Norwegian University of Life Sciences
Faculty of Biosciences
Department of Plant Sciences

Philosophiae Doctor (PhD)
Thesis 2021:32

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Planteparasittiske nematoder hos enset (*Ensete ventricosum*) - Forekomst, karakterisering, distribusjon og forvaltning av nematodene idenne kulturplanten som er viktig for matvaresikkerheten i Etiopia

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Glory be to God! እግዚአብሔር ይመስገን።

Selamawit A Kidane

Ås, February 2021

Summary

To confront the challenges of eradicating hunger and improve food security, agricultural growth needs to be driven by intensification. This in turn increases the threat of pests and diseases, including plant-parasitic nematodes. This calls for an approach that integrates various disciplines in order to implement sound pest management strategies. On top of this, the impact of climate change on agriculture is immense, in terms of bringing more uncertainty to agricultural productivity. Our staple crops are facing major challenges, therefore diversification from over-reliance on a few major crops is much needed. Here is where the underutilised, neglected or orphan crops come to the picture, these crops have great potential to circumvent global food insecurity. Enset (*Ensete ventricosum* (Welw.) Cheesman) is one of those orphan crops serving as a major starch staple for about 20 million people in southern Ethiopia.

The overall aim of this work is to provide a comprehensive study associating the rarely recognised plant-parasitic nematodes and the orphan food security crop enset. We provide the most comprehensive study to date, relating nematode infection and distribution with enset in Ethiopia. This thesis is comprised of four papers each addressing specific objectives. The first objective was to review plant-parasitic nematodes associated with banana, enset and abaca. This book chapter (Paper I) summarises and highlights researches conducted on the biology, disease cycle, host reaction, symptoms and management strategies of the most widespread and important nematodes associated with banana, enset and abaca, namely the burrowing, lesion, spiral and root-knot nematodes. Most of the reviewed studies focus either on bananas or plantains, with only a few studies associating nematodes with enset. This knowledge gap has led us to undertake a more in-depth study in this area (paper II and IV).

In order to assess the distribution, population density and incidence of plant-parasitic nematodes associated with enset a survey was conducted in August 2018 (Paper II). A total of 308 samples were collected and 11 plant-parasitic nematode genera were identified: *Pratylenchus*, *Meloidogyne*, *Helicotylenchus*, *Scutellonema*, *Tylenchorhynchus*, *Rotylenchulus*, *Aphelenchoides*, *Cephalenchus*, *Pratylenchoides*, *Trophurus* and *Hoplolaimus*. With the genus *Pratylenchus* being the most prominent one, occurring in 100% of samples at densities as high as 25,000 per 10g roots in samples obtained from enset roots growing in the highlands of Guraghe (2200-3000 m.a.s.l.). The lesion nematode was found causing dark purple lesions on the enset corm and roots. Using morphometric and molecular data, all *Pratylenchus* populations were identified to be *Pratylenchus goodeyi*. During the study we found out that differences in the number of *P. goodeyi* extracted from the roots of different enset cultivars, indicating possible

resistance of the cultivars to the nematode. As a separate study (Paper IV), we conducted a survey to assess the nematode infection levels of enset planting materials. It is evident that banana and plantain suckers are key sources of nematode infection and spread. One to two-year-old enset suckers were collected from markets and farmers' fields and nematodes were extracted from the roots. *P. goodeyi* was recovered from 100% of root samples, at densities ranging from 10 to 190 per 10 g roots. We observed that younger suckers appeared to be less infected and lesions were prominently visible on the corms and roots of the older suckers. This has led us to further investigate the host response of enset; we screened nine enset cultivars for resistance against *P. goodeyi* and assessed the pathogenicity of *P. goodeyi* using four inoculum densities on three enset cultivars (Paper III).

The third objective of our project was to provide the first proper assessment of the reaction of enset to *P. goodeyi* infection. So far, the damage caused or how different enset cultivars react to the nematode has not been determined. Identifying resistance is an important task and the best sought management strategy for plant parasitic nematodes. In a screenhouse, nine enset cultivars were inoculated with a mix of adult and juvenile *P. goodeyi* to evaluate host response and identify potential sources of resistance. After 9 months of incubation, significant differences in the final population densities (P*f*) and reproduction factor (RF) were observed amongst the cultivars. The cultivar 'Gefetanuwa' was the most susceptible (P*f* = 25799 and RF = 12.9) and similarly in a repeat experiment for 4.5 months (P*f* = 126534 and RF = 63.3). On the other hand, cultivars 'Maziya', 'Heila' and 'Kellisa' demonstrated resistance. In the pathogenicity experiment, four inoculum densities used significantly affected the P*f* and RF but there was difference among the three cultivars used ('Maziya', 'Heila' and 'Arkiya'). From the screening experiment, we can see that cultivars 'Maziya' and 'Heila' are both resistant and cultivar 'Arkiya' also had similar traits. Such studies help to provide information on various enset cultivars and detect resistance that can be used for breeding improved, pest and disease resistant cultivars (Paper III).

Key words: enset, plant-parasitic nematodes, population density, reproduction factor, resistance, suckers, *P. goodeyi*, lesion

Sammendrag

For å møte utfordringene med å utrydde sult og forbedre matsikkerheten, må produksjonen på jordbruksarealene intensiveres. Dette til gjengjeld øker trusselen fra skadedyr og sykdommer, inkludert planteparasittiske nematoder. Dette krever en tilnærming som integrerer ulike fagområder for å frambringe en sunn skadedyrsstrategi. På toppen av dette er virkningen av klimaendringer på jordbruket enorm, som øker usikkerheten til landbruksproduksjonen. Våre basisvekster står overfor store utfordringer, og det er derfor stort behov for diversifisering i hva bøndene dyrker, for å redusere risikoen for matmangel. Det er her er underutnyttede og forsømte kulturvekster kan komme inn i bildet, disse vekstene har stort potensiale for å bidra til bedre global matsikkerhet. Enset (*Ensete ventricosum* (Welw.) Cheesman) er en av de uutnyttede vekstene som bidrar som en viktig stivelseskide for rundt 20 millioner mennesker i Sør-Etiopia.

Det overordnede målet med dette arbeidet er å gjennomføre en omfattende studie som ser på samhandlingen melleom de sjeldent anerkjente planteparasittiske nematodene og denne uutnyttede planten som er så viktig for matsikkerheten i Etiopia. Vi har utført den mest omfattende studien i nematoder hos enset til dags dato, knyttet til nematodeinfeksjon og spredning av nematoder med enset i Etiopia. Denne oppgaven består av fire artikler som hver tar for seg spesifikke mål. Det første målet var å gjennomgå plante-parasittiske nematoder assosiert med banan, enset og abaca. Dette bokkapittelet (Artikkel I) oppsummerer og fremhever undersøkelser utført rundt biologi, sykdomssyklus, påvirkning av vertsplanten, symptomer og bekjempelsesstrategier for de mest utbredte og viktige nematodene assosiert med banan, enset og abaca, nemlig har ikke norsk navn "gravende" er ikke så bra navn egentlig, rotsår, spiral og rotgall nematoder. De fleste av de studiene vi så på fokuserer enten på bananer eller plantains, siden det finnes bare noen få studier som forbinder nematoder med enset. Dette kunnskapsgapet har ført til at vi har gjennomført grundige studier på dette området (Artikkel II og IV).
For å vurdere utbredelsen av nematoder hos enset i Etiopia, ble populasjonstetthet og forekomst av planteparasittiske nematoder forbundet med enset gjennømført i en undersøkelse i august 2018 (Paper II). Totalt ble 308 prøver samlet og 11 vi identifiserte planteparasittiske nematodeslekter: *Pratylenchus, Meloidogyne, Helicotylenchus, Scutellonema, Tylenchorhynchus, Rotylenchulus, Aphelenchoides, Cephalenchus, Pratylenchoides, Trophurus* og *Hoplolaimus*. Slekten *Pratylenchus* er den mest fremtredende, og den forekommer i 100% av prøvene med tettheter så høye som 25.000 per 10g røtter i prøver hentet fra ensetrøtter som vokser i høylandet i Guraghe-området (2200-3000 m.o.h.). Rotsår nematode forårsaket mørklilla lesjoner på knoller og røtter. Ved hjelp av morfologiske og molekylære metoder ble alle *Pratylenchus*-populasjoner identifisert som *Pratylenchus goodeyi*. Vi fant forskjeller i antall *P. goodeyi* ekstrahert fra røttene hos forskjellige enset-kultivarer, noe som peker på mulig motstandskraft av ulike sorter mot denne

nematoden. Som en egen studie (Artikkel IV) gjennomførte vi en undersøkelse for å vurdere formeringsmateriale av enset som er på markedet i Etiopia. Det er tydelig at banan- og plantain-avleggere er viktige kilder til nematodeinfeksjon og spredning. En til to år gamle avleggere ble samlet inn fra markeder, og hos bønder, og nematoder ble ekstrahert fra røttene. *P. goodeyi* ble funnet i 100% av røttene i en tetthet fra 10 til 190 per 10 g røtter. Vi observerte at yngre avleggere så ut til å være mindre infiserte, og lesjoner var tydelig synlige på knollene og røttene til de eldre avleggerne. Dette fikk oss til å undersøke vertsresponsen hos enset med dyptgående; vi screenet ni enset-kultivarer for motstandskraft mot *P. goodeyi* og vurderte patogenisiteten til *P. goodeyi* ved hjelp av fire inokulat-tettheter på tre enset-sorter (Artikkel III).

Det tredje målet med prosjektet vårt var å gi den første gjennomførte vurderingen av samspillet mellom enset og *P. goodeyi*-infeksjon. Ingen har så langt sett på skadene forårsaket av, eller hvordan forskjellige ensettkulturer reagerer på, denne nematoden. Å identifisere motstandskarft er en viktig oppgave og den beste bekjempelsesstartegien for å kontrollere nematodeinfeksjoner. I et 'screenhouse' ble ni enset-kulturer inokulert med en blanding av adulte og yngre *P. goodeyi* for å evaluere vertsrespons og identifisere potensielle kilder til motstandskraft hos enkelte sorter. Vi observerte signifikante forskjeller blant sortene med hensyn til populasjonstetthet (P*f*) og reproduksjonsfaktor (RF) etter 9 måneders inkubasjon.. Kultivaren 'Gefetanuwa' var den mest utsatte (P*f* = 25799 og RF = 12,9) og tilsvarende i et annet gjentaketter 4,5 måneder (P*f* = 126534 og RF = 63,3). På den annen side var sortene 'Maziya', 'Heila' og 'Kellisa' motstandsdyktige. I patogenisitetseksperimentet påvirket fire inokulumtettheter P*f* og RF i betydelig grad, men det var forskjell mellom de tre sortene som ble brukt i dette forsøket ('Maziya', 'Heila' og 'Arkiya'). Fra forrige screeningseksperiment kunne vi se at 'Maziya' og 'Heila' begge var resistente og 'Arkiya' hadde også lignende egenskaper. En slik type undersøkelse bidrar til informasjon om ulike sorter og oppdage kilder til motstandskraft som kan brukes i fremtidig foredling av enset (Artikkel III).

Stikkord: enset, planteparasittiske nematoder, populasjonstetthet, reproduksjonsfaktor, motstandskraft, avlegger, *P. goodeyi*, lesjon

List of papers

Paper I: Coyne, D. and Kidane, S. (2018). Nematode Pathogens. In: Jones, D.R. (Ed) *Handbook of Diseases of Banana, Abacá and Enset.* 2nd edition. CAB International, Wallingford, UK, pp. 429-461.

Paper II: Kidane, S.A., Meressa, B.H., Haukeland, S., Hvoslef-Eide, A.K., Magnusson, C., Couvreur, M., Bert, W., and Coyne, D.L. (2020). Occurrence of plant-parasitic nematodes on enset (*Ensete ventricosum*) in Ethiopia with focus on *Pratylenchus goodeyi* as a key species of the crop. *Nematology* 1, 1-13. https://doi.org/10.1163/15685411-bja10058.

Paper III: Kidane, S.A., Meressa, B.H., Haukeland, S., Hvoslef-Eide, A.K., and Coyne, D.L. (2021).The Ethiopian staple food crop enset (*Ensete ventricosum*) assessed for the first time for resistance against the root lesion nematode *Pratylenchus goodeyi*. *Nematology* 0, 1-9. https://doi.org/10.1163-15685411-bja10075

Paper IV: Kidane, S.A., Meressa, B.H., Haukeland, S., Hvoslef-Eide, A.K., and Coyne, D.L. (2021). Planting material of enset (*Ensete ventricosum*), a key food security crop in Ethiopia, is a key element in the dissemination of plant-parasitic nematode infection. (Submitted to Frontiers in Plant Science)

Contents

1. General introduction

In 2050 the world population is predicted to reach nine billion; this increase puts agriculture under pressure to produce a greater quantity of food, feed, and biofuel using the limited land resource we have, to maintain biological diversity in the non-agricultural areas (Godfray *et al.*, 2010). In order to cope with the estimated 40% increase in the world population by 2050, agricultural production must increase by 70% (Bruinsma, 2009). More than 50% of the predicted global population growth between now and 2050 is expected to happen in Africa. Africa's share of the global population is projected to increase from 17% in 2017 to 26% in 2050 and could possibly reach 40% by 2100 (United Nations, 2017). According to the 2019 Global Hunger Index, Ethiopia falls under the serious category with a score of 28.9 and ranking 97th out of 117 countries in the world; between 2016-18, 20.6% of the population was undernourished (Von Grebmer *et al.*, 2019).

Globally about 8.9% of the world population - about 690 million people sleep on an empty stomach every night. Statistics show that global hunger is on the rise since 2014. The hunger sustainable development goal that calls for ending hunger in the world by 2030 will likely not be met, even without considering the effects of the COVID-19 pandemic that might add 83-132 million more hungry people. To provide for the 690 million undernourished people in our world and the additional 2 billion people our world will have by 2050, we need change in the global food and agriculture system. An increase in agricultural productivity and sustainable food production is critical to mitigate the perils of hunger (United Nations, 2020).

Growing populations, urbanisation, and low agricultural productivity have reduced per capita food availability, which calls for agricultural intensification. However, such intensification of agricultural practices can result in additional issues to deal with, such as increases of pest and disease pressure on crops (Coyne *et al.*, 2018). Climate change has a notable impact on both biotic and abiotic stresses in agriculture, threatening yields and sustainability. Over-reliance on selected major staple crops has agronomic, ecological, nutritional, and economic risks (Keatinge *et al.*, 2014). In order to achieve food security, crop diversification will be a necessity. Neglected or underutilised crops are often ancient crop species cultivated in local communities contributing significantly to these communities' livelihoods but are barely recognised, if not wholly unknown, outside their cultivation regions. However, they have great potential to contribute to global food security at large (Mayes *et al.*, 2012).

1.1.Enset in Ethiopia

Ethiopia is a center of biological diversity and domestication for a range of crops, including coffee (*Coffea arabica* L.), tef (*Eragrostis tef* (Zucc.) Trotter), khat (*Catha edulis* Forsk), noog (*Guizotia abyssinica* (L.f) Cass.), finger millet (*Eleusine coracana* L.) and enset (*Ensete ventricosum* (Welw.) Cheesman). According to anthropologists, the domestication of enset dates back approximately 10,000 years (Brandt *et al.*, 1997). Agronomists and biogeographers consider the Ethiopian highlands to be the primary center of origin of enset agriculture; the fact that domesticated enset has a very narrow geographic distribution and divergence in present-day enset agricultural systems supports this theory (Harlan, 1969; Harlan 1971; Sauer, 1952). Despite wild species occurring in eastern, central and southern Africa (Baker and Simmonds, 1953) enset is only found as a cultivated crop in Ethiopia (Brandt *et al.*, 1997).

Enset is a large perennial monocarpic, single-stemmed herbaceous plant belonging to the family *Musaceae*, along with banana and plantain. Both enset and banana have an underground corm, a bundle of leaf sheaths that form the pseudostem and large leaves (Fig. 1A-F). Although enset resembles the banana plant, it is larger, reaching up to 10 m, a diameter up to 1 m and does not usually produce suckers at the plant's base. Unlike bananas, enset does not produce edible fruits, instead, it is grown for its carbohydrate-rich food obtained from the pseudostem, leaf sheaths and underground corm harvested 3-12 years after planting (Brandt *et al.*, 1997).

Wild enset is reproduced sexually via seeds, while domesticated enset is exclusively propagated vegetatively through suckers emerging from a well-prepared corm of a young plant. Since enset plants are usually harvested before emergence of the inflorescence, seeds are not available for planting, although vegetative propagation is preferred due to increased vigour of suckers (Alemu and Sanford, 1991). Domesticated enset seeds also have low germination rates (Negash, 2001).

In order to produce suckers for planting, a farmer harvests the pseudostem of a desired 2-4-year-old enset plant and saves the corm. Unlike banana natural sucker formation is not common on enset due to strong apical dominance, the apical bud needs to be physically destroyed to initiate multiple suckers. After removing the apical meristem, the corm can be used entirely or split into two or four smaller pieces, kept under shade or exposed to sunlight for 2-4 days depending on the producing zone and finally planted into 20-30 cm deep soils mixed with manure (Yemataw *et al.*, 2014). Depending on the cultivar, size and age of the mother plant, cultural practices, and environmental conditions, 40-200 suckers will emerge within 2-3 months after planting. Multiple suckers arising from the buried corm are kept intact for about a year and then are replanted in a well-manured nursery for another year. These are further transplanted up to four times into wider spacings (Tsegaye and Struik, 2002; Shumbulo *et al.*, 2012). Farmers produce their own suckers

for planting and there is also exchange of planting materials between villages and beyond. Several landrace suckers of different ages are also sold in markets. Various methods for rapid propagation have been studied, such as zygotic embryo culture (Negash *et al.*, 2000; Negash *et al.*, 2001; Diro *et al.*, 2003), shoot tip culture (Afza *et al.*, 1996; Zeweldu, 1997; Negash *et al.*, 2000) and callus culture and somatic embryogenesis (Mathew *et al.*, 2000; Mathew and Philip, 2003). These technologies can provide a large number of disease and pest free planting materials within a short period of time.

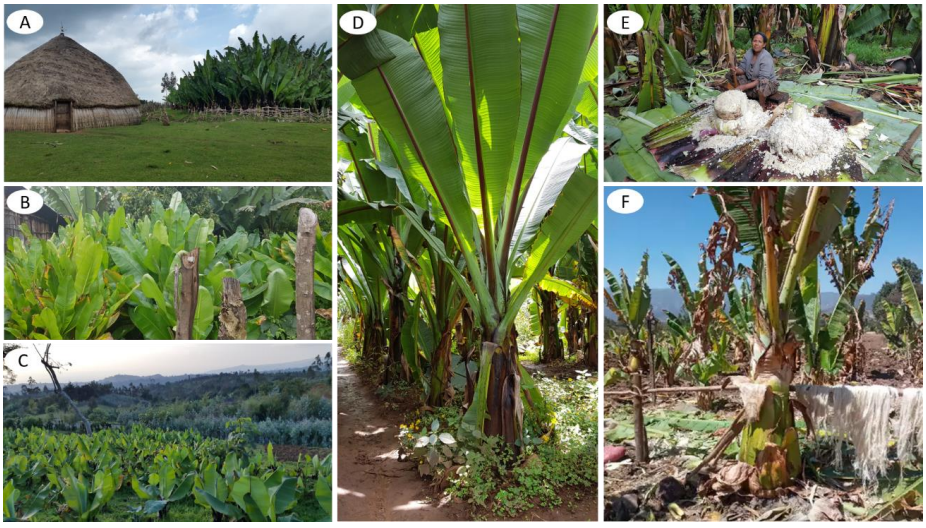

Figure 1:(A) Enset garden around a traditional hut in Hadiya zone; (B) young enset plantlets arising from corms; (C) young enset plants at second transplating stage. Spacing and number of transplanting varies across different zones; (D) mature enset plant ready for harvest; (E) harvested enset plant, parts of the pseudostem and corm being processed for food by a woman; (F) fiber produced by scrapping the pseudostem of enset

Enset has even been described as the "tree against hunger" by Jeronimo Lobo, a Portuguese priest who travelled to Ethiopia in 1640 (Costa and Lockhart, 1984). Enset is a major starch staple food for over 20 million people in South and Southwestern Ethiopia (Brandt *et al.*, 1997). It is an important food security crop sustaining the lives of many people, which was evident during the harsh famine in Ethiopia in the 1980's, where enset growing communities were not affected at all (Dessalegn, 1995). Enset farming has several attributes contributing to food security. It is perceived to be drought-tolerant, withstands flooding and heavy rain. Enset can be harvested at any maturity stage during the year, at any growth stage and the fermented products can be stored for long periods. This combination of characters makes enset an important food security crop and has earned its name "The Tree Against Hunger" (Brandt *et al.*, 1997). In addition to these factors, enset can be harvested 3-12 years after planting; leaving the tree for seven years is believed to be the optimum. The enables the farmers to harvest their enset ahead of the optimal seven years, if another staple crop fails due to drought or flood, securing the food situation for crop failure years.

Agricultural research in Ethiopia is mainly focused on cereal-based systems, while the highly localised enset-based farming system has received only limited development or research attention, despite it being an important crop in various regards (Brandt *et al.*, 1997). The consequences of the inadequate efforts have resulted in under exploitation of the crop and put the genetic resources of enset, along with its associated indigenous knowledge, at a greater risk of erosion (Olango *et al.*, 2014).

1.2.Genetic variation and breeding of enset

The wide geographical range of enset cultivation and various cultural influences within Ethiopia has led to an extensive genetic variation in enset landraces. The germplasm collection at Areka Agricultural Research Center, Ethiopia, maintains 623 enset landraces sourced from 12 major enset growing zones to preserve the genetic variability. These landraces fall into clusters differing in quantitative and qualitative traits, such as maturity time, plant height, pseudostem height, circumference, leaf number and end-product yield. A significant phenotypic variation has been confirmed based on 387 accessions of enset (Yemataw *et al.*, 2017a). Farmers have developed local naming and classification systems for enset, based on morphological characteristics, yield, susceptibility to diseases, ease of processing and end-uses of the plant (Tesfaye and Lüdders, 2003). Unlike other essential food crops, only a few studies use molecular markers for enset germplasm characterisation and genetic diversity. Birmeta *et al.* (2002) and Negash *et al.* (2002) used random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphisms (AFLP), respectively, to assess the genetic diversity of more than a hundred enset clones in each study, reporting a high level of genetic variation among the studied clones. The authors also discovered duplication of vernacular names, with the same name assigned to genetically different but morphologically similar cultivars. In a later study, Birmeta *et al.* (2004) compared wild and domesticated enset populations using RAPD markers and found that populations clustered separately. A subsequent study by Tobiaw and Bekele (2011) evaluating 71 enset clones from Keffa and Dawro zones using inter simple sequence repeat (ISSR) markers showed that clones clustered in two groups aligned with their collection zones. Olango *et al.* (2015) also developed the first set of genomic simple sequence repeats (SSR) markers in enset, used for studying genetic diversity and developing strategies for conservation and breeding. Harrison *et al.* (2014) presented a draft genome-wide sequence of enset with an approximate size of 547 Mb (GenBank accession number AMZH02), which will be influential in stimulating future research on this neglected crop. Furthermore, 17 enset accessions were re-sequenced using the Illumina HiSeq and MiSeq platforms and raw reads aligned against the published reference genome sequence by Yemataw *et al.* (2018). Gerura *et al.* (2019) assessed the genetic diversity of 83 enset germplasm accessions from Guraghe zone using polymorphic SSR markers, confirming

high genetic diversity; outcomes of the study are useful for further conservation, breeding and development of resistant cultivars.

Despite these efforts on the genetic diversity of enset, there has been no published work on the genetic improvement of enset through traditional plant breeding. This is hardly surprising, due to long generation time and since cultivated enset does not set fertile seeds. A recent review from Merga *et al.* (2019) assessed the possibilities of using the somatic embryogenesis-based protocol established for transformation of banana (Tripathi *et al*., 2014a and 2014b) to obtain genetically modified enset with resistance to bacterial wilt, *Xanthomonas campestris* pv. *musacerum.* These genetically modified (GM) bananas have shown resistance both to artificial inoculation in the greenhouse (Tripathi *et al*., 2010) as well as long-term field trials in Uganda (Tripathi *et al*., 2014b). Merga *et al.* (2019) concluded that the same methods could be used in the close relative of banana, enset, based on the regeneration protocols by Tripathi *et al.* (2017a). This could also give the opportunity to transform enset for nematode tolerance/resistance since the successful field trials with GM plantain in Uganda also included assessing nematode resistance (Tripathi *et al*., 2015). Many other species have also been successfully transformed to convey resistance against several severe nematode pathogens (Tripathi *et al*., 2017b). The future could hold nematode-resistant enset for Ethiopian farmers, whether through conventional genetic engineering or gene editing.

1.3. Uses of enset

The cultivation of enset plays an important cultural role in many enset growing communities, and it is a symbol of their identity (Olango *et al.*, 2014). Within the household, women play a significant role in the labour-intensive processing, cooking and sale of enset products. Major starchy foods obtained from enset are kocho, bulla and amicho, prepared from the decorticated pseudostem and grated corm that are fermented in pits. Other than being a major starch staple food, enset is a good source of animal fodder and is used in traditional medicine. It also provides good quality fiber obtained from decorticated leaf sheaths, which can be used for construction, making ropes, mats and baskets. The leaves of enset are also used as a packaging material. Around the home garden, enset provides protection from the sun and wind, its thick dark green canopy beautifying the landscape and protecting against soil erosion on the steep hillsides. Enset cultivation has a positive impact on the environment in terms of maintaining soil fertility; accumulation of litter and organic farming (manure and domestic waste) improve soil quality; reduced soil erosion and surface runoff in enset fields; and increased water infiltration (Brandt *et al.*, 1997).

1.4. Enset production status

Data analysis from the Ethiopian Central Statistical Agency (CSA, 1995-2017) shows that enset production has increased by approximately 46% over the previous two decades, with over 400,000 hectares of land covered by enset and a national estimated yield of 5.3 million tons (CSA, 2016). However, the CSA data shows that this increase is through an increased area of coverage, while several studies demonstrate enset productivity to be declining (Abebe *et al*., 2013; Yemataw *et al*., 2017b). This period (1996 to 2017) also coincides with an increasing Ethiopian population of 77%, from 59 to 105 million (Center for International Earth Science Information Network, 2017). There is also no evidence on policies and crop development-based drivers that could have attributed to the reported increase in productivity of enset (Borrell *et al*., 2019). Cochrane and Bekele (2018) have also raised concerns in terms of the quality, methods and politicisation of CSA data. Another source of disparity in terms of estimating enset yield is the problems related to the survey methods applied to other annual and perennial crops. Unlike other crops enset is a multi-year crop harvested from the 2nd year onwards until maturity (Borrell *et al*., 2020).

Borrell *et al.* (2020) summarise the challenges and sources of variation in estimating the area under production and yield for enset in Ethiopia. The area under enset production is influenced by successive transplanting of enset, agronomic practices differing among regions, intercropping, differences in harvesting times and mortality due to pests and diseases. Estimating yield is affected by varying performance of genetically distinct landraces, differences in agroecological zones, age at harvest, yield and ratio of different food products, local unit names and weights for products, and lack of quantification of the water content of different products.

1.5. Production constraints of enset

Pests and diseases affecting enset growth and yield are a major challenge to enset production. Enset bacterial wilt disease *Xanthomonas vasicola* pv. *musacearum* (Xvm) (previously named *X. campestris* pv. *musacearum* (Xcm)) (Studholme *et al.*, 2020) is the most important disease threatening enset production (Yemataw *et al.*, 2016). The enset root mealybug (*Cataenococcus ensete*) is another major production constraint causing severe damage to the roots and corm, reducing crop vigour and production (Addis *et al.*, 2010). *Sclerotium* sp. root and corm rot disease and *Acremonium* inflorescence spot are fungal diseases causing moderate damage (Tesera and Quimio, 1994; Quimio and Tesera, 1996). Leaf streak disease is a newly reported disease caused by a new *Badnavirus* species (Abraham *et al.*, 2018; Abraham, 2019). A few studies have associated plant-parasitic nematodes with enset, with the lesion nematode *Pratylenchus goodeyi* being the most predominant species (Peregrine & Bridge, 1992; Tesera & Quimio, 1994; Speijer & Fogain, 1998; Mandefro & Dagne, 2000; Swart *et al.*, 2000; Bogale *et al.*, 2004) along with

root-knot nematodes (*Meloidogyne incognita, M. javanica* and *M. ethiopica*) and *Aphelenchoides ensete* (Mandefro and Dagne, 2000; Swart *et al.*, 2000). The major dissemination of these diseases and pests is through infected farm tools and planting materials.

East African agriculture will be substantially impacted by climate change, resulting in yield reduction (Adhikari *et al.*, 2015). Climate change and declining farm diversity are major threats to enset cultivation, which will result in shifting of environmental conditions in the long run. Despite the projections of climate change in Ethiopia, there have been no studies assessing the impact on enset cultivation. The lack of nationally and internationally secure germplasm collections, both *in vivo* and long-term seed storage, are a threat to the germplasm diversity. Regardless of its potential enset is underrepresented in *ex situ* germplasm collections, limiting the potential for breeding and crop improvement. In the future, with climate change, agricultural intensification, biotic constraints, habitat loss, and introduction of high-yielding genotypes, both wild and domestic enset are at risk of losing diversity along with the adaptive traits of the crop (Borrell *et al.*, 2019).

1.5.1. Plant-parasitic nematodes

Nematodes are the most abundant animals representing 80-90% of all animals on Earth that are dominant components of the soil community, filling all trophic levels in the soil food web (Van Den Hoogen *et al.*, 2019). Despite being ubiquitous and present in every habitat (Cobb, 1915), nematodes are essentially aquatic animals that require moisture for survival (Decraemer and Hunt, 2013). They occupy all ecological niches, including being parasites of other animals and plants (Blaxter and Bird 1997). Plant-parasitic nematodes feed on plant cells by inserting their spears or stylets and sucking the contents, causing numerous diseases (Agrios, 1997; Hussey *et al.*,2002). Currently, just 4100 species of plant-parasitic nematodes have been described, which is about 15% of the total number of nematode species known, causing substantial losses in agriculture (Decraemer and Hunt, 2013).

In order to reduce the constraints imposed on agriculture, identification and understanding the biology of nematode pests is very crucial. Presently identification relies on morphological characters and is completed with molecular approaches (Decraemer and Hunt, 2013). In sub-Saharan Africa (SSA), pests and diseases, including plant-parasitic nematodes, pose a major threat to crop production systems. The region is located within the tropics and covers various agro-ecological zones with a diversity of nematode species having multiple generations per season within a range of cropping seasons, threatening crop production (Sikora *et al.*, 2018). Although nematodes are important globally and cause significant loss, they are overlooked and highly neglected, especially in tropical agriculture, where smallholder systems often dominate (Coyne *et*

al., 2018). A diversity of plant-parasitic nematode species present numerous challenges to crop production in SSA, with root-knot nematodes (RKNs) (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) being the two most important groups (Jones *et al.*, 2013). The tropical root-knot nematode *Meloidogyne incognita* is considered the most destructive pathogen (Trudgill and Blok, 2001). Smallholder systems are characterised by a lack of improved technologies, limited inputs and access to improved cultivars, poor infrastructure and poor disease diagnostics. These, along with the poor understanding and limited knowledge of nematodes by farmers and agricultural staff, create difficulties for nematode management (Coyne *et al.*, 2018; Cortada *et al.*, 2019).

Estimating crop losses due to nematodes is difficult, with global estimates ranging from $80 billion (Nicol *et al.*, 2011) to $157 billion USD per year (Abad *et al.*, 2008). In the United States alone, nematodes have been estimated to cause crop losses of $10 billion USD compared with $6.6 billion USD loss caused by insect pests (Gianessi and Carpenter, 1999). As a specific example, the potato cyst nematode in the United Kingdom is estimated to cause losses of approximately $70 million USD, which accounts for about 9% of the value of UK potato production. However, there are no reliable data indicating losses due to nematodes in SSA (Coyne, *et al.*, 2018).

1.5.2. Nematode management and control on enset

In most cases, the control of nematodes is not feasible. Control involves using specific measures to reduce or kill nematodes. In contrast management strategies are implemented to suppress nematode populations below economic thresholds using several measures that consider the whole system and the impact on biodiversity (Thomason and Caswell, 1987). The impact of the pest management strategy on the ecological balance in soil and biodiversity should be assessed (Viaene *et al.*, 2013). Several species of plant-parasitic nematodes are associated with enset in Ethiopia (Kidane *et al.*, 2020; Addis *et al*., 2006; Bogale *et al.*, 2004; Mandefro & Dagne, 2000; Peregrine & Bridge, 1992; Speijer & Fogain, 1998; Swart *et al.*, 2000; Tesera & Quimio, 1994), with the lesion nematode *Pratylenchus goodeyi* appearing to be the most prevalent. However, the lack of awareness of plant-parasitic nematodes by farmers and extension service providers has made it difficult to know the underlying problems caused and thus determine the most suitable management strategies. Coyne *et al.* (2018) have emphasised how critical the under-representation of nematology expertise is, given the immense losses caused by nematodes under intensification agriculture.

1.6. Objectives of the study

Our study focuses on the very important but neglected food security crop enset and the rarely recognised major production constraints from nematodes. In order to fulfill the aims of the study, a review on nematode pests was undertaken, as well as a survey on the distribution of nematodes in key production areas and an assessment of their damage. The overall objective of this study was to identify nematode pests and the damage they cause on enset, their distribution across key enset growing agro-ecological zones, with an emphasis on *P. goodeyi* and the response of enset. Activities conducted included:

- Review on nematode pathogens of banana, enset and abaca
- Survey on the occurrence and distribution of plant-parasitic nematodes associated with enset
- Morphological and molecular characterization of *P. goodeyi* collected from different enset growing zones in Ethiopia
- Screening and evaluation host plant response of enset cultivars to inoculation of *P. goodeyi* under screen house condition
- Assessing the pathogenicity of *P. goodeyi* on selected enset cultivars under screen house condition
- Assessing the infection status of enset planting materials used by farmers

2. Material and methods

- Enset root and soil samples were collected from the southern part of Ethiopia, from administrative zones where enset is most commonly grown (Sidama, Hadiya, Kembata, Keffa, Guraghe and Jimma) in August 2018 (Paper II: Fig. 1).
- Additional *P. goodeyi* populations were collected from Kenya and Uganda, and others supplied from Canary Islands (courtesy of Javier López-Cepero), which were included in the molecular assessment, for comparison with Ethiopian populations (Paper II: Fig. 5).
- Nematodes were extracted from both soil and roots using a modified Baermann method over a period of 48 h (Hooper *et al.*, 2005) (Fig. 2A). Nematode suspensions were reduced to 10 ml and densities were counted from 1 ml aliquots under a compound microscope. Nematode densities were calculated for each root and soil sample and expressed as the number of nematodes per 10 g root or 100 ml soil.
- The extracted nematodes were subsequently heat-killed, and half the quantity was preserved in triethanolamine formalin (TAF) to prepare permanent slides for morphological analyses and the remainder preserved in ethanol (97%) for molecular analysis.

2.1. Screenhouse experiments

- For the screenhouse experiments, one-year-old enset seedlings of known cultivars were obtained from Areka Agricultural Research Centre, Areka, Wolaita (Fig. 2B).
- Nine cultivars were selected and assessed for resistance to *P. goodeyi*: Gewada, Zereta, Maziya, Heila, Kellisa, Gefetanuwa, Yanbule, Messana and Endale.
- Three enset cultivars (Maziya, Arkiya and Heila) were used to assess *P. goodeyi* pathogenicity.
- *P. goodeyi* used for inoculation (Fig. 2C) were isolated from infected enset roots collected from a high infection 'hotspot' highland area in Agena, Guraghe, identified during a recent study (Kidane *et al.*, 2020).
- Prior to planting, the roots were removed, and the corms peeled before sanitising in boiling water treatment for 20 seconds (Coyne *et al.*, 2010) (Fig. 2D). All experiments were conducted in the screen house located in Jimma University College of Agriculture and Veterinary Medicine, Jimma, Ethiopia 7˚42' N; 36˚50' E at an altitude of 1710 m.a.s.l.
- Morphological characterisation was conducted at the nematology laboratory in Nibio, Norway and the molecular analysis was carried out at the Nematology Research Unit in Ghent University.

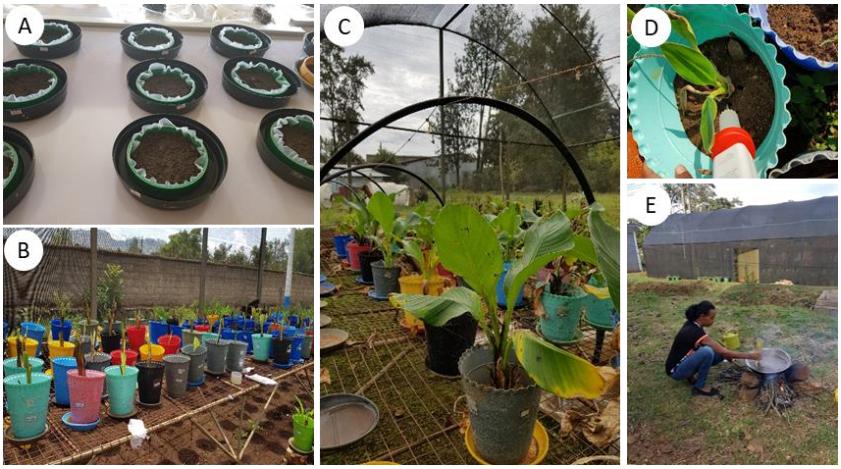

Figure 2: (A) extraction of nematodes using a modified Baermann method; (B & C) one-year-old enset seedlings in pots; (D) nematode inoculation of seedlings (E) boiling water treatment of seedlings prior to planting in pots

3. Main results and discussions

3.1. Nematode pathogens of banana, enset and abaca (Paper I)

Many plant-parasitic nematodes are associated with banana, enset and abaca. Some are known to cause significant damage in terms of growth reduction and yield loss. This book chapter summarises the biology, disease cycle, host reaction, symptoms and control measures of the most widespread and important nematodes associated with banana, enset and abaca, namely the burrowing, lesion, spiral and root-knot nematodes.

The burrowing nematode (*Radopholus similis*), a migratory endoparasite, is one of the main nematode pests of banana in the lowland tropics, causing huge losses in commercial banana plantations (Gowen *et al.*, 2005). The current geographic distribution of the nematode is attributed to the exchange of planting materials and the temperature requirements of the nematode (Price, 2006). Populations of *R. similis* are diverse in terms of host preference, reproductive fitness, pathogenicity and morphology; this nematode is known for its pronounced sexual dimorphism in which males have an atrophied stylet and are considered to be non-parasitic (Chabrier *et al.*, 2010). The nematode causes reddish-brown lesions on roots and corms (Paper I: Plate 7.1). The infected roots wither, blacken and eventually die; a weakened root system results in the toppling of the whole plant, especially during strong winds. The presence of other organisms, such as fungi and bacteria accelerate the necrosis of corms and tissues (Pinochet and Stover, 1980; Shehabu *et al.*, 2010). This nematode, however, has not been found infecting and causing damage to enset plantations to date.

The root-lesion nematodes (*Pratylenchus* spp.) occur widely on bananas across the tropics (Bridge *et al.*, 1997; Gowen *et al*., 2005). Like the burrowing nematode, their distribution likely increased through the movement of infected clonal planting material. The damage in roots and corms is identical to that caused by *R. similis*. Root-lesion nematodes feed on the cytoplasmic contents of cells in the cortex and migrate between and within cells, causing the formation of cavities within the root tissue and results in characteristic, dark purple lesions and necrotic patches (Bridge *et al*., 1997). The two groups of nematodes can be differentiated by the position of the vulva, which is near to the tail in *Pratylenchus* spp. and at mid-length of the body for *R. similis* females. *Pratylenchus goodeyi* is not so widely distributed and is generally associated with cooler climates. It is predominantly found in the cooler highlands in Africa (Price and Bridge, 1995). In Ethiopia, *P. goodeyi* is the predominant nematode species found on enset across agro-ecologica1 zones (Bogale *et al.*, 2004; Addis *et al.*, 2006).

The spiral nematode species important on banana is *Helicotylenchus multicinctus*. It occurs almost wherever banana is grown and almost exclusively in combination with other important nematode species (McSorley and Parrado, 1986). Like the burrowing and root-lesion nematodes, *H. multicinctus* is likely to have been distributed widely on infected planting material. Spiral nematodes have also been found on abacá (Bridge, 1976) and enset (Addis *et al*., 2006).

Root-knot nematodes (*Meloidogyne* spp.) are ubiquitous pathogens with global distribution and infect a wide range of host plants (Perry *et al*., 2009). Root-knot nematodes have been found in association with banana in all producing areas. They have also been identified as infecting abacá (Ocfemia and Calinisan, 1928) and enset (O'Bannon, 1975; Bogale *et al.*, 2004; Addis *et al*., 2006). On banana, galls and swellings on primary and secondary roots are the most obvious symptoms of root-knot nematode infection. In general, root-knot nematodes have not been considered important banana pathogens in the past. However, as with the spiral nematode, their importance may be underestimated due to a limited understanding of their role in disease as they regularly occur in combination with other damaging nematode species. The endodermis is penetrated by vermiform infective juveniles, which enter the stele and induce the vascular parenchyma or differentiating vascular cells in the central part of the stele to form multinucleate giant cells. The nematode becomes sedentary and feeds from these giant cells as it develops into a mature female and reproduces. In banana roots, *Meloidogyne* spp. often occur together with fungi capable of colonising weakened or wounded tissue. In Yemen, Sikora (1980) observed higher levels of root rot in banana plantations where *M. incognita* and root-rot fungi (*Fusarium* and *Rhizoctonia* spp.) were present together in the soil.

Pre-planting measures, such as strict quarantine systems on the movement of planting materials, fallowing with non-host plants, rotation, flooding and fumigation, can reduce nematode populations prior to planting. Cleaning planting materials or using *in vitro* micro propagated plantlets free of nematodes and other infections reduce the risk of contaminating fields. Paring, accompanied by immersion of suckers in boiling water for 30 s, is an effective method to disinfect planting materials (Coyne *et al.*, 2010). Exposing plantlet roots to beneficial microorganisms in growth media (Sikora *et al.*, 2008), such as using bio-enhancers (endophytes), has been shown to protect against nematode damage and improve yields (Waweru *et al.*, 2014). Post-planting control measures have mainly relied on the application of nematicides through granular application or drip irrigation to banana plantations (Gowen *et al.*, 2005). However, most chemicals are banned from being used due to environmental and human concerns due to their toxic and hazardous nature. Botanicals (plant extracts) and biological control agents are effective control strategies against nematodes. Breeding for resistance is the most efficient management strategy, especially where knowledge of nematode pests and their management is poor, and access to or availability of quality inputs is limited (Coyne *et al.*, 2009). There are new cellular and molecular banana

improvement techniques, which continuously reduce the challenges of traditional breeding (Ortiz, 2011). Genetic modification of banana cultivars is an option for nematode management with a successful generation of resistant lines (Roderick *et al.*, 2012) with confirmed resistance in fields in Uganda (Tripathi *et al.*, 2015).

This review highlights the overall impact of plant-parasitic nematodes on *Musa* species. Most of the reviewed studies are focused on bananas and plantains, with just a limited number of studies on nematodes associated with enset, which clearly limits the information on their occurrence and impact on enset. Given the rudimentary understanding of nematodes, lack of expertise in this area, the magnitude of the devastating effect of nematodes and the importance of enset to Ethiopia, we undertook a thorough study on nematodes associated with enset, which can then serve as a baseline for future studies.

3.2. Occurrence of plant-parasitic nematodes on enset (*Ensete ventricosum*) in Ethiopia with focus on *Pratylenchus goodeyi* as a key species of the crop (Paper II)

Our study provides an extensive assessment of nematodes associated with enset, the latest and most comprehensive since Addis *et al.* (2006) with 98 farms sampled in 2004 and Bogale *et al.* (2004), who assessed 49 farms in 1999. Eleven plant-parasitic nematode genera (*Pratylenchus, Meloidogyne, Helicotylenchus, Scutellonema, Tylenchorhynchus, Rotylenchulus, Aphelenchoides, Cephalenchus, Pratylenchoides, Trophurus* and *Hoplolaimus*) were identified from the 308 samples collected from various enset growing zones, out of which *Pratylenchus, Meloidogyne* and *Aphelenchoides* were recovered from roots only. In the soil samples, *Pratylenchus* and *Helicotylenchus* species had a frequency of occurrence (FO%) of 100% and 52%, respectively, while *Pratylenchus, Meloidogyne* and *Aphelenchoides* species occurred in 100%, 8% and 4% of root samples, respectively.

In the highlands of Guraghe zone (2200-3000 m.a.s.l.) *Pratylenchus* spp. mean densities of 16,050 and 12,217 per 10 g roots were observed in Meskan and Ezha woredas/districts respectively, although densities as high as 25,000 per 10 g root were recorded from individual fields. These high densities exceed those previously recorded from enset (Bogale *et al.*, 2004) and banana roots in other African countries (Bridge *et al.*, 1995; Kashaija *et al.*, 1994).

All investigated *Pratylenchus* species are unmistakably *P. goodeyi*, which is confirmed by morphology, D2-D3 sequences and a putative species-specific pseudogene (Paper II: Fig. 5). Our study shows *P. goodeyi* to be present in every enset field sampled. The roots appeared to be dry and when split longitudinally, extensive black or purple necrotic cortical tissue was evident. Large

portions of the corm were also covered in lesions to the point of being rotten even, which had to be removed during processing, resulting in smaller corm size and poor food quality (Paper II: Fig. 3). In line with previous studies (Bogale *et al.*, 2004; Addis *et al.*, 2006), our study shows the predominance of *P. goodeyi*, indicating this as the major nematode threat to enset production.

We also observed a difference in *P. goodeyi* densities amongst different enset cultivars. This indicates that there is a possible difference in resistance to the nematode among cultivars. During the field visits, we observed that none of the farmers had any awareness of nematode pests, regardless of the huge threat they pose. This survey shows the status of nematode pests on enset. It also provides information on major hotspot areas, that can be used to collect nematode inoculum to be used in future controlled experiments.

3.3. The Ethiopian staple food crop enset (*Ensete ventricosum*) assessed for the first time for resistance against the root lesion nematode *Pratylenchus goodeyi* (Paper III)

Pratylenchus goodeyi appears to be the most prevalent nematode pest of enset in Ethiopia, where it can occur in extremely high densities (Kidane *et al.*, 2020). So far, the damage caused by the nematode has not been established. The current study is the very first assessment of the reaction of different enset cultivars to this nematode pest (*P. goodeyi*).

Nine enset cultivars were inoculated with 2,000 *P. goodeyi* (mixed juvenile and adult stages) and maintained for 4.5 and 9 months after inoculation in two separate experiments. In both sets of experiments, the enset cultivars significantly differed ($P < 0.001$) in their host suitability to *P. goodeyi*. Each cultivar showed different levels of susceptibility to *P. goodeyi* based on the final population density (*Pf*) and reproduction factor (RF). The cultivar Gefetanuwa had the highest *Pf* of 25,799 with a RF = 12.9, followed by cv. Zereta (*Pf* = 11,196; RF = 5.6) and cv. Endale (*Pf* = 3573; RF = 1.8) and cultivars with the lowest density were Maziya (*Pf* = 455; RF = 0.2) and Heila (*Pf* = 350; RF = 0.2). Similarly, in the second experiment, Gefetanuwa had the highest *Pf* of 126,534 with a RF = 63.3, followed by cv. Yanbule (*Pf* = 22525; RF = 11.3) and cv. Zereta (*Pf* = 20085; RF = 10) and cultivars with the lowest density were Heila (*Pf* = 5255; RF = 2.6), Kellisa (*Pf* = 3529; RF = 1.8) and Maziya (*Pf* = 2746; RF = 1.4).

With regards to pathogenicity, *P. goodeyi* multiplied on all three tested cultivars (Maziya, Arkiya and Heila) after 4.5 months but with no differences in *Pf* or RF among them. However, significant differences ($P < 0.001$) were observed, based on the four levels of inoculation densities used within each cultivar. All three cultivars supported the reproduction of the nematode. Our study reveals that there is indeed a difference in the susceptibility of enset cultivars to *P. goodeyi*. Information

from screening studies, such as ours, provides information on the resistance of cultivars that can be used in future breeding programs.

3.4. Planting material of enset (*Ensete ventricosum*), a key food security crop in Ethiopia, is a key element in the dissemination of plant-parasitic nematode infection (Paper IV)

Domesticated enset is exclusively propagated vegetatively, using suckers that are purposely cultivated from corms prepared for this use. Such corms are removed from harvested plants, cut in half and buried in a shallow excavation. Suckers emerging from these corms are removed and successively transplanted into nurseries with increasing spacing for 2-4 years. Suckers are then used for planting new fields and are traded at markets or exchanged between farmers. It is well known that a key source of infection of new fields of banana is via infected banana suckers, exchanged between farmers. There is no information available on the levels of nematode infection of enset suckers or whether they represent a source of infection. A study was therefore undertaken to determine the likelihood of this, and if so, to what extent. A total of 340 enset sucker samples were assessed during the study. *Pratylenchus goodeyi* was recovered from the roots of 100% of sucker samples, in densities ranging between 10 and 295 per 10 g roots. Although the age of the suckers was not specifically recorded for each sample, in general, younger suckers appeared less infected, than larger, older suckers. On some suckers, especially the older ones, lesions and necrotic tissue were clear on their roots and corms. We observed a 100% infection of suckers, which are transported between farms, from region to region and are planted without any further treatment, other than some limited trimming of the roots and parts of the corms. Growers are not aware of nematodes, resulting in the dissemination of infected suckers to all enset growing areas. Interventions regarding improving the awareness of nematodes by farmers, the damage nematodes cause, and suitable management strategies are required.

4. Discussion

Since 2014, there has been a gradual increase globally in the number of people affected by hunger. Assessment of food security and nutrition shows that the world will not achieve Zero Hunger by 2030. Putting into consideration the health and socio-economic impact of the COVID-19 pandemic, the food security and nutritional status of at-risk populations over the world will be tremendously affected (FAO *et al.*, 2020). The impact of increasing human population, urbanisation, climate change, soil degradation and drought on food security, hunger and malnutrition will be immense, especially in marginal areas of the tropics and subtropics (Sikora *et al.*, 2018).

Enset is an important staple crop providing food to approximately 20 million Ethiopians. It is a drought-tolerant crop grown across a wide range of agro-ecological zones and can be harvested at any maturity stage, after several year's growth. This almost equals having a food bank on the farm; in cases of crop failure, a farmer is still able to provide food for the family by harvesting enset. In regions where enset is grown, famine is rare due to the ever-present enset (Dessalegn, 1995). Other than being an important dietary source, enset provides fiber, medicine, animal feed and packaging (Borrell *et al.*, 2019). Enset cultivation is a sustainable form of agriculture in terms of maintaining soil fertility, reducing soil erosion and surface runoff with minimal use of inorganic fertilisers (Brandt *et al.*, 1997). Despite its potential, enset has received minimal research attention and is greatly underexploited. Research in areas such as agronomy, genetic diversity, breeding, pathology, and conservation is very much needed in order to optimise and maximise the benefits from this multi-purpose crop that is so important for Ethiopians (Borrell *et al.*, 2019).

Enset production is threatened by various pests and diseases, bacterial wilt disease being the most destructive. Plant-parasitic nematodes affecting bananas and plantains are considered as major constraints (Sikora *et al.*, 2018). However, they have received limited attention on enset, with just a few studies associating nematodes with enset, including those within this thesis (Coyne and Kidane 2018; Kidane *et al.*, 2020; Kidane *et al*., 2021).

We provide a comprehensive review of various studies on plant-parasitic nematodes associated with banana, enset and abaca that summarise the biology, disease cycle, host reaction, symptoms and control measures of the most widespread and important nematodes. From the review in the book chapter, it is clear that just a few studies on enset are available. This has inspired us to further study enset nematodes. Our studies serve to provide comprehensive baseline information on plant-parasitic nematodes associated with enset, to screen and evaluate the response of enset to the key nematode species, *P. goodeyi*, and to assess the role of planting materials as sources of nematode infection. We provide the most comprehensive study to date; relating nematode infection and

distribution with enset in Ethiopia. For the first time, we evaluate resistance in enset against the key nematode threat, *P. goodeyi,* and assess the role of commonly used planting materials in the dissemination of nematode pests. All these are important prerequisites for the successful management of nematode pests in enset in the future.

5. Challenges encountered

While we are pleased with the outcome of the study, the data collected and the outputs achieved, a few challenges were encountered in due course of this study, which set us back and created some delays. We feel that it is useful to outline the major elements of these challenges here; in order to provide some additional insight into this study and help explain the extended period of study.

- During the period of study, a number of civil conflicts in the study area led to significant travel restrictions, over extended periods of time, for security reasons, interfering with our ability to travel for sampling, fieldwork or assessing pot experiments.
- Securing a plot to conduct my experiments was very important. We were fortunate to be offered an abandoned screenhouse at Hawassa Research Center, even though it required substantial renovation. After much effort to repair and sourcing appropriate germplasm material from another regional Research Center (Areka), we set up and got the pot experiments established. Unfortunately, without prior notice, this area of Hawassa Research Center was sold, the screenhouse demolished and an industrial park was created over the site. Our experiments were destroyed while I was in Addis Ababa, unable to travel due to security travel restrictions. This proved a major setback, resulting in substantial delay as it had taken much time to renovate the screenhouse and secure the planting material, which was only available at one specific period during each year. We therefore had to find another site and start all over again.
- Limited equipment and/or access to lab facilities in Hawassa created some difficulties for sample assessment and experiment preparations. Consequently, after some time, alternative venues were assessed for me to be based at. Addis Ababa University was considered initially, which eventually did not work out, before re-establishing all activities at Jimma University under the supervision of Beira Hailu, who generously accepted to host me.
- Enset suckers for planting pot experiments were collected from Areka Research Center, adjusting the time of collection and inoculation depended on the availability of the suckers at one specific period each year.
- As there has been no similar research related to nematodes on enset, testing of and adjusting protocols to suit enset took some time, such as establishing suitable timings for hot water treatment of suckers and inoculation of nematodes, which proved quite time-consuming.
- Rearing of *P. goodeyi* cultures in the lab proved unsuccessful after much effort and as such, the use of naturally infected root material was relied upon. Waiting for the unsuccessful cultures delayed pot experimentation, while tedious extraction and counting of nematodes until desired amounts were obtained from sites identified in the survey was time consuming.
- It was unfortunate that during the period of study, medical issues prevented me from attending to my studies and activities for a couple of months.

6. Conclusions

- Enset is an important staple crop for about a fifth of the Ethiopian population. However, it is not much known outside of its cultivation area in the southern part of the country. Enset cultivation currently faces challenges due to climate change, urbanisation, emerging pests and diseases and the danger of the rich indigenous knowledge associated with the crop being lost, which will have a toll on the future of this food security crop. Pests and diseases represent the most significant threat to enset, which require immediate response in order to reduce their impact on production. In this study, we have tried to provide the baseline information on the status of plant-parasitic nematodes associated with the crop, the damage they cause and the reaction of enset to the key nematode species *P. goodeyi*. Our study area included different agro-ecological zones suitable for enset cultivation, which is representative of the entire enset growing areas in southern Ethiopia, we have painted a picture of what the current situation is and we believe that our results are very instrumental for future studies.
- This thesis is a compilation of four studies. A review on plant-parasitic nematodes of banana, enset and abaca; a survey to assess the occurrence and distribution of nematodes associated with enset; screening the resistance of different enset cultivars against *P. goodeyi* and the pathogenicity of the nematode; and the status of enset planting materials and their role as disseminating nematodes.
- The review summarised the biology, disease cycle, host reaction, symptoms and control measures of the most widespread and important nematodes associated with banana, enset and abaca, namely the burrowing, lesion, spiral and root-knot nematodes.
- The survey revealed that a range of plant-parasitic nematodes are associated with enset across the different agro-ecologies but that the lesion nematode of the genus *Pratylenchus* is the most prominent nematode occurring in each sampled enset field. It was also present in densities higher than had previously been recorded, which were extremely high, especially in higher altitudes. The damage caused by this nematode manifested as purple lesions and necrotic tissues over the whole corm and roots. Morphological and molecular analysis revealed that all populations were identified as *P. goodeyi*, making it the key plant-parasitic nematode species. Differences observed in population densities of the nematode across the survey indicate possible resistance amongst enset cultivars.
- In the first proper assessment of nematode resistance in enset, a range in susceptibility to *P. goodeyi* among enset cultivars was revealed. *P. goodeyi* had a much greater multiplication on cultivar 'Gefetanuwa' while cultivars 'Maziya', 'Heila', and 'Arkiya' had lower multiplication of the nematode with good levels resistance indicated. Such studies help to identify sources of resistance that can be used for future breeding activities. During this controlled study, we pre-

treated enset planting materials by briefly immersing in hot water prior to planting, which is an effective method to reduce nematodes.

- Enset sucker planting materials were shown to clearly act as a widespread source of contamination of *P. goodeyi* into new fields. Differences in infection levels were observed. Susceptibility of cultivars and age of planting materials contribute to these differences. Once again, *P. goodeyi* was the key nematode present in the enset planting materials. Experiences drawn from the sanitation of banana and plantain planting materials can be used in enset towards the development of healthy planting material systems.
- During the course of the study, we have seen that farmers and extension personnel are not aware of nematodes and the damage they cause. We hope that our study will change this scenario. We provide a framework for future research on this important but neglected crop and plant-parasitic nematodes.

7. Future prospects and needs

- Awareness and knowledge of plant-parasitic nematodes in Ethiopia is very minimal. Incorporating nematology as a training and research discipline in university curricula is crucial; although some have included courses at the MSc level, greater promotion of nematology as a discipline is necessary, especially at a national level in terms of policy changes.
- Delivery of basic nematology techniques for farmers and extension workers is essential for creating awareness on nematodes, their impact and how to better manage them.
- Adapting the single root resistance screening method on enset, to assess the resistance of many enset cultivars against dominant plant-parasitic nematodes could be employed to increase knowledge on enset resistance.
- Standardisation of a hot water treatment therapy for enset planting material, as a simple disinfestation technique to reduce nematode transmission on planting materials, towards the development of healthy enset seedling systems.
- Use of molecular markers for the identification of nematode resistance to aid breeding programmes towards nematode management, especially where transferable from other related species of the genus *Musa*.
- There has been no study conducted so far on the management of nematodes on enset. Therefore, it is very important to check the effectiveness of potential management options suitable for smallholder systems, such as botanicals and biological control methods (e.g. endophytes).
- Tissue culture protocols for enset have been developed; management options should be considered during mass propagations, such as the incorporation of beneficial endophytes.
- Bacterial wilt disease is the main production constraint on enset; it is evident from banana that the presence of plant-parasitic nematodes can predispose it to bacterial wilt disease. The nematode-bacteria complex needs, therefore, to be assessed and addressed on enset.
- Undertake genetic improvement through breeding programmes, for nematode resistance to complement conventional management strategies.
- Collaboration and communication with other enset researchers working on different aspects of the crop. Data and reports should be readily available and accessible to all.

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Paper I

Handbook of Diseases of Banana, Abacá and Enset

Edited by David R. Jones

7 Nematode Pathogens

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Overview

Many different plant parasitic nematodes are found in association with banana, abacá and enset, but only a relatively small number cause significant damage. These nematodes often occur in mixed populations, which can create difficulties in assessing the damage caused by each species and thus establishing their relative importance. The effect of one species on the host may be similar to that of another, resulting in a general reduction in crop growth and loss of yield. However, each species can cause specific symptoms and may require different control strategies. Conversely, a number of management options may form a common basis for the control of more than one nematode pathogen.

Burrowing Nematode

Introduction

The burrowing nematode, first described from banana in Fiji (Cobb, 1893), is generally viewed as one of the most important root parasites of banana in tropical areas (Stover, 1986; Gowen *et al*., 2005; Jones, 2009) and the cause of a costly disease affecting commercial plantations growing cultivars in the AAA Cavendish subgroup (Sarah, 1989; Stanton, 1994). It is not found on banana growing at altitude, such as in the highlands of Central and East Africa, or in the higher latitude zones, such as the Mediterranean area, Canary Islands, Madeira, the Cape Province of South Africa and Taiwan (Stover, 1972; Bridge, 1988; Sarah, 1989; Gowen *et al*., 2005), though it may be present in these regions under greenhouse cultivation. The present geographical distribution of the burrowing nematode is a reflection of historical movements of infected planting material, especially corms of Cavendish cultivars, and of the temperature preference of the pathogen (Price, 2006). It also causes black head rot or tip-over disease of abacá (Anderson and Alaban, 1968; Davide, 1972; Castillo *et al*., 1974).

The burrowing nematode destroys root and corm tissue, which reduces water and nutrient uptake. It also has a deleterious effect on root anchorage, which results in the uprooting or toppling of heavily affected plants, particularly during windstorms and heavy rain once bunches have developed. The damage also reduces plant growth and development. In banana, this may lead to severe reductions in bunch weight and a significant lengthening of the crop cycle (Gowen, 1975; Stanton, 1994; Coyne *et al*., 2005).

Crop losses depend on several factors, including the pathogenicity of local burrowing nematode populations, associated pathogens (including other nematode species), banana cultivar, climatic conditions and soil factors, especially fertility. In commercial plantations of

Cavendish cultivars in areas of Côte d'Ivoire where soils are poor, reduced bunch weights and toppling have been reported to cause losses of over 75% (Sarah, 1989). In such circumstances and without nematode control, banana plants become virtually unproductive after the first harvest. In the more fertile peat soils of Côte d'Ivoire and in the volcanic soils of Cameroon, cumulative crop losses are generally below 30% (Melin *et al.*, 1976; Sarah, 1989). In South Africa, losses have reached 75% (Jones and Milne, 1982). In Central America (Costa Rica and Panama) and South America (Colombia), crop losses estimated by counting uprooted plants fluctuate between 12% and 18%. Losses have been recorded as around 5% in the Sula Valley in Honduras (Pinochet, 1986).

The actual economic impact of the burrowing nematode on smallholder cultivation is difficult to estimate. However, severe symptoms have been observed in cultivars in the AAB Plantain subgroup in Côte d'Ivoire growing near Cavendish plantations (Sarah, 1985) and damage has reached 50% in experimental plots (Sarah, 1989; Coyne *et al.*, 2013). In Honduras, Stover (1972) reported that there was considerably more uprooting, which resulted in complete loss of yield, in burrowing nematode-infested plots of 'Horn' (AAB, Plantain subgroup) than in control plots. In Cameroon, cumulative losses of 60% were recorded in 'French Sombre' (AAB, Plantain subgroup) planted in a field naturally infested with the burrowing nematode (Fogain, 2001). In south-western Nigeria, yield losses averaging 29% were recorded for 17 banana, plantain and bred hybrid cultivars grown for two cropping cycles in soil with a burrowing nematode-dominated population of nematodes (Dochez *et al.*, 2009). A combined burrowing and spiral nematode infestation reduced yields and caused toppling of 'Obino l'Ewai' (AAB, Plantain subgroup) in Nigeria with production losses of up to 90% being reported (Speijer and Fogain, 1999). Although not introduced into the 'lowlands' of East and Central Africa until the 1960s, there is compelling evidence that the burrowing nematode has contributed significantly to the decline of the AAA East African highland banana in this region (Speijer and Kajumba, 1996; Speijer *et al.*, 1999; Price, 2006).

Symptoms

Infection of banana corm and root tissues by the burrowing nematode results in a reddish-brown necrosis. On corms, this is clearly visible after the surface has been washed free of dirt and lightly peeled (Plates 7.1 and 7.2) with the necrosis usually focused around the points where roots leave the corm. Depending on the level of infection, the size of lesions varies from small spots to large areas of necrotic tissue. Damage caused by the banana weevil borer (*Cosmopolites sordidus*) is superficially similar to larger lesions, but extends much further into the corm tissue as tunnels (Plate 7.3).

Infected roots have dark patches on the surface, which gradually coalesce as the nematode damage advances (Plate 7.4). The root eventually

Plate 7.1. Banana corm showing necrotic patches caused by the burrowing nematode (photo: D. Coyne, IITA).

Plate 7.2. Peeled banana corm showing damage caused by the burrowing nematode (left) with a healthy uninfested corm (right). Lesions extend from the root bases on the infested corm (photo: D. Coyne, IITA).

withers, blackens and dies. Uninfected roots are pale and firm. If infected roots are cut in half and sliced longitudinally, the symptoms of the burrowing nematode can easily be identified as reddish-brown necrotic patches extending from the surface towards the centre, but do not affect the central stele (Plate 7.5). Symptoms of burrowing nematode can be distinguished from those of Fusarium wilt as the latter are confined to vascular tissue and do not extend to the root surface.

As discussed earlier, the main impact of the burrowing nematode is to weaken root systems so that plants easily topple during strong winds. Severe nematode damage will be observed in the corm and root tissue of such plants, which usually appear unthrifty with thin pseudostems and small bunches. Leaf cover is also reduced (Roderick *et al.*, 2012a) and pseudostem turgidity can be affected, especially under periods of water stress. This leads to pseudostems snapping more easily (Coyne *et al.*, 2013).

Plate 7.3. Cross-section of the base of the banana pseudostem showing tunnel damage caused by the banana weevil (*Cosmopolites sordidus*) (photo: D. Coyne, IITA).

Causal agent

Burrowing nematode is the common name for the species *Radopholus similis* (Plate 7.6). It normally feeds at the advancing edge of necrotic lesions and can be isolated from the reddish tissue that is found here. Large numbers of the nematode can be obtained by teasing affected tissues in a dish of water or using a simple plate extraction method to quantify population densities (Coyne *et al.*, 2014). More sophisticated methods, such as centrifugal flotation and mist extraction, allow for a more accurate quantitative evaluation (Hooper, 1986).

It has been demonstrated that populations of *R. similis* are biologically diverse in terms of their host preference, reproductive fitness, pathogenicity and/or morphology. They can also differ biochemically and molecularly. Two 'races' of the nematode, one attacking banana, but not citrus, and the other attacking banana and citrus were demonstrated by Ducharme and Birchfield (1956). Later, Huettel *et al.* (1984) controversially proposed *Radopholus citrophilus* as the name of the

Plate 7.4. Lesions caused by the burrowing nematode on banana roots (photo: J.L. Sarah, CIRAD).

Plate 7.5. Cross-section of banana roots showing damage caused by the burrowing nematode. Lesions extend from the exterior of the root to the central cylinder (photo: J.L. Sarah, CIRAD).

Plate 7.6. Infestation of *Radopholus similis* in banana root tissue. All stages of the life cycle of the nematode from egg to adult are present (photo: M. Boisseau, CIRAD).

nematode attacking banana and citrus. The concept that they are indeed two pathotypes of the same species has since been supported by further morphological (Valette *et al.*, 1998) and genomic (Kaplan *et al.*, 1998, 2000; Haegeman *et al.*, 2010) studies.

Using different criteria, such as chromosome number, pathogenicity, reproduction rate and host preference, three pathotypes of *R. similis* were distinguished from Central America and the Caribbean (Edwards and Wehunt, 1971; Pinochet, 1979, 1988a; Tarté *et al.*, 1981; Rivas and Roman, 1985). More recently, pathogenic diversity was reported to be worldwide and clearly linked to reproductive fitness in plant tissues. Isolates from Uganda, Côte d'Ivoire, Costa Rica and Guinea were shown to have higher multiplication rates than those from Martinique, Guadeloupe, Sri Lanka and Queensland in Australia (Sarah *et al.*, 1993; Fallas and Sarah, 1995a; Fallas *et al.*, 1995; Hahn *et al.*, 1996).

Enzymatic phosphoglucose isomerase (PGI) and randomly amplified polymorphic DNA (RAPD) analyses revealed two genomic groups of burrowing nematode that are not related to pathogenicity (Fallas *et al.*, 1996).

In Africa, damage to banana by *R. similis* appears more severe than elsewhere (Pinochet, 1979, 1988a, 1988b; Marin *et al.*, 1999). Populations from Uganda seemed to cause the most damage (Fallas *et al.*, 1995). Diversity in the reproductive fitness and virulence of *R. similis* populations from Uganda on banana was later demonstrated, with some isolates being particularly aggressive (Dochez *et al.*, 2005). Further assessment established that some of these aggressive populations were able to reproduce and damage roots on banana carrying the two widely confirmed sources of genetic resistance against *R. similis* (Plowright *et al.*, 2013). The distribution of these genomic groups appears to be linked to historical movements of planting material. In the case of Uganda, the aggressive populations appear, on the basis of phylogenetic analysis, to originate from Sri Lanka (Plowright *et al.*, 2013).

Disease cycle and epidemiology

Penetration occurs preferentially at the root apex, but *R. similis* is able to invade any portion of the root length. The nematode migrates in and between cells in the root cortex, where it feeds on the cell cytoplasm. This results in collapsed cell walls, cavities and tunnels (Blake, 1961, 1966; Valette *et al.*, 1997). On corms, lesions develop where infected roots are attached and then spread outwards. Necroses can extend to the whole cortex of corms (black-head disease) and roots, but the root stele is usually not damaged, except occasionally on very young roots (Mateille, 1994b; Valette *et al.*, 1997).

Radopholus similis is a migratory endoparasite, which completes its life cycle in 20–25 days under optimal conditions. Embryonic development takes 4–10 days and the four juvenile stages are completed in 10–15 days, depending on temperature (Van Weerdt, 1960; Loos, 1962). This species has a pronounced sexual dimorphism, in which males present an atrophied stylet and are

considered to be non-parasitic. Males also survive longer than females, an attribute that apparently enables them to fertilize females after becoming adults without competing for food (Chabrier *et al.*, 2010). Juveniles and adult females are actively mobile. They may migrate into the soil under adverse conditions and move towards new roots. The temperature range for R. similis development lies between 24°C and 32°C, with optimum reproduction occurring at around 30°C (Loos, 1962; Fallas and Sarah, 1995a). It does not reproduce below 16–17°C or above 33°C (Fallas and Sarah, 1995a, b; Pinochet *et al.*, 1995).

Necrosis of root and corm tissues is accelerated if other organisms, such as fungi and bacteria, are present. Fungi commonly associated with burrowing-nematode lesions are *Cylindrocarpon musae*, *Acremonium stromaticum*, *Fusarium* spp. and *Rhizoctonia solani* (Laville, 1964; Pinochet and Stover, 1980). Fungi of the genus *Calonectria* have been found to be pathogenic on banana in the French Antilles and Cameroon, causing lesions similar to those of *R. similis.* In association with the nematode, they can cause severe damage (Loridat, 1989). Studies on the interaction between *R. similis* and *Xanthomonas campestris* pv. *musacearum*, the cause of a bacterial wilt of banana, show that root wounds caused by the nematode act as entry points for the bacterium present in the surrounding soil (Shehabu *et al.*, 2010).

Environmental factors and stages of plant development will influence nematode population densities. As a rule, *R. similis* is less influenced by soil conditions than other species, and this may be due to its strictly endoparasitic habit (Quénéhervé, 1988). Rainfall appears to be the main factor (Melin and Vilardebo, 1973; Jaramillo and Figueroa, 1976; Vilardebo, 1976; Jones and Milne, 1982; Hugon *et al.*, 1984; Sarah *et al.*, 1988; Quénéhervé, 1989a, b); too little or too much water suppresses nematode densities in the roots. Temperature also limits development, with *R. similis* generally absent in cooler banana-growing areas. During the crop cycle, *R. similis* densities increase gradually until after the emergence of the flower bud (Melin and Vilardebo, 1973; Vilardebo, 1976; Sarah, 1986). The increase is faster in the roots of suckers (Sarah, 1986), especially those that are not pruned (Mateille *et al.*, 1984).

Host reaction

Radopholus similis is able to attack almost all banana cultivars, as well as abacá and other seeded *Musa* species (Gowen *et al.*, 2005). At least 250 host plants have been listed as susceptible, among which there are many weeds and several crops of economic importance, such as black pepper, coconut, tea, tuber crops, fruit trees and ornamentals (Milne and Keetch, 1976; O'Bannon, 1977; Bridge, 1987).

Parameters used to measure the reaction of *Musa* to *R. similis* include the number of nematodes on each plant, the number of nematodes in known weights of root, the percentage of infected roots and assessments of lesion damage on roots and corms (Wehunt *et al.*, 1978; Pinochet, 1988b; Sarah *et al.*, 1992; Fallas *et al.*, 1995; Fogain, 1996; Speijer and Gold, 1996; Price and McClaren, 1996). Trial designs, sampling strategies and methods of statistical analysis have been reviewed by Price and McClaren (1996). Taking into consideration the above methods, Speijer and De Waele (1997) published a manual containing standardized protocols for the assessment of nematode damage on banana.

In an attempt to simplify the measurement of parameters useful for identifying nematode resistance in banana under field conditions, Hartman *et al.* (2010) used an index that included the percentage of dead roots, the number of large lesions and nematode population density.

In pot experiments using plants derived from *in vitro* propagation, root damage measured 12 weeks after inoculation correlated well with nematode infestation levels measured at 6–8 weeks (Fallas *et al.*, 1995). Marin *et al.* (2000) developed a standard method for screening for genetic resistance using 200 nematodes in pots, while De Schutter *et al.* (2001) devised a single-root method for evaluating banana germplasm using an inoculum of 50 nematodes per 8 cm root section, with final nematode numbers measured after 12 weeks. This method optimizes the use of inoculum and has been modified to simultaneously assess resistance to a number of nematode species (Coyne and Tenkouano, 2005).

A pot evaluation of wild *Musa* species has shown that *Musa acuminata* ssp. *banksii* is quite susceptible (Wehunt *et al.*, 1978). In contrast, most accessions of *Musa balbisiana* tested have been very resistant (Fogain, 1996) or partially

resistant (Dochez *et al*., 2006). *Musa acuminata* ssp. *malaccensis*, *microcarpa*, *zebrina* and *burmannica* (accession 'Calcutta 4') have been found generally to have moderate to good resistance (Wehunt *et al*., 1978; Fogain, 1996; Dochez *et al*., 2006). However, one accession of *M. acuminata* ssp. *microcarpa* has been rated as moderately susceptible (Wehunt *et al*., 1978). *Musa textilis* is moderately resistant (Price and McClaren, 1996). Consequently, *R. similis* is not reported as a major problem in abacá crops (Anunciado *et al*., 1977). *Radopholus similis* has not been recovered from enset.

Diploid cultivars vary in their reaction to *R. similis*. 'Pisang Mas' (AA, Sucrier subgroup) and 'Pisang Lidi' (AA, syn. 'Pisang Lilin') have been found to have moderate resistance (Wehunt *et al*., 1978; Fogain, 1996). 'Pisang Batuau' (AA), 'Pisang Oli' (AA) and many accessions of 'Pisang Jari Buaya' (AA) are viewed as highly resistant (Wehunt *et al*., 1978; Fogain, 1996). However, 'Pisang Jari Buaya' has been found to be susceptible to some aggressive populations from Uganda (Plowright *et al*., 2013). 'Guyod' (AA) and 'Tuu Gia' (AA) are very susceptible (Wehunt *et al*., 1978; Fogain, 1996). 'Safet Velchi' (AB, syn. 'Ney Poovan') appears to be very resistant (Price and McClaren, 1996). In a screening study of 55 banana accessions, it was found that some AA cultivars, from the Pisang Jari Buaya and Pisang Batuau subgroups, had good resistance to *R. similis*. In addition, 17 diploid accessions were observed to have partial resistance (Quénéhervé *et al*., 2009).

Many cultivars in the Cavendish subgroup (AAA) have been estimated to be moderately susceptible to *R. similis*. 'Gros Michel' and its dwarf mutant 'Cocos' are less susceptible (Wehunt *et al*., 1978; Price and McClaren, 1996). 'Yangambi Km 5' (AAA, Ibota subgroup) has very strong resistance to *R. similis* (Sarah *et al*., 1992; Price, 1994b; Fogain, 1996; Price and McLaren, 1996; Fogain and Gowen, 1998) and is often used as the resistant check in experiments. However, it has shown susceptibility to some Uganda populations (Plowright *et al*., 2013).

Cultivars in the AAB Plantain subgroup are, on the whole, very susceptible to *R. similis* (Price, 1994b; Fogain, 1996; Price and McLaren, 1996, Coyne *et al*., 2005; Dochez *et al*., 2009). 'Popoulou' (AAB, Mai'a Maoli–Popoulu subgroup) is also very susceptible (Quénéhervé *et al*., 2009). This susceptibility may be linked to *M. acuminata* ssp. *banksii*, the wild banana, which may have contributed both A genomes to these subgroups (Carreel, 1995; Fogain, 1996). 'Focanah' (AAB, Pome subgroup), 'Figue Pomme Ekonah' (AAB, Silk subgroup), 'Pisang Kelat' (AAB) and 'Pisang Ceylan' (AAB, Mysore subgroup) all have good resistance (Fogain, 1996; Price and McClaren, 1996).

Not many ABB cultivars have been screened against *R. similis*. Of those that have, 'Bluggoe' and 'Cardaba' accessions appear moderately susceptible and 'Pelipita' moderately resistant (Price and McLaren, 1996; Dochez *et al*., 2009).

Control

In general, the control of burrowing nematode is not consciously practised in most smallholdings. This is largely because of a limited understanding of nematodes as the causal agents of the damage that farmers experience. As a consequence, most control methods discussed are those practised in commercial plantations. Chemicals have traditionally been relied upon to keep the burrowing nematode in check. However, with the withdrawal from use of many nematicides over recent years, because of environmental and human health concerns, there has been more research into identifying suitable alternative options.

Pre-planting measures

Reducing nematode densities in the soil before planting and the use of cleansed or nematode-free plant material are of primary importance in the control of *R. similis*. Eradication of *R. similis* from the soil is virtually impossible. After the first detection of *R. similis* in South Africa, some drastic measures, which included roguing, burning, soil fumigation with methyl bromide and fallowing, were introduced without total success (Jones and Milne, 1982). However, by implementing a strict quarantine system on the movement of plant material from areas where *R. similis* was present in South Africa, the spread of *R. similis* was contained (Willers *et al*., 2002).

Population densities of *R. similis* may be reduced by fallowing with non-host plants, of which a number have been identified, including

some cash crops (Milne and Keetch, 1976; Gowen *et al.*, 2005). Using *Panicum maximum* (Poaceae) in Queensland, Australia (Colbran, 1964) and *Chromolaena odorata* (Asteraceae) in West Africa (Sarah, 1989) for 1 year proved successful in reducing populations to non-detectable levels. In Côte d'Ivoire, rotation with pineapple (*Ananas comosus*) helped to reduce *R. similis* populations (Sarah, 1989) and rotation with sugarcane (*Saccharum officinarum*) also met with some success in Central America (Loos, 1961). In Panama, an 18-month clean fallow period did not eradicate *R. similis* (Loos, 1961).

An alternative method to fallow is soil cleansing. Loos (1961) reported that *R. similis* was eliminated after land in Honduras and Panama was flooded for 5–6 months. Flooding has also been used in Suriname (Maas, 1969). In Côte d'Ivoire, 6–7 weeks of complete flooding was as effective as 10–12 months of fallow for reducing nematode populations (Sarah *et al.*, 1983; Mateille *et al.*, 1988). However, this method is rarely practical, as land needs to be level and continued treatment requires a permanent water-supply.

Chemical fumigation, such as with dichloropropene or methyl bromide, has been quite efficient for soil cleansing, However, this method is now generally not used, because of environmental hazards (WHO, 2006).

Nematodes may be introduced into clean soil in new growing areas through infected corms and suckers. Even if visually clean, low, undetectable infections will multiply and spread, ultimately affecting the crop. This risk is overcome by the use of *in vitro* micropropagated plantlets that are free of nematode and other infections. Most banana planting material for commercial production is now supplied as tissue-cultured material, which should be the only type used when banana is grown in virgin soil. The uptake and use of plantlets derived from tissue culture is also increasing amongst some smallholders, especially those located near weaning nurseries (Dubois *et al.*, 2006, 2013).

The use of macropropagation techniques can substitute for tissue-cultured material if undertaken correctly and if safeguards are met. One improvised method has been shown to be very effective in producing and supplying healthy planting material to farmers, especially smallholders (Lefranc *et al.*, 2010). The principle relies on the use of corm material, sourced from healthy plants. Roots are removed by trimming and any necrotic areas are cut out by paring. The corm is then disinfected with hot/boiling water before being incubated, often split into two halves, in a nematode-free medium, such as sawdust. A wooden frame covered in polythene sheeting helps to maintain high humidity (Plate 7.7). The sprouting plantlets can be removed when of a suitable size and weaned in pots until ready for use.

For larger suckers, a simple method of disinfection consists of paring the corm tissue to remove necrotic tissue. However, nematodes located deep within the cortex may escape removal. Storing pared material for 2 weeks may further reduce the nematode population (Quénéhervé and Cadet, 1985b), but such techniques cannot be applied to small suckers, which are quite sensitive and need to be replanted rapidly.

Paring, followed by hot-water treatments (52–55°C for 15–20 min), has been a common and effective practice in Latin America and Australia (Blake, 1961; Stover, 1972; Pinochet, 1986). In commercial settings this can work well. However, hot-water treatments are cumbersome and require careful monitoring of temperature and immersion times to prevent the death of tissues. A recent modification involving

Plate 7.7. Macropropagation of banana plantlets under locally constructed units (photo: D. Coyne, IITA).

the immersion of suckers in boiling water for 30 s (Coyne *et al.*, 2010) simplifies the treatment and has been shown to be effective and accurate, especially for smallholder farmers (Tenkouano *et al.*, 2006; Hauser and Messiga, 2010).

Planting material can also be disinfected using chemicals. Dipping plant material in a nematicide has proved effective (Jones and Milne, 1982). Another method consists of immersing the planting material in a nematicide–mud mixture, which adheres to the surface, forming a nematicidal coat, and is known as as 'pralinage' (Vilardebo and Robin, 1969).

Of increasing interest and development is the use of biologically based products for the treatment of planting material, especially plantlets derived from tissue culture. When plantlet roots are exposed to certain beneficial microorganisms in water suspension or by drenching the growing medium, the microorganisms enter the plants and become endophytic (Sikora *et al.*, 2008). The bio-enhanced plants have been shown to be protected to a greater extent against nematodes in the field than plants that have not been treated, reducing nematode damage and improving yields (Waweru *et al.*, 2014).

Tissue-cultured planting material is an ideal candidate for enhancement with beneficial microorganisms. Although free of many pests and diseases, *in vitro* plantlets are also free of beneficial endophytes. Bio-enhancement returns the natural equilibrium to some degree (Dubois and Coyne, 2006). A range of beneficial endophytes, such as non-pathogenic *Fusarium oxysporum*, *Trichoderma* spp. and *Bacillus* spp., have been identified that offer protection (Sikora *et al.*, 2008). However, there is as yet limited knowledge on the persistence of endophytes and how long they may provide protection.

Post-planting measures

In most cases where contaminated planting material has been used to initiate new plantations, or where clean planting material has been planted in infested soil, *R. similis* will multiply quite rapidly. Yield losses can be reduced through propping up or guying pseudostems to prevent toppling. Improved drainage is also an important factor in reducing nematode damage in high-rainfall regions, such as parts of Central America (Pinochet, 1986). Similarly, any measures that improve fertility and root development may increase plant tolerance to nematodes. Such measures include ploughing before planting, incorporation of organic matter in the soil, fertilization and irrigation. In smallholder plots in West Africa where cultivars in the AAB Plantain subgroup are grown, Coyne *et al.* (2005) demonstrated the beneficial effect of mulching with organic matter to reduce losses to nematodes and improve crop performance. Their conclusion was that any mulch was better than no mulch.

Post-planting nematode control has primarily relied on the applications of nematicides to banana plants through granular applications or drip irrigation (Gowen *et al.*, 2005; Jones, 2009). In some locations, such as in the Canary Islands, Martinique and Colombia, emulsifiable compounds are applied as liquid sprays or through irrigation systems, and generally on the basis of two to three applications/year. The optimum application time, dose and frequency of applications are determined by nematicide efficiency, environmental conditions, population dynamics and the pathogenicity of local strains. In some banana-growing countries, nematicides have traditionally been applied on a regular basis with no attempt made to determine if treatments were necessary or not. Ideally, nematode levels should be checked periodically to determine treatment needs. The threshold for 'triggering' nematicide application will depend on local parameters, such as climatic and soil conditions, as well as aggressiveness of pathotypes. For this exercise to be worthwhile, the check needs to be based on accurate nematode counts. Nematodes must be extracted from plant material and surrounding soil using proven protocols (Sarah, 1991; Speijer and De Waele, 1997).

Chemical control has in the past relied heavily upon the regular and repetitive use of the same nematicide. However, this resulted in the rapid microbial degradation of the active ingredients and/or the build-up of resistance in the nematode population rendering the treatment inefficient (Anderson, 1988; Hugo *et al.*, 2014). Most of the previously relied-upon nematicides were labelled as being either extremely hazardous or highly hazardous. Many have been progressively removed from use (WHO, 2006), resulting in the search for less hazardous and more environmentally friendly products (Zum Felde *et al.*, 2009).

Management of *R. similis* in the French West Indies traditionally relied upon the repeated application of carbamate or organophosphate nematicides, but an environmentally sound scheme based upon the use of tissue cultures, fallow and intercropping with non-hosts has since been implemented (Risède *et al*., 2009). A similar scheme in Hawaii includes the incorporation of crop residue into the soil (HBIA, 2010).

Synthetic nematicidal products that were developed taking into consideration a greater need for environmental safety continue to be released for use on banana. One such product is based on the fungicide fluopyram, which is marketed as being environmentally friendly. Another is a nematicide utilizing fosthiazate even though it is an organophosphate. As markets and developments evolve and fluctuate, the current status of such products and their efficacy in relation to local conditions need to be assessed and evaluated locally.

Soil sanitation can be achieved through a cleansing system based on injections of the herbicide glyphosate into banana plants before uprooting (Risède *et al*., 2009). Emphasis is also being increasingly placed on efforts to identify suitable biologically based solutions, such as the use of mycorrhizae, endophytes and bio-pesticides (Sikora *et al*., 2008; Viaene *et al*., 2013).

Applications of plant extracts, some of which appear to provide good control options, while other data and assessments are less consistent or convincing, have received much attention. Of particular note are neem (*Azidirachta indica*) formulations, which, provided that they originate from reliable sources, can give very good nematode management on banana (Bartholomew *et al*., 2014). Products based on sesame oil, blends of essential plant oils that include sesame or garlic, furfuraldehydes and products based on *Myrothecium verrucaria* have been shown to be highly toxic to nematodes and can provide very promising reduction of *R. similis*.

Biological control

Plant-parasitic nematodes have many natural enemies and a number have been considered as possible biological control agents, including omnivorous and predatory nematodes, nematode-trapping fungi, nematode-parasitic fungi and bacteria. Plant health-promoting rhizobacteria and arbuscular mycorrhizal fungi are also receiving increasing interest for their additional plant-host protection qualities, as are endophytes (see section above). However, difficulties with mass production, shelf-life, and efficiency in regard to host specificity and quite precise soil or environmental conditions (pH, organic-matter content, composition of microfauna/flora) have hampered their development (Stirling, 1991; Cayrol *et al*., 1992; Davide, 1994).

Formulations based on the parasitic fungus *Purpureocillium lilacinus* (previously known as *Paecilomyces lilacinus*) are probably the most widely used against *R. similis* on banana, with a number of products and formulations commercially available. The fungus parasitizes eggs, juveniles and adults. Results vary depending on conditions, but in general are favourable and economically viable. Species of *Bacillus*, such as *B. firmus* and *B. subtilus*, and strains of the bacterium *Pseudomonas fluorescens* have been demonstrated to inhibit the invasion of roots of banana by *R. similis* (Aalten *et al*., 1998; Mendoza and Sikora 2009).

The obligate nematode parasitic bacteria *Pasteuria* spp. differ in their host range and pathogenicity to nematodes. *Pasteuria penetrans* has been found parasitizing *R. similis* (Wang and Hooks, 2009; Sharmila *et al*., 2012), but has yet to be developed fully for use against this pest.

The plant growth-promoting effects of arbuscular mycorrhizal fungi not only provide potential benefits to banana, but have also been shown to reduce *R. similis* infection and damage (Elsen *et al*., 2001, 2008). The total *R. similis* density was reduced by 60% and root necrosis by 56%, in banana plantlets colonized by arbuscular mycorrhizal fungi under greenhouse conditions (Koffi *et al*., 2012). However, as with many observations when assessing and applying biological control agents, reactions may be quite specific depending on host cultivar and control agent species or strain (Elsen *et al*., 2003). Combinations of microbial control agents have also been assessed on a number of occasions. Although results can be variable, and often depend on local conditions, combined applications have demonstrated better control of *R. similis* than individual applications (Zum Felde *et al*., 2009). Combined application of *F. oxysporum* and *B. firmus* was more effective in controlling *R. similis* on banana than either alone or in combination with *P. lilacinus* (Mendoza and Sikora, 2009).

Breeding for resistance

Fogain and Gowen (1997) demonstrated in field trials that population levels of *R. similis* were higher on the root systems of nematicide-treated susceptible cultivars than on an untreated resistant cultivar. Their work showed that genetic resistance can effectively control *R. similis*.

Because of differences in pathogenicity among *R. similis* populations, as well as the other nematode species able to parasitize and damage banana roots, efforts in breeding banana for broad resistance against all these variants will be extremely difficult (De Waele, 1996). Nevertheless, potentially valuable banana cultivars have been evaluated against local populations of the burrowing nematode (Frison *et al*., 1997). Techniques for the early screening of germplasm in small pots have been developed (Pinochet, 1988b; Sarah *et al*., 1992; Fogain, 1996; De Schutter *et al*., 2001; Coyne and Tenkouano, 2005). Such methods allow susceptible germplasm to be rapidly identified and inferior lines eliminated, retaining only the most promising germplasm for final evaluation in more costly field trials (Price and McLaren, 1996).

Several clones of 'Pisang Jari Buaya' (AA) have long been recognized as an exploitable source of resistance to burrowing nematode (Pinochet and Rowe, 1978, 1979; Wehunt *et al*., 1978; Pinochet, 1988a; Ortiz and Swennen, 2014). The resistance of 'Pisang Jari Buaya' has been incorporated into breeding lines and this has led to the production of hybrids of commercial interest (Rowe and Rosales, 1994; Quénehervé *et al*., 2009). Screening studies have shown that sources of resistance that may be useful in breeding programmes are present in many genotypes (Sarah *et al*., 1992; Price, 1994b; Price and McLaren, 1996; Fogain, 1996; Ortiz and Swennen, 2014). Pot tests in Honduras have shown that the bred hybrid 'Goldfinger'/'FHIA-01' (AAAB) has resistance to *R. similis*, as have the synthetic AA diploids 'SH-3142', 'SH-3362', 'SH-3648' and 'SH-3723' (Viaene *et al*., 2003). Diploid banana hybrids bred for black leaf streak resistance in Nigeria have resistance to *R. similis* (Tenkouano *et al*., 2003). Similarly, three leaf spot-resistant banana hybrids (AAA) bred by CIRAD and designated 'FB918', 'FB919' and 'FB924' have also proved resistant to *R. similis* in Martinique (Quénehervé *et al*., 2008).

It has been suggested that clones with large numbers of roots may exhibit a higher tolerance to nematode attack and selection for this character should be a worthwhile breeding objective (Gowen, 1996).

Research of resistance mechanisms to *R. similis* have shown that phenolic compounds, especially some tannins and flavonoids, could be involved, reducing the inroads that nematodes make into banana tissues and their multiplication within these tissues (Mateille, 1994b; Valette *et al*., 1996, 1997). Dochez *et al*. (2009) found that resistance to *R. similis* is controlled by two dominant genes with additive and interactive effects, where one recessive genotype in one locus suppresses the dominant allele in the other locus.

Resistance to *R. similis* through genetic improvement has long been hindered by difficulties associated with conventional banana breeding (Menendez and Shepherd, 1975; Pinochet, 1988a). Generating hybrids combining host-plant resistance with desired agronomic and quality traits from the cultivars remains a challenge. Nevertheless, good progress has been made in introgressing resistance to burrowing nematode in elite selections (Quénehervé *et al*., 2009; Lorenzen *et al*., 2010).

New cellular and molecular banana improvement techniques continuously enable the natural limitations of traditional plant breeding to be circumvented (Ortiz, 2013). The genetic modification of existing cultivars is now presenting a realistic option for nematode management with the successful generation of resistant lines (Roderick *et al*., 2012b), which have confirmed resistance in the field in Uganda (Tripathi *et al*., 2015). Flow cytometry protocols, DNA markers, resulting genetic maps and the recent sequencing of the banana genome offer yet greater insights and help to identify useful genes (Ortiz and Swennen, 2014).

Root-lesion Nematodes

Introduction

Root-lesion nematodes occur widely, but not universally, on banana throughout the tropics (Bridge *et al*., 1997; Gowen *et al*., 2005). Like the

burrowing nematode, their distribution likely increased through the movement of infected clonal planting material. However, they are not found so commonly in commercial plantations of cultivars in the AAA Cavendish subgroup, where *R. similis* has traditionally been the most important nematode pest. Root-lesion nematodes have, in particular, been reported in association with the AAB Plantain subgroup (Ogier and Merry, 1970; Pinochet and Stover, 1980; Bridge *et al.*, 1995; Speijer *et al.*, 2001) with some evidence to indicate that plantain is more susceptible to these nematodes than are other banana types (Perez *et al.*, 1986). Root-lesion nematodes have also been recorded on abacá (Davide, 1972). One of the root-lesion nematodes found on banana also attacks enset in Ethiopia (Addis *et al.*, 2006).

Symptoms

It is possible that root-lesion nematodes are overlooked when they occur in mixed populations with *R. similis* or are mistaken for that species. The damage in roots and corms is identical to that caused by *R. similis*. Root-lesion nematodes feed on the cytoplasmic contents of cells in the cortex and migrate between and within cells. This causes the formation of cavities within the root tissue and results in characteristic, dark purple lesions and necrotic patches (Plate 7.8). Symptoms are usually confined to the cortex, while the stele tissue is generally unaffected – a useful diagnostic character when examining necrotic roots. Infected plants become stunted, bunch weight is decreased and the production cycle is extended. Damage leads to a reduction in the size of the root system and toppling of plants. Plant toppling may be more prevalent in poor soils.

Reduced plant growth, a diminished leaf cover and toppling can increase the exposure of soils to sunlight. This results in a rise in soil temperatures and a reduction in the organic content. Nutrient leaching and erosion may also occur in soils exposed to direct rainfall (Bridge *et al.*, 1997).

Causal agent

Root-lesion nematodes are species of *Pratylenchus*, which can be confused with *R. similis* when nematodes are viewed under a dissection microscope (× 50). However, unlike *R. similis*, *Pratylenchus* males have functional stylets. To the experienced technician the genera can be differentiated by the position of the vulva, which is near to the tail in *Pratylenchus* spp. and at mid-length of the body for *R. similis* females.

Many reports in the banana literature do not identify *Pratylenchus* to species level. The most widely reported is *P. coffeae*, with *P. goodeyi* recognized as probably the second most significant species. Differences in tail morphology help to separate the two species.

Pratylenchus coffeae (Plate 7.9) infects a number of important crops, which include potato, yam, citrus, coffee, ginger (Luc *et al.*, 2005), abacá and some ornamental plants. It is the most important nematode pest of banana in the Pacific

Plate 7.8. Damage to a banana root caused by *Pratylenchus goodeyi*, a root-lesion nematode (photo: B. Pembroke, UR).

Plate 7.9. *Pratylenchus coffeae*, a root-lesion nematode that attacks banana (photo: B. Pembroke, UR).

and is significant in parts of Southeast Asia, especially in Thailand on 'Klaui Namwa' (ABB, Pisang Awak subgroup). This nematode is also reported as the most damaging on cultivars in the AAA Cavendish subgroup in Honduras.

Recent surveys have shown an increased incidence of *P. coffeae* in West Africa, where it is also regarded as aggressive and displacing *R. similis* (Brentu *et al*., 2004; Coyne, 2009; Dubois and Coyne, 2011). In Ghana, it was found to be one of the two most widespread species (Afreh-Nuamah and Hemeng, 1995; Schill *et al*., 1996) and in south-western Nigeria it has been recognized as the most important biotic constraint to plantain production (Speijer *et al*., 2001). In the Cavendish plantations in South Africa, *P. coffeae* is reported as endemic and in the Limpopo Province responsible for up to 60% losses (De Villiers *et al*., 2007). *Pratylenchus coffeae* is also now being observed more often in East Africa (Coyne, 2009) where it seems to be gaining prominence. In Zanzibar, it has been found in 68% of banana fields (Rajab *et al*., 1999). Its localized distribution and rising status in other African countries indicate that it may have only recently been introduced (Bridge *et al*., 1997; Coyne, 2009). However, caution is needed in the interpretation of surveys, as some nematodes identified as *P. coffeae* in Ghana (Brentu *et al*., 2004) have since been identified by molecular characterization as *P. speijeri*, a morphologically similar but separate species (De Luca *et al*., 2012).

Pratylenchus goodeyi is not so widely distributed as *P. coffeae* and seems adapted to cooler climates. Banana, abacá and enset are economically important hosts (O'Bannon, 1975; Peregrine and Bridge, 1992; Tessera and Quimio, 1994). The nematode is found predominantly in Africa and its general absence from commercial plantations of Cavendish cultivars in lowland areas and its presence only on smallholder banana crops indicate that it may be indigenous to this continent (Price and Bridge, 1995). It can occur in extremely high densities, such as on banana in Tanzania (Speijer and Bosch, 1996) and enset in Ethiopia (Peregrine and Bridge, 1992).

In Cameroon, *P. goodeyi* is the most serious nematode pathogen at elevations above 700 m and in the East African highlands it replaces *R. similis* as the dominant species above 1400 m altitude (Speijer and Fogain, 1999). The nematode has also been associated with banana losses in coastal Kenya (Seshu Reddy *et al*., 2007), where prevailing temperatures tend to be higher than is optimal for this species. In Rwanda, no correlation could be established between incidence of *P. goodeyi* and cooking banana losses (Gaidashova *et al*., 2009).

Pratylenchus goodeyi is regarded as a major pest in commercial Cavendish plantations in the Canary Islands (De Guiran and Vilardebo, 1962) and has also been recorded in Madeira, Egypt and Crete (Gowen *et al*., 2005). In Australia, *P. goodeyi* was as pathogenic as *R. similis* in commercial Cavendish plantations in the subtropics with the former nematode species being more prevalent in the cooler months and the latter in the warmer months (Pattison *et al*., 2002). It is also common on banana in Hainan Province, China (Zhang *et al*., 2015).

Large numbers of *P. coffeae* were extracted from abacá roots in Ecuador. Severe damage was observed on the plants in the form of root necrosis, yellowing of leaves and stunted growth (Bridge, 1976).

In Ethiopia, *P. goodeyi* is the predominant nematode species found on enset across agroecologica1 zones (Bogale *et al*., 2004; Addis *et al*., 2006). From a total of 71 enset cultivars sampled, all were infected to varying degrees with the nematode.

Disease cycle and epidemiology

The optimum temperature for invasion and development of *P. coffeae* is 25–30°C, which is the same as for *R. similis* and appears similar for *P. speijeri*. With *P. goodeyi*, it is nearer 20°C. The life cycle of *P. coffeae* is completed in about 4 weeks under optimum conditions.

The level of damage caused to banana by *P. coffeae* varies geographically (Dubois and Coyne, 2011). In some areas of Uganda, very high densities of the nematode have been observed on old banana stands, which still remain productive, and yet in Tanzania lower densities are associated with a high incidence of plant toppling (Bridge *et al*., 1997). The variability in reported yield reductions caused by *P. coffeae* has been attributed to the existence of different pathotypes or strains of the nematode and to misidentification of the pathogen (Duncan *et al*., 1999; De Luca *et al*., 2012). The question of

whether there are biotypes of *Pratylenchus* spp. with different host preferences is under continued investigation.

Population densities are usually expressed on the basis of 100 g of fresh roots and results vary according to the extraction technique used. In plantations where damage is obvious, either as uprooting or on visual inspection of roots, densities greater than 10,000 nematodes/100 g roots may be common.

In Cameroon, at altitudes over 900 m, the population densities of *P. goodeyi* on plantations averaged 15,000 with a maximum of 56,000 nematodes for every 100 g of roots (Bridge *et al.*, 1995). In Uganda, average densities on East African highland banana cultivars in the AAA, Lujugira–Mutika subgroup were 25,000 nematodes/100 g roots at ten farms at altitudes over 1600 m, but only 680 nematodes/100 g roots on a similar number of fields at altitudes of 500 m or lower (Kashaija *et al.*, 1994). However, banana plants on these lower-altitude farms were suffering no less, because roots were supporting densities of 32,000 *Helicotylenchus multicinctus* (spiral nematode) and 6500 *R. similis* (burrowing nematode) per 100 g roots. These two species were not present at the higher elevation. This illustrates the complexity of determining the relative importance of nematodes in mixed populations and in different environments.

Host reaction

Pratylenchus coffeae is a significant pest on cultivars in the Cavendish, Plantain and Pisang Awak subgroups. In Africa, *P. goodeyi* is an important pest on cultivars in the Plantain and Lujugira–Mutika subgroups (Bridge *et al.*, 1997; Coyne *et al.*, 2005). The occurrence of *Pratylenchus speijeri* is associated with severe damage to 'Apantu-pa' (AAB, Plantain subgroup) (Brentu *et al.*, 2004).

Resistance (or decreased susceptibility) to *Pratylenchus* spp. has been demonstrated in glasshouse experiments. In one study, 12 diploids, including the 'Long Tavoy 1', 'Long Tavoy 2' and 'Calcutta 4' accessions of *Musa acuminata* ssp. *burmannica* (AAw), exhibited partial resistance to *P. coffeae* (Quénéhervé *et al.*, 2009). 'Yangambi Km 5' (AAA, Ibota subgroup), 'Paka' (AA), 'Kunnan' (AB) and 'Pisang Ceylan' (AAB, Mysore subgroup) have been shown to have significant resistance to *P. coffeae* (Collingborn and Gowen, 1997). 'Yangambi Km 5' has also been shown to have some resistance to *P. goodeyi* in pots (Pinochet *et al.*, 1998) and in the field in Cameroon (Fogain and Gowen, 1998). Potted plants of 'Tjau Lagada' (AA), 'Pisang Bungai' (AA) and 'Pisang Mas' (AA) were found to have lower infection levels than 'Grande Naine' (AAA, Cavendish subgroup), the susceptible check, after inoculation (Moens *et al.*, 2005).

Control

Cultural, biological and chemical methods of control that are effective against *R. similis* are in general effective against *Pratylenchus* spp. These include planting nematode-free suckers or plantlets derived from tissue culture in land free of nematodes and paring suckers to remove roots and infested areas of the corm followed by a hot-water treatment for 20 min at 53–55°C or immersion in boiling water for 30 s.

Nematicides that are currently used for the control of *R. similis* in commercial banana plantations are equally effective on *Pratylenchus* spp. Similarly, biologically based management options for *R. similis* are likely to be suitable for *Pratylenchus* spp. but have in general been less studied than for *R. similis*.

Host-range information is important for developing a management strategy based on healthy planting material. Several crops and common weeds will support reproduction of *P. coffeae* (Gowen *et al.*, 2005) and a few alternative hosts of *P. goodeyi* have been discovered in East Africa (Mbwana, 1992). In glasshouse experiments, early inoculation with arbuscular mycorrhizal fungi appeared to increase the tolerance of 'Grand Naine' (AAA) to *P. goodeyi* by reducing the number of lesions on roots and enhancing plant nutrition (Jaizme-Vega and Pinochet, 1997).

In the long term, conventional banana breeding, perhaps coupled with genetic transformation, should contribute towards a partial management of *Pratylenchus* spp. Sources of resistance are currently being identified in Honduras, Cameroon, Uganda and the Canary Islands (Tenkouano and Swennen, 2004; Lorenzen *et al.*, 2010). Screening of leaf spot-resistant banana

hybrids with AAA genomes in Martinique showed four, designated 'FB918', 'FB919', 'FB920' and 'FB924', to have resistance to *P. coffeae* (Quénéhervé *et al.*, 2008). As reported earlier, three of these four hybrids were also resistant to *R. similis*. However, resistance against *R. similis* will not necessarily confer resistance against *P. coffeae*. It is important to establish the specific *Pratylenchus* species involved in causing damage to banana in order to be able to screen accurately for resistance.

Spiral Nematodes

Introduction

There are several species known collectively as spiral nematodes. Their name comes from the characteristic manner in which they coil when relaxed or heat-killed. Only one is a significant pathogen on banana and, unlike other spiral nematodes, feeds on the crop as an endoparasite. It occurs almost wherever banana is grown and almost exclusively in combination with other important nematode species (McSorley and Parrado, 1986). Because of this, opinions have differed as to its importance as a pathogen of banana However, evidence for its role in causing an important disease, often at the edge of the climatic range for banana, is gradually accumulating. In Israel, the spiral nematode has been shown to cause serious damage (Minz *et al.*, 1960) and in Ghana it is regarded as being of equal significance to *P. coffeae* (Schill *et al.*, 1996).

Spiral nematodes have also been found on abacá (Bridge, 1976) and enset (Addis *et al.*, 2006).

Symptoms

Like other migratory endoparasites, the spiral nematode feeds on the cell contents in the root cortex, causing necrotic lesions. However, unlike *R. similis* and *Pratylenchus* spp., feeding is often restricted to the outer parenchymal cells of the cortex (Zuckerman and Strich-Harari, 1963; Blake, 1966; Mateille, 1994a). In roots where the spiral nematode is the only parasite, lesions are often superficial (Plate 7.10). However, in severe infections, necrosis may extend to the stele causing root death. Therefore, the spiral nematode can also cause toppling of infected plants.

Plate 7.10. Superficial lesions on banana roots caused by *Helicotylenchus multicinctus*, a spiral nematode (photo: S.R. Gowen, UR).

Causal agent

The spiral nematode species important on banana is *Helicotylenchus multicinctus,* although others may be present, especially *H. dihystera*. *Helicotylenchus multicinctus* occurs frequently in roots that are infected with *R. similis*, *Pratylenchus* spp. or *Meloidogyne* spp. In extracts from root samples, *H. multicinctus* can be readily distinguished from these other genera by comparison of the lengths of the stylet (which are longer) and by the shape of the body when killed. Dead specimens are curved in the form of a letter C, whereas other spiral nematodes die in a coiled position. Those of *Radopholus similis* and *Pratylenchus* spp. are generally straight when at rest. Juveniles of *Meloidogyne* spp. are straight when at rest and smaller in size than *H. multicinctus.*

Helicotylenchus dihystera, *H. multicinctus* and another unidentified species have been found on enset, though not frequently (1–5% incidence).

Disease cycle and epidemiology

Unlike other spiral nematodes, which are ectoparasites, *H. multicinctus* is entirely endoparasitic. Like the burrowing and root-lesion nematodes, *H. multicinctus* is likely to have been distributed widely on infected planting material. All stages

(juveniles and adults) are infective and can be found in roots and adjacent soil.

When occurring in mixed populations, numbers of *H. multicinctus* may be greater than those of *R. similis* (Kashaija *et al*., 1994). On commercial plantations of 'Robusta' (AAA, Cavendish subgroup) in St Lucia, densities reached 24,000 spiral nematodes/100 g fresh roots, three times greater than that of *R. similis* (Gowen, 1977b). In Venezuela, where *H. multicinctus* was found coexisting with *Meloidogyne incognita* on the roots of a cultivar in the Cavendish subgroup, densities of 35,000 spiral nematodes/100 g roots were reported (Crozzoli *et al*., 1995). Population densities in Côte d'Ivoire averaged up to 53,000/100 g root (Adiko and N'Guessan, 2001) and up to 40,000/100 g root in Burkina Faso (Sawadogo *et al*., 2001).

Host reaction

There are few reports on differential susceptibility to this nematode in *Musa* because it has in the past been considered a less serious pathogen than other nematodes. This is an omission that is beginning to be corrected. However, techniques for mass-culturing *H. multicinctus* have also proved an obstacle as they are not as well established as for *R. similis* and *P. coffeae* and which, to a certain extent, constrains critical experimental work.

Ssango *et al*. (2004) were able to separate the effects of different nematode species on cultivars of the AAA Lujugira–Mutika subgroup in Uganda and demonstrate that *H. multicinctus* caused damage. Evidence from field trials on 'Agbagba' (AAB, Plantain subgroup) in Nigeria also indicated that *H. multicinctus* was responsible for much production damage when in mixed populations with other nematodes (Coyne *et al*., 2013). From surveys in plantain fields in the Democratic Republic of the Congo, root necrosis was positively and significantly correlated to population densities of *H. multicinctus* (Kamira *et al*., 2013). However, compared with non-inoculated plants, *H. multicinctus* caused no reduction in bunch weight of 'Grande Naine' (AAA, Cavendish subgroup) in microplots in Costa Rica, whereas losses were caused by *R. similis*, *Meloidogyne incognita* and *P. coffeae* (Moens *et al*., 2006). In West Africa, *H. multicinctus* is highly prevalent on cultivars in the AAB Plantain subgroup and regularly associated with necrotic root systems and toppled plants (Caveness and Badra, 1980; Adiko and N'Guessan, 2001; Speijer *et al*., 2001; Brentu *et al*., 2004).

In pot experiments, 'Poyo' (AAA, Cavedish subgroup) and 'Gros Michel' (AAA) were both found to be equally susceptible to *H. multicinctus* (Mateille, 1994a). In Costa Rica, most of the 31 *Musa* cultivars assessed by Moens *et al*. (2005) supported similar or higher densities of *H. multicinctus* as the susceptible check 'Grande Naine' (AAA, Cavendish subgroup), while 'Yangambi Km 5' (AAA, Ibota subgroup) supported low densities. 'Tjau Lagada' (AA) and 'Pisang Bungai' (AA) appeared resistant, but this finding needs further confirmation. Of 19 bred hybrids (primary tetraploids and improved diploids) screened in India against *H. multicinctus* in inoculated pot trials and field studies, 'H 531' ('Mysore' (AAB) × 'Pisang Lilin' (AA)) exhibited resistance. 'H-02-34', 'H-03-05', 'H-03-13', 'H-04-12', 'H-03-17', 'H-04-24 , NPH-02-01 and H 510 were classed as tolerant (Das *et al*., 2014a).

Control

Chemical, biological and cultural control methods used in the management of *R. similis* will also mostly apply to *H. multicinctus*.

Root-knot Nematodes

Introduction

Root-knot nematodes have been found in association with banana in all producing areas. They have also been identified as infecting abacá (Ocfemia and Calinisan, 1928) and enset (O'Bannon, 1975; Bogale *et al*., 2004; Addis *et al*., 2006).

In general, root-knot nematodes have not been considered important banana pathogens in the past. However, as with the spiral nematode, their importance may be underestimated due to a limited understanding of their role in disease as they regularly occur in combination with other damaging nematode species. On cultivars in the AAA Cavendish subgroup, the burrowing nematode is usually more successful and tends to dominate in situations where both types of

nematode are found. Root-knot nematodes are more likely to cause problems in areas where Cavendish cultivars have not been introduced or where the climate is too cold for *R. similis*.

Early reports, such as those from Honduras (Pinochet, 1977), Colombia (Zuniga *et al*., 1979) and the French Antilles (Kermarrec and Scotto la Massese, 1972), did not place any great significance on root-knot nematodes as important pathogens of banana in the Latin American–Caribbean region. However, they are now viewed as having greater importance (Cofcewicz *et al*., 2005), such as in Brazil, where root-knot nematodes occurred in 79% of root samples (Lima *et al*., 2013).

In East Africa, root-knot nematodes do not appear to be of great significance (Nsemwa, 1991; Kashaija *et al*., 1994; Speijer and Kajumba, 1996; Speijer *et al*., 1999). However, they are recorded as a problem in the northern Cavendish-growing districts of South Africa, where they have been implicated, in part, in a condition known as false Panama disorder (Deacon *et al*., 1985; de Beer *et al*., 2001).

Root-knot nematodes and *Fusarium oxysporum* (but not f. sp. *cubense*) are associated with this disorder. Treatment with nematicides can prevent the appearance of symptoms (A. Severn-Ellis, Australia, 1999, personal communication with D. De Waele). They have also been regularly recovered during field surveys in Central Africa. Root-knot nematodes were the second most frequently occurring nematodes in the Democratic Republic of the Congo after *H. multicinctus*, being present in 48% of fields in the lowlands of Bas Congo and 61% of fields in the highlands in South Kivu (Kamira *et al*., 2013). In West Africa they can also be common, occurring in association with *H. multicinctus* in 90% of fields or more (Caveness and Badra, 1980; Adiko and N'Guessen, 2001; Sawadogo *et al*., 2001). In North Africa, root-knot nematodes have been recognized as a problem and are believed likely to contribute significantly to production losses (Gowen *et al*., 2005).

In the Philippines, root-knot nematodes are also found on Cavendish cultivars. Large and widespread populations have been detected in commercial growing areas around Davao in Mindanao. The average population density in 82% of plantations examined was 3539 nematodes/100 g fresh roots. Large root-knot nematode densities were also found on the roots of all local cultivars sampled (Davide *et al*., 1992).

In Southeast Asia, root-knot nematodes are widely distributed on local diploid and triploid dessert cultivars, and also on cooking-banana cultivars. In West Malaysia, they were widespread in a commercial Cavendish plantation, presenting extensive root galls and average densities of 2300 individuals/200 ml soil (Razak, 1994). They were also the most predominant species recovered from banana roots (Rahman *et al*., 2014). Root-knot nematodes are commonly found on local banana cultivars in Thailand (Prachasaisoradej *et al*., 1994), Malaysia (Razak, 1994) and Indonesia (Hadisoeganda, 1994), where they have largely been regarded as being of minor importance. In North Vietnam, root-knot nematodes along with *P. coffeae* are the two major nematode species associated with banana (Van den Bergh *et al*., 2006). Root-knot nematodes are very common throughout the banana-growing regions of Australia, but were not shown to cause yield loss (Stanton, 1994).

Root-knot nematodes have been described as common and abundant on banana in Mediterranean countries, such as Crete (Vovlas *et al*., 1994) and Lebanon (Sikora and Schlosser, 1973).

Studies on interactions between banana and root-knot nematodes to determine production and yield losses are relatively few compared with other nematode species. In field experiments in the Philippines, Davide and Marasigan (1985) reported a yield loss of 26.4% after 'Giant Cavendish' (AAA, Cavendish subgroup) was inoculated with 1000 juveniles per plant. A 45.4% yield loss was caused by inoculations with 10,000 juveniles and a 57.1% yield loss by 20,000 juveniles. In Costa Rica, bunch weights of 'Grande Naine' (AAA, Cavendish subgroup) were reduced by 32% after inoculations of 1000 root-knot nematodes per plant. This loss was greater than that caused by the same numbers of burowing, root-lesion and spiral nematodes (Moens *et al*., 2006). In North Vietnam, field studies showed that 'Chuối Ngu Tien' (AA) and 'Grande Naine' (AAA, Cavendish subgroup) inoculated with 8700 root-knot nematodes per plant suffered yield reductions of 23% and 19%, respectively (Van den Bergh *et al*., 2006). In a field study in Nigeria using 'Agbagba' (AAB, Plantain subgroup), bunch weights in the plant crop were reduced by 50% following an inoculation of 2000 root-knot

Plate 7.11. Swollen and necrotic banana roots caused by root-knot nematodes (photo: D. Coyne, IITA).

nematodes per plant and by a similar amount in the following two crop cycles (Coyne *et al.*, 2013).

Symptoms

On banana, galls and swellings on primary and secondary roots are the most obvious symptoms of root-knot nematode infection (Plate 7.11). Sometimes, the root tips are invaded and there is little or no gall formation, but growth ceases and new roots proliferate just above the infected tissues. Infected plants may have a much lower number of secondary and tertiary roots and root hairs (Claudio and Davide, 1967).

Dissection of galls reveals the typical swollen females in various stages of development (Plate 7.12). At maturity, the females are saccate. Eggs are laid within a gelatinous matrix to form an external egg sac or egg mass. In thick, fleshy primary roots the egg masses may be contained within the root, resulting in swollen roots. On banana roots grown under *in vitro* conditions, protruding egg masses were observed 28 days after inoculation (Coosemans *et al.*, 1994). Different root-knot nematode species may occur in the same gall (Pinochet, 1977; Cofcewicz *et al.*, 2005). They may also colonize the outer layers of the corm up to 7 cm deep (Quénéhervé and Cadet, 1985a).

Above-ground symptoms caused on banana by root-knot nematode in Pakistan included yellowing and narrowing of leaves, stunting, reduced plant growth and less fruit production (Jabeen *et al.*, 1996). Stunted growth has also been attributed to root-knot nematodes in India (Sudha and Prabhoo, 1983) and Taiwan (Lin and Tsay, 1985).

Plate 7.12. A swollen banana root caused by root-knot nematodes in longitudinal section. White females are clearly present at the centre of the dark-coloured areas (photo: D. Coyne, IITA).

On abacá, galls on roots have been reported to be 3–10 mm in diameter and may run together to form an irregular club-shaped body up to 5 cm long and over 1 cm in thickness. Infected roots become brown and then almost black in colour. The surface of the galls crack with age and become rough to the touch. Leaves turn pale green or yellowish. The youngest leaf is generally the worst affected. Later, leaves become narrower and shorter. Plants appear stunted and leaves tend to bunch.

Galls on the primary and secondary roots of enset are associated with root-knot nematodes. Infected plants become stunted and have yellow leaves, which may wilt in the dry season. Young seedlings can be seriously affected.

Causal agents

Root-knot nematodes (*Meloidogyne* spp.) are ubiquitous pathogens with a global distribution

and infect a wide range of host plants (Perry *et al.*, 2009). Identification to species level is often not undertaken because of a lack of diagnostic expertise or facilities. However, this is changing and the list of species found infecting *Musa* is expanding.

The species most commonly recorded on banana are *Meloidogyne incognita* and *M. javanica*. *Meloidogyne arenaria* and other *Meloidogyne* species are also variously reported. In the Caribbean region, *M. arenaria* was the most frequently occurring species on banana at 62% of sampled sites in Guadeloupe, French Guiana and Martinique. In the same survey, *M. cruciani* and *M. hispanica* were infrequently found (Cofcewicz *et al.*, 2005). *Meloidogyne graminicola* was recorded causing damage on 'Tianbao' (AAA, Cavendish subgroup) in Fujian Province, China (Zhou *et al.*, 2015), while *M. enterolobii* was additionally recorded causing galling damage on 'Baxi' (AAA, Cavendish subgroup) in the same area (Zhou *et al.*, 2016).

The *Meloidogyne* species found on abacá have not been identified, but are reported in combination with *R. similis* and *Helicotylenchus* spp. (Bridge, 1976). Root-knot nematodes so far identified from enset roots include *M. incognita, M. javanica* and *M. ethiopica* (Peregrine and Bridge, 1992; Tessera and Quimio, 1994; Mandefro and Dagne, 2000).

Disease cycle and epidemiology

The life cycle of *Meloidogyne* spp. on banana is similar to its life cycle on other hosts. The endodermis is penetrated by vermiform infective juveniles, which enter the stele and induce the vascular parenchyma or differentiating vascular cells in the central part of the stele to form multi-nucleate giant cells. The formation of these giant cells disturbs or blocks the surrounding xylem vessels (Dos Santos and Sharma, 1978; Sudha and Prabhoo, 1983; Vovlas and Ekanayake, 1985; Jabeen *et al.*, 1996). The nematode becomes sedentary and feeds from these giant cells as it develops into a mature female and reproduces. They have spherical bodies with slender necks. Multiple life cycles can be completed within the same root, depending on the longevity of the root and the severity of the necrosis. The males are vermiform and generally rare.

Root-knot nematodes may require much more time to become established in banana roots than root-lesion nematodes. In Cuba, *Meloidogyne* spp. needed 24–30 months to establish themselves on 'Dwarf Cavendish' (AAA, Cavendish subgroup) (Fernandez and Ortega, 1982).

Root-knot nematodes are influenced by rainfall and soil conditions, such as temperature, texture and pH. After establishment, soil moisture and temperature are mainly responsible for fluctuations in populations (McSorley and Parrado, 1981; Fernandez and Ortega, 1982; Mani and Al Hinai, 1996; Youssef and Aboul-Eid, 1996). Regardless of inoculum levels, *M. incognita* usually reach highest densities in the soil during the rainy season and then decline to reach lowest levels during the dry season. The climate also affects the host. During the dry season, not enough new roots are available for nematodes to infect, resulting in low nematode densities. In Egypt, the highest densities of *M. incognita* on banana were also positively correlated with the highest soil temperatures (26–30°C) observed at the experimental site (Youssef and Aboul-Eid, 1996). In the Philippines, Davide (1980) reported that the highest population densities of *M. incognita* were observed in sandy loam soils and at pH 5–5.6.

Meloidogyne spp. and *R. similis* can jointly infect banana. However, root-knot nematodes are usually reduced or completely replaced by *R. similis*, as the latter species destroys the roots, which provide the feeding sites for the root-knot nematodes (Santor and Davide, 1992). In West and Central Africa, it is quite common to find *Meloidogyne* spp. and *H. multicinctus* in combination, often with lower densities of other nematodes.

In banana roots, *Meloidogyne* spp. often occur together with fungi capable of colonizing weakened or wounded tissue. In Yemen, Sikora (1980) observed higher levels of root rot in banana plantations where *M. incognita* and root-rot fungi (*Fusarium* and *Rhizoctonia* spp.) were present together in the soil. Synergistic effects of *M. incognita* and *Fusarium oxysporum* f. sp. *cubense*, the cause of Fusarium wilt, on roots of 'Rasthali' (AAB, Silk subgroup) have also been reported (Jonathan and Rajendran, 1998).

Root-knot nematodes are often dispersed in run-off water and can also be spread with irrigation water and contaminated planting material.

Host reaction

In general, the widely grown banana cultivars tend to be susceptible to root-knot nematode. Often, inconclusive results have been obtained when banana genotypes have been screened for resistance to *Meloidogyne* spp. on a large scale. In Brazil and India, of numerous *Musa* genotypes screened against *M. incognita* and *M. javanica*, none were found resistant or even moderately resistant (Zem and Lordello, 1981; Patel *et al*., 1996).

In the Philippines, Davide and Marasigan (1985) screened 90 different *Musa* genotypes for reaction to *M. incognita*. They reported that the response of cultivars varied considerably, ranging from mild to severe root-gall formation. 'Viente Cohol' (AA), 'Dakdakan' (AA, syn. 'Viente Cohol'), 'Pogpogon' (AA), 'Alaswe' (AAA), 'Inambak' (AAA), 'Pastilan' (AAA), 'Sinker' (AAA), 'Mai'a Maole' (AAB, Mai'a Maoli–Popoulu subgroup) and 'Pa-a Dalaga' (ABB) showed some resistance to *M. incognita* with generally only a few nematodes infecting the roots, which had trace to slight gall formation.

In Malaysia, the popular cultivars 'Pisang Mas' (AA, Sucrier subgroup), 'Pisang Embun' (AAA, Gros Michel subgroup), 'Pisang Nangka' (AAA), 'Pisang Berangan' (AAA, Lakatan subgroup), 'Pisang Rastali (AAB, Silk subgroup) and 'Pisang Tandok' (AAB, Plantain subgroup) were susceptible to root-knot (Razak, 1994). Using 26 Vietnamese banana accessions from the AA, AAA, AAB, ABB and AB genome groups and some wild accessions, no source of resistance was found against a mixture of *Meloidogyne* spp. (Van den Bergh *et al*., 2002). Of 31 *Musa* accessions screened for nematode resistance in Costa Rica, no resistance was observed against *M. incognita* (Moens *et al*., 2005). Nor was any source of resistance to *M. incognita* or *M. arenaria* found from 55 *Musa* accessions screened for resistance in Martinique (Quénéhervé *et al*., 2009). In India, Das *et al*. (2014b) screened 19 bred hybrids in inoculated pot trials and in the field against *M. incognita*. 'H 531' was found to be resistant and 'H-02-34', 'H-03-05', 'H-03-13', 'H-04-12', 'H-04-24' and 'NPH-02-01' were classified as tolerant.

To date, no assessment appears to have been made on abacá and enset.

Control

Chemical, biological and cultural options utilized in the management of the burrowing nematode will also mostly apply to *Meloidogyne* spp.

Meloidogyne spp. can be disseminated with infected planting material. Risks can be minimized by using healthy planting material derived from tissue culture or by removing/peeling the outer tissues of the corm or sucker followed by a hot-water, boiling-water or nematicide treatment before planting (Haddad *et al*., 1973). In Yemen, heavy banana losses, associated with severe infection by *M. incognita*, were reduced through the use of *Meloidogyne*-free propagative stocks (Ibrahim, 1985).

Root-knot nematodes have a wide host range and associations with other plant hosts, including numerous weeds, are far more numerous than for the other banana nematode pests. Special attention should be given to the maintenance of weed-free fallow and the selection of cover crops in rotation systems and intercrops.

In India, intercropping with *Coriandrum sativum*, *Sesamum indicum*, *Crotalaria juncea*, *Tagetes erecta* and *Acorus calamus* have significantly reduced *M. incognita* on 'Robusta' (AAA, Cavendish subgroup) in field trials (Charles, 1995). The same effect on *Meloidogyne* spp. was obtained in crop rotation trials with Pangola grass, maize and sugarcane in Cuba (Stoyanov, 1971) and with *Tagetes patula* in South Africa (Milne and Keetch, 1976). Rotation with paddy rice can also drastically reduce root-knot nematode densities, though this was a result of flooding (Sivakumar and Marimuthu, 1986). Fallowing to eradicate root-knot nematodes may, however, be ineffective, as *Meloidogyne* spp. have been shown in Cuba to persist in soil in the absence of banana for up to 29 months (Stoyanov, 1971).

Numerous field experiments have shown the effectiveness of nematicide in the control of root-knot nematodes. Dipping banana corms in a solution of nematicide for 10 min before planting may protect the plants for a few months against nematode infection. Immersion of peeled corms in a 1% solution of sodium hypochlorite (NaOCl) for 5 or 10 min also controlled *Meloidogyne* spp. and was considered by Lordello *et al*. (1994) as an effective, low-cost and non-toxic pre-planting treatment. By knowing the seasonal fluctuation in nematode population densities,

an effective nematicide application strategy can be developed (Badra and Caveness, 1983). Control is most effective when treatments are timed to coincide with the build-up of nematode populations that usually occurs at the onset of the rainy season. In Puerto Rico, oxamyl applied to leaf axils of 'Giant Cavendish' (AAA, Cavendish subgroup) four times at 30-day intervals during the growing season effectively controlled *M. incognita* (Robalino *et al.*, 1983).

Hoan and Davide (1979) reported that root extracts of 11 plant species showed nematicidal effects when tested against *M. incognita* in the Philippines. Root extracts from African marigold (*Tagetes erecta*), ipil-ipil (*Leucaena leucocephala*), Bermuda grass (*Cynodon dactylon*) and makahiya (*Mimosa pudica*) prevented eggs from hatching. The performance of these root extracts was comparable to that of commercially produced chemical nematicides. Results of another study revealed that leaf extracts from kaatoan bangkal (*Anthocephalus chinensis*) and water lily (*Eichornia crassipes*) and extracts of garlic (*Allium sativa*) and onion (*Allium cepa*) bulbs were also effective against *M. incognita* (Guzman and Davide, 1992). Characterization of the active nematicidal principle showed a phenolic aldehyde from kaatoan bangkal, a carboxylic acid from water lily and a ketone from onion.

Culture extracts of 17 species of microorganisms have been evaluated under laboratory and greenhouse conditions in the Philippines for nematicidal activity against *M. incognita* infesting 'Giant Cavendish' (Molina and Davide, 1986). Purified extracts of *Penicillium oxalicum*, *P. anatolicum* and *Aspergillus niger* showed high nematicidal activity. *Purpureocillium lilacinus* and *Penicillium oxalicum* have been very successful in controlling *Meloidogyne* spp. and other nematodes on banana (Davide, 1994).

Arbuscular mycorrhizal fungi are also being investigated as biological control agents. Inoculation of microprapagated 'Grande Naine' (AAA, Cavendish subgroup) with two isolates of *Glomus mosseae* suppressed gall formation and build-up of *M. incognita* in roots under greenhouse conditions. The presence of nematodes had no effect on the colonization of roots by these fungi (Jaizme-Vega *et al.*, 1997). Inoculation of the same banana cultivar with *Glomus intraradices* did not affect the build-up of *M. incognita* in the roots, but increased plant growth by enhancing plant nutrition (Pinochet *et al.*, 1997).

Following the commercial production of *Pasteuria penetrans* for control of *Meloidogyne* spp. (Smith *et al.*, 2004), the prospects of improving the management of root-knot nematodes through biological control moves closer.

Other Nematodes

In addition to the burrowing nematode, root-lesion nematodes, spiral nematodes and root-knot nematodes, numerous other nematode species have been recovered from banana. Most are of little consequence and occur in relatively low numbers. Some, however, although not generally viewed as key pathogens, have a localized significance and require mention.

Rotylenchulus reniformis, or reniform nematode, has been found in association with banana in all producing areas throughout the world. Documented reports come from South America (Zuniga *et al.*, 1979; Crozzoli *et al.*, 1993), Hawaii (Wang and Hooks, 2009), the Caribbean (Oramas and Roman, 1982), Africa (Fargette and Quénéhervé, 1988; Adiko and N'Guessen, 2001; Kamira *et al.*, 2013; Daneel *et al.*, 2015), Asia (Chau *et al.*, 1997; Rahman *et al.*, 2014) and the Mediterranean (Aboul-Eid and Ameen, 1991). In St Lucia, densities of up to 2500 juvenile and infective immature female nematodes were found in 100 cm^3 samples of soil taken from around the fine secondary roots in which mature adult females were permanently lodged (Gowen, 1977b). In West Malaysia, *R. reniformis* were the most prevalent nematode species recovered from the banana rhizosphere (Rahman *et al.*, 2014). In Guangdong Province in China, *R. reniformis* was present in 61% of fields and identified as a major nematode parasite of banana in the area (Shaomei *et al.*, 2006). However, although *R. reniformis* is believed to cause damage to the root system (Edmunds, 1968), little quantitative data on the effect of this nematode species on growth and yield of banana have been reported.

Rotylenchulus reniformis penetrates the cortex of banana roots perpendicularly to the stele and establishes a permanent feeding site in the endodermis. Nematode feeding induces the fusion of endodermal, pericycle and vascular

parenchymal cells to form a syncytium, with hypertrophied nuclei and prominent nucleoli (Vovlas and Ekanayake, 1985). These permanent feeding sites are generally located on the secondary roots (Ayala, 1962; Edmunds, 1968).

Rotylenchulus reniformis is usually found in association with other pathogenic nematode species. Most nematicides effective against root-knot nematodes, including oxamyl applied to leaves (Gowen, 1977a; Robalino *et al.*, 1983), were also effective against *R. reniformis*. Information on either cultural or biological control is limited. In India, intercropping with *Coriandrum sativum*, *Sesamum indicum*, *Crotalaria juncea*, *Tagetes erecta* and *Acorus calamus* significantly reduced *R. reniformis* densities on 'Robusta' (AAA, Cavendish subgroup) in field trials (Charles, 1995). The nematode has also been reported to infect the roots of abacá (Davide, 1972).

Hoplolaimus pararobustus, which can be found in relatively high densities on banana (1000–18,000 nematodes/100 g roots) (Gueroout, 1974; Hunt, 1977; Price, 1994b), appears to occur only in the subepidermal cortex (Mateille, 1994a). The potential of this nematode to cause damage has been questioned, but, if large populations of this relatively large migratory endoparasite are present, it was believed that they must be having some effect on plant development (Price, 1994a). One of the few studies to have assessed the damage this species causes was conducted on 'Agbagba' (AAB, Plantain subgroup) in the field in Nigeria. Following inoculation with 2000 *H. pararobustus* nematodes/plant, bunch weights were reduced by 53% in the plant crop cycle with significantly more stems snapping than in the controls as a result of reduced water uptake (Coyne *et al.*, 2013).

Heterodera oryzicola occurs on banana in southern India and its incidence is probably related to the cropping system, where banana is grown in rotation with paddy rice. Pathogenicity studies suggest that this nematode could cause yield loss (Charles and Venkitesan, 1993).

Pratylenchus spp., such as *Pratylenchus minutus*, and *Paratrichodorus* spp., such as *Paratrichodorus minor*, have been mentioned in some studies (Daneel *et al.*, 2015), but remain of limited and possibly local importance, with little information on their pathogenicity on *Musa*.

Nematode black leaf streak disease of enset was first recorded in 1991 in Ethiopia (Tessera and Quimio, 1994), where it occurs in most growing areas. The disease is caused by *Aphelenchoides ensete* (originally reported as an *Ektaphelenchoides* species) (Swart *et al.*, 2000) and can severely damage enset suckers and seedlings. The most characteristic symptom is small black streaks on leaves (Plate 7.13). Streaks sometimes coalesce to form long necrotic stripes. Severe streaking can cause the premature death of leaves (Plate 7.14). The nematode lives and multiplies in leaf tissue (Plate 7.15) and spreads to neighbouring healthy leaves by rain splash or during watering operations. The nematode is carried to new farms on infected plants. Most enset clones seem susceptible. The early removal of infected leaves helps to control the disease and minimize the chance of spread.

An *Ektaphelenchoides* sp. was also recovered from 30% of enset roots of surveyed fields (Addis *et al.*, 2006). During the same survey, other plant-parasitic nematodes, recorded mostly from the root rhizosphere, included *Scutellonema paralabiatum*, *Scutellonema* sp., *Rotylenchulus* sp., *Tylencholaimellus* sp. and *Tylenchorhynchus leviterminalis*.

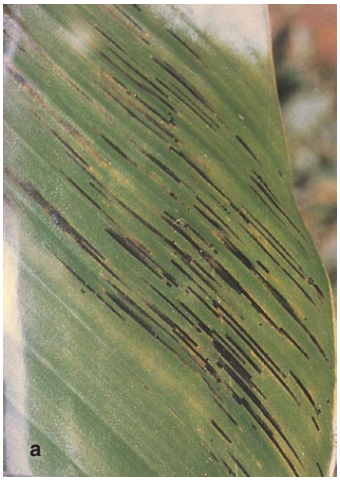

Plate 7.13. Symptoms of nematode black leaf streak disease on the leaf of a young enset seedling (photo: M. Tessera and A.J. Quimio, IAR).

Plate 7.14. Leaf of an enset sucker with severe symptoms of nematode black leaf streak disease caused by *Aphelenchoides ensete* (photo: M. Tessera and A.J. Quimio, IAR).

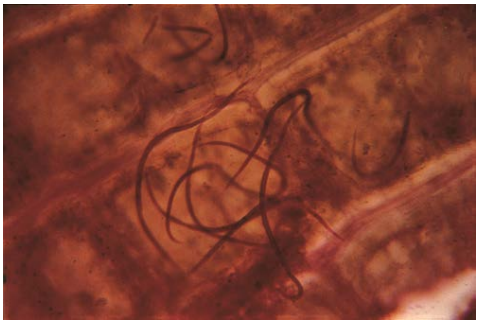

Plate 7.15. *Aphelenchoides ensete* in leaf tissue of enset (photo: M. Tessera and A.J. Quimio, IAR).

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Paper II

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Occurrence of plant-parasitic nematodes on enset (*Ensete ventricosum*) in Ethiopia with focus on *Pratylenchus goodeyi* as a key species of the crop

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Summary – Enset (*Ensete ventricosum*) is an important starch staple crop, cultivated primarily in south and southwestern Ethiopia. Enset is the main crop of a sustainable indigenous African system that ensures food security in a country that is food deficient. Related to the banana family, enset is similarly affected by plant-parasitic nematodes. Plant-parasitic nematodes impose a huge constraint on agriculture. The distribution, population density and incidence of plant-parasitic nematodes of enset was determined during August 2018. A total of 308 fields were sampled from major enset-growing zones of Ethiopia. Eleven plant-parasitic nematode taxa were identified, with *Pratylenchus* (lesion nematode) being the most prominent genus present with a prominence value of 1460. It was present in each sample, with a highest mean population density per growing zone of 16 050 (10 g root)$^{-1}$, although densities as high as 25 000 were observed in fields at higher altitudes in Guraghe (2200-3000 m a.s.l.). This lesion nematode is found in abundance in the cooler mountainous regions. Visible damage on the roots and corms was manifested as dark purple lesions. Using a combination of morphometric and molecular data, all populations were identified as *P. goodeyi* and similar to populations from Kenya, Uganda and Spain (Tenerife). Differences in population densities amongst cultivars indicate possible resistance of enset to *P. goodeyi*.

Keywords – altitude, food security, lesion nematode, molecular data, morphology, prominence value.

Ensete ventricosum, commonly known as enset, is a large perennial herbaceous plant belonging to the Musacea family, together with banana and plantain. Unlike banana and plantain, however, enset does not produce bunches but instead produces a large underground corm that is harvested. The pseudostem is formed from a bundle of leaf sheaths and large leaves, which may reach up to 10 m high and 2 m diam. (Westphal & Stevels, 1975). Wild enset species are found distributed over sub-Saharan Africa and Asia, but in Ethiopia, where it has been domesticated, it is cultivated as an important food crop grown on approximately 400 000 ha (CSA-Ethiopia, 2016). As a key starch staple food source, enset provides food security for over 20 million people, or at least 20% of the Ethiopian population. Furthermore, it is also used for animal feed, fibre, construction and medicine (Brandt *et al*., 1997). The crop grows best at cooler, higher altitudes and is found mostly between 1200-3100 m a.s.l., in the south and southwestern areas of the country. Enset-based farming systems represent a traditional and sustainable form of agriculture, which includes a diverse range of crops that are cultivated alongside enset (Cheesman, 1947; Sim-

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monds, 1962; Brandt *et al*., 1997). Enset is a perennial crop that takes, on average, 7 years to mature; however, as with most crops, the period to maturity is likely to be lengthened under challenge from biotic and abiotic threats. Identifying the biotic threats that challenge enset, and consequently addressing them, has received limited attention.

A number of constraints challenge enset production, with bacterial wilt disease caused by *Xanthomonas vasicola* pv. *musacearum* (Xvm) (previously named *X. campestris* pv. *musacearum* (Xcm)) (Studholme *et al*., 2020) receiving most attention (Addis *et al*., 2004, 2008; Nakato *et al*., 2018). The enset root mealy bug (*Cataenococcus ensete*) can cause severe damage to the roots and corm, reducing crop vigour and production (Addis *et al*., 2010). Also, fungal diseases such as a *Sclerotium* sp. root and corm rot, and *Acremonium* inflorescence spot, causing necrosis on flower bracts and leaves, can affect production, although they appear not to be widespread (Tesera & Quimio, 1994; Quimio & Tesera, 1996). A newly reported leaf streak disease, caused by a new *Badnavirus* species, has also recently been identified (Abraham *et al*., 2018; Abraham, 2019). Plant-parasitic nematodes, well known as major production constraints to banana and plantain production (Sikora *et al*., 2018), have received only limited attention on enset (Coyne & Kidane, 2018; Coyne *et al*., 2018). A few studies have associated various nematode species with the crop, with the lesion nematode *Pratylenchus goodeyi* appearing to be the most prevalent (Peregrine & Bridge, 1992; Tesera & Quimio, 1994; Speijer & Fogain, 1998; Mandefro & Dagne, 2000; Swart *et al*., 2000; Bogale *et al*., 2004). The root-knot nematodes *Meloidogyne incognita*, *M. javanica* and *M. ethiopica*, and *Aphelenchoides ensete* have also been reported as potential production constraints (Mandefro & Dagne, 2000; Swart *et al*., 2000).

Compared to other pathogens, nematodes are, in general, poorly recognised in sub-Saharan Africa (Coyne *et al*., 2018) and Ethiopia in particular (Abebe *et al*., 2015). Despite a handful of studies associating nematode species with enset, there has been no concerted effort to establish the pest potential of nematodes on enset. The current study serves to provide a basis for more focused studies towards understanding the pest potential of nematodes on the crop. A comprehensive sampling of nematodes was undertaken in southern Ethiopia to establish the current situation regarding nematode incidence across the region, in relation to commonly cultivated cultivars and the influence of altitude (temperature) on their occurrence, with emphasis on the most prevalent nematode genus, *Pratylenchus*. This study also served to identify 'hot spots' where material could be collected for use in trials.

Materials and methods

Survey area

Enset root and soil samples were collected from the southern part of Ethiopia, from administrative zones where enset is most commonly grown (Sidama, Hadiya, Kembata and Keffa) in August 2018. Based on the Ethiopian administrative structure a total of 308 fields were sampled; 72 fields were selected randomly from each of the four zones and an additional ten fields each from Guraghe and Jimma zones (Fig. 1). In some fields where multiple cultivars were present, samples were collected separately from different cultivars. For each sample, the location, geographical coordinates, altitude and enset cultivar were recorded. Enset thrives best in slightly acidic, well-drained and fertile soils (Brandt *et al*., 1997). Specific soil characteristics were not assessed for each site; however, we have observed that in each farm enset was grown in soils rich in organic matter. Root and soil samples were removed using a spade by excavating a hole *ca* 0.5 m distance from the stem, from 3-4 plants of each cultivar per field and placed in plastic bags, labelled and stored in a cooler box for transport to the laboratory. Additional *P. goodeyi* populations were collected from Kenya and Uganda, and others supplied from Canary Islands (courtesy of Javier López-Cepero), which were included in the molecular assessment for comparison with Ethiopian populations.

Processing of samples

Soil and root samples were processed separately. Enset roots were carefully washed, cut longitudinally and chopped into *ca* 0.5 mm-sized pieces and a 10 g sub-sample was used for nematode extraction. For soil samples, a 100 ml sub-sample was extracted after fully mixing the soil for each sample. Nematodes were extracted from both soil and roots using a modified Baermann method over a period of 48 h (Hooper *et al*., 2005). Nematode suspensions were decanted and nematodes collected on a 38 μm sieve, rinsed into beakers, reduced to 10 ml and densities counted from 1 ml aliquots under a compound microscope. Nematode densities were calculated for each root and soil sample and expressed as the number of ne-

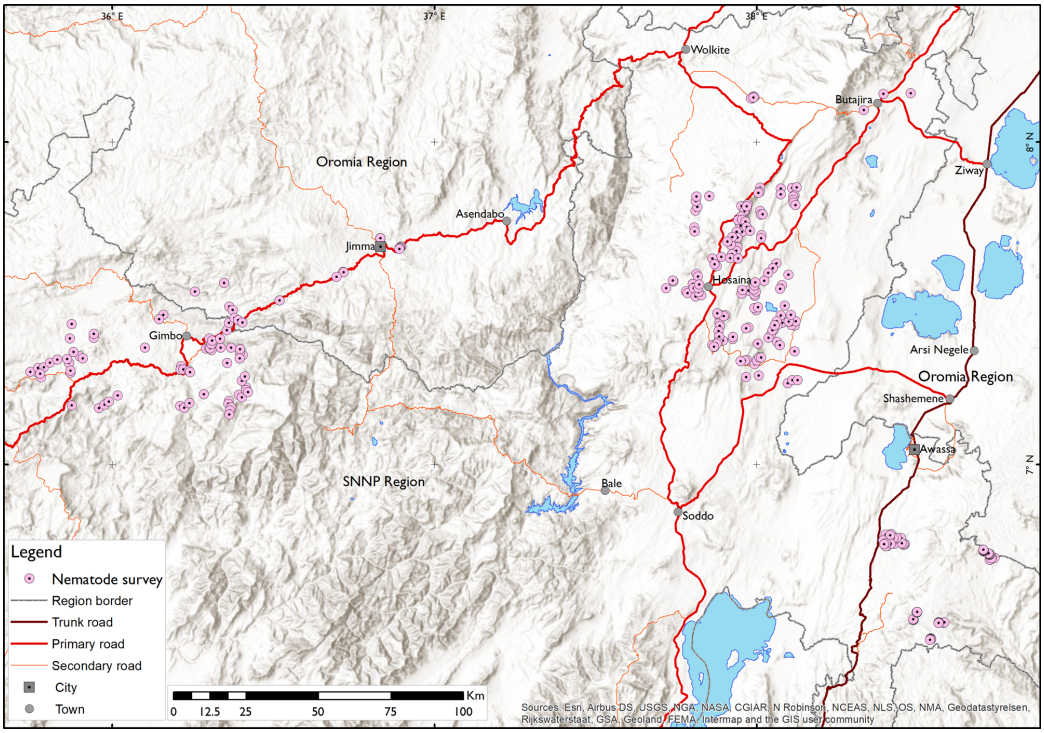

Fig. 1. Map showing nematode survey fields in southern Ethiopia.

matodes in 10 g root or 100 ml soil. The extracted nematodes were subsequently heat-killed, with half the quantity preserved in triethanolamine formalin (TAF) to prepare permanent slides for morphological analyses and the remainder preserved in ethanol (97%) for molecular analysis.

Morphological characterisation

Nematodes fixed in TAF were processed to anhydrous glycerin for permanent slides, following a modified glycerin-ethanol method (De Grisse, 1969). Morphological features were observed and measurements made using a Leica DM 6000 B compound microscope equipped with Leica Application Suite (LAS) version 4.6.1 fitted with an Andor iXon 885 EMCCD camera. *Pratylenchus* specimens were identified to species level based on available keys (Sher & Allen, 1953; Castillo & Vovlas, 2007).

Molecular characterisation

The ethanol-preserved nematodes were washed three times in 400 μl of sterile water for 10 min. DNA extraction was done by cutting an individual specimen and transferring the two pieces to an Eppendorf tube containing 20 μl of WLB (50 mM KCl;10 mM Tris pH 8.3; 2.5 mM $MgCl_2$; 0.45% NP-40 (Merck Life Sciences); 0.45% Tween-20). The samples were frozen for at least 10 min; 1 μl proteinase K (1.2 mg ml^{-1}) was added and the samples were incubated for 1 h at 65°C and 10 min at 95°C. Finally, the samples were centrifuged for 1 min at max speed 20 (800 *g*). They were stored at −20°C before running the PCR. The supernatant was taken as a template for PCR reaction; 2 μl was transferred to an Eppendorf tube containing 25 μl master mix (Derycke *et al.*, 2010) and PCR amplification was performed using a Bio-Rad T100™ thermocycler. For the D2-D3 expansion segment of the large sub-unit (LSU) rDNA primers MalF

(5′-GGATAGAGCCRACGTATCTG-3′) (Wiśniewska & Kowalewska, 2015) and 1006R (5′-GTTCGATTAGTCTT TCGCCCCT-3′) (Holterman *et al*., 2008) were used. The PCR amplification conditions were: initial denaturation of 5 min at 94°C, 35 cycles of (94°C for 1 min; 52°C for 90 s; 68°C for 2 min), and a final extension of 10 min at 68°C. The cytochrome c oxidase subunit 1 (*COI*) gene fragment was amplified using the forward primer JB3 (5′-TTTTTTGGGCATCCTGAAGTCTAT-3′) (Derycke *et al*., 2010) and the reverse primer JB4prat (5′-CCTATTCTTAAAACATAATGAAAATG-3′) adapted from Bowles *et al*. (1992) with an initial denaturation of 5 min at 94°C, 40 cycles of (94°C for 1 min; 48°C for 30 s; 72°C for 100 s), and a final extension of 10 min at 72°C. The PCR products were separated by electrophoresis on agarose gel stained with GelRed (Biotium) and visualised under UV light. Sanger sequencing of purified PCR fragments was carried out in forward and reverse direction by Macrogen (Europe). Contigs were assembled using Geneious 2019.0.4 (Biomatters; http://www.geneious.com). All contigs were subjected to BLAST searches to check for possible contaminations. The resulting sequences were analysed with other relevant sequences available in GenBank. Multiple alignments of the different DNA sequences were made using MUSCLE with default parameters and followed by manual trimming of the poorly aligned ends using Geneious 2019.0.4. Phylogenetic trees were created by using MrBayes 3.2.6 add-in of Geneious with the GTR + I + G model. The Markov chains for generating phylogenetic trees were set at 1×10^6 generations, four runs, 20% burn-in and sub-sampling frequency of 500 generations (Huelsenbeck & Ronquist, 2001).

Statistical treatment of data

Nematode population densities were calculated for each genus and/or species per field. Nematode count data were $\log(x + 1)$ transformed before analysis to minimise variation and conform data to normal distribution (Zuur *et al*., 2010). The percentage frequency of occurrence was calculated as (FO = (number of sites where a genus was detected/total number of sites) × 100), and prominence values (PV = population density × frequency of occurrence/10) (De Waele & Jordaan, 1988) were also calculated for each nematode genus and/or species (identified from both soil and root samples) across the sampled fields. PV is an indication of the relationship of population density and frequency. The association between nematode density and enset cultivar and the association between nematode density and altitude was analysed using RStudio® and Pearson's correlation analysis. Using the GIS coordinates for each farm sampled, distribution maps were created for the key nematode species *P. goodeyi*.

Results

A total of 308 enset field samples were collected from six administrative zones. Eleven plant-parasitic nematode genera were identified: *Pratylenchus*, *Meloidogyne*, *Helicotylenchus*, *Scutellonema*, *Tylenchorhynchus*, *Rotylenchulus*, *Aphelenchoides*, *Cephalenchus*, *Pratylenchoides*, *Trophurus* and *Hoplolaimus* (Table 1). The genera *Pratylenchus*, *Meloidogyne* and *Aphelenchoides* were recovered from roots (Table 2). With regard to frequency of occurrence (FO%), *Pratylenchus* and *Helicotylenchus* species were present in 100 and 52% of the soil samples, respectively, followed by *Tylenchorhynchus* (16%), *Scutellonema* (10%) and *Meloidogyne* (13%). *Pratylenchus*, *Meloidogyne* and *Aphelenchoides* species occurred in 100, 8 and 4% of root samples, respectively (Table 2). *Pratylenchus* was the most prominent nematode taxa across the enset-growing areas with a prominence value of 1460, followed by *Meloidogyne* and *Aphelenchoides* with PVs of 20 and 4, respectively. *Pratylenchus* spp. densities were highest in the highlands of Guraghe, where mean densities of 16 050 and 12 217 (10 g root)$^{-1}$ were observed in Meskan and Ezha woredas/districts, respectively, although densities as high as 25 000 (10 g root)$^{-1}$ were recorded from individual fields. The elevation of these areas ranged between 2200 and 3000 m a.s.l. (Fig. 2). Roots from these locations appeared dry and, when split longitudinally, extensive black or purple necrotic cortical tissue was evident (Fig. 3A), which was also observed on the corms (Fig. 3B). Roots from locations infected with *Meloidogyne* presented visible galling damage (Fig. 3C) but no obvious damage was associated with *Aphelenchoides* species.

This study found *P. goodeyi* to be present in every farm sampled and thus widely distributed, but alongside a range of species associated with enset and the cooler climate at the highest altitudes (>2200 m a.s.l.). There was a positive correlation ($r = 0.11$, $P = 0.08$) between altitude and population densities of *P. goodeyi* (Fig. 4). The correlation of *P. goodeyi* root density with cultivar showed that densities varied from 20 ('Bedo') to 4600 ('Birdo') (10 g root)$^{-1}$, but no statistically significant differences in

Table 1. Frequency of occurrence, population density and prominence value of major plant-parasitic nematode genera recovered from enset soil in Ethiopia.

Zone	District* and elevation (m a.s.l.)	*Praty-lenchus*	*Meloido-gyne*	*Helicoty-lenchus*	*Scutel-lonema*	*Tylenchor-hynchus*	*Rotylen-chulus*	*Aphelen-choides*	*Cepha-lenchus*	*Pratylen-choides*	*Trophurus*	*Hoplo-laimus*
Sidama	Dale 1700-1800	+	+	+	+	+			+		+	
	Arbegona >2600	+	+	+	+			+				
	Hula >2600	+	+	+	+							
Hadiya	Misha 2300-2600	+	+	+		+	+		+			
	Lemo 2300-2600	+	+	+		+	+		+			
	Duna 2300-2600	+	+	+		+			+			
Kembata	Angacha 2000-2500	+		+	+		+			+		
	Doyo Gena 2200-2700	+		+	+					+		
	Kedida Gamella 2000-2200	+		+	+						+	
Keffa	Chenna 1700-2100	+		+	+	+						+
	Decha 1700-2100	+		+	+	+						+
	Gimbo 1700-2100	+		+	+	+						
FO %		100	13	52	10	16	9	1	3	3	5	5
PD		84	26	45	34	21	35	10	17	110	47	58
PV		84	9	32	11	8	11	1	3	19	11	13

* Each district has 24 sampled fields.
FO % = Frequency of occurrence (FO %), i.e., number of fields where a genus is detected/total number of fields sampled ×100.
Population density (PD) = Mean number of individuals of a genus over the sampled fields where the genus was detected; densities per 100 ml soil.
Prominence value (PV) = Mean population density × (Frequency of occurrence)$^{1/2}$ × 10^{-1}.

Table 2. Frequency of occurrence, population density and prominence value of major plant-parasitic nematode taxa recovered from enset roots in Ethiopia.

Zone	District* and elevation (m a.s.l.)	*Pratylenchus*	*Meloidogyne*	*Aphelenchoides*
Sidama	Dale 1700-1800	+	+	
	Arbegona >2600	+	+	
	Hula >2600	+	+	
Hadiya	Misha 2300-2600	+	+	+
	Lemo 2300-2600	+	+	
	Duna 2300-2600	+	+	
Kembata	Angacha 2000-2500	+		+
	Doyo Gena 2200-2700	+		+
	Kedida Gamella 2000-2200	+		
Keffa	Chenna 1700-2100	+		
	Decha 1700-2100	+		
	Gimbo 1700-2100	+		
FO (%)		100	8	4
PD		1460	69	22
PV		1460	20	4

* Each district has 24 sampled fields.
FO % = Frequency of occurrence (FO %), i.e., number of fields where a genus is detected/total number of fields sampled $\times 100$.
Population density (PD) = Mean number of individuals of a genus over the sampled fields where the genus was detected; densities per 100 ml soil.
Prominence value (PV) = Mean population density $\times$ (Frequency of occurrence)$^{1/2} \times 10^{-1}$.

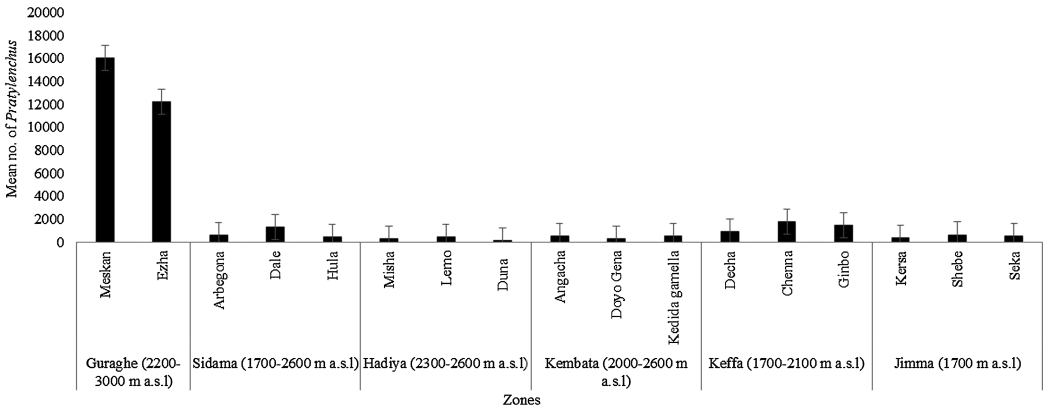

Fig. 2. *Pratylenchus goodeyi* densities per 10 g root across enset-growing zones of Ethiopia.

densities amongst the cultivars were observed. The number of samples for each cultivar also differed, reflecting farmer and/or geographical preferences for different cultivars.

Pratylenchus was the most frequently occurring nematode genera in soil samples with mean soil density of 84 nematodes (100 ml soil)$^{-1}$ followed by *Helicotylenchus* (45) and *Scutellonema* (34). The genera *Pratylen-*

A

B

C

Fig. 3. A: Longitudinal section of enset roots showing lesions caused by the lesion nematode; B: Purple lesions caused by the lesion nematode on enset corm; C: Galling on enset roots caused by root-knot nematodes, *Meloidogyne* spp.

choides, *Hoplolaimus* and *Trophurus*, which occurred in fewer sites, had densities of 110, 58 and 47 nematodes (100 ml soil)$^{-1}$ (Table 1).

Morphological and molecular characterisation of *Pratylenchus*

Using a combination of morphometric and molecular data with *Pratylenchus* specimens, *P. goodeyi* was the only species of the genus identified. Morphologically, the Ethiopian populations displayed typical diagnostic characteristics of *P. goodeyi*, including four lip annuli, four inconspicuous lateral field lines, stylet 16-18 μm long with pronounced, anteriorly flattened stylet knobs, large spermatheca filled with sperm, tail conoid, ventrally concave with dorsal contour sinuate just prior to tail tip, which matched the characterisation described by Castillo & Vovlas (2007). Seven *Pratylenchus* specimens (five females and two males) were measured: female; L = 0.56 mm; a = 32.88; b = 4.12; c = 17.93; V = 73.42; stylet = 16.44 μm; male: L = 0.55 mm; a = 29.48; b = 4.36; c = 23.1; T = 55.52; stylet = 16.1 μm.

Pratylenchus goodeyi populations from Ethiopia, Kenya, Uganda and the Canary Islands were used for molecular analysis. Eighty-one D2-D3 of 28S rDNA (GenBank accession numbers of selected sequences: MT569985, MT569991-94) and 101 mtDNA *COI* sequences with a maximum intraspecific variability of, respectively, 3 (0.5%) and 16 (4.1%) nucleotides were obtained. The D2-D3 phylogenetic tree (based on 652 bp long alignment with 116 sequences) revealed that all obtained sequences are in a maximally supported clade together with virtually identical *P. goodeyi* sequences from GenBank (0-3 bp difference), but without internal resolution (Fig. 5). For *COI*, sequences were obtained with premature stop codons that appeared difficult to align with other *Pratylenchus COI* sequences. This indicates that the used primers appeared not to have targeted the genuine *COI* region, but mitochondrial fragments into the nuclear genome (nuclear mitochondrial pseudogenes). Subsequent attempts with several other primers (Folmer *et al*., 1994; Kanzaki & Futai, 2002; Derycke *et al*., 2010) did not alleviate this pseudogene problem, *i.e.*, always the same pseudogene was obtained. Nevertheless, the resulting phylogenetic tree (based on 360 bp long alignment with 102 sequences) clearly clustered all our sequences with a very similar (0-16 bp different) *COI* reference of *P. goodeyi* (unpublished sequence in the framework of the study of Janssen *et al*. (2017a)), which confirms the species identity. However, the internal resolution in this *P. goodeyi* clade was limited and without relation to host or location, impeding statements related to intraspecific relations (separated analyses of the pseudogene sequences). Despite the evidence of nuclear pseudogenes that com-

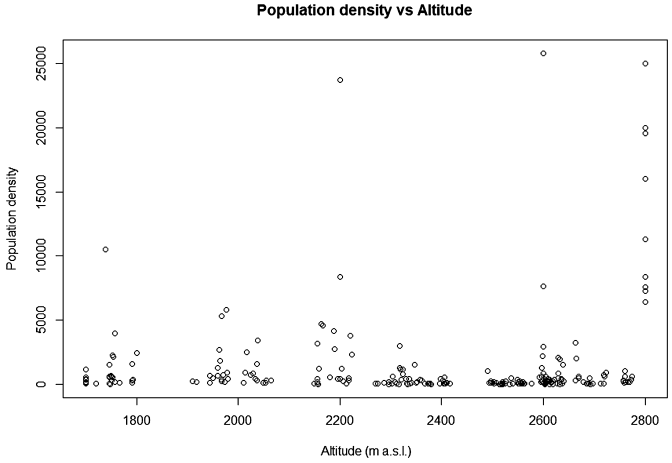

Fig. 4. Correlation between altitude and *Pratylenchus goodeyi* density on enset roots in Ethiopia.

plicated this study, it was evident that all investigated *Pratylenchus* species are unmistakably *P. goodeyi*, which is confirmed by morphology, D2-D3 sequences and a putative species-specific pseudogene (Fig. 5).

Discussion

The present study shows that although a range of plant-parasitic nematode species are associated with enset in the major producing zones in south and south western Ethiopia, *P. goodeyi* dominates strongly and is the most prominent species. Our study also represents the most extensive assessment of nematodes on enset to date, and the latest since Addis *et al*. (2006) with 98 farms sampled in 2004 and Bogale *et al*. (2004) who assessed 49 farms in 1999. The predominance of *P. goodeyi* in the previous studies and in our study identifies this nematode as probably the greatest nematode threat to enset. Root-knot nematodes (*Meloidogyne* spp.) were recovered from a few root samples, with relatively lower PV scores and densities; galling damage was observed on enset roots in the current study, which indicates it is becoming more problematic as this is the first time this appears to have been observed. Previously *Meloidogyne* spp. was found in 9% of 98 enset samples (Addis *et al*., 2006) and 60% in a smaller study (Bogale *et al*., 2004), which shows some variability in the recovery of these nematodes between studies. The current comprehensive study therefore demonstrates the incidence of *Meloidogyne* spp. associated with the crop across the region and supports the growing concern of this pest becoming more serious on crops across sub-Saharan Africa (Coyne *et al*., 2018). *Aphelenchoides* spp. were isolated from the roots of enset, reflecting previous studies, although no discoloured leaves were observed, which has previously been associated with *A. ensete* infection (Swart *et al*., 2000; Bogale *et al*., 2004; Addis *et al*., 2006). Although *Aphelenchoides* spp. have been associated with damage to enset, this does not appear to be prominent (PV = 4). No *Helicotylenchus multicinctus* were recorded from the roots, even though this nematode is common on banana in Ethiopia and was recorded from 5% of enset roots by Addis *et al*. (2006). Neither was any *Radopholus similis* recorded on enset, in line with previous studies (Bogale *et al*., 2004; Addis *et al*., 2006), even though it was present on banana in the previous studies (Addis *et al*., 2006). Enset therefore, may not be a suitable host for *H. multicinctus* or *R. similis*, unlike banana. However, environmental factors may not be suitable for *R. similis*, which is known to be thermophilic and present at warmer, lower altitudes than enset is generally cultivated (Kashaija *et al*., 1994).

In line with previous studies (Bogale *et al*., 2004; Addis *et al*., 2006), *Meloidogyne*, *Helicotylenchus*, *Scutellonema*, *Tylenchorhynchus* and *Rotylenchulus* were among the plant-parasitic nematodes associated with enset soil samples. Our study also detected species of *Cephalenchus*, *Pratylenchoides*, *Trophurus* and *Hoplo-*

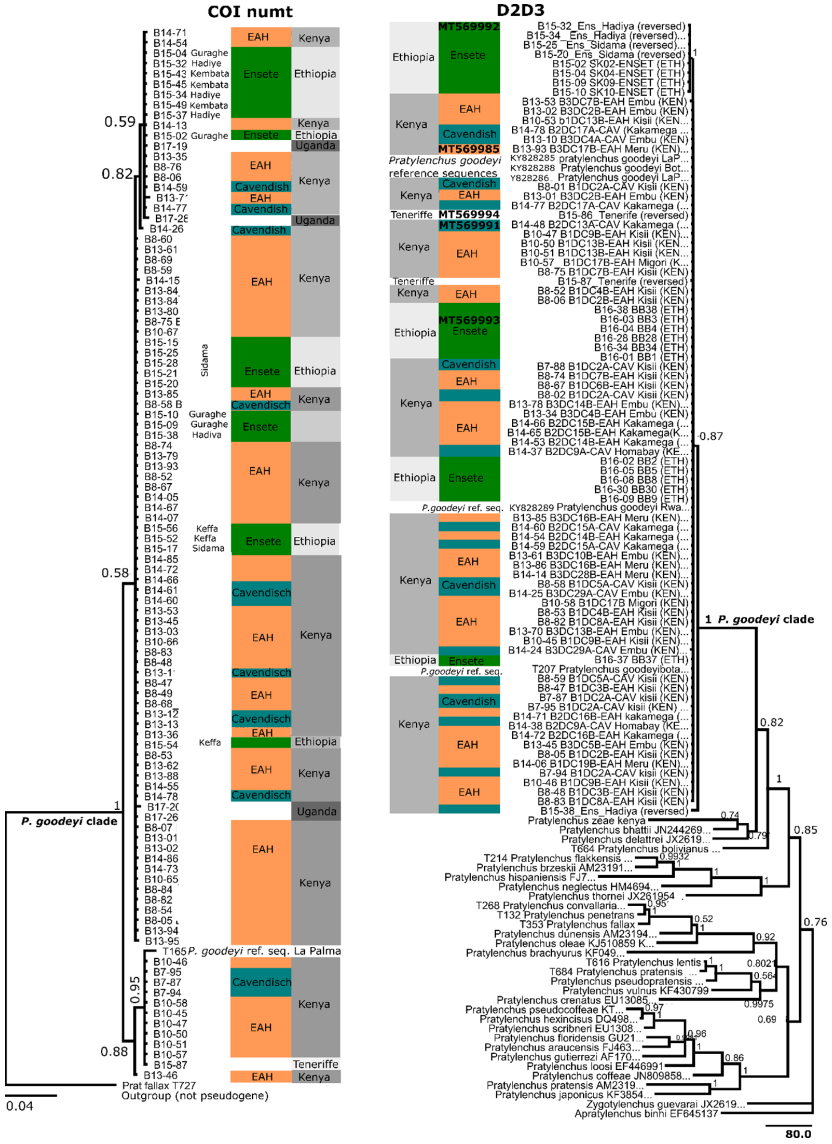

Fig. 5. Bayesian 50% majority-rule consensus tree inferred from *Pratylenchus goodeyi COI* and D2-D3 (pseudogene) sequences, obtained from enset in southern Ethiopia, and from banana in Kenya and Tenerife.

laimus from enset soil samples, but all were in relatively low densities and do not appear to be causing any major threat to the crop.

The incidence and distribution of *P. goodeyi* was prominent in higher altitudes (2000-3000 m a.s.l.), such as the extremely high densities observed in some farms from the Guraghe mountains, with over 20 000 (10 g root)$^{-1}$ recorded and even up to 26 000 (10 g root)$^{-1}$. This by far exceeds the density of *P. goodeyi* (5484 (10 g root)$^{-1}$) previously recorded by Bogale *et al*. (2004) from enset rhizome tissue and the 15 000 recorded by Peregrine & Bridge (1992). A maximum of 5600 nematodes (10 g root)$^{-1}$ was recovered from banana in Cameroon (Bridge *et al*., 1995) and mean densities of 2500 nematodes from East African Highland banana roots in Uganda (Kashaija *et al*., 1994). Enset, therefore, appears to be able to tolerate high densities of *P. goodeyi*. There are no reports on how this affects crop growth and production; therefore, it remains speculative as to the level of damage being caused to enset. A case study on banana in Rwanda showed that the highest *P. goodeyi* densities and root necrosis were present in the best performing banana plants, a possible explanation being that the negative impact of the nematode was masked by the fact that the plants were receiving better nutrient inputs (Gaidashova *et al*., 2009). However, it is assumed that at such high densities as observed during our study, substantial damage is being caused. Roots with high *P. goodeyi* densities were associated with root necrosis and purple lesions, while the outer cortex of corms at times presented severe necrotic lesioning, especially on planting materials (Fig. 3b). When visiting farms, substantial portions of the corm with lesions and rotten areas were observed being removed during the preparation of corm material for food processing, resulting in much reduced corm size and food quantity. The wide range in *P. goodeyi* densities could indicate possible variations in the biology or pathogenicity of geographic populations. Populations of *P. goodeyi* from elsewhere within Africa were also shown to be similar to the Ethiopian populations, indicating a relatively recent distribution of the species within Africa (Bridge *et al*., 1997). Difference in pathogenicity between geographic populations or 'pathotypes' has been speculated, given the contradictory evidence of damage observed by *P. goodeyi* on bananas and the uniformity of *P. goodeyi* populations (Speijer & Bosch, 1996; Coyne, 2007). Populations occurring in Tanzania appeared similar to those from other countries (Mgonja *et al*., 2019), even though some of these populations were recovered from tropical lowland areas, which is atypical for the species. Similarly, populations of *P. goodeyi* are being recovered from other tropical lowland locations (Coyne, 2007; Sikora *et al*., 2018). As yet, there is no conclusive evidence to demonstrate differences between populations.

In the current study, both morphometric and molecular techniques were used to identify the *Pratylenchus* populations. In general, morphological identification of *Pratylenchus* species is difficult due to the low number of diagnostic features, high morphological plasticity and incomplete taxonomic descriptions (Castillo & Vovlas, 2007; Janssen *et al*., 2017a). DNA-based identification tools are therefore important for *Pratylenchus* species (Waeyenberge *et al*., 2000), but also a strong link between morphology and DNA sequences is of crucial importance to prevent sequence-based misidentifications (Janssen *et al*., 2017b). However, the morphological characterisation of the Ethiopian *P. goodeyi* all corresponded closely to the documented characteristics (Sher & Allen, 1953). *Pratylenchus goodeyi* is also one of the few *Pratylenchus* species that can be relatively easily identified based on morphology alone. The molecular assessment of *P. goodeyi* populations, based on the D2-D3 and *COI* region, and including specimens from countries other than Ethiopia, did not reveal informative differences. As expected, intraspecific resolution of the D2-D3 region is limited for *Pratylenchus* (Janssen *et al*., 2017a). For *COI* a higher resolution can be expected; however, sequences that are most likely nuclear pseudogenes have complicated our analyses. Pseudogenes have been detected in several eukaryotes and impede the usefulness and dependability of DNA (Leite, 2012). Nonetheless, for nematode taxonomic and phylogenetic studies, pseudogenes are not well recognised as being problematic. Furthermore, the *COI* region of several *Pratylenchus* has been sequenced (Janssen *et al*., 2017a, b), but the pseudogene problem only appears to be present in *P. goodeyi*, in all globally distributed populations. How specifically *P. goodeyi* differs in this respect remains to be investigated.

Although there was no significant difference in *P. goodeyi* population densities among the cultivars, variations in levels of infection across cultivars from the current study show that possible differences in resistance exist in enset against *P. goodeyi*. The assessment of 111 cultivars using Random Amplified Polymorphic DNA (RAPD), demonstrated that each cultivar had unique DNA (Birmeta *et al*., 2002). However, given the difference in infection levels between geographic and altitudinal locations, this needs proper assessment through controlled inoculation studies. Differences in nematode densities between 71 en-

set cultivars, sampled from 98 farms, showed possible differences in resistance to *P. goodeyi* (Bogale *et al*., 2004) but this again requires verification.

During our survey, we perceived that very few farmers were aware of, or had any knowledge of, nematode pests. To some extent, they were aware of the bacterial wilt problem on enset and other foliar diseases but not of nematodes. As in the case of many smallholder farmers and agricultural agents even in sub-Saharan Africa, there remains a huge gap in the awareness of nematodes as pests and their management, even though nematodes are regarded as economically important pests of most crops in the region (Coyne *et al*., 2018). With such a high frequency of occurrence of *P. goodeyi* on enset in Ethiopia, and with such high densities recorded, it is assumed that this nematode is causing damage to crop growth and production. With a lack of information on the damage potential of this nematode to enset, this survey will provide a basis for identifying hotspots for nematode material for use in assessing the efficacy of the nematode on enset, potential on-farm assessment and interaction of nematodes with other organisms.

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Paper III

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The Ethiopian staple food crop enset (*Ensete ventricosum*) assessed for the first time for resistance against the root-lesion nematode *Pratylenchus goodeyi*

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Summary – *Pratylenchus goodeyi* appears to be the most prevalent nematode pest of enset in Ethiopia, where it can occur in extremely high densities. However, the damage to yield or how different enset cultivars react to the nematode has yet to be determined. The current study therefore sought to establish a first assessment of these reactions by enset to *P. goodeyi* infection. Determining pest-resistant cultivars is an important task in developing management strategies. Our study evaluated nine enset cultivars to establish host response and identify potential sources of resistance. In addition, the pathogenicity of *P. goodeyi* was assessed on three enset cultivars. After 9 months' growth, significant differences in final population densities (P_f) and reproduction factor (RF) were observed amongst the nine cultivars, with 'Gefetanuwa' the most susceptible ($P_f = 25\,799$ and RF = 12.9), and similarly in a repeat experiment for 4.5 months ($P_f = 126\,534$ and RF = 63.3). 'Maziya' and 'Heila' were the most resistant in the first experiment ($P_f < 455$ and RF < 0.2) as well as in the repeat, together with 'Kellisa' ($P_f < 5255$ and RF < 2.6). In the pathogenicity experiment four inoculum densities significantly affected the P_f and RF but not among the three cultivars 'Maziya', 'Arkiya' and 'Heila'. This is the first known study to assess genotype reaction to *P. goodeyi*, which shows that there are significant differences in the reactions of different cultivars and that resistance appears to be present in enset.

Keywords – cultivar, Ethiopia, food crop, management, pathogenicity, reproduction factor.

Ensete ventricosum is a large herbaceous plant that belongs to the Musaceae family, the same as bananas. The genus *Ensete* comprises seven species (*E. ventricosum*, *E. homblei*, *E. livingstonianum*, *E. superbum*, *E. perrieri*, *E. lecongkietii* and *E. glaucum*) (Cheesman, 1947; Simmonds, 1962; Luu *et al*., 2012). Wild *E. ventricosum* species are found distributed in sub-Saharan Africa and Asia, but it is domesticated and cultivated as a food crop only in Ethiopia. Unlike banana, enset does not produce edible fruit, but rather the pseudostem and corm are harvested after 3-12 years and processed into food products. Major food products prepared from enset are kocho (obtained through fermentation of decorticated leaf sheath and corms), bulla (powder from the liquid squeezed out of leaf sheath and pulverised corm) and amicho (boiled corms) (Brandt *et al*., 1997). In the south and southwestern part of the country, enset serves as a key staple food crop for about 20% of the Ethiopian population (Borrell *et al*., 2019). It is also important as the key signature crop of the complex enset-based cropping systems in this area, creating stability in relation to food security, as well as the agroecology. As a perennial crop that can be harvested at any time of the year, enset offers food security when other crops are less available, providing a year-round availabil-

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ity of nutritious food. It is also generally perceived to tolerate drought, with a broad agroecological distribution and is easily cultivated around the home with low input and management requirements. Consequently, the crop represents an important position in household food security. In Ethiopia, enset is reported to be more productive per unit area than other starch crops (Tsegaye & Struik, 2001). In addition to food, enset is also used for a multitude of other purposes, such as for feed, medicine, building and fibre. As an orphan crop, with restricted geography, it has received relatively limited attention in terms of crop improvement. This is beginning to change, however, as the importance of this crop becomes better understood, with a few genetic diversity studies being undertaken, as well as research to identify pest and disease resistance (Brandt *et al*., 1997; Harrison *et al*., 2014; Borrell *et al*., 2020).

More than 600 enset landraces collected from major enset-growing areas in Ethiopia have been conserved *ex situ* in the gene bank in the Areka Agricultural Research Center (Yemataw *et al*., 2017). Molecular characterisation of enset landraces using amplified fragment length polymorphism (AFLP) (Negash *et al*., 2002), random amplified polymorphic DNA (RAPD) (Birmeta *et al*., 2002, 2004), simple sequence repeat markers (SSR) (Olango *et al*., 2015; Gerura *et al*., 2019) and inter-simple sequence repeat (ISSR) (Tobiaw & Bekele, 2011) techniques have revealed high genetic diversity amongst various landraces. Despite the progress in genetic studies and the potential of the crop, genetic improvement and conservation are based on conventional methods and have remained stagnant (Olango *et al*., 2015). To date, breeding enset using conventional or biotechnology applications has yet to materialise in improved varieties for any trait (Merga *et al*., 2019). Its perennial life cycle, with its extended duration to flowering and seed set, its complex vernacular naming, the absence of known traits such as disease resistance and reliance on vegetative propagation make genetic improvement tedious, expensive and time consuming (Olango *et al*., 2015). Consequently, enset is by far the least studied food security crop (Borrell *et al*., 2019).

Despite its resilience and versatility, several production constraints, including plant-parasitic nematodes, challenge enset. Studies have shown that although a range of nematode species are associated with the crop, the root-lesion nematode *Pratylenchus goodeyi*, root-knot nematodes (*Meloidogyne* spp.) and the foliar nematode *Aphelenchoides ensete* appear the most important nematode threats (Peregrine & Bridge, 1992; Swart *et al*., 2000; Bogale *et al*., 2004; Addis *et al*., 2006). However, *P. goodeyi* is by far the most common and prevalent species, occurring in all fields sampled, at densities as high as 25 000 (10 g soil)$^{-1}$ (Bogale *et al*., 2004; Addis *et al*., 2006; Kidane *et al*., 2020). When challenged with densities this high, the crop might undergo considerable stress, with roots straining to maintain water and nutrient supply to the plant. However, the damage potential to enset by these nematodes is yet to be determined, as is the susceptibility to nematodes of the various land races and cultivars used by farmers.

Of the various strategies for the management of nematodes, the use of resistance is most suited for smallholder farmers in Africa, but knowledge of nematode pests and their management is poor and access to, or availability of, quality inputs is limited (Coyne *et al*., 2009). Commercial banana plantations have mainly relied on chemical nematicides, which are not an option for smallholder enset farmers. Exploiting resistance is an alternative management strategy against nematodes (Speijer & De Waele, 1997). Traditional breeding for genetic traits in members of the Musaceae, however, is fraught with numerous obstacles based on inherent sterility, low genetic base and the long-term nature of the crop (Ortiz, 2011). A first step for the development of a management option is to identify cultivars that are resistant to pests and diseases (Speijer & de Waele, 1997; Pinochet *et al*., 1998; Coyne & Tenkouano, 2005). To date, there has been no known screening for resistance of enset against plant-parasitic nematodes. Resistance is defined as the ability of a host plant to suppress nematode reproduction and development. Whereas nematodes will reproduce on a susceptible host and cause damage, a tolerant host will support nematode reproduction but suffer limited injury even in the presence of high infection levels, while a sensitive host cannot support even a light infection of nematodes (Bos & Parlevliet, 1995).

The objective of our study was to screen and evaluate the host plant response of nine enset cultivars to inoculation with *P. goodeyi*, in order to identify sources of resistance in the enset germplasm for potential use in nematode management, as well as to assess the pathogenicity of *P. goodeyi* on three selected enset cultivars.

Materials and methods

All experiments were conducted in the screenhouse located at Jimma University College of Agriculture and Veterinary Medicine, Jimma, Ethiopia, 7°42′N, 36°50′E,

at an altitude of 1710 m a.s.l. The area receives an annual rainfall of 1250 mm, average maximum and minimum temperatures of 26°C and 11°C, and an average maximum and minimum relative humidity of 91.4 and 37.9%, respectively.

Enset cultivars

One-year old enset seedlings, of known cultivars, were obtained from Areka Agricultural Research Centre, Areka, Wolaita. Suckers for each cultivar were regenerated from a single corm to ensure the purity of each cultivar. Prior to planting, roots were removed and the corms peeled before sanitising in boiling water treatment for 20 s (Coyne *et al*., 2010). The suckers were then trimmed in order to ensure uniformity in size prior to planting. The waste root and corm material was assessed for nematodes before and after boiling water treatment.

Nematode inoculum

Pratylenchus goodeyi was isolated from infected enset roots collected from a high infection 'hotspot' highland area in Agena, Guraghe, identified during a recent study (Kidane *et al*., 2020). A combination of morphometric and molecular data revealed that *P. goodeyi* was the only species of the genus identified from this area (Kidane *et al*., 2020). Due to there being no monoxenic cultures of *P. goodeyi* available, naturally infected roots were used as inoculum, which has previously been shown to be a successful alternative (Coyne *et al*., 2010). Monoxenic culturing of some species of *Pratylenchus* is also not always successful using the conventional method on carrot discs (Santos *et al*., 2012), and *P. goodeyi* has proved difficult to date (Coyne, pers. comm.). Nematodes used for inoculation (P_i) were extracted from a 10 g sub-sample of chopped enset root and corm material using a modified Baermann extraction method over 48 h (Hooper *et al*., 2005). Nematodes were collected on a 38 μm sieve, rinsed into beakers, the suspension was reduced to 10 ml, and counted from 1 ml aliquots under a compound microscope.

Resistance screening

Nine cultivars were selected and assessed for resistance to *P. goodeyi*: 'Gewada', 'Zereta', 'Maziya', 'Heila', 'Kellisa', 'Gefetanuwa', 'Yanbule', 'Messana' and 'Endale'. These cultivars are among the 623 enset accessions maintained in Areka Agricultural Research Centre, obtained from single corms of each cultivar. These cultivars have distinct phenotypic variations. They are among the released cultivars for desired characteristics, such as yield and bacterial wilt disease tolerance. The experiments were conducted on raised benches in the screenhouse using 2 l pots containing oven-sterilised sandy soil, arranged in a randomised complete block design (RCBD) with six plants per treatment (cultivar). Suckers were maintained for 2 months to enable enough root development before inoculation with nematodes. At 2 months after planting (MAP) 2000 *P. goodeyi* (mixed juvenile and adult stages) were added to the pots in a 7 ml suspension into three holes made using a pencil around the base of the suckers and then covered. The plants were watered as needed and fertiliser applied as urea, once at 3 months after inoculation (MAI). The experiment was terminated at nine MAI and repeated once; the repeat was terminated at 4.5 MAI (due to the availability of seedlings at a later time and timeline of the study period).

Pathogenicity assessment

Three enset cultivars ('Maziya', 'Arkiya' and 'Heila') were used to assess *P. goodeyi* pathogenicity. These cultivars are among the cultivars released for their desirable traits and they were also selected, based on results from previous nematode surveys, for supporting either high or low *P. goodeyi* densities. Enset suckers were planted into 2 l pots and inoculated with 500, 1000 and 2000 *P. goodeyi* in a 10 ml suspension and compared with a non-inoculated water control. The pots were prepared and maintained as outlined above in the screening experiment, arranged in a RCBD with four plants per treatment (cultivar × inoculum density) on raised benches in the screenhouse. The experiment was terminated at 4.5 MAI; unavailability of seedlings prevented a repeat.

Growth and damage parameters assessed

At harvest the plant height, shoot fresh weight and root fresh weight were recorded for each plant. Plant height was measured from the soil surface to the tip of the youngest growing leaf. Plants were carefully removed from pots, rinsed free of soil and dabbed dry with paper towels. The roots were removed with a knife and the shoot (including leaves) and roots weighed separately. Roots were chopped into *ca* 0.5 cm pieces and nematodes extracted from a 10 g sub-sample per plant. The soil from each pot was thoroughly mixed before removing a 100 ml sub-sample. Nematodes from roots and soil were

extracted using a modified Baermann method for 48 h (Hooper *et al*., 2005). Nematodes were collected on a 38 μm sieve, rinsed into beakers, suspensions reduced to 10 ml and densities assessed from 3 × 1 ml aliquots under the microscope. The overall nematode root and soil densities per plant were calculated by multiplying the density per g root by the total root weight and per ml soil by soil volume (2000 ml). Final nematode population density (P_f) per plant was calculated as the sum of the root and soil factions. The reproduction factor (RF) was calculated by dividing P_f by the initial nematode population density (P_i).

Statistical treatment of data

All data were analysed using RStudio®. The least significance difference was calculated for separation of means with $P \leqslant 0.05$. Nematode population densities were $\log(x+1)$ transformed prior to analysis of variance in order that data conformed to a normal distribution. Mean nematode population density data were back-transformed for presentation.

Results

Resistance screening

All enset cultivars tested showed different levels of susceptibility to *P. goodeyi* based on the P_f and RF. In the first experiment, the enset cultivars differed significantly ($P < 0.001$) in their host suitability to *P. goodeyi*. 'Gefetanuwa' had the highest P_f of 25 799 with a RF = 12.9, followed by 'Zereta' (P_f = 11 196; RF = 5.6) and 'Endale' (P_f = 3573; RF = 1.8). Cultivars with the lowest density were 'Maziya' (P_f = 455; RF = 0.2), 'Heila' (P_f = 350; RF = 0.2) and 'Yanbule' (P_f = 335; RF = 0.2). Similarly, in the second experiment, although terminated earlier, there was a significant difference ($P < 0.001$) amongst the enset cultivars. 'Gefetanuwa' had the highest P_f of 126 534 with a RF = 63.3, followed by 'Yanbule' (P_f = 22 525; RF = 11.3) and 'Zereta' (P_f = 20 085; RF = 10). Cultivars with the lowest density were 'Heila' (P_f = 5255; RF = 2.6), 'Kellisa' (P_f = 3529; RF = 1.8) and 'Maziya' (P_f = 2746; RF = 1.4) (Table 1). Both experiments showed a similar trend except for 'Yanbule', which had low P_f in the first experiment, possibly because of low root weight and development, hence resulting in few nematodes. When 'Yanbule' was removed from the analysis, there was no significant difference ($P = 0.02$) between the two sets of experiments (Fig.1; Supplementary Table S1).

Pathogenicity assessment

Results showed that in the pathogenicity study *P. goodeyi* multiplied on all three cultivars ('Maziya', 'Arkiya' and 'Heila') after 4.5 months but with no differences in P_f or RF among them. Significant differences ($P < 0.001$) on the P_f and RF were observed, based on the four levels of inoculation densities used within each cultivar. We also found that the RF of *P. goodeyi* was low in all three cultivars compared to susceptible cultivars such as 'Gefetanuwa' as seen in the cultivar screening experiment (Table 2). No differences in plant growth parameters were observed between the controls and inoculated plants (Table 3).

Discussion

Our study represents the first proper assessment of nematode resistance in enset. Although data from a small number of survey studies indicate possible differences in susceptibility or resistance to plant-parasitic nematodes among enset cultivars (Bogale *et al*., 2004), there is as yet no information available from any controlled studies. Indeed, there is only limited information on the resistance of enset cultivars against the various pest and diseases. Our results reveal that there does appear to be quite a range in susceptibility to *P. goodeyi* among enset cultivars. The low multiplication of *P. goodeyi* on 'Maziya', 'Heila' and 'Arkiya' also demonstrates a good level of resistance, with a low population build-up, while 'Gefetanuwa' was highly susceptible, with a much greater *P. goodeyi* multiplication.

There are over 600 enset cultivars maintained in the Areka gene bank, with a number of studies underway to characterise enset germplasm for genetic and phenotypic variability amongst accessions (Yemataw *et al*., 2017; Gerura *et al*., 2019). Screening activities, such as the current study, help contribute to building up the information on the various accessions, towards detecting sources of resistance across a range of constraints and identifying suitable sources for breeding. The current study initiates information gathering for nematode resistance and shows some promising results that provide a basis for further large-scale screening studies. However, the process is time consuming and subject to sensitivity and error, while ambiguity of accession names can be misleading. Conse-

Table 1. *Pratylenchus goodeyi* reproduction on nine enset cultivars.

9 MAI			4.5 MAI		
Cultivar	Final nematode density (P_f)	Reproduction factor (RF)	Cultivar	Final nematode density (P_f)	Reproduction factor (RF)
'Gefetanuwa'	25 799 a	12.9 a	'Gefetanuwa'	126 534 a	63.3 a
'Zereta'	11 196 ab	5.6 b	'Yanbule'	22 525 ab	11.3 b
'Endale'	3573 bc	1.8 b	'Zereta'	20 085 b	10 b
'Kellisa'	1623 cd	0.8 b	'Endale'	9396 bc	4.7 b
'Messana'	1153 cd	0.6 b	'Gewada'	8455 bc	4.2 b
'Gewada'	591 cd	0.3 b	'Messana'	7691 bc	3.8 b
'Maziya'	455 cd	0.2 b	'Heila'	5255 bc	2.6 b
'Heila'	350 d	0.2 b	'Kellisa'	3529 c	1.8 b
'Yanbule'	335 d	0.2 b	'Maziya'	2746 c	1.4 b

P_f analysis was undertaken using log-transformed data; back-transformed data are presented. MAI = months after inoculation. Mean *Pratylenchus goodeyi* densities and RFs in a column with the same letter are not significantly different ($P \leqslant 0.05$).

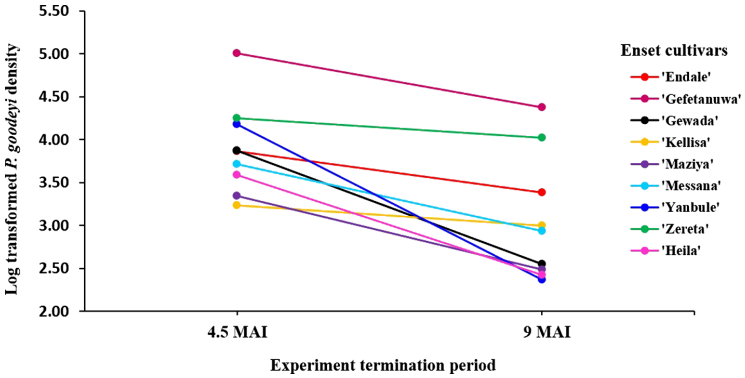

Fig. 1. Position of enset cultivars based on log-transformed mean densities of *Pratylenchus goodeyi* at 4.5 and 9 months after inoculation (MAI).

Table 2. *Pratylenchus goodeyi* reproduction on three cultivars of enset following inoculation at four levels in pots.

Inoculum density[1]	Cultivar[2]						Final population density (P_f)	Reproduction factor (RF)
	'Arkiya'		'Maziya'		'Heila'			
	P_f	RF	P_f	RF	P_f	RF		
0	0 a	0 e	0 a	0 e	0 a	0 e	0 a	0 a
500	666 b	1.33 f	582 b	1.16 f	892 b	1.78 f	713 b	1.43 ab
1000	2033 c	2.03 g	2974 c	2.97 g	1297 c	1.29 g	2107 c	2.11 c
2000	5745 d	2.87 h	4143 d	2.07 h	6354 d	3.17 h	5414 d	2.71 d

[1] *P. goodeyi* inoculum (juveniles and adults) per 2 l pot.
[2] Final nematode density analysis was undertaken using log-transformed data; back-transformed data are presented. Mean *P. goodeyi* densities and RF of each cultivar in a row with the same letter are not significantly different ($P \leqslant 0.05$). Mean *P. goodeyi* densities and RF across three cultivars in a column with the same letter are not significantly different ($P \leqslant 0.05$).

Table 3. Plant growth parameters of three enset cultivars following inoculation with *Pratylenchus goodeyi* in pots after 4.5 months.

Inoculum density[1]	'Arkiya'			'Maziya'			'Heila'		
	Root weight (g)	Shoot weight (g)	Plant height (cm)	Root weight (g)	Shoot weight (g)	Plant height (cm)	Root weight (g)	Shoot weight (g)	Plant height (cm)
0	110 a	67 b	25 c	109 d	95 e	27 f	66 g	113 h	20 i
500	114 a	57 b	17 c	108 d	124 e	28 f	119 g	85 h	26 i
1000	135 a	83 b	22 c	110 d	114 e	30 f	48 g	48 h	21 i
2000	146 a	121 b	26 c	95 d	99 e	29 f	89 g	86 h	28 i

Plant growth parameter measurements in a column with the same letter are not significantly different ($P \leqslant 0.05$).
[1] *Pratylenchus goodeyi* inoculum (juveniles and adults) per 2 l pot.

quently, suitable protocols need to be established, based on the use of accessions that are genetically characterised for conformity of names. Rapid screening procedures targeting single roots and assessing nematode multiplication adopted for banana (De Schutter *et al*., 2001; Coyne & Tenkouano, 2005) can also be used to screen enset germplasm. The development of tissue culture-based *in vitro* propagation protocols for enset (Negash *et al*., 2000) could also improve the efficiency and speed of propagating disease-free planting materials for distribution to farmers.

Determining germplasm with good resistance to key pests, diseases and abiotic constraints is important for improving crop productivity, especially in Africa, where losses are particularly large (Coyne *et al*., 2018). Identifying accessions that have multiple resistance is therefore of even greater value when determining germplasm for use in breeding programmes, or providing recommendations to farmers. For instance, 'Maziya' is regarded as less susceptible to bacterial wilt disease (*Xanthomonas vasicola* pv. *musacearum*), whilst 'Gefetanuwa', which supported the highest reproduction of *P. goodeyi*, also supports rapid *X. vasicola* pv. *musacearum* development, as does 'Arkiya', which has been used as a susceptible control in evaluation studies (Muzemil *et al*., 2019). Although 'Arkiya' was regarded as one of the cultivars with higher densities of *P. goodeyi* in a previous survey (Bogale *et al*., 2004), the P_f and RF were similar to the other two cultivars ('Maziya' and 'Heila'). As nematode infection is known to predispose banana to bacterial wilt (Shehabu *et al*., 2010) and fusarium wilt diseases (Almeida *et al*., 2018), it further serves a purpose to have nematode resistance traits in banana, as well as enset. Studies such as ours can be very important to identify cultivars to use for studying the relationship of nematodes and bacterial wilt disease.

In our study we found that infection with *P. goodeyi* did not result in any decrease in growth parameters of the enset suckers over the 4.5 months of assessment, as compared to similar studies with banana (Van den Bergh *et al*., 2002; Dochez *et al*., 2009; Coyne *et al*., 2013). This could be explained by the long perennial nature of the enset crop, with about 7 years to maturity, and the period of assessment being too short to detect differences. Alternatively, it may be that the enset cultivars assessed in the current study all exhibit a level of tolerance to the nematodes. This may also explain the high *P. goodeyi* densities experienced on enset roots during recent surveys (Bogale *et al*., 2004; Addis *et al*., 2006; Kidane *et al*., 2020). Similarly, unlike other studies on banana, root necrosis damage was not readily observed or visualised, possibly due to the thin enset roots, combined with the short duration of the experiment, or possibly due to host tolerance. Infection of enset roots with *P. goodeyi* does result in necrosis, however, which can be considerable, as seen during field studies (Bogale *et al*., 2004; Addis *et al*., 2006; Kidane *et al*., 2020) and which is undoubtedly detrimental to growth and production of enset. In any case, it is clear that further investigations are necessary to determine more effectively host damage potential by *P. goodeyi*, possibly over a longer duration, and with a greater range of germplasm assessed using methods such as the single-root inoculation (Coyne & Tenkouano, 2005), which should be repeated to confirm results.

Although the current study screened a few cultivars from the enset germplasm and over a short duration compared to the perennial nature of the crop, this study demonstrates that there are indeed differences in the resistance of cultivars to *P. goodeyi*. Being the first study conducted on enset resistance against nematodes, it can be used as a base for further studies such as screening and interaction of other nematodes and other pathogens.

Most synthetic chemical nematicides have been removed from the market due to environmental and human health concerns and so it is important to select the best performing cultivars in terms of resistance to nematodes and other diseases. Chemical pesticide use on enset is currently very low under the predominantly subsistence manner of production around homesteads. Therefore, the identification of cultivars resistant to the predominant nematode species is a first step towards using those in future breeding efforts.

Despite the importance of enset in Ethiopia, there has been little attention given to the genetic improvement of the crop. Baseline studies on the identification of nematode resistance, such as ours, accompanied by information on the molecular characterisation and genome-wide sequence data of enset (Harrison *et al*., 2014) will enhance research on this important but neglected crop towards its genetic improvement. Having established tissue culture propagation and *in vitro* conservation protocols for enset will additionally provide a basis for extending such screening work (Negash *et al*., 2000; Birmeta, 2004).

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Supplementary Table S1. Summary of analysis of variance of log-transformed mean densities of nine enset cultivars in the two sets of experiments (4.5 and 9 months after inoculation).

	df	Sum squares	Mean squares	*F* value	Pr(> *F*)
Cultivar	7	25.7	3.7	24.4	3.62e-16***
Experiment repeat	1	10.1	10.1	67.1	1.09e-11***
Cultivar × Experiment repeat	7	2.8	0.4	2.7	0.018*
Residuals	67	10.1	0.2		

Paper IV

Planting material of enset (*Ensete ventricosum*), a key food security crop in Ethiopia, is a key element in the dissemination of plant-parasitic nematode infection

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Orphan Crops: Breeding and Biotechnology for Sustainable Agriculture, Food and Nutrition

Enset (*Ensete ventricosum*), is a perennial herbaceous plant belonging to the family Musaceae, along with banana and plantain. Despite wild populations occurring in eastern, central and southern Africa, it is only in Ethiopia that the crop has been domesticated, where it is culturally and agriculturally symbolic as a food security crop. Although a little-known orphan crop, enset serves as a staple food for about 20% of the Ethiopian population, comprising more than 20 million people, demonstrating its value in the country. Similar to banana and plantain, enset is heavily affected by plant-parasitic nematodes, with recent studies indicating record levels of infection by the root lesion nematode *Pratylenchus goodeyi*. Enset is propagated vegetatively using suckers that are purposely initiated from the mother corm. However, while banana and plantain suckers have proven to be a key source of nematode infection and spread, knowledge on the infection levels and role of enset suckers in nematode dissemination is lacking. Given the high levels of plant-parasitic nematodes reported in previous surveys, it is therefore speculated that planting material may act as a key source of nematode dissemination. To address this lack of information, we assessed enset planting material in four key enset growing zones in Ethiopia. A total of 340 enset sucker samples were collected from farmers and markets and analyzed for the presence of nematodes. The root lesion nematode *Pratylenchus goodeyi* was present in 100% of the samples, at various levels of infection. These conclusive results show that planting material is indeed a key source of nematode infection in enset, hence measures taken to ensure clean suckers for planting will certainly mitigate nematode infection and spread. The effect of nematode infection on yield and quality on enset remains to be investigated and would be a way forward to complement the nematode/disease studies conducted so far and add valuable knowledge to the current poorly known impact of pests and diseases.

Key words: lesion nematode, *Pratylenchus goodeyi*, orphan crop

INTRODUCTION

Described as the "tree against hunger" (Costa and Lockhart, 1984), enset (*Ensete ventricosum*) is a perennial monocarpic single-stemmed herbaceous plant belonging to the family Musaceae, along with banana and plantain. Although wild species occur in eastern, central and southern Africa (Baker and Simmonds, 1953) enset is cultivated in, and solely unique to, Ethiopia, where it is culturally and agriculturally symbolic; cropping systems in the south and southwest are based around this pivotal, yet little-known orphan crop. Unlike bananas, enset does not produce edible fruits, instead, it is grown for its carbohydrate-rich food obtained from the pseudostem, leaf sheaths and underground corm, which are harvested and processed into food products. Harvest can be at any time during the year, at any growth stage and the fermented products can be stored for long periods, a combination of characters that make it an important food security crop, upon which millions depend. Its value was prominently highlighted during the harsh Ethiopian famine in the 1980's when enset growing communities were unaffected by the calamity (Dessalegn, 1995). However, on a regular basis, approximately 20% of the Ethiopian population depends on enset as a key staple food crop, primarily in the south and southwestern part of the country (Borrell *et al.*, 2019; Borrell *et al.*, 2020). Furthermore, it is used for several other purposes, such as animal feed, fibre, construction material and in traditional medicine. The crop best grows at cooler, higher altitudes and is found mostly between 1200-3100 m above sea level (Brandt *et al.*, 1997).

Harvest commonly occurs after 4 to 6 years, but there is variability in when plants are harvested, with indications as early as three years and up to 12 years (Brandt *et al.*, 1997; Borrell *et al.*, 2020). Enset is vegetatively propagated using suckers that are produced through a succession of growth stages. Unlike banana, it does not produce suckers aside the mother plant, instead suckers are purposely initiated from a mother corm, obtained from harvested

plants, after cutting off the pseudostem and roots and removing the apical dominance. Corms are then buried in the ground, just below the surface, and from which multiple suckers sprout and develop. Depending on the genotype and the size of the corm, between 20-100 suckers will arise (Brandt *et al.*, 1997). After approximately one year, these suckers are transplanted into a well-manured nursery and repeatedly replanted, up to four times, into increasingly wider spaced nurseries until the suckers are removed for use as planting material. Seedlings aged two to four years are used for planting into the field, many of which are sold at designated local seedling markets each year between December and February (Olango *et al.*, 2014). Farmers also raise their own suckers or exchange planting materials between themselves.

Similar to banana and plantain, enset is heavily affected by plant-parasitic nematodes (Coyne and Kidane, 2018). Several plant-parasitic nematodes are associated with enset, with the lesion nematode, *Pratylenchus goodeyi*, considered the most important threat to the crop (Peregrine & Bridge, 1992; Bogale *et al.*, 2004; Addis *et al.*, 2006; Kidane *et al.*, 2020). For banana and plantain, the use of infected planting material (suckers) represents a key source of nematode dissemination and the perpetuation of the problem. Farmers exchange planting materials, which is responsible for the continuous distribution of nematodes to new fields. The use of healthy planting materials, therefore, is essential to arrest the spread of nematodes and prevent losses due to the pests. A range of techniques are used in order to create healthy planting materials, such as through the use of *in vitro* tissue cultured material, macro propagation and sucker sanitation by paring and hot water treatment (Tenkouano *et al.*, 2006; Coyne *et al.*, 2010). The use of clean and healthy banana and plantain planting material plays a crucial role in averting the spread of nematodes and other root-borne pests and diseases and the damage they cause, especially in smallholder farming systems, where expensive management strategies are avoided (Coyne *et al.*, 2006).

Given the sparse knowledge by farmers of nematodes, as well as the current high incidence and levels of *P. goodeyi* infection on enset (Kidane *et al*., 2020), it is speculated that, similar to banana and plantain, nematodes are being disseminated to newly planted farms through the use of infected enset sucker seedlings. To date, there appears to be no information available or studies conducted to assess the level of nematode infection of enset suckers. The current study was undertaken to assess the infection status of enset planting materials as a basis for developing suitable nematode management options.

MATERIALS AND METHODS

Enset suckers aged between 1-2 years were collected from farmers (Fig. 1A & 1B) and markets (Fig. 2) in four key enset growing zones in Ethiopia (Dawro, Keffa, Guraghe and Wolayita). In each of these administrative zones, 13 locations were randomly selected and 24-40 enset suckers collected at each site. The altitude was recorded for each site. The suckers were transported to the laboratory, where roots were carefully washed, cut longitudinally, and chopped roughly into ~0.5 mm-size pieces and a 10 g sub-sample used for nematode extraction. Nematodes were extracted using a modified Baermann method over a period of 48 h (Coyne *et al.*, 2018). Nematode suspensions were decanted, collected on a 38 μm sieve, rinsed into beakers, reduced to 10 ml and densities counted from 1 ml aliquots using a counting slide under a compound microscope. Nematode densities were calculated for each root sample and expressed as the number of nematodes per 10 g root. *Pratylenchus* specimens were identified to species level based on available keys (Sher and Allen, 1953; Castillo and Vovlas, 2007).

Figure 1: Enset suckers (A) (B) collected from farmers' nurseries in Ethiopia

Nematode root density data were analyzed for any differences in infection levels between the regional zones. All data were analyzed using RStudio® after log(n+1) transformation so that the data conformed to normal distribution (Zurr *et al.*, 2010). The association between nematode density and altitude was analyzed using Pearson's correlation analysis.

Figure 2: Enset suckers collected from markets in Ethiopia

RESULTS

A total of 340 enset sucker samples (2-3 suckers) were assessed during the study. *P. goodeyi* was recovered from the roots of 100% of sucker samples in densities ranging between 10 and 190 per 10 g roots (Table 1). Apart from a few non-parasitic nematodes in some samples, *P. goodeyi* was the only plant-parasitic nematode recovered from roots. Although the age of the suckers was not specifically recorded for each sample, in general younger suckers appeared less infected, than larger, older suckers (Kidane pers. obs.). On some suckers, especially the older ones, lesions were clearly evident on their roots and corms (Fig. 3).

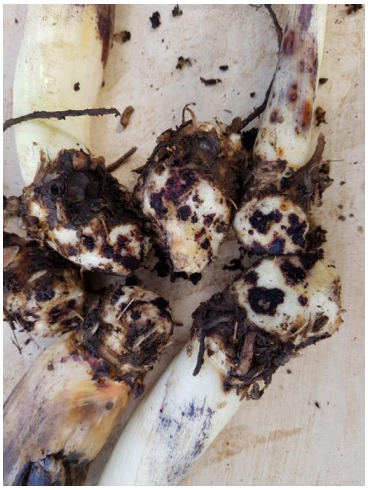

Figure 3: Extensive lesioning on enset suckers collected from markets and farms in Ethiopia

ANOVA revealed no difference (P = 0.31) in *P. goodeyi* root infection levels of sucker samples between sites. Neither was there any correlation (r = 0.014; P = 0.85) in nematode infection with altitude, across all locations.

Table 1: *Pratylenchus goodeyi* root density on enset sucker planting material collected from key enset production zones in Southern Ethiopia

Zone	Site/ elevation (m.a.s.l.)	Number of samples	*Pratylenchus goodeyi* mean density per 10 g root
Dawro	Tercha (1400)	24	141
	Maraka (2100-2200)	24	137
	Marimansa (1800-2000)	24	120
		Total = 72	
Keffa	Gimbo (1600-1900)	24	93
	Decha (1700-2100)	24	190
	Shishenda (1700-2200)	24	174
		Total = 72	
Guraghe	Ezha (>2400)	25	140
	Meskan (2200)	22	70
	Abeshge (1600-1700)	16	69
	Silte (2000)	30	124
		Total = 93	
Wolayita	Boloso soro (1700-1800)	40	141
	Damot gale (2000)	40	10
	Sodo zuria (2000)	23	295
		Total = 103	

DISCUSSION

Infection of enset planting material with *P. goodeyi* is clearly widespread across the main enset growing zones in Ethiopia, and consequently acting as a key source of contamination of new fields. The nematode-infected suckers, often visibly affected with lesions on their roots and corms, are planted into new fields. Other than trimming the roots and parts of the corms, which is a common procedure performed during transplanting, there is no further treatment undertaken to reduce the nematode infection. With 100% infection incidence of planting material during the study, it is highly likely that this reflects the situation across all enset production systems in Ethiopia. Sucker infection levels were relatively high in some cases, and although infection levels varied between samples, this did not differ significantly between zones. This variability could be attributed to differences in susceptibility of the cultivars (Kidane *et al.*, 2021), while the lack of difference in infection levels between zones could be a consequence of the high diversity of cultivars (Kidane *et al.*, 2020), each with varying levels of resistance against *P. goodeyi*. The current study aimed to assess the planting material most commonly available and used by farmers, which was suckers aged 1-2 years. However, when processing the suckers for nematode extraction, the older, larger suckers appeared to be relatively more infected, with more apparent lesions and damage observed in general (Fig. 3). The variability in sucker age across samples may have additionally contributed to the high variability of nematode densities.

Interestingly, just one nematode pest species was recovered during the study. While several species of plant-parasitic nematodes are associated with enset in Ethiopia, *P. goodeyi* is the principal and most prevalent species (Bogale *et al.*, 2004; Addis *et al.*, 2006; Kidane *et al.*, 2020). This is unlike other members of Musaceae, such as banana and plantain, upon which several species often occur in combination (Sikora *et al.*, 2018; Coyne and Kidane, 2018). As

it appears that nematode pests are being constantly disseminated through contaminated planting material that is exchanged between farmers, the implementation of interventions that can avert this should be sought. Given the similarities with banana and plantain, experiences drawn from successful seedling sanitation practices will be helpful for implementation of techniques for enset (Tenkouano *et al.*, 2006).

In the current study, we observed almost total ignorance of enset farmers to nematodes and the possible damage that they cause. This is despite a common practice of trimming necrotic sections from suckers before transplanting. Although the suckers are trimmed and cleaned to some degree, large amounts of necrotic tissue often remained on the transplanted suckers (Fig. 3), indicating a lack of awareness of the importance of this damage by farmers. To date, there is no information available on the levels or extent of the damage being caused to enset production by *P. goodeyi.* It is effectively present in all plantations, to varying degrees of infection, but can be present at extremely high densities (Kidane *et al.*, 2020). This blanket contamination of enset crops in Ethiopia has undoubted consequences to production and quality, which requires attention. Interventions regarding improving awareness of nematodes, the damage they cause, and suitable management strategies are required. However, the implementation of simple and effective options for the establishment of healthy seedling systems and sucker sanitation need to be prioritized. It is not surprising that a principal mode of nematode transmission on enset is through the dissemination of contaminated planting material. The current study confirms this and provides a basis for developing management options to amend this. Despite it being an important crop in various regards, the highly localized enset-based farming system has received only limited research attention, which needs to be rectified to ensure and improve the productivity of this neglected orphan crop (Brandt *et al.*, 1997; Borrell *et al.*, 2020).

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