Terrestrial non-parasitic nematode assemblages associated with glyphosate-tolerant and conventional soya bean-based cropping systems

By Akhona Mbatyoti, Mieke Stefanie Daneel, Antoinette Swart, Dirk de Waele and Hendrika Fourie

Commercial production of genetically modified crops (GM), either herbicide or insect tolerant, commenced in the 1990s. Herbicide tolerance of GM crops to broad spectrum herbicides containing glyphosate (N-phosphonomethyl) as the active substance is the predominant trait of these crops. Among glyphosate tolerant crops grown globally, soya bean (*Glycine max L.*) dominates in terms of hectares planted (54,2 million ha), followed by maize (*Zea mays L.*) (13,2 million ha), cotton (*Gossypium hirsutum L.*) (5,1 million ha) and canola (*Brassica napus L.*) (2,3 million ha).

In South Africa, glyphosate became commercially available more than a decade ago and is now widely used in soya bean and maize-based cropping systems in particular. It is estimated that more than 90% of soya bean (630 000ha) and 16% of maize (284 000ha) grown in South Africa is glyphosate tolerant (Dlamini *et al*., 2014; James 2015).

The driving force behind the rapid adoption of glyphosate is because producers prefer to use a single herbicide to control a broad spectrum of weeds and grasses, resulting in minimal crop injury and great economic benefits to producers.

Glyphosate and nematodes

Glyphosate is often regarded as an environmentally friendly pesticide due to its low mammalian toxicity, relatively short environmental half-life and very low activity in soil due to its binding to soil minerals. However, the increasing cultivation of glyphosate tolerant crops has raised a wide range of concerns such as its effects on non-target microorganisms, e.g. nematodes in the soil.

Nematodes play a crucial role in important ecosystem services such as nutrient recycling and decomposition, suppression of pathogenic microorganisms, and biodegradation of harmful compounds. As a result, changes in nematode community composition (assemblage) may have a substantial impact on the ecosystem functioning.

Information about the non-target effects of glyphosate on soil nematodes is scarce and not well documented. More important, often inconclusive and/or conflicting effects of glyphosate on nematode assemblages are reported. Only six scientific reports could be found that dealt with the effects of glyphosate on nematodes. The majority of these focused on the effects that glyphosate has on plant-parasitic nematodes. Liphadzi *et al*. (2005), however, reported that different glyphosate dosages had no effect on non-parasitic nematode densities in a growth chamber experiment.

No information on the effects of glyphosate on, or its association with, either plant-parasitic or terrestrial non-parasitic nematodes (generally referred to as beneficial or free-living), is available for South African agricultural production areas. Therefore, the main aims of this study were to identify terrestrial, non-parasitic nematode assemblages in commercial soya bean fields where glyphosate has been applied regularly versus not applied for at least five years prior to this study and examine whether glyphosate application affected such nematode assemblages in a two-year soya bean/maize cropping system.

Commercial soya bean field study

Thirty-two non-parasitic nematode genera were collectively identified from soils of the three ecosystems, with 65% identified from soils of glyphosate-tolerant soya bean fields, 72% from conventional soya bean fields, and 88% from natural vegetation sites. The genera identified were represented by different feeding groups and functional guilds, and included bacterivores, fungivores, predators and omnivores.

The predominant non-parasitic nematodes from glyphosate-tolerant soya bean sites for the 2011/12 season were *Aphelenchus, Acrobeles*, and *Acrobeloides*. *Aphelenchus* occurred in soils from all of the glyphosate-tolerant soya bean sites while *Acrobeles* and *Acrobeloides* occurred in only 50%. For conventional soya beans, the predominant genera were *Panagrolaimus*, *Acrobeloides*, and *Aphelenchus*. *Panagrolaimus* occurred at 75% of the sites, with *Acrobeloides* and *Aphelenchus* occurring in 50%. In soils from natural vegetation sites, the predominant genera were *Acrobeles*, *Aphelenchus*, and *Acrobeloides*. *Acrobeles* occurred at all sites, while *Aphelenchus* and *Acrobeloides* were found at 75% of the sites.

For the 2012/13 season the predominant genera identified from soils of glyphosate-tolerant soya bean sites were *Aphelenchus*, *Acrobeles*, and *Eucephalobus*. *Aphelenchus* occurred at all sites and *Acrobeles* and *Eucephalobus* at 89% and 78%, respectively. For conventional soya bean, the predominant genera were *Aphelenchus*, *Eucephalobus*, and *Acrobeloides*. *Aphelenchus* occurred at all sites, with *Eucephalobus* and *Acrobeloides* occurring at 44% and 67%, respectively. In soils from natural vegetation sites the predominant genera were *Aphelenchus*, *Eucephalobus* and *Panagrolaimus*. *Aphelenchus* and *Panagrolaimus* occurred at all sites and *Eucephalobus* at 83%.

Although the abundance of the predominant genera (*Acrobeles*, *Acrobeloides*, *Aphelenchus*, *Eucephalobus*, and *Panagrolaimus*) varied substantially for the three ecosystems, it did not differ significantly between ecosystems according to t-test analyses.

Significant interactions

Mixed Models analysis showed significant ($P \leq 0,05$) interactions for fungivores, omnivores, and predators for season locality and for predators for season ecosystem locality. Due to relatively low F-ratios for this interaction for fungivores and the absence or very low numbers for predators and omnivores (ranging between two and seven for omnivores and two and four for predators), further discussion of the data is abstained from.

Season significantly ($P \leq 0,05$) affected the abundance of all four nematode trophic groups (bacteri-, fungi-, omnivores, and predators). The abundance of bacterivores (873 ± 426 vs 120 ± 430 nematodes/200g soil), fungivores (283 ± 150 vs 88 ± 152 nematodes/200g soil) and omnivores (1,6 ± 0,9 vs 0,5 ± 0,9 nematodes/200g soil) was significantly higher in Season 2 compared with Season 1. By contrast, predator abundance was significantly higher ($P \leq 0,05$) in Season 1 (0,8 ± 0,15 nematodes/200g soil) than Season 2 (0,38 ± 0,12 nematodes/200g soil). However, due to either the absence or very low numbers for predators and omnivores, discussion of the data for these two trophic groups is abstained from.

Ecosystem affected only predator abundance significantly ($P \leq 0,05$), with significantly higher population densities in glyphosate-tolerant (1 ± 0,2 nematodes/200g soil) compared with conventional soya bean (0,3 ± 0,2 nematodes/200g soil) and natural veld (0,2 ± 0,1 nematodes/200g soil). However, the very low predator numbers recorded for all three ecosystems warrants no further discussion.

Locality significantly ($P \leq 0,05$) affected omnivore and predator abundance but warrants no further discussion due to very low population densities recorded for these to trophic groups.

According to CCA analyses, no differences were apparent for the nematode assemblages present in soils from the three ecosystems when data for the sites were combined. However, when the three ecosystems were plotted per site, distinct variations existed among the respective nematode communities for the three ecosystems with the cumulative explained variation for the different locations for both seasons ranging from 22 to 82%.

An example is that of Edenville, with a cumulative explained variation of 48,9%. For the other localities, similar differences between the nematode communities for the three ecosystems were observed, although the nematode assemblages associated with each ecosystem differed among the localities.

Presence of fungivores

According to faunal analysis, soils from the majority of the sites (54%) of the three ecosystems due to their Enrichment Index (EI) and Structural Index (SI) being <50% for both seasons were dominated mainly by the presence of fungivores, especially Fu2. Of the sites, 46% plotted in Quadrant A due to their EI being >50% and SI being <50%. These soils were dominated by bacterivores, mainly belonging to Ba1 and Ba2.

The metabolic footprints (data pooled for sites from each ecosystem for each season) for the three ecosystems were small. The EI for the three ecosystems was intermediate (38%) to moderately high

(68%) and the SI very low (<10%) for both seasons. Small differences were evident for both natural vegetation for the two respective seasons and glyphosate-tolerant for the two respective seasons' ecosystems.

However, for the conventional soya bean ecosystem the difference for the 2011/12 growing season and the 2012/13 growing season was more pronounced. This phenomenon was probably due to a higher percentage of Fu2 being present in soils during the 2013 season.

Soya bean/maize cropping experiment

All nematode genera identified from the experimental plot were present in soil samples taken before the study commenced. Their numbers were, however, low and ranged between two and seven per 200g soil.

Fourteen non-parasitic nematode genera were identified from rhizosphere soil samples. In general, higher numbers of non-parasitic nematodes were recorded during the 2014/15 season compared with the 2013/14 growing season. *Aphelenchus* was most abundant and always occurred in higher numbers in glyphosate-treated plots.

Aphelenchoides only occurred in the glyphosate-treated half of the plot while Tylenchus only occurred in non-treated halves of both crops. *Acrobeloides*, Cephalobus, *Eucephalobus*, and *Panagrolaimus* always occurred in higher numbers in the glyphosate-treated compared with the non-treated half of the plots.

Faunal analysis

Substantial differences were apparent for non-parasitic nematode assemblages present in soils of the soya bean/maize cropping system for the glyphosate-treated compared with the non-treated plot halves. Data for the non-treated soil of all sampling dates plotted, with EI >45% due to domination by bacterivores (Ba2 in particular representing *Acrobeles*, *Acrobeloides*, and *Eucephalobus*).

One sample from the non-treated maize plants plotted with a high SI (86%) due to the presence of predators (Pr5) belonging to the genera *Aporcelaimellus* and *Discolaimium*. By contrast, all samples from the glyphosate-treated plot half, except for one, plotted in Quadrants C and D with a low EI (<35%). This was substantiated by the presence of fungivores, Fu2 in particular, belonging to *Aphelenchus* and *Aphelenchoides* while Fu4 was also present and was represented by *Tylencholaimus*.

The increasing cultivation of glyphosate tolerant crops has raised a wide range of concerns such as its effects on non-target microorganisms. (ID 121201752 © Adisak Krongsawat | Dreamstime.com)

Discussion

The 32 non-parasitic nematode genera identified from the commercial soya bean field study and adjacent vegetation, and an experimental site where a soya bean/maize rotation was done, represent novel information for South Africa. Previous studies in such agricultural areas only focused on plant-parasitic nematodes.

Various abiotic factors are known to impact on nematode development and survival, with season significantly shown to affect the abundance of the four non-parasitic nematode trophic groups recorded in our study. This scenario implies that prevailing environmental conditions played a pronounced role during the two seasons this study was conducted.

Although soils from the commercial glyphosate tolerant fields were dominated by the fungivore genus *Aphelenchus* during both seasons of the study, this genus also dominated in soils from conventional soya bean and natural vegetation ecosystems in the second season. In the soya bean/maize cropping experiment, it dominated in the second season in both plots.

These results agree with those by Neher *et al*. (2014) who recorded higher abundance of fungivores in soils from Bt maize compared with those from their near-isolines. Also, it is to a certain extent in agreement with those by Liphadzi *et al*. (2005) who stated that fungivores dominated in soils treated with various herbicides. These authors, however, did not refer to glyphosate-treated soils as was done in the present studies.

Variation across ecosystems

The abundance and dominance of the non-parasitic nematode genera, however, varied among the three ecosystems sampled during the extensive field study, and for the two-year experimental soya bean/maize cropping study. For the field study, the glyphosate tolerant soya bean ecosystems supported the least number of genera (21), while the natural vegetation supported the most (27), followed by the conventional soya bean ecosystem (23).

This trend is in agreement with reports by Bekker (2016) that natural vegetation ecosystems adjacent to maize fields in South Africa supported a higher diversity of non-parasitic nematodes than conventional and conservation maize ecosystems. Also, the general trend that nematode communities in soya bean fields and natural vegetation sites were dominated by bacterivore genera of the families *Acrobelidae*, *Cephalobidae*, and *Panagrolaimidae* and fungivores of the families *Aphelenchidae* and *Aphelenchoididae* is in agreement with results by Bekker (2016) who did a similar study for commercial maize fields.

The dominance of bacterivores in terms of the genera diversity in soils sampled during the present studies is also in agreement with reports by Djigal *et al*. (2004) and Xu *et al*. (2015). These authors suggested that bacterial feeding nematodes are the most abundant metazoans in soil substrates.

Prevalence of fungivores

Fungivores were the second most prevalent group in soils sampled in the present studies, which is in agreement with a recent study by Renčo and Čerevková (2017). These authors reported that fungivores are the second most abundant in soil after bacterivores nematodes. The lower abundance and occurrence of predators and omnivores in the commercial field study was not surprising since these two groups are regarded as being very sensitive to soil disturbances.

A similar trend was reported by Bekker (2016) for a commercial maize field study. Hence, despite that ecosystem significantly affecting predator abundance, the very low population densities and/or absence of this trophic group at various sites are suggested to have caused this effect and hence discussion of the data is abstained from. The absence of omnivores in soils of the two-year experimental soya bean/maize study is another interesting observation and cannot be explained at this stage.

Nematode communities generally differ and fluctuate substantially among different locations in terms of abundance, diversity, and occurrence. This tendency, although not significant, was apparent for the three ecosystems sampled during the commercial field study. When the three ecosystems were, however, analysed per site using the nematode trophic groups, each generally had different nematode communities and was separated from each other according to CCA analyses. However, no trend existed where a specific nematode genus/genera was exclusively associated with either of the three ecosystems.

Although it was not possible to deduct the impact of each ecosystem on the nematode communities, our study showed that glyphosate-tolerant soya bean had no deleterious effects on non-target beneficial nematodes. This is in agreement with those for other genetically modified crops, e.g. Al-Deeb *et al*. (2013) and Neher *et al*. (2014) demonstrating that genetically modified Bt maize had no significant adverse effects on non-target, beneficial, and plant-parasitic nematodes.

Also, Chen *et al*. (2017) concluded that Bt rice had no remarkable impact on beneficial soil nematode communities and was pest specific. However, Neher *et al*. (2014) suggested that rhizosphere soil from Bt maize may contain more complex and successfully mature nematode communities opposed to those from non-Bt near isolines, which may be applicable to our study where fungivores generally dominated in soil from glyphosate-treated soya bean crops. This phenomenon may be an indication that less disturbance in the glyphosate-treated soya bean fields probably can contribute to nematode communities being more matured.

Sub-optimal soil quality

According to faunal analysis, all soya bean sites sampled were disturbed and degraded, indicating that the quality of these soils is not optimal in terms of the presence of beneficial nematodes. This situation is often associated with management practices such as repeated tillage and pesticide application, which are typical practices in local soya bean production areas.

Contrary to annual crop fields, natural vegetation ecosystems are usually regarded as stable and structured due to either no or minimal disturbances. However, in the current study all-natural vegetation sites were also degraded or disturbed. This might be explained by the vegetation type that was represented by mainly grasses. Often natural vegetation consists of woody, perennial plants that are mostly considered less disturbed than grassland vegetation. The latter vegetation probably experiences periods during which the organic content of the soil is high compared to periods when substantially less organic material is present.

Results from the soya bean/maize cropping experiment, however, showed that glyphosate applied as a leaf spray twice per season during two consecutive growing seasons generally affected the abundance and diversity of non-parasitic nematodes. This was substantiated by soil food web analysis of the different nematode sampling dates that showed that the majority of the glyphosate-treated plots for both seasons were degraded and depleted opposed to the non-treated plots that were disturbed but enriched.

These results are not in agreement with those of the commercial field study and also those reported by Liphadzi *et al*. (2005), who found that glyphosate application had no effect on the abundance and diversity of non-parasitic nematodes in glyphosate-treated plots during a three-year study.

It is worth mentioning that the non-treated plot was hoed, implying some disturbance in the upper soil, while organic material was also added to the soil. Both these activities might have had an effect on non-parasitic nematode communities and probably favouring bacterivore genera.

Ultimately, results from the two South African studies conducted showed similarity in terms of *Aphelenchus* domination. However, glyphosate application did not affect the general abundance of non-parasitic nematodes compared with those from conventional soya bean fields and natural vegetation sites where no glyphosate had been applied for at least five years prior to this study, or never before.

For more information, send an email to MbatyotiO@arc.agric.za.