Jenkins (1958) showed that in tall fescue infected with *Paratylenchus projectus*, the average number of tillers produced on plants inoculated with 1,000, 5,000 and 10,000 nematodes was 17, 16 and 29, respestively compared with 13 in the uninfected control. A significant increase in petiole length in sugarbeet plants 8 and 9 wks after inoculation with *Heterodera schachtii* larvae was observed by Doney and his co-workers in 1971.

Plants inoculated at 2 and 4 wks after planting generally had lower vine weight (Table 5). This indicated that greater damage by the nematodes occurred when inoculated at an earlier stage. Likewise, plants inoculated earlier were exposed longer to nematode infection and therefore exhibited stunting and reduced top growth. Similar observation was reported by Olasiman (1981) on the reduced weight of vines of sweetpotato inoculated with 20,000 eggs of *M. incognita* at 2 wks after planting.

Plants inoculated earlier regardless of inoculum density significantly had heavier root weight compared to plants inoculated at later stage. Likewise, plants inoculated with 5,000 or more eggs had heavier root weight compared to the uninoculated control (Table 6). The heavier root weight of plants inoculated with 5,000 or more eggs at earlier stage may be attributed to more galls produced on these plants (Table 7). The roots of younger plants were still succulent and easily penetrated by root-knot larvae which resulted in the production of more galls. These galls apparently have contributed to the total weight of the roots; likewise, vine weight (Table 5) was affected. Roots of infected plants developed large galls which are primarily due to formation of giant cells and large masses of nodule-like cells and this in effect have interfered with the uptake of nutrients thus reducing vine weight. Galls and giant cell formation changes the physiologic activities of the plant system (Taylor and Sasser, 1978).

Weight of marketable and non-marketable fruits and total fruit yield

The marketable and non-marketable fruits and total fruit yield of ampalaya as influenced by inoculum density of *M. incognita* inoculated at different growth stages of the plant is presented in Table 1. Total fruit yield (g) was higher in 8-wk-old plants regardless of inoculum density compared to the other treatment

Table 7. Marketable and non-marketable fruits and total fruit yield of ampalaya as influenced by different inoculum densities of *Meloidogyne incognita* inoculated at different growth stages of the plant.

Inoculum Density (Eggs)	Growth Stage, wks	Fresh Weight, g[1]		Total Fruit Yield, g	Percent Yield Reduction[2], %
		Marketable Fruits	Non-Marketable Fruits		
1,000	2	60.32fg	8.00d	68.32	69.89
	4	59.30fg	7.94d	67.24	70.37
	6	58.26fg	9.78cd	68.04	70.02
	8	161.42ab	20.78ab	182.20	19.71
5,000	2	37.46fg	8.72d	46.18	79.65
	4	43.06fg	11.54cd	54.60	75.94
	6	65.56efg	6.90d	72.46	68.07
	8	153.98bc	7.14d	161.12	29.00
10,000	2	31.56g	11.22cd	42.78	81.15
	4	34.94g	7.74d	42.68	81.19
	6	85.12def	14.24bcd	99.36	56.22
	8	109.72cde	7.82d	117.54	48.20
20,000	2	28.80g	12.50bcd	41.30	81.80
	4	33.60g	5.92d	39.52	82.58
	6	84.82def	18.58abc	103.40	54.44
	8	113.42cd	8.86d	122.28	46.12
0 (Control)		203.48a	23.45a	226.93	

[1]Mean of 5 replications: Means followed by a common letter are not significantly different at 5% level based on Duncan's Multiple Range Test (DMRT).

[2]Percent yield reduction is computed following the formula below:

$$\text{Yield reduction (\%)} = \frac{\text{(Yield of uninoculated control - Yield of treatment)}}{\text{Yield of uninoculated control}} \times 100$$

combinations. However, this was significantly lower from the yield of control plants which gave a total fruit yield of 226.93 g.

Yield reduction ranged from 68-70% on 2-, 4- and 6-wk-old plants inoculated with 1,000 and 5,000 eggs and percent yield reduction increased when plants were inoculated with 10,000 and 20,000 at 2 and 4 wks after planting. Yield reduction in 8-wk-old plants increased as the inoculum density was increased (Table 7).

The result of this experiment clearly indicated that root-knot nematode, *M. incognita,* could reduce yield of ampalaya even at low inoculum density of 1,000 eggs particularly when plants were inoculated at an earlier stage. Roots of ampalaya plants inoculated earlier were exposed longer to infection by root-knot nematodes and were damaged as shown by higher gall index and this contributed to low yield. Galls in roots are pathologically developed cells, tissues or organs of plants that have arisen mostly by hypertrophy (over-growth) and hyperplasia (cell proliferation). Heavily galled roots caused disruption of the xylem and phloem vessels thereby reducing translocation of food material and water, consequently reducing photosynthetic activity (Loveys and Bird, 1973; Meon et al., 1978). Reduction in yield in several crops by root-knot nematodes,*Meloidogyne* spp. have been reported to range from 10 to 50% (Ducusin and Davide, 1972). Yield reduction also varies when crop is inoculated at different growth stages.

The result of the experiment also indicated that control of root-knot nematode in ampalaya should be initiated at pre-planting density of 1,000 larvae in the soil. Since ampalaya is seeded directly in the soil, root-knot nematode infection upon emergence may result in heavy losses or total loss in yield.

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