International Journal of Plant Breeding and Crop Science Research
Vol. 1, No. 1, June 2013, PP: 01 - 09
Available online at http://acascipub.com/Journals.php

Research article

Testing impacts of endoparasitic nematode *Meloidogynejavanica*on crop productivity, using tomato cultivar "Gboko" as a case study in Nigeria

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ABSTRACT

Two weeks aged tomato seedlings of the cultivar "Gboko" were inundated with 500 to 2000 eggs/juveniles (egg/J) of *Meloidogynejavanica*to determine the effects of different rhizosphere densities of *M. javanica* on tomato productivity. We found that high infestation significantly reduces tomato fruit productivity. The threshold value of infestation was 250egg/J. The mean productivity at low level of infestation (< 250 egg/J) was 20.130 fruits. However, at high level of infestation (> 250 egg/J), reproductive factor (RF) was the most significant variable influencing fruit productivity. At high value of RF (> 1.14), the mean productivity was 6.857 fruits; but when RF was low, soil nematode density was important factor that determined productivity level. At high soil nematode density, productivity was 5 fruits, but this decreased to 2.7 fruits at low soil nematode density. We suggest that infestation likely stimulates tomato plants in a way that plant metabolism is diverted towards growth, so reducing energy spent on fruit production. This finding has important agricultural consequences, as it can help control for level of soil nematode density before any agricultural activities in a specific soil in Nigeria.

Key Words: Crop production, reproduction factor, nematodes, linear-mixed effect model.

INTRODUCTION

Food security is a major problem globally but with severe acuity in developing world (Inter Academy Council, 2004). Although several factors such as political and social instability are important drivers of food insecurity especially in Africa, the root of the problem remains a lack of sound understanding of biological factors that control crop production especially within communities of local farmers where agriculture is mainly traditional (Mwaniki, 2003). Parasitic infestation, especially by plant nematodes, is one of the most important biological factors limiting crop production all over the world (Barker *et al.*,1985; Fawole*et al.*,1992; Williamson and Hussey, 1996; Olowe, 2009). Due to a wide range of hosts, nematodes (e.g. *Meloidogyne* spp.) are regarded as the most economically important plant parasitic nematode in existence (Sasser *et al.*,1984; Imafidor and Nzeako, 2007).

Generally, nematodes require very close association with host plants for efficient and effective parasitism (Adesiyan*et al.*, 1990). Their impactsare determined by several factors including host physiology, initial rhizosphere population, edaphic conditions and cultural farming practices (Goverse*et al.*, 2000). Infested plants exhibit multiple symptoms such as stunting, wilting, chlorosis, and untriftly appearance (Agrios, 1997; Abubakar*et al*., 2004). Such crop damages are ill afforded in developing countries especially in Africa where food security is still of great concern (Adeoye*et al*., 2009; Olaniyi*et al*., 2010).

Tomato is one of the world's largest vegetable crops in term of production (Olaniyi*et al*., 2010). It is found cultivated in 85% of farms in Nigeria (Olaniyi*et al*., 2010), and regarded as the most important vegetable after onions and pepper (Fawusi, 1978), certainly due to its richness in qualitative nutrients (Vilareal, 1980; Olaniyi*et al*., 2010). In Nigeria alone, the production of tomato has been estimated to 114 tones/ha (FAO 1983), compared to 175 and 178 tones/ha in USA and Taiwan respectively (Bowen and Kralky, 1982). This relatively high productivity of tomato at country level could be misleading. For instance, the average in western or northern parts of Nigeria is estimated to only 5 and 20 tones per hectare respectively (Quinn, 1980; Adelana, 1978). One of the major causes of this low productivity at local scale is found to be linked to a lack of fructification of about 50 % of tomato flowers (Adelana, 1975). This difficulty in fructification is a stimulus for investigating underlying factors. In this study, we focus on infestation of tomato plants with nematode *Meloidogynejavanica*, and test how this nematode affects fruit productivity, using the Nigerian local cultivar "gboko" as a model.

MATERIALS AND METHODS

Experimental design

Two-week aged seedlings of the indigenous tomato cultivar "gboko" were planted in polyethylene bags filled with soil mixture of sand and loam (1:1) steam sterilized. Three treatments were applied to the seedlings depending on the level of infestation with eggs of *M. javanica*: low infestation (500-1500 eggs/J), high infestation (1500-2000 eggs/J) and control (no infestation). Five replicates per treatment were considered, making it to 15 replicates in total. Bioassays of 50g of soil samples were carried out for all replicates at 30 days intervals for a period of 90 days to determine the rhizosphere density of *M. javanica*. Density of nematodes in roots of tomato plants was also evaluated using 5g of roots as bioassay. Further, growth parameters were also assessed, including plant height, wet root weight, and stem girth of tomato plants. Finally, fruit yield (henceforth referred to as productivity) was evaluated by counting the number of fruits on each plant tomato after 90 days.

Data analysis

In our experimental design, measurements were performed on each individual plant tomato three times (30, 60 and 90 days after inoculation). There is therefore a temporal pseudoreplication to account for in our analysis. To address the pseudoreplication effect, we fitted a linear-mixed effect model defining fixed and random effects as follows. We defined a single fixed effect termed "infestation" with three levels (low, high and control) and five replicate plants in each treatment, with each plant measured on three occasions (30, 60 and 90 days after inoculation). We defined a random effect termed "period" with three levels (30, 60 and 90 days after inoculation). We then reconstructed time series plots for each of individual plant on the basis of the mean of six response variables: productivity, height, girth, root weight, soil nematode density, and root nematode density.

In addition, we investigated interactions between all measured variables that might explain dispersion in productivity, using a tree-based model. We also conducted a principal component analysis (PCA) to determine the principal axes that best explain the amount of total variation in parameters measured on tomato plants, and identified the parameters that correlate with each axis.

RESULTS AND DISCUSSION

First, we applied the principal components analysis method (PCA; Figure 1) to extract major gradients of parameter variation from the tomato-plant's mean parameter values (centred to zero mean and standardised to unit variance). We revealed two major principal components (PC 1 and PC2) collectively accounting for 69.26% of the total variation, with PC1 explaining alone 46.29% and PC2 22.97%. The search for the meaning of each axis indicated that PC1 axis was associated with decreasing infestation whilst the second axis PC2 was related to increase in soil nematode density (Figure 2). PC 1 could then be identifies as infestation axis, and PC 2 as soil density axis. These findings suggest that infestation level and soil nematode density were likely two important factors that might correlate with productivity.

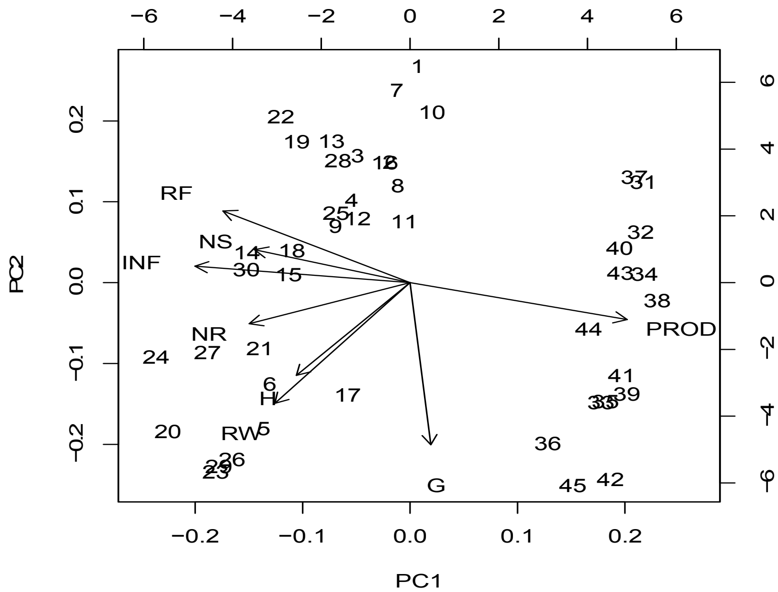

Figure 1: First two principal components (PC 1 and PC 2) of nematode-tomato interaction parameters. The first and second principal components accounted for 46.29% and 22.97% of the total variation, respectively. Arrows indicate relative loadings of each parameter on each principal component. RF, Reproductive factor; NS, Nematode density in soil; NR, Nematode density in tomato roots; INF, Infestation levels; PROD, Productivity; RW, Root weight, G, Growth (indicating variation in height).

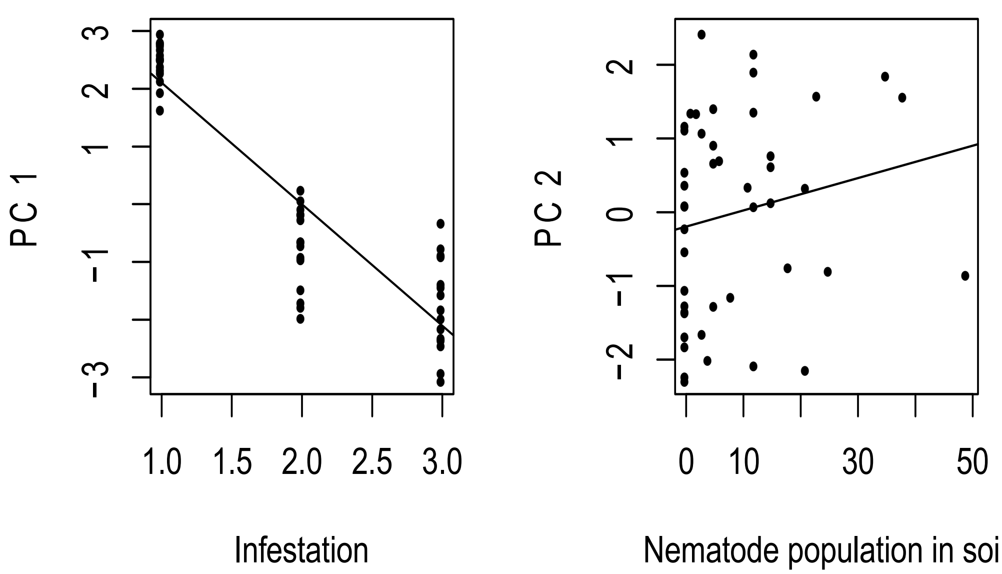

Figure 2: Correlation between the first axis and infestation level (Left) and the second axis and soil nematode density (Right)

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Second, to test this hypothesis, we conducted a multiple one-way ANOVA using productivity as response variable, and testing separately all other factors as explanatory variables. We found that nematode infestation had strong influences on productivity of tomato-plants. Specifically, we found that the productivity was significantly higher in control treatments than in infested treatments (ANOVA, $F = 42.69$, $p < 0.001$; Figure 3; Table 1).

Third, to further test the hypothesis, we performed a tree regression analysis. This analysis confirmed that infestation was the most important explanatory variable of tomato-plant fruit productivity. In addition, tree regression analysis revealed that the threshold value separating low and high infestation was 250 eggs/J, and that the mean productivity for low level of infestation was 20.130 fruits. However, for high level of infestation, the tree showed that nematode reproductive factor (RF) was the second variable that had significant impact on productivity. At high value of RF (> 1.14), the mean productivity was 6.857 fruits; but when RF was low (< 1.14), soil nematode density was the third important factor that determined productivity level. At high soil nematode density, productivity was 5 fruits, but this decreased to 2.7 fruits at low soil nematode density (Figure 4). Our findings indicate that nematode infestation reduces significantly tomato fruit production (see Williamson and Hussey, 1996; and also Esmenjaud*et al*., 1997 for effect of nematode on *Prunus* spp.)

Given the importance of tomato as crop production, the high density of nematodes (Imafidor and Nzeako,2007, 2008) and low nutrient-content (Adeoye and Agboola, 1985)in Nigerian soils,this finding raises a serious concern from a crop production perspective, and an adequate solution to control soil nematode density is required. Several methods are currently proposed. These include crop rotation (Dong *et al*., 2007), soil fumigation (Bridge, 1996) and application of nematicides (Onifade*et al*., 2008). However, limitations of these methods have recently been identified (Li *et al*.,2011). For instance, the use of nematicides is likely to increase environmental pollution with its detrimental effect of underground biodiversity. Crop rotation technique can be difficult to implement especially for perennial fruit plants (Li *et al*.,2011). An important solution could be the search for cultivars resistant to nematode negative effects (Djian-Caporalino*et al*., 1999; Li *et al.* 2011). The gene *Mi*-1.2 (Vos*et al*., 1998) is well-known as conferring nematode resistance to tomato(Goggin*et al*., 2006), but the breeding technology is still poorly developed especially in Africa where the need for such technology is urgent for food security.

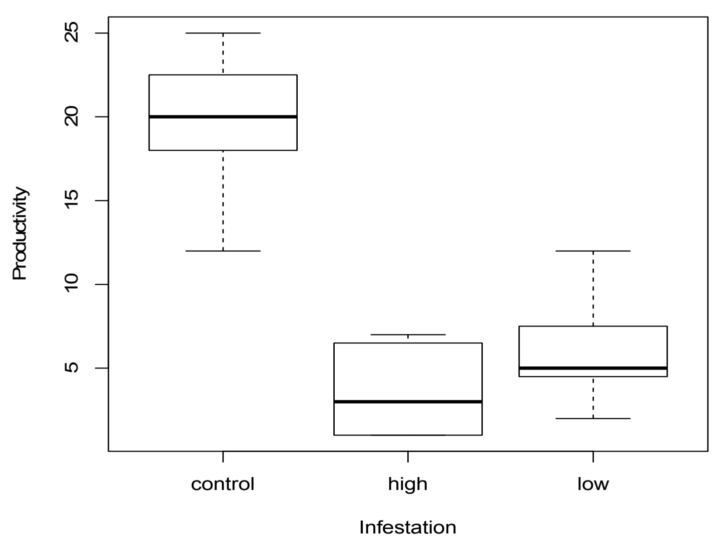

Figure 3: Variation in productivity (number of tomato fruits) and infestation levels.

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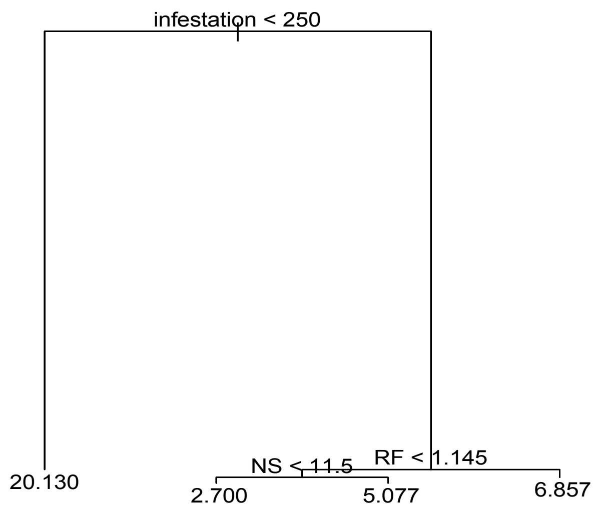

Figure 4: Tree regression analysis. RF, Reproductive factor; NS, Nematode density in soil;

How such negative interactions could be explained? To get more insights into nematode interactions with tomato plants, we investigated the effects of infestation on growth parameter such as plant height. We found that the higher the infestation level, the longer the plant (Figure 5), but the lower the productivity (Figure 3). In light of this result, we conclude that, nematode infestation stimulates plant growth, but simultaneously inhibit metabolic reactions necessary for fruit production (Tsay*et al.*, 2004). Such metabolic inhibition might have indirect effect on the lack of pollination of about 50% of tomato flowers that was reported over the past few decades in Nigeria (Adelana, 1975; Olaniyi*et al.*, 2010). This hypothesis needs however to be tested. The mean increase in height associated with high and low infestation was +14.71 cm (SE = 8.98, 12 df), and +1.53 cm (SE = 8.98, 12 df) respectively, but the increase was not significantly different from that of the control treatment (p = 0.12 vs. p = 0.86 respectively).

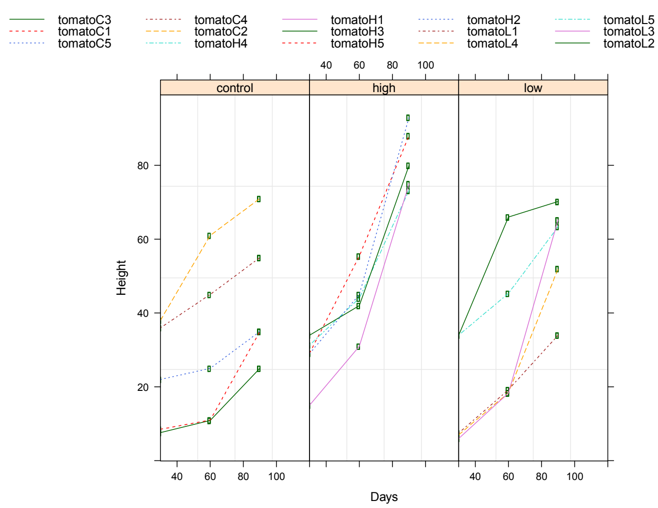

Figure 5: Time-series plots indicating the variation of plant height over time. All the 15 replicate plants are indicated.

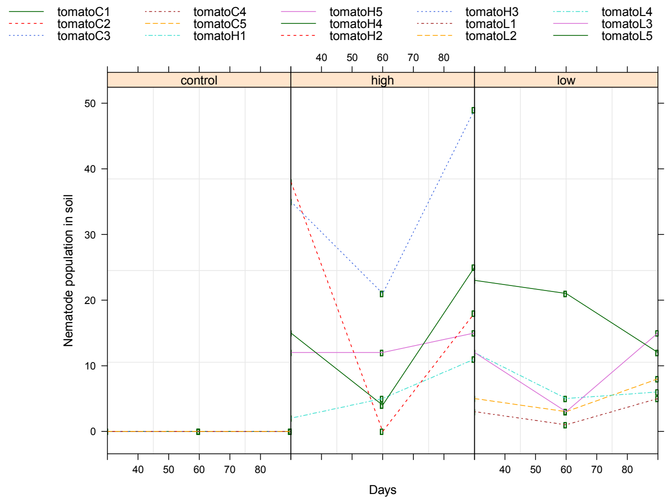

Figure 6: Time-series plots indicating the variation of nematode density in soil over time. All the 15 replicate plants are indicated.

We also looked at variation over time in soil nematode density, root nematode density as well as nematode reproductive factor. As expected, we found that increase in infestation level resulted in increase in soil nematode density (Figure 6). The mean increase in soil nematode density associated with high level of infestation was +17.47 nematode/50 g soil (SE = 4.52, 12 df) and this increase was significantly different from that of control treatment ($p = 0.0023$). Low infestation level also leads to increase in soil nematode density: mean increase = +8.93 nematodes/50g soil (SE = 4.52, 12 df), but this was not significantly different from the control ($p = 0.0717$). Furthermore, we found also significant increase of nematode density within roots (Figure 7): the mean increase in root nematode density associated with high level of infestation was +39.87 nematodes/5g roots (SE = 9.45, 12 df, $p = 0.0012$), whereas the increase associated with low infestation level was +26 nematodes/5g roots (SE = 9.45, 12 df, $p = 0.017$). Interestingly, we noted that when nematode density increases in soil over time, nematode density within roots decrease and vice-versa (Figure 6 vs. Figure 7). This finding is indicative of the movement of nematode from soil into plants and from plants into soil.

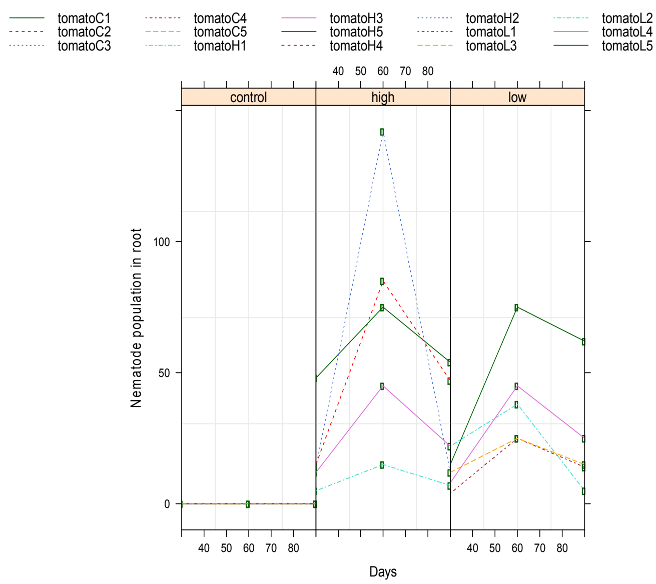

Figure 7: Time-series plots indicating the variation of nematode density within roots over time. All the 15 replicate plants are indicated.

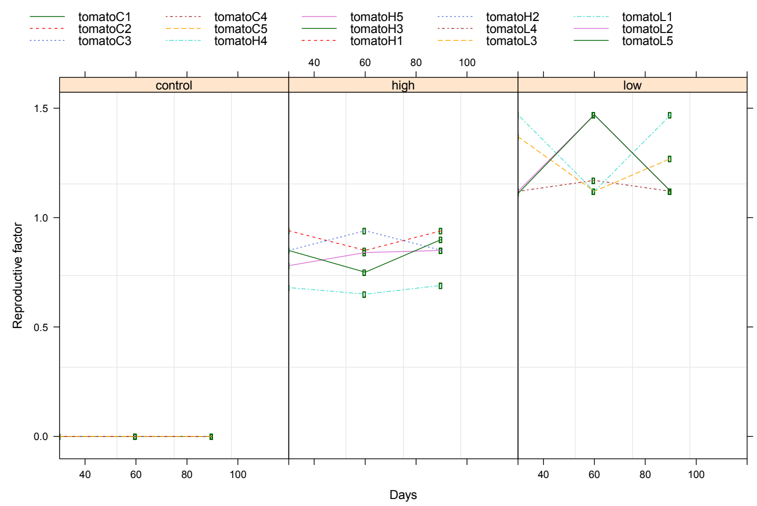

Figure 8: Time-series plots indicating the variation of nematode reproductive factor over time. All the 15 replicate plants are indicated.

However, reproductive factor (RF) decreases with increase in infestation level (Figure 8). At high infestation level, the mean RF equals 0.82 (SE = 0.043, 12 df, $p < 0.001$), but at low level, RF equals 1.24 (SE = 0.04, 12 df, $p < 0.001$). To explain this, we suggest that at low infestation level where nematode soil density is low, competitive interactions between nematodes might be weak, allowing high reproduction ability (Khan *et al*., 1987; Stanton, 2001). This may be the driver of higher RF at low inoculum level. However, at high infestation, nematode soil density is high, and density-dependant effect occurs, leading to low RF values (Khan *et al*., 1987; Stanton, 2001).
Overall, we found that nematode infestation is a key factor that reduces considerably tomato fruit production. We also revealed how multiple parameters interact to lead to this low production of infested tomato-plants. We suggest that nematode infestation might cause inhibition of pollination of tomato flowers, leading to low fructification, but this hypothesis requires further investigation. More importantly, we found a threshold of nematode density in soil beyond which a strong negative impact could be felt on tomato productivity. This finding has important agricultural consequences. Before the cultivation of tomato at least in our study area, nematode soil density must first be assessed, and compared to the threshold we found in this study. Such comparison is necessary to decide whether preliminary actions are needed to control for nematode levels in soil before cultivation takes place.

ACKNOWLEDGEMENT

We would like to thank Mavis Siakpere, Ugochukwu Okoroafor, Barister J. Uddoh and the entire laboratory crew of the Department of Animal and Environmental Biology, at the University of Port Harcourt for their support during the study; We appreciate the University of Port Harcourt, the Association of Common Wealth Universities (TITULAR, 2011) Fellowship and collaborators from the University of Johannesburg in South Africa.

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