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The Development and Life Cycle of Meloidogyne incognita in sweetpotato (Ipomoea batatas) cv TIS 4400 -2

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Abstract: *The life cycle and development of root-knot nematode, Meloidogyne incognita, was studied in the roots of sweetpotato (CV TIS4400-2) in a screen house. Three-week old sweetpotato seedlings grown in 16 litre polyethylene pots containing 15 litre steam-sterilized sandy loam soil were each inoculated with 5,000 eggs of M. incognita. Twenty four hours later, and subsequently on a daily basis, two seedlings were randomly uprooted and the roots were cleaned and stained using lactoglycerol method and were examined for nematode penetration and stages of nematode development. The development of M. incognita spanned 30 days at a temperature range of 21.33±0.13°C to 28.36±0.26°C : egg to second stage juvenile (J2) (2 days); J2 to third stage juvenile (J3) (10 days); J3 to fourth stage juvenile (J4) (2 days); J4 to young adult (2 days) and young adult to adult females with 441±9.7 eggs (14 days).*

Key words: *Life cycle, development, Meloidogyne incognita, sweetpotato,*

I. Introduction

Sweetpotato, Ipomoea batatas (L) Lam, is the only member of the family Convolvulaceae that is of economic significance (Purseglove, 1968; Onwueme, 1978; Odebode, 2008). It originated from North Western South America or Central America from where it spread to other regions of the world (Onwueme, 1978; Austin, 1988; Odebode, 2008). Currently, it is widely cultivated throughout the tropical and sub-tropical world and in warmer parts of the temperate countries where it forms an important part of the diet of these communities (Darrel and Donald, 1980; Lenne, 1991). It is one of the important food crops in Africa and it plays a major role in alleviating food crisis in the region (Odebode, 2008).Many sweetpotato varieties have been developed that can serve as alternative to other root and tuber crops like yam and cassava (Odebode, 2004). The tuber is rich in carbohydrate, vitamins A and C, riboflavin, thiamin and significant proportions of calcium and iron and magnesium (Onwueme, 1978; Ozerol, 1984; Odebode, 2008). The leaf is much richer than the tuber in protein and vitamins and the peels have higher protein, minerals and non-carbohydrate contents than the tuber and leaf. (Onwueme and Sinha, 1991; Odebode, 2008). Tubers are utilized boiled, baked, roasted, fried, pounded (mixed with yam) into fufu or peeled, chopped, sliced, parboiled, sun-dried, and stored or milled into flour. This is used as composite flour with wheat in the preparation of bread, doughnut, pastries, cakes etc (Odebode, 2008; Akoroda, 2009). The vines are edible and the leaves are useful in preparing soups and stews. The sweetpotato starch is used industrially in textile manufacture and for the production of alcohol. About a third of the sweetpotato produced in the United States is fed to animals (Onwueme, 1978; Onwueme and Charles, 1994). Some processed products from sweetpotato include starch, noodles, candy, desserts, flour and jam (Zandrata, 2000; Chivinge et al., 2000; Odebode, 2008).

One of the major pest groups that cause considerable damage to sweetpotato are the plant-parasitic nematodes (Oversweet, 2009). Many genera of plant-parasitic nematodes have been found to be associated with sweetpotato. The most important ones are the root-knot nematodes, Meloidogyne spp. and the reniform nematode, Rotylenchulus reniformis (Anon, 1978; Lenne, 1991; Fawole and Claudius-Cole, 2000). Onwueme (1978); Anon (1978); Lenne (1991) and Scurrah et al. (2005) gave the lists of other sweetpotato nematodes which could adversely affect the growth when present in large populations. M. incognita is widely distributed in sweetpotato growing areas and constitutes a major biotic factor militating against sweetpotato production (Anon, 1978). Root-knot nematode infection in primary roots causes knotting or swelling of the entire root and heavy infestation can inhibit apical growth (Anon, 1978). However, galls or knots are not usually well developed on tubers. The most obvious symptoms on tubers are development of longitudinal cracks and /or blister-like bumps causing rough appearance and decay of the root system (Clark and Moyer, 1988; Oversweet, 2009). Gapasin and Validez (1979) reported that M. incognita could reduce tuber production by as much as 47.7% depending on the nematode population. In the Philippines, losses of about 50% have been recorded but could reach 100% with three continuous croppings in infested fields (Gapasin 1984, 1986). Theberge (1985) also reported that yield reduction of 20-30% or more may occur depending on the cultivar grown, soil and environment. Lenne (1991) reported a yield loss of 18% in the Southern Highlands of Papua New Guinea In a recent study, Osunlola (2011) reported a yield reduction of 48.8-50.6% in Nigeria.

The knowledge of biology of any pest is very essential in the formulation of appropriate management strategies. With this information, it is possible to determine the generation time of the pest, number of generations of the pest per season, estimate the population of the pest at the end of the growing season and identify the vulnerable stage of the pest. From this, decision can be taken on the necessity or otherwise for control, the timing and the type of control measure(s) to adopt. This research elucidated the development and life cycle of M. incognita in the roots of sweetpotato (Ipomoea batatas cv. TIS 4400 -2)

II. Materials And Methods

Eighty (80) polyethylene pots perforated at the bottom that were each filled with 15-l steam-sterilized soil obtained from the Crop Garden of the Department of Crop Protection and Environmental Biology, University of Ibadan were used for this study. The pots were arranged in 20 x 4 rows on concrete floor of the screen house of the Department of Crop Protection and Environmental Biology, University of Ibadan and spaced at one meter within and between the rows. A 30cm long vine of a M. incognita susceptible sweetpotato cultivar (CV TIS 4400-2) was planted in each pot. The nematode eggs used for this work were extracted from twelve-week old M. incognita-galled roots of Celosia argentea using the method of Hussey and Barker (1973). Each sweetpotato seedling was inoculated with 5,000 freshly extracted M. incognita eggs at three weeks after planting by exposing the roots and smearing the eggs on the roots with the aid of a plastic hypodermic syringe (without the needle). The roots were covered with the soil inside the pots immediately after inoculation.

On a daily basis, as from 24 hours after inoculation, two inoculated plants were randomly and carefully uprooted and the roots were washed in gentle stream of water, dried between paper towels and stained for observation using lactoglycerol method (Bridge et al., 1982). The roots were cut into small pieces (4-5 cm), wrapped in muslin cloth and tied with a piece of cotton string. The staining solution consisted of equal amounts of glycerol, lactic acid, and distilled water plus 0.05% acid fuchsin, while the clearing solution was made up of equal volumes of glycerol and distilled water acidified with a few drops of lactic acid. The root pieces were dropped in boiling staining solution for three minutes and were allowed to cool and then washed in water. The stained roots were left overnight in clearing solution.

The roots were examined on a daily basis for nematode penetration and development after penetration. The relative humidity as well as the soil and atmospheric temperatures of the experimental environment were monitored throughout the period of study. Photomicrographs of the major life stage of the nematode were taken and the measurements of these stages were made by the use of eye-piece graticule calibrated against stage micrometer. The observation and measurements continued on a daily basis until eggs laid by developed adult females were once more observed. The number of eggs laid by adult females were also counted.

III. Results

The soil temperature (at 15cm depth) at 8.00am ranged between 23.3^{o}C and 29.9^{o}C with 25.4±0.29^{o}C as mean. At 1.00pm., it ranged between 24.4^{o}C and 30^{o}C with 26.7±0.27^{o}C as mean. The minimum atmospheric temperature ranged between 19.5^{o}C and 22.5^{o}C with 21.33±0.13^{0}C as mean while the maximum temperature for different days ranged between 25^{o}C and 31.5^{o}C with 28.36±0.26^{o}C as mean. The minimum Relative Humidity (RH) of the study environment ranged between 50% and 70% with 63.27±0.93% as mean. The maximum RH for different days ranged between 84% and 95% with 91.27±0.56% as mean.

The mean length and width of the eggs which were used to inoculate sweetpotato roots were 93.75±2.7µ and 37.83±1.6 µ respectively. The mean length of the second stage juveniles that hatched from the eggs was 405.0±20.6µ and the width was 13.51±0.0µ.

No nematodes were seen in the roots of sweetpotato 24 hours after inoculation. However, at 48 hours after inoculation, second stage juveniles were observed penetrating the roots behind the root cap. Some were already inside the roots but many had not fully entered the roots. They resembled those that hatched from the eggs that were used for inoculation. Their mean length and broadest width were 405.6±15.9 µ and 13.50±0.8µ, respectively.

From the third day after inoculation, most of the juveniles were observed in the stele, though some were still entering the roots (Plate 1). At this stage, the juveniles had started to increase in size and this was very noticeable on the eighth day. The developing juvenile, now fusiform (swollen) in shape, measured an average of 475.0±2.3 µ in length. Increase in size continued and by the ninth day, the developing juvenile measured 486.50±5.6µ. Moulting was first noticed at 12 days after inoculation which gave rise to the third stage juvenile (Plate 1).

A second moult occurred 14 days after inoculation which gave rise to the fourth stage juveniles. Two types of developing juveniles were seen at this stage, one type with rounded posterior region which would eventually become females and the other, fusiform in appearance with spiked tail which would develop into adult male (Plate 1). The former were very many while only one of the latter was observed. A third moulting was observed 16 days after inoculation and this produced a young female nematode.

By 17 days after inoculation,the vulva and the perineal patterns of young female nematodes were observed in the posterior part of the nematodes (Plate 1). The young females now possessed stylet with knobs and large median bulb. The head of the nematode was observed to be attached to the stele of the root and other parts of the body were observed in the cortex. The female had a mean length of 527.0±2.6µ.The young females continued to increase in size and number by 27 days after inoculation, the developing females had started producing gelatinous matrix or egg sac which had no eggs in them. Eggs were observed for the first time in the egg sac at 30 days after inoculation (Plate 1). The mature and egg producing females measured an average of 608.10±2.9µ in length and 405.40±5.7 µ at their broadest width. The mature adult female nematode laid 441±9.7 eggs. The eggs measured a mean of 95.00±3.4 µ in length and 39.18±2.6 µ in width.

The development of male could not be monitored easily because only one one adult male was observed throughout the duration of this experiment apart from the only male fourth stage juvenile observed 14 days after inoculation. The male nematode was seen around sweetpotato root 32 days after inoculation. It had a well developed stylet with knobs and the spicule was posteriorly located. It measured 1210±6.2 µ in length and 54.9±2.6 µ in width.

IV. Discussion

The course of life cycle and development of M. incognita in the root of sweetpotato as observed in this investigation corroborates the reports of earlier workers (Taylor and Sasser, 1978; Nwauzor, 1979; Nwauzor and Fawole, 1992; Fatoki 2001; Claudius-Cole, 2005). The point of entry of the second stage juveniles was just behind the root cap. The root cap corresponds to the meristematic regions of plant roots which Nwauzor and Fawole (1992) reported in their studies as the point of entry for Meloidogyne juveniles in white yam. In this investigation, second stage juveniles were recovered from sweetpotato roots 48 hours after exposure. Other workers reported juvenile penetration within 6-48 hours of exposure. The difference in penetration time could be due to the host crop used, the species of the Meloidogyne and the inoculum used. Nwauzor (1979), Nwauzor and Fawole (1992) used second stage juveniles as inoculum while eggs were used for inoculation in this study. The eggs had to hatch first before juvenile penetration and that was probably the reason why juveniles were not recovered from the root of test plant within 24 hours. However, the penetration time still falls within the range (6-48 hours) reported by earlier workers. The observation of fusiform shaped juveniles on the 8^{th} and 9^{th} day after inoculation in this study is in line with the reports of other researchers. Nwauzor (1979); Nwauzor and Fawole (1992) made this observation nine and six days after inoculation respectively. The rapid growth and development of nematode which started with the appearance of third stage juvenile conforms to the reports of other researchers (Nwauzor, 1979; Adesiyan et al, 1990; Nwauzor and Fawole, 1992; Claudius-Cole, 2005). The third and fourth stage juveniles lacked stylet (feeding structure) which had been cast off during the first moult and as such they did not feed. They must have relied on the food and energy reserves during moulting. The moulting process must therefore be quickly completed with regeneration of a new stylet for nematode to resume feeding.

For most species of root-knot nematodes the life cycle is completed between 25 and 40 days at temperatures between 25 and 30^0C (Adesiyan et al., 1990). Nwauzor (1979), Nwauzor and Fawole (1992) observed laying of eggs 22 days after inoculation (DAI) in tomato at a mean minimum temperature of 22.2±0.55^{o}C and mean maximum temperature of 29.08±0.99 ^{o}C and 28 DAI in white yam at a mean minimum temperature of 22.5±1.2 ^{o}C and a mean maximum temperature of 37.6±3.2^{o}C respectively using second stage juveniles as inoculum in both cases. Claudius Cole (2005) using eggs to inoculate observed that eggs were laid in the roots of Centrosema, Stylosanthes and Vigna unguiculata 31, 29 and 22 DAI, respectively. The laying of eggs 30 DAI observed in this investigation is, therefore, in agreement with reports of these workers. In conclusion, the time taken for the development of M. incognita in sweetpotato (CV TIS 4400-2) from the time of inoculation as egg to egg producing adult female nematode was 30 days at a mean minimum temperature of 21.33±0.13^{o}C and a mean maximum temperature of 28.38±0.26^{o}C. Therefore, the time for completion of a life cycle as observed in this study was 30 days. The dearth of adult male nematode in this study is not unusual as Nwauzor and Fawole (1992) made a similar observation on yam. This is because root-knot nematode reproduces more commonly by the process of parthenogenesis in which case the spermatozoa produced by adult males are not necessary for egg development (Adesiyan et al., 1990).

Furthermore, when food is in abundance, most juveniles develop into females (Taylor and Sasser, 1978). In this case, sweetpotato roots appear to be serving as a very good source of food for this nematode, most of the juveniles developed into adult females. It should be noted that this experiment was conducted between the months of July and September. The prevailing environmental conditions reported above were adequate and suitable for the growth and development of root-knot nematode and that sweetpotato (CV TIS 4400 2) is a favourable host providing abundant food supply for the nematode.

In conclusion, it took thirty days for M. incognita to complete its life cycle in the roots of sweetpotato cv. TIS 4400-2 .This suggests that three or more generations of the nematode are possible in a sweetpotato farm

in a growing season depending on the cultivars grown. The short generation cycle would lead to a quick population build up of the nematode which would severely attack sweetpotato causing yield and quality reduction. In addition this would leave a large population of nematode for subsequent crops.

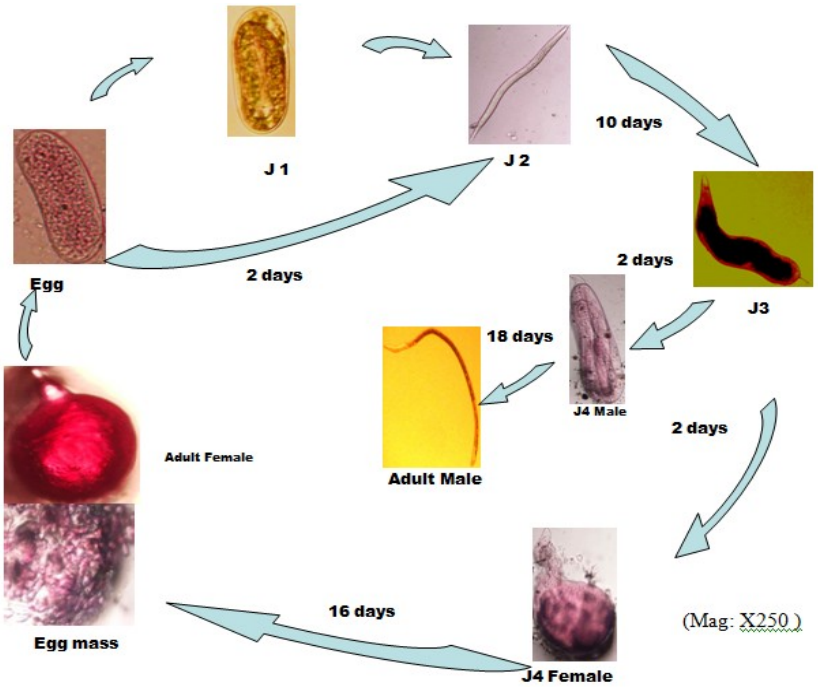

Plate 1**:** Life cycle of Meloidogyne incognita in Sweetpotato (CV: TIS4400-2)

J1 =First stage juvenile
J2 =Second stage juvenile
J3 =Third stage juvenile
J4=Fourth stage juvenile

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