Developmental Stages and Morphological Changes of *Heterodera sacchari* in Upland NERICA1 Rice Cultivar in Screen House Conditions

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ABSTRACT

Heterodera sacchari Luc and Merny (1963) is widely distributed and has been found to notoriously devastate upland New Rice for Africa (NERICA). The life cycle of *H. sacchari* on susceptible NERICA rice has not been assessed. Therefore, the time taken for it to develop from second-stage juvenile (J_2)-to-J_2 in NERICA1 rice was investigated under screen house conditions at the International Institute of Tropical Agriculture (IITA), Ibadan. Three-week old NERICA1 rice seedlings were planted 1/pot in 5L pots (diameter: 20.5 cm), 160 pots were each inoculated with 5,000 J_2 of *H. sacchari*. Four plants were uprooted and roots rinsed with tap water at 24 hour intervals for 38 days. Roots were stained in acid fuchsin in lacto-glycerol and observed for the presence, development and morphological changes of *H. sacchari* under a stereo microscope (Mag. x100). Measurement of maximum body length and broadest diameter were taken at each stage of development with an eye-piece graticule and calibrated against stage micrometer. *H. sacchari* penetrated into rice roots within 24 hours after inoculation with emergence of spindle shaped 3rd, 4th juvenile stages and lemon shaped white females occurring at 11, 17 and 21 days, respectively. Mature females with large deposits of embryonated eggs were observed 24 days after inoculation (DAI). The life cycle of the nematode from infective J_2-to-mature females was completed within 24 hrs and J_2 emerged 29 DAI at a minimum and maximum soil temperature range of 23.9°C and 30.0°C, relative humidity of 50.3% and 95.0%, respectively. Female body wall changed colour from milky white to brown cyst 38 DAI. This result indicated that *H. sacchari* could complete at least 2-3 generations in the crop life of susceptible NERICA1 rice. Therefore, an integrated pest management option could be adopted to control the pre-plant population of *H. sacchari.*

Keywords: cyst nematode, embryonated eggs, life cycle, NERICA rice, penetration

INTRODUCTION

The cyst nematode, *Heterodera sacchari* Luc and Merny (1963) is a serious pest of rice in both upland and hydromorphic environments of West Africa, India and other parts of Asia (Coyne *et al.,* 1998; Bridge *et al.,* 2005; Coyne and Plowright, 2000a). The time taken for the development of egg to adult female in cyst-forming nematodes depends on several factors such as low temperature which is known to prolong development (Brody, 1976). *Heterodera sacchari,* an important member of the family Heteroderidae (Hunt *et al.,* 2005) has been reported to be an

important parasite of sugarcane in Nigeria (Caveness, 1967; Jerath, 1968). According to Jerath (1968), sugarcane plants attacked by *H. sacchari* were stunted measuring 1.5 meters in height and remained thin as compared to the healthy plants which were 3 to 4 m tall. Merny (1970); Fortuner and Merny (1973), Babatola (1983), Coyne and Plowright (2000b) reported the pathogenic effects of *H. sacchari* on rice. *H. sacchari* is reported to complete its life cycle on sugarcane in 30-35 days at a soil temperature of 24-35ºC from second stage-stage juvenile to adult female (Salawu 1994). The study of Hajihassani *et al*. (2011) found that the cereal cyst nematode, *Heterodera latipons* completed its life-cycle in 145-150 days in wheat and mature females containing embryonated eggs were observed at the soil temperature of 14.2-15.3ºC. Furthermore, the fourth-stage juveniles of *H. latipons* were observed 55-80 days after the penetration of second-stage juvenile when the mean soil temperature dropped from 0ºC to -15ºC. Vovlas and Inserra (1983) reported that *Heterodera mediterranea* completed its life cycle from second stage-juvenile to brown cysts in 42-50 days at a soil temperature of 24-30ºC on olive (*Olea europaea* L.) and pistachio (*Pistacia vera* L.) in the glasshouse. The life cycle of soybean cyst nematode, *Heterodera glycines* on Soybean was completed in 24 days and 41 days at an average soil temperature of 23.3ºC and 17.8ºC, respectively in a pot trial (Ichinohe, 1955). *Heterodera sacchari* reproduced parthenogenetically and males are rare and many have degenerated copulatory apparatus (Ferris (1999). *H. sacchari* has been reported to be an important pest of upland New Rice for Africa (NERICA) in Ivory Coast and Nigeria (Coyne and Plowright, 2000a; Akpheokhai, 2013). NERICA rice was derived from the hybridisation of two cultivated rice, *Oryza glaberrima* and *Oryza sativa* (Semagn *et al.,* 2007). The value of these interspecific hybrids for increasing yield and ability to thrive in harsh environment in rice systems in Africa, India and Asia is rapidly being recognised. The knowledge of biology of any pest is very essential in the formulation of sustainable management strategies. It is therefore pertinent to investigate the life cycle of *Heterodera sacchari,* as well as the developmental stages in the roots of NERICA1 rice after penetration of second-stage juvenile.

MATERIALS AND METHODS

Source and extraction of cyst nematode

The cyst nematode population was obtained from experimental rice plots of Africa Rice Centre (ARC), Ikenne (Lat. 2^0 67´N and 7^0 97´N, Long. 6^0 32´E, and 4^0 40´E), Ogun State in Nigeria. The cyst nematode culture was initiated from single cyst and multiplied on a known susceptible rice cultivar OS6 (Salawu, 1992) for twelve weeks for the experiment in the screen house, International Institute of Tropical Agriculture (IITA), Ibadan. Cysts were picked from infected OS6 rice roots and identified as *Heterodera sacchari* at Biosystematics, Agricultural Research Council - Plant Protection Research Institute, Pretoria, Republic of South Africa.

Mature cysts of *H. sacchari* were collected from OS6 rice soil by floating organic debris, extracted from soil using a jet

of water. This was decanted to a 250 µm aperture sieve and collected in fluted filter papers. Cysts were picked with a pair of forceps from the debris into distilled water in a 200 ml glass beaker. Cysts were individually pierced and crushed using a dissecting needle while viewing under a dissecting microscope. Broken cysts were washed into a 500 ml beaker and the suspension agitated for five minutes to free eggs and juveniles. The liberated eggs and juveniles were subsequently collected on a stack of sieves of 60 µm, 38 µm and 25 µm. Where aperture size 60 µm trapped the cyst cuticle, 38 µm and 25 µm trapped the emerging juveniles and eggs, respectively (Coyne, 1999). The eggs in distilled water were kept for 48 hours to hatch into second-stage juveniles (J_2) in an incubator set at 27±0.5°C. Unhatched eggs were separate from the J_2 suspension using 38 µm sieve and thereafter, the number and viability of J_2 in suspension was confirmed with the aid of a stereomicroscope. The J_2 suspension was adjusted to a concentration of 1,000 J_2 per ml of distilled water.

Sowing of NERICA1 rice seeds and inoculation of *Heterodera sacchari* second-stage juveniles (J_2)

Two seeds of *H. sacchari*-susceptible NERICA1 rice cultivar were sown into 5-litre plastic pots (diameter of 20.5 cm) filled with steam-sterilized soil. Two weeks after sowing, seedlings were thinned to one plant per pot and at three weeks after sowing, seedlings were inoculated with 5,000 J_2 of *H. sacchari* per pot with the aid of a 1 ml Eppendorf pipette. Nematodes were dispensed from the pipette on 10 points around the exposed roots of each plant. Thereafter, the roots were immediately covered with the soil inside the pots after inoculation. Inoculated seedlings were arranged in a row of 40 seedlings repeated 4 times in the screen house, at, IITA, Ibadan. Inoculated plants were watered daily throughout the duration of the experiment.

Four seedlings were randomly and carefully upturned at 24 hour intervals for 38 day duration of the experiment. Rice roots were then separated from the soil, rinsed in a gentle stream of water and allowed to drain between paper towels to remove excess water. Rinsed roots were cut into 1-2 cm pieces wrapped in muslin cloth and tied with a piece of cotton string. The staining solution was prepared by adding equal volumes 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 chopped and wrapped roots were immersed in the boiling staining solution for three minutes removed and rinsed in cool tap water to remove excess stain (Coyne *et al.* (2007). The stained roots were then left overnight in clearing solution (made up of equal volumes of glycerol and distilled water acidified with a few drops of lactic acid) before examination. Stained roots were teased with a dissecting needle and examined for nematode penetration and stages of development after penetration. Percentage of penetration of J_2 in roots was calculated at 24, 48 and 72 hours after inoculation using the formula: $\{100 - (Pi - X)/Pi\ (100)\}$, where Pi = initial nematode population, X = number of penetrated nematodes. Photomicrographs of the life stages of the nematode were taken,

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and measurements of maximum body length and broadest body width were made by the use of eye-piece graticule fitted on to a compound microscope calibrated against stage micrometer at the magnification of X 100. The soil temperature in pots (at 10 cm depth) at 9.00 am and 3.00 pm were taken using a soil thermometer, atmospheric temperature and relative humidity (RH) of the study environment were monitored using Oregon Scientific Weather forecast station with atomic time, model Bar 206 throughout the duration of the experiment. This experiment was repeated without any modifications.

Data were collected on meteorological parameters, nematode length and nematode broadest body diameter were collected. Where necessary, means and Standard error of data collected were calculated with Microsoft Excel (MS Office, 2003)

RESULTS

The environmental conditions presented in Table 1 supported the life cycle and developmental stages of *Heterodera sacchari* on NERICA1 rice. The data collected on the environmental conditions in the two trials were not significantly different ($P \leq 0.05$), therefore, the data were combined for analysis and means presented (Table 1). The temperature range of 23.9 – 30.0^{o}C and relative humidity of 50.3% - 95.0% was favourable for *H. sacchari* penetration and completion of J_2-to-J_2 life cycle of the nematode in the screenhouse, 29 days after inoculation (DAI). At 24 and 48 hours after inoculation, 62.2% and 71.3% of second-stage juveniles (J_2) of *Heterodera sacchari* were observed and extracted from the meristematic region (root tips) of the root system of NERICA1 rice (Table 1). Also, the environmental conditions recorded, adequately supported the various stages of development from vermiform J_2, fusiform third-stage juvenile and lemon shaped adult female with eggs in NERICA1 rice roots as presented in Plate 1. The development of *H. sacchari* consisted of four moults and five stages, typical of endoparasitic phytonematodes (Plate 1). At 24 hours after inoculation of J_2, there was no appreciable increase ($P \leq 0.05$) in the mean length (718.1 µm ±20.2) and broadest diameter (18.4 µm ±0.8) of 45 individual nematodes recovered from the root tips of NERICA1 rice root (Table 2). From the third day after infestation 71.5% (3755.0 nematodes) of the juveniles were observed in the stele, although some juveniles were observed still entering the roots. At the 7th DAI, the mean length (734.2 µm ±17.2) and the broadest diameter (25.6 µm ±1.3) of 25 individual J_2 recovered had increased in shape and size. Moulting was first noticed on the 9th DAI, before the emergence of the third-stage juvenile. The old stylet was observed enclosed in the anterior part of the old cuticle. The third-stage juvenile, emerged 11 DAI when the old cuticle had completely been replaced by the new one. The 20 fully developed fusiform

Table 1: Environmental conditions of the study area and percentage of penetrated second-stage juveniles (J_2) in NERICA 1 rice root

Environmental Observations	Mean ± SE	Range
Soil temperature at 9.00 am	25.4±0.29 ºC	23.3 - 29.9 ºC
Soil temperature at 3.00 pm	26.7±0.27 ºC	24.4 - 30.0 º C
Minimum atmospheric temperature	21.3±0.13 ºC	19.5 - 22.5º C
Maximum atmospheric temperature	28.4±0.26 ºC	25.0 - 31.5 º C
Minimum relative humidity (%)	63.3±0.93%	50.3 – 70.0%
Maximum relative humidity (%)	91.3±0.56%	84.0 – 95.0%
Penetrated J_2 24 hours after inoculation (%)	62.2 ±0.21%	3025.0 – 3190.0
Penetrated J_2 48 hours after inoculation (%)	71.3±0.37%	3030.0 – 4100.0
Penetrated J_2 72 hours after inoculation (%)	71.7±0.37%	3125 - 4040.0

Percentage of penetrated J_2= {100 – (Pi – X)/Pi (100)}, where Pi = initial nematode population, X = number of penetrated J_2.

shaped third-stage juveniles measured had no stylet. The body length (747.1 µm ±11.5) and diameter (134.9 µm ±8.0) of the nematodes increased over time.

Gonads were observed in 25 developing forth-stage juveniles which is an indication of nematodes eventually emerging as females however, the males were not observed. The average body length and broadest body diameter was 827.1 µm±54.9 and 575.4 µm ±71.7, respectively. A third moult was observed on the 19th DAI and the white female nematodes emerged 21 DAI. The posterior portion of white females protruded out of the roots, splitting the roots. The average length and the broadest diameter of 25 individual nematodes measured and observed by a compound microscope was 875.4 µm ± 117.1 and 615.9±54.4 µm, respectively. Some eggs in their gastrula stage of embryonic development were observed within the body of the nematode at this stage.

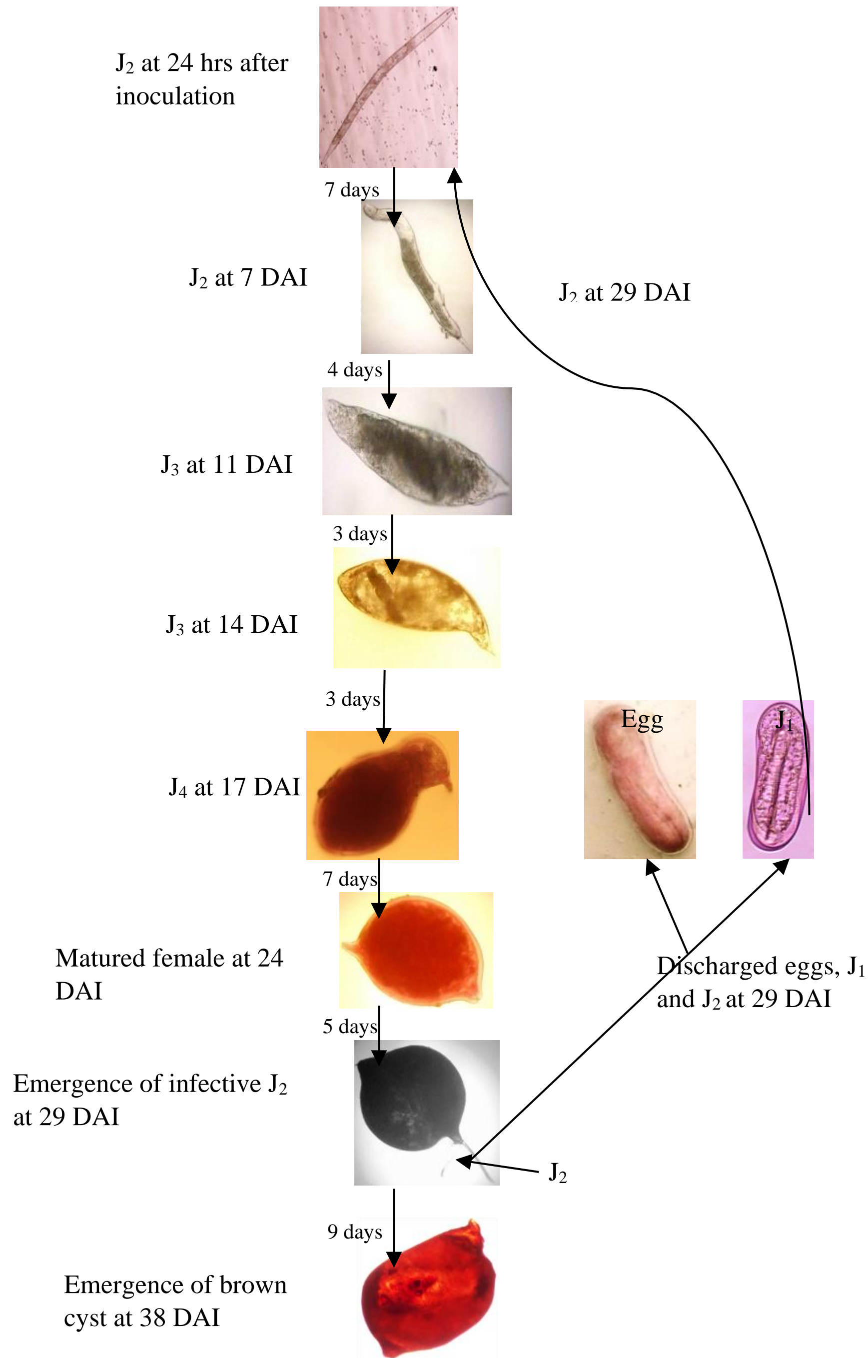

Plate 1: Life Cycle of *Heterodera sacchari* on NERICA 1 Rice Cultivar.

Where; J_1 = First-stage juvenile, J_2 = second-stage juvenile, J_3 = third-stage juvenile, J_4 = fourth-stage juvenile, DAI = days after inoculation

A second moult started 15 DAI and the fourth-stage juveniles emerged 17 DAI. The shedding of cuticle occurred first at the head region and moulting was completed 17 DAI. Two

Twenty-four DAI, fully developed lemon-shaped, creamy white females were recovered from the roots. The average length and average broadest body diameter of 40 individual females recovered was 1029.4 µm ±57.3 and 626.9 µm ±37.9, respectively. Females were squashed on glass slides and examined under a compound microscope (x 10 objective magnification), at this stage, majority of the eggs were fully developed and some first-stage juveniles (J_1) curled within the egg shell were observed. The average number of eggs and juveniles recovered from 80 gravid females in pot culture contained an average of 316 eggs and juveniles per cyst 29 DAI. Furthermore, second-stage juveniles were observed emerging from the posterior region of the nematode. The colour of the body wall of 528 females obtained from rice roots changed from milky white to tough, leathery brown cyst 38 DAI (Table 2).

Table 2: The body length and broadest body diameter of *Heterodera sacchari* at different developmental stages recovered from NERICA1 rice root cultivar

DAI	NGS	body length (µm)	broadest body diameter of nematode stage (µm)
1	J_2	718.1±20.2*	18.4±0.8
7	Enlarged J_2	734.2±17.2	25.6 ±1.3
11	J_3	747.1±11.5	134.9± 8.0
17	J_4	827.1±54.9	575.4±71.7
21	White female	875.4±117.1	615.9±54.4
24	Female with eggs	1029.4±57.3	626.9±37.9
38	Cysts	938.1±47.2	540.2±23.2

DAI = days after inoculation, NGS = nematode growth stage, * = mean± standard error

Values with the same letters in the same column are not significantly different (P>0.05) by DMRT.

DISCUSSION

The life cycle of *H. sacchari* on susceptible upland NERICA1 rice from J_2-to- adult females with eggs was completed in 24 days after penetration of rice roots and J_2 emerged 29 days after inoculation. Eggs at gastrula stage of embryonic development were first observed from 21 DAI at a mean soil temperature range of 23.9 – 30.0^{o}C under screen house conditions. Temperature in both temperate and tropical regions of the world is one of the several factors necessary for the rate of juvenile development in *Heterodera* species (Brigde *et al.,* 2005). In the temperate region with lower soil temperature, the rate of development of *H. glycine*s on soybean, and *H. latipons* on wheat was slower than the rate of *H. sacchari* development on sugarcane in the tropical region (Ichinohe, 1961; Vovlas and Inserra, 1983; Salawu, 1994; Hajihasani *et al*., 2011). The development of *H. latipons* was only one prolonged generation of 145-150 days on wheat at a temperature range of 14.2-15.3^{o}C in a microplot trial in Iran (Hajihasani *et al*., 2011). The life cycle of *H. glycines* on Soybean under gnotobiotic conditions was completed 21 DAI upon hatching of the eggs and emergence of second-stage juvenile at a temperature of 25°C in United States of America (USA) (Lauritis *et al.,* 1983). On sugarcane, the life cycle of *H. sacchari* from J_2 to adult females was completed 30-35 days after penetration at a soil temperature of 24-35°C in Ibadan (Salawu, 1994). However, in this current study, the life cycle of *H. sacchari* was shorter on NERICA1 rice at a temperature slightly lower than what Salawu (1994) reported on Sugarcane in Nigeria. This may probably be due to the type of crop on which the nematode developed and the high relative humidity observed in the environment. Furthermore, the generation time of *H. sacchari* on NERICA rice in this study was shorter given that the experimental site is tropical where temperature is higher and suitable for activities than the temperate region with temperature as low as -15°C (Hajihassani, 2011) . In this investigation, 70% of the inoculated J_2 had completely penetrated rice roots within 48 hrs after infection This was slightly higher than the percentage (42-61%) penetration range of *Heterodera glycines* J_2 in soybean cv. Kent axenic root explants under gnotobiotic conditions reported by Lauritis *et al*. (1983) and higher than 35-45% of *H. schachtii* penetrating sugar beet root explant. The difference in penetration may be due to crop type, nematode species and the environmental conditions such as suitable temperature range of 23.9^{o}C – 30.0^{o}C in which the studies were carried out. The penetration of *H. sacchari* via the root cap of NERICA rice in this study corroborates the work of Salawu (1994) who reported that the second-stage juveniles of *H. sacchari* entered through the meristematic root zone of sugarcane within 24 hours. Taylor and Sasser (1978); Fatoki (2001); Claudius-Cole (2005); Osunlola (2010) reported that the point of entry of the second-stage juveniles of *M. incognita* was behind the root cap and the penetration of J_2 was within 6-48 hours after inoculation. According to Ekanayake (2004) who observed that many juveniles of *M. graminicola* congregated near the tip of rice roots at 10 days after inoculation. This may further explain why 70% penetration of *H. sacchari* into rice roots was observed in 48 hrs after infestation since the root tips are succulent, active (root secretions) and also attractive to the nematode. Halbrendt (1992) explained that fewer number of J_2 of *H. glycines* were observed within the decapitated root tips than in intact roots of soybean, this could result

from reduced attractiveness of decapitated root and more difficult for the J_2 to penetrate in the absence of root tips and also the root exudate secretions which is very active at the root cap.

In contrast with the observations made by Salawu (1994) who recovered third juvenile stage of *H. sacchari* on sugarcane between 16 to 21 days after inoculation, the fusiform shaped third-stage juveniles emerged on the 12th day after inoculation in this study. Vovlas and Inserra (1983) observed the emergence of 3rd juvenile stages 8 days after juvenile penetration of *H. mediterranea* on Olive and *H. schachtii* on sugarbeet at the soil temperature of 24-30ºC. This difference in the time of emergence of the third-stage juvenile may be due to the host crop used or differences in host parasite relationships. Furthermore, the differences may be due to differences in the time of juvenile penetration, pathotype or the species of cyst nematode. In this investigation, *H. sacchari* was observed to develop rapidly on upland NERICA1 rice. In fact, 29 days after infestation, some J_2 were observed emerging from cysts this is probably due to suitable soil temperature and host which in particular, are some of the factors that enhance survival and rapid development of *H. sacchari* on upland NERICA rice in the tropics (such as Nigeria). Rice plants develop profuse fibrous roots on which the nematodes feed, reproduce and multiply. Therefore, source of suitable food and suitable warm soil temperature are responsible for the rapid rate of juvenile development to adult female.

In this investigation, the J_2 of *H. sacchari* had a mean length of 758.5 µm; mean broadest diameter of 18.4 µm and the matured cysts had an average length and broadest diameter was 1029.5 µm and 626.9 µm, respectively. These values are slightly different from the morphometric data provided by Luc and Merny (1963) and Salawu (1994). They obtained values for length which ranged from 504 µm to 508 µm and diameter of 17.3 µm to 17.5 µm for the J_2 and the adult female length, ranged from 614.0 µm to 817.0 µm with body diameter of 502.0 -525.0 µm, respectively. With this additional information, the *H. sacchari* in this study was larger and may be a pathotype of sugarcane cyst nematode.

It can be noted that NERICA1 rice, a widely cultivated interspecific hybrid rice in Nigeria (Nwilene, pers. comm.) is a favourable host providing abundant food supply for the development of cyst nematode. In addition, *H. sacchari* can be a major biotic limitation where *H. sacchari*-susceptible rice is cultivated. The ability of the nematode to complete at least two generations in a cropping season of susceptible NERICA rice cultivars, implies that a management strategy need to be developed. It is abundantly clear that control and eliminating damage caused by *H. sacchari* to rice is dependent on the knowledge of the biology, behaviour, generation time in the crop life and pathogenicity of the nematode. Nwauzor and Fawole (1992) reported that to formulate a reasonable control strategy for *M. incognita* on *Discorea rotundata* var. Okwocha, a good knowledge of the life history is essential, possible vulnerable stages of the nematode identified and the number of generations of the nematode in the crop life is estimated. Thus, the population density of the nematode at the end of a growing season could be projected. The development and life cycle of *Heterodera sacchari* in upland NERICA rice 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 susceptible upland NERICA rice causing yield reduction or total crop failure in subsequent rice growing seasons. In addition, leaving a large population of nematode in the soil. Therefore, this information is essential for taking appropriate decisions on time and also to formulate an appropriate and sustainable management strategy in controlling the pre-plant population of this nematode on rice.

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