# Survey and Management of Potato Pests in Uganda

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## Summary

Potato (*Solanum tuberosum* L.) is an important food and income generating crop for its growers. The crop is also nutritionally rich in carbohydrates, vitamins (C and B), proteins, minerals (potassium) among other nutritional components. In Uganda, potato has been recognized as a crop with potential for nutrition security and income generation. Despite these benefits, average potato yield (4.8 t/ha) in Uganda is still comparatively lower than attainable global average yield (30 to 40 t/ha), owing to several biotic and abiotic production constraints. A study was conducted to establish the status of potato pests and diseases in north eastern and south western Uganda to include districts such as Mbale, Namisindwa, Kween, Kapchorwa and Kabale, Rubanda, Kisoro, respectively. The study was aimed at assessing the incidence and prevalence of key pests and diseases affecting potato in Uganda, with special emphasis on potato cyst nematode. Several pests and diseases including leaf miner flies, aphids, potato tuber moths, whiteflies, viruses, several nematode species, bacterial wilt (*Ralstonia solanacaerum*), and *Fusarium* spp. were observed to be affecting potato. Through the prospections conducted in this survey, the potato cyst nematode (*Globodera rostochiensis*) was identified in north eastern and south western areas of Uganda. Male farmers were more engaged in decision-making activities for potato production than their female counterparts in Eastern Uganda. An inclusive and multi institutional team was tasked to conduct this potato disease survey; this exercise was led by the International Institute of Tropical Agriculture (IITA) and the International Fertilizer Development Centre (IFDC-GIZ), with the special cooperation of the International Potato Centre (CIP), and the active engagement of the BugiZARDI-NARO and the District Production Office of the District Local Governments and Ministry of Agriculture, Animal Industry and Fisheries-Department of National Crop Certification Services.



**Keywords:** insects, nematodes, bacteria, virus, potato cyst nematode, bacterial wilt, Mbale, small-holder farmers.

# Introduction

Potato (*Solanum tuberosum* L.) is an important crop for food security and income generation. The crop is also nutritionally rich in carbohydrates, vitamins (C and B), proteins, minerals (potassium) among other nutritional constituents and surpasses cereals in terms of the amount of dry matter and protein per cropping unit area (VIB, 2019). In Uganda, potato has been recognized as a crop with potential for nutrition security and income generation (PASIC, 2016), as it is in most of the highlands of Africa (East and Central Africa). Despite these benefits, potato yields have suffered substantial fluctuations over the last two decades (FAOSTAT, 2018), and currently average potato yield (4.8 t/ha) in Uganda is significantly lower than average yields in Kenya (8 – 10 t/ha) and far below than attainable global average yield (30 to 40 t/ha), owing to several biotic and abiotic production constraints, from poor agronomic practices (i.e. limited use of fertilizer by farmers), to lack of quality potato seed, and/or high prevalence of pests and diseases.

Pests including, but not limited to, potato tuber moth, root-knot nematodes and diseases such as early blight, late blight and bacterial wilt have been reported to severely affect potato production in Uganda (Coyne et al., 2014; Okonya et al., 2019). Recent reports on insect pests of economic importance in potato include the potato tuber moth (PTM) (*Phthorimaea operculella* Zeller), leaf miner flies (*Liriomyza* spp.), aphids (*Aphis gossypii*, *Aphis fabae*, *Macrosiphum euphorbiae*, and *Myzus persicae*) in Uganda and Kenya (Okonya and Kroschel, 2015; Okonya et al., 2019). The PTM is a major insect pest of potato affecting potato yield through infestations of leaves, stems, and harvested tubers. Harvested tubers infested by PTM often initiate tuber infestation in stores causing losses of stored tubers of up to 70 % (Raman and Radcliffe, 1992; Kroschel and Schaub, 2013). Traditionally, nematodes have been reported to play a significant role in yield reduction amongst tuber and root crops. Nevertheless, due to its microscopic nature and the scarcity of nematologists, this type of pests remains overlooked while its detrimental impact on crops' productivity is largely underestimated, particularly in sub-Saharan Africa (Coyne et al., 2018). Among all the pest affecting potato, the potato cyst nematode (PCN) has been considered the most detrimental pest for this crop and it is subject to strict quarantine regulations in over 100 countries (EPPO, 2017); the presence of the two species of PCN (*G. rostochiensis* and *G. pallida*) has been recently reported in Kenya (Mwangi et al., 2015; Mburu et al., 2018) and in Rwanda *G.*

*rostochiensis* has been reported for the first time in 2019 (Niragire *et al.*, 2019). In both cases, PCN has been isolated in areas neighbouring south-west (from Rwanda) and north-east Uganda (from Kenya), indicating that these detrimental pests could be widespread across the East Africa region. Amidst these challenges, it is imperative to direct research towards reducing the negative impact of these pests and diseases on potato production and productivity. Therefore, in order to develop and implement an effective plant health strategy to assist farmers to manage pests and diseases of potato in Uganda, is crucial to establish the current prevalence and distribution of the same.

The current study was aimed at **assessing the incidence and prevalence of key pests and diseases affecting potatoes in Uganda.** The results from the current survey should assist IFDC to identify areas in eastern and western Uganda that would be likely to have low pest and disease pressure, which could be suitable for healthy seed potato production; in addition, this new survey adds on the survey commissioned by IFDC to IITA in 2014 (Coyne et al., 2014) related to the situation on invasive pests, such as PCN, after the findings of Kenya and Rwanda. The results of this survey shall also shade light on the potential implications that the presence of PCN should have on the potato seed industry in Uganda.

## Objectives and Project Activities

**Project objectives**

1. Conduct a survey of the major pests (diseases, nematodes, and insects) present in eastern Uganda;
2. Conduct a rapid appraisal of potato fields in SW Uganda, with focus on establishing the presence of PCN; and
3. Evaluate the potential of banana fibre paper to mitigate potato pest and disease losses.

Project activities

1. Provide training on pest and disease assessment to key personnel;
2. Visual assessment of pest problems, recording incidence and severity, and collecting plant samples;
3. Diagnose plant samples in the laboratory to confirm the visual assessment during the field survey; and

4. Analyse pest incidence/severity by geographic region and agroclimatic division:
    a) Record incidence and severity of PCN and other key threats in potato fields; and
    b) Conduct field trials in E Uganda to assess yield differences when using banana fibre.

# Materials and Methods

## Survey Area

Three surveys scattered between 2018 and 2019 were conducted. The first survey was conducted between 20th - 23rd of November 2018 (north-east Uganda) and the second one was conducted between 9th - 23rd June 2019 (north-east Uganda), both in Mount Elgon region; the third one was conducted between 26th - 30th October 2019 (south-west Uganda). The surveys were aimed at establishing the incidence and prevalence of the major biotic constraints (diseases, nematodes and insects) of potato and to have a better understanding of farmers' practices. In the north -eastern (NE) region, the surveys were conducted in the districts of Mbale, Kween, Namisindwa and Kapchorwa; and in Kisoro, Kabale and Rubanda for the south-western (SW) region. Namisindwa was specially selected due to its proximity to the Kenyan border town of Lwakhakha known to have high cross border agricultural trade and thus a gateway for introduction of pests and diseases of potato. In NE region, a comprehensive epidemiologic study to determine the incidence of pests and diseases took place, leading to the collection of both soil and plant samples (i.e. stems, leaves, roots) for microbiological and molecular analyses at the laboratory. In the SW region, only soil samples were collected in a bid to re-ascertain the potential presence of PCN since the last survey conducted this side in 2014. This was particularly important after a report from neighbouring Rwanda confirmed presence of this group of nematodes in countries directly bordering Uganda. A total of 132 fields were surveyed (**Table 1**), including 99 from the NE region (Mount Elgon bordering Kenya), with and 33 from south western region bordering Rwanda (**Figure 1**).

## Field Sampling

Potato fields at least 2 km apart from each other were selected, with the support of IFDC technical staff and NARO extension officers. Sample collection within each field followed a zig-zag transect for both soil and plant sample collection. During collection of plant samples, 10 plants were randomly collected along the same Z transect from each field. From each of the 10 plants uprooted,

a pulled sample of eight stem portions were collected, and wrapped in parafilm to prevent dryness; three leaves were hand picked off individual plants, and pooled together for a composite sample, and, finally, 3 tubers per field were also collected for analyses; for each field, all these samples were kept individually in paper bags for microbiological and molecular analyses for detection of bacteria, virus and fungi. The same procedure was used for collection of root and tuber samples meant for nematode analyses. Root samples from individual plants were also pooled together to form a composite sample, alongside with the potato tubers. For soil collection, up to 20 soil cores were drawn per each farm using a hand trowel from the upper 30 cm along the same Z-transect. Soil cores were pooled together to form a composite soil sample (2 Kg) per field.

**Table 1**: Summary of sampled farms per geographic area (North East and South West) and district during potato pest and disease management survey.

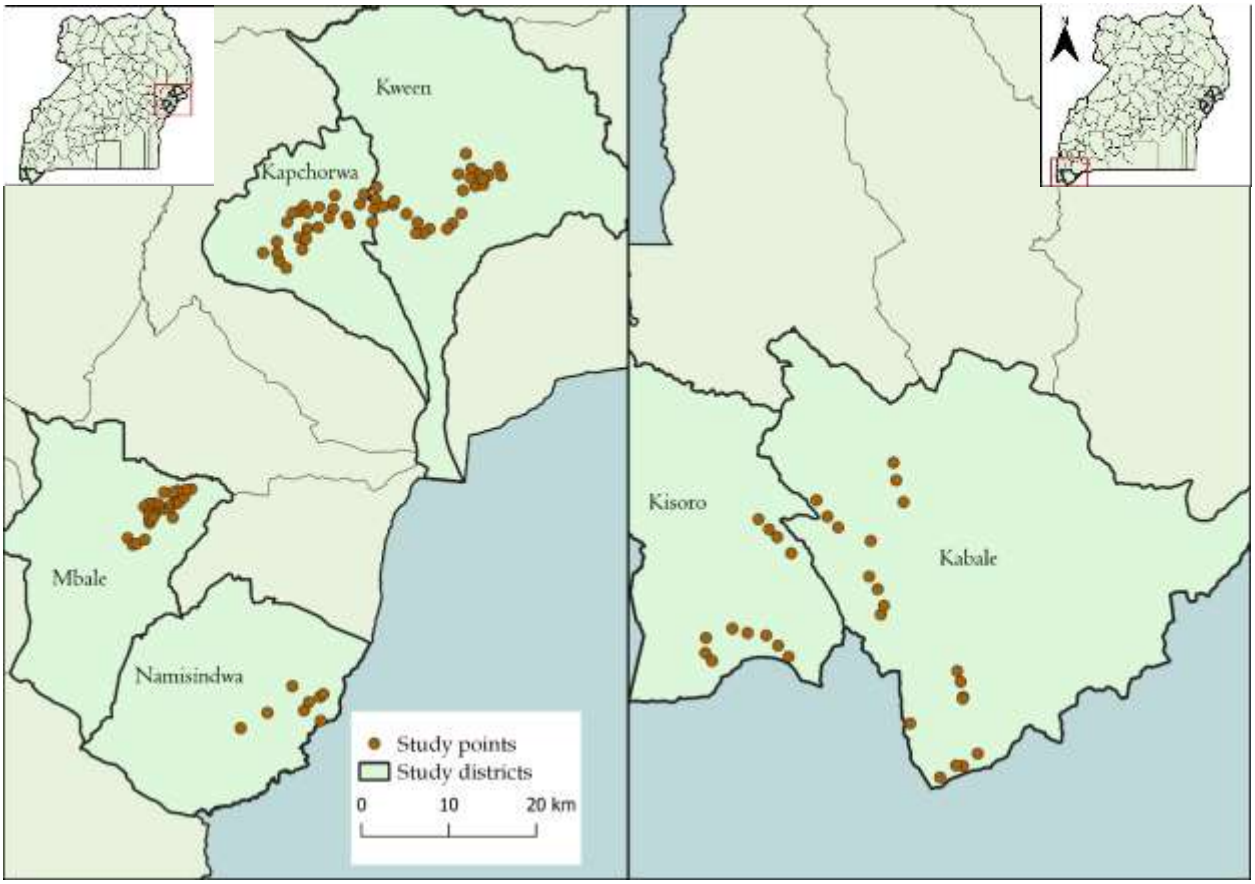
| Region | District | Number of fields surveyed | Number fields where soil samples were collected | Number fields where plant samples were collected * |
|---|---|---|---|---|
| South Western | Kabale | 9 | 9 | 0 |
| | Kisoro | 13 | 13 | 0 |
| | Rubanda | 11 | 11 | 0 |
| North eastern | Kapchorwa | 29 | 29 | 25 |
| | Kween | 30 | 30 | 25 |
| | Mbale | 32 | 32 | 31 |
| | Namisindwa | 8 | 8 | 7 |
| | **Overall** | **132** | **132** | **88** |

* From each plant, samples from leaves, stems, tubers and roots were collected for analyses in the laboratory.

Following each sampling event, all the collected samples were kept in cool-boxes for preservation until analysis. Samples, especially leaves, tubers, stems and roots were transported to IITA analytical laboratories in Kawanda and Sendusu every 2 days of sample collection, for advanced processing and preservation, and to prevent degradation of the same. Geographical coordinates (GPS sampling points) were recorded all the study sights and data downloaded to excel. Also, farms' physical perimeter was obtained by walking around the limits of the fields, in order to have an accurate estimate of the size of each of the sampled farms. Geo-referencing was done for all

study points using QGIS version 3.8.3-Zanzibar (2019). Finally, a structured questionnaire[1] was administered in every farm, after prior consent of the interviewee. Questions captured farmers' practices related to potato cultivation (i.e. use of fertilizers, implementation of rotation systems, type of potato cultivars planted) or elements related to house-hold (HH) decision making, including questions to better understand role of men and women related to potato cultivation (i.e. who responsible for selecting potato varieties, who does pest scouting at HH level).

**Figure 1.** Distribution of study points – potato pest and disease survey 2018-2019, Uganda.




## Sampling preparation and processing for assessment of nematode presence

Soil, root, and tuber samples were subjected to nematode extraction; root samples were cleaned under running water to free the of earth material. Roots where chopped into smaller pieces which

[1] Questionnaire has been shared as Annex 3 during the interim report presented in April 2019. The metadata of this survey is presented in the final report as an excel matrix with all the information available in a softcopy.

were eventually homogenized to create a composite sample. Similarly, tuber samples were also gently washed under running water before peeling them with a knife prior to nematode extraction; the peels from each composite sample were chopped into smaller pieces and homogenized. For nematode extraction, 20 g of homogenised roots and potato peels were measured and use to prepare a modified Baermann tray (Coyne et al., 2007), for the extraction of the mobile infective larval stages (J2) present within the root tissues and on the potato peels.

Prior to extraction of nematode cysts from soil, samples were thoroughly mixed, air-dried, homogenized and sieved (1 mm mesh). Cysts were extracted from three 200 cc of soil sub-samples per field from the already sieved and dried soil, using a Fenwick can (EPPO 2013). For extraction of other types of nematodes present in the soil, this is J2s as well as other free-living nematodes, 100 cc per sub-sample of the processed soil per field were used to set up a Baermann, following the extraction protocol for roots and potato peels. Baermann trays were left 48-72 h at room temperature and the nematodes collected in the Baermann traps were sieved with a 38 µm mesh and concentrated in suspension of 10 ml of water; morphological identification to the genus level was conducted under a compound microscope. Prevalence and Simpson's diversity index – D of the various nematode genus identified were analysed for, based on the following formulas:

$$Prevalence = \frac{Number\ of\ fields\ infested\ with\ nematodes}{Total\ number\ of\ fields\ sampled} * 100$$

$$Simpson's\ diversity\ index, D = \frac{1}{\left(\frac{\sum n(n-1)}{N(N-1)}\right)}$$

n = number of individuals in a given nematode species

N = total number of individuals in all species

## Sampling preparation and processing for assessment of potato diseases

In this report we present the analyses of the 68 samples (Table 1) that were collected from the field during the survey conducted between 9th-23rd June 2019 (north-east Uganda)[2]. Samples received

[2] The results from the samples collected during the November 2018 survey were presented in the Interim Report April 2019.

from the field were kept in a refrigerator at -20 $^0$C (stems and leaves) while tuber samples were kept on shelf at room temperature. Composite samples were obtained from the tubers, stems and leaves respectively collected from each field. For detection by culture on media, tuber, stem, and leaf tissues were surface sterilized for 30 seconds in 1.5% sodium hypochlorite, one minute in 70% ethanol and, finally rinsed thrice in distilled water to remove any trace ethanol. Each composite sample was mixed and crushed in 9 ml sterile distilled water. The extract solutions were then made into two serial dilutions.

### Detection of *Ralsonia solanacearum*

Microbiological detection of bacterial wilt (*Ralstonia solanacearum*) was done on Kelman's Tetrazolium medium, 2% peptone agar medium, and by ELISA detection. Kelman's Tetrazolium Medium was prepared by dissolving to a volume of 1000 ml with distilled water: Casamino acids (1 g), Bacto-Peptone (10 g), Dextrose (5 g) and, Bacto-Agar (15 g). The medium solution was sterilized by autoclaving at 121 $^0$C for 15 minutes. The solution was cooled to about 40-45 $^0$C and filter sterilized; Tetrazolium Chloride was added to obtain a final concentration of 50 mg per 1000 ml. Peptone agar was prepared by mixing 20 g of Peptone agar in 1000 ml of distilled water. The solution was then sterilized by autoclaving at 121 $^0$C for 15 minutes. Thereafter, 10 ml of the media was poured onto 12 cm diameter sterile Petri dishes. To culture the cells, 20 µL of sample from dilutions $D_0$ and $D_2$ were pipetted onto the two media plates. The culture plates were sealed with cling film and incubated on open shelf for two days at room temperature. Following the two-day incubation, plates were placed under a light microscope and the bacterial colonies were visualized at x10 magnification. Each plate was scored separately for the presence (1) or absence (0) of *R. solanacearum*. Detection of *R. solanacearum* by ELISA was conducted using the Enzyme Linked Immunosorbent Assay on Nitrocellulose Membrane (NCM-ELISA) kit developed by the International Potato Center (CIP) for detection of bacterial wilt. Tuber samples pooled from individual fields were crushed respectively and, analysed according to the kit procedure.

### Detection of *Phytopthora infestans*, *Fusarium* spp., *Rhizoctonia solani*, and *Alternaria* spp.

*Phytopthora infestans*, *Fusarium* spp., *Rhizoctonia solani*, and *Alternaria* spp. were investigated on Potato Dextrose Agar (PDA) medium. The medium was prepared by mixing 40 g of PDA in distilled water and then autoclaving at 121 $^0$C for 15 minutes, and 10 ml plated; 20 µL of sample

from dilutions $D_0$ and $D_2$ were pipetted and plated on the media. The plates were sealed with cling film and incubated for two days at room temperature. Following the two-day incubation, plates were placed under a light microscope and microbial growth was visualized at x10 magnification. Each plate was scored separately for the presence (1) or absence (0) of *Phytopthora infestans*, *Fusarium* spp., *Rhizoctonia solani*, and *Alternaria* spp. respectively. Typical fungal growth characteristics of each pathogen were used for observation.

### Detection of Potato Virus diseases

The double-antibody sandwich ELISA (DAS-ELISA) kit developed by the CIP was used to detect for potato viruses Potato Virus X (PVX), Potato Virus M (PVM), Potato Virus Y (PVY), Potato Virus S (PVS), Potato Virus A (PVA), and potato leafroll virus (PLRV). Leaf samples that were pooled from individual plants of the same field, were crushed in a mortar with a pestle. Serological tests were performed on the extracts following procedures outlined in the DAS-ELISA kit. Each viral antigen was detected using a specific antibody with each virus detection completed on a separate plate. Following final incubation, presence of the different type of virus was revealed by colour change from colourless to yellow. The intensity of the colour positively corresponded to the amount of the antigen (virus) in the sample. Each plate was scored separately. A positive well was scored 1 for the presence of virus and negative wells were scored 0 for absence of virus. Quantification of viral load was achieved using the ELISA plate reader.

### Extraction of DNA for further molecular detection

Genomic DNA was extracted from tubers, stems and leaves using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol; 200 mg of leaf, stem and tuber tissue were crashed with little sand and 700 µL of extraction buffer (CTAB buffer). Samples were placed in 1.5 ml tubes and vortexed to obtain a uniform mixture. The samples were incubated at 65 $^0$C for 30 minutes in a water bath with occasional mixing. An equal volume of chloroform: isoamyl alcohol (24:1) was added and the tubes gently mixed and then centrifuged at 10000 rpm for 10 minutes. The upper aqueous phase was pipetted out and transferred to a clean tube and, an equal volume (500 µL) of chloroform: isoamyl alcohol was again added. This procedure was repeated twice. The tubes were incubated at -20 $^0$C over night to precipitate the DNA. The DNA was pelleted by centrifuging at 13000 rpm for 10 minutes in a chilled centrifuge. The supernatant was discarded, and the DNA

pellet was washed with 800uL of cold 95% ethanol. The tubes were invented on a clean paper towel for 1hour to air dry the pellet. 50 µL of 1x TE buffer was added to dissolve the pellet. Finally, 2 µL of (10 mg/ml) RNase was added and, the mixture was incubated at 37 $^0$C for 30 minutes in the water bath to degrade (RNA). The quality of DNA was checked by electrophoresis on 1 % *w/v* agarose gel. Its quantity and purity were determined using the NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA). The DNA was stored at -80 $^0$C for future pathogen analysis.

Data were recorded on the scores of *Ralstonia solanacearum*, *Phytopthora infestans*, *Fusarium* spp., *Rhizoctonia solani*, *Alternaria* spp. and potato viruses. Scores were summed for districts and tissues respectively. The frequencies and distribution were computed and analyzed using MicroSoft Excel.

## Sampling of insect pests and assessment of specie's incidence and prevalence

During field surveys, ten plants per field were assessed for signs of damage by the following insects and diseases:

1. **Potato tuber moth (PTM) foliar damage:** Ten plants were assessed per field for PTM damage (infestation rate). The number of infested leaves per plant and number of PTM larvae or mines per plant were recorded (infestation intensity).
2. **Leaf miner flies (LMF) foliar damage**: Ten plants per field were assessed visually for LMF larval damage. Percentage of leaf area for the upper, middle and lower part per plant affected by LMF was estimated.
3. **Aphid and whitefly abundance assessment**: Ten plants were searched for both aphids and whiteflies. The number of aphids and whiteflies per plant were counted.

Infestation rates, incidence and prevalence were assessed visually and calculated based on the equations listed below. Data for infestation rates/incidence, prevalence and severity were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute Inc, 2008).

$$Prevalence(\%) = \frac{\left(\begin{matrix}Number\ of\ fields\ with\ atleast\ one\ plant\ showing \\ disease\ symproms\ or\ pest\ damage\end{matrix}\right)}{Total\ number\ of\ surveyed\ fields} \times 100$$

$$Incidence\ or\ Infestation\ rate\ (\%) = \frac{\left(\begin{matrix}Number\ of\ plants\ showing \\ disease\ symptoms\ or\ pest\ damage\end{matrix}\right)}{Total\ number\ of\ sampled\ plants} \times 100...$$

$$Severity\ or\ Infestation\ Intensity(\%) = \frac{\left(\begin{matrix}Leaf\ area\ damaged\ by\ a\ pest \\ or\ disease\end{matrix}\right)}{Total\ leaf\ area\ on\ a\ plant} \times 100.$$

# Results from the epidemiologic survey

## Results from nematology analyses

Higher diversity of plant parasitic nematode (PPN) species was observed in NE region, compared to SW (Table 2). Just like in the NE region, soil samples registered higher PPN diversity than root samples. Soils act as reservoir for pathogenic nematode species just waiting for a suitable host to infect. These observations in part may be evidence for survival of these species on alternative weed hosts in absence of their preferred hosts, multiplying inoculum for crops in subsequent seasons.

**Table 2:** Diversity of nematode species across regions and samples

| Region/sample | Simpson's diversity index (D) |
|---|---|
| South western | 1.88 |
| North Eastern region | 2.11 |
| Root samples | 1.23 |
| Soil samples | 4.03 |

Root-knot nematodes (RKN) (*Meloidogyne* spp.) were the most prevalent species recovered from soil in 70 out of 32 fields (53%) sampled, with Kabale and Kween districts recording the highest prevalence at 77 and 76%, respectively; by regions, the fields sampled in SW registered the highest prevalence of RKN at 57%, followed by the NE region (51% prevalence) (Figure 2). Spiral nematodes (*Helicotylenchus* spp.) were recovered from 103 out of the 132 fields (78%) with Kabale, Kapchorwa, Mbale and Namisindwa districts registering the highest prevalence of over 80% within field soils. Lesion nematodes (*Pratylenchus* spp.) and burrowing nematodes (*Radopholus* spp.) were recovered at 17% and 6%, respectively, across all the fields surveyed (Figure 3). Other nematode genera, including lance nematodes (*Hoplolaimus* spp.) and *Rotylenchulus* spp. also appeared in minor proportion in Kisoro, Kabale and Rubanda districts.

Related to nematode infestation observed in plants' roots and tubers, in the NE region, 35% of the potato fields plants were infested with RKN, followed by lesion and spiral nematodes at 14%

prevalence (Figure 3). Mbale district registered the highest number of fields with plants infested with nematodes (61% prevalence), while Kapchorwa district recorded the least number of fields with plants infested with nematodes (24% prevalence). Overall, 44% of the 88 fields from which root samples were collected in the eastern region had their plants infested with nematodes and showed symptoms in their tubers (Figure 3) or in the roots (Figure 4).

**Figure 2**. Soil prevalence (%) of PPN species in all the potato growing districts of sampled in Uganda.

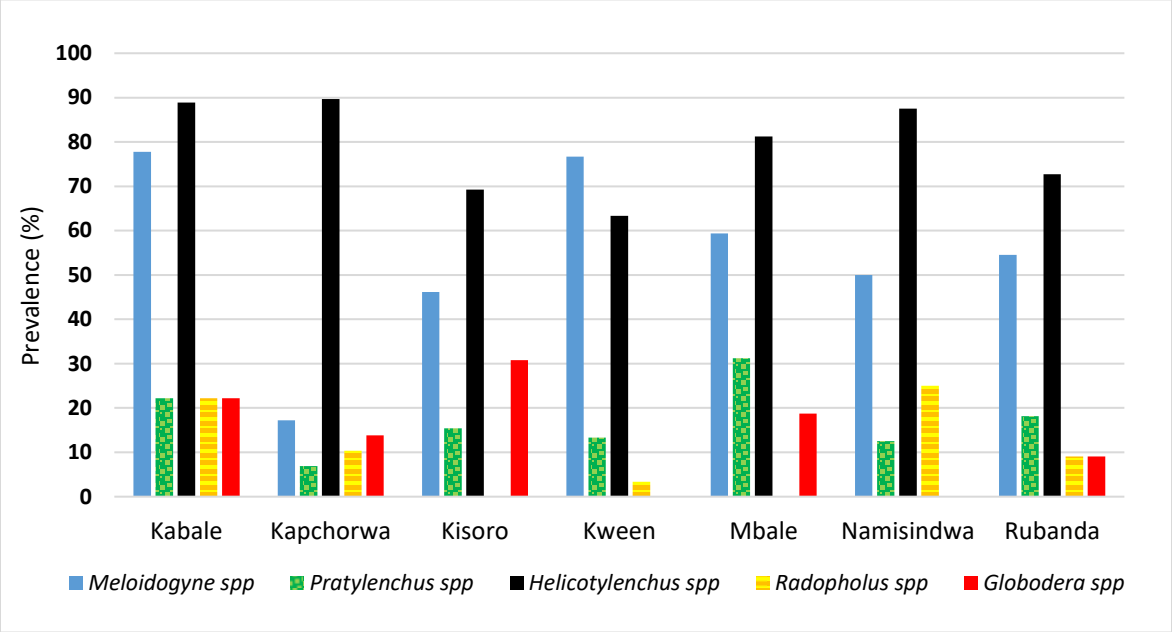
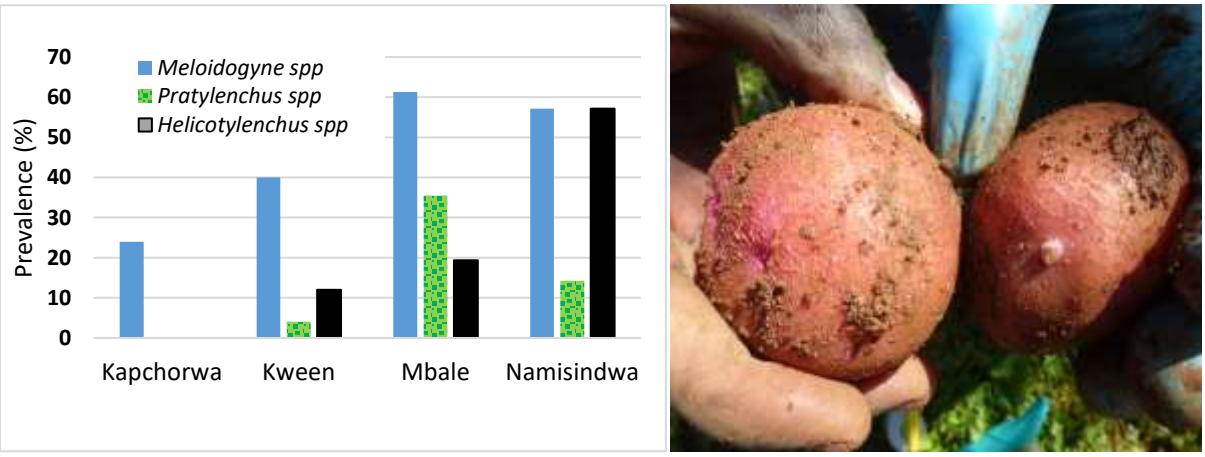



**Figure 3.** (Left) prevalence (%) of PPN species observed in roots and tubers of potato collected from field samples across Uganda; (Right) an example of damage caused by RKN in potato tubers.

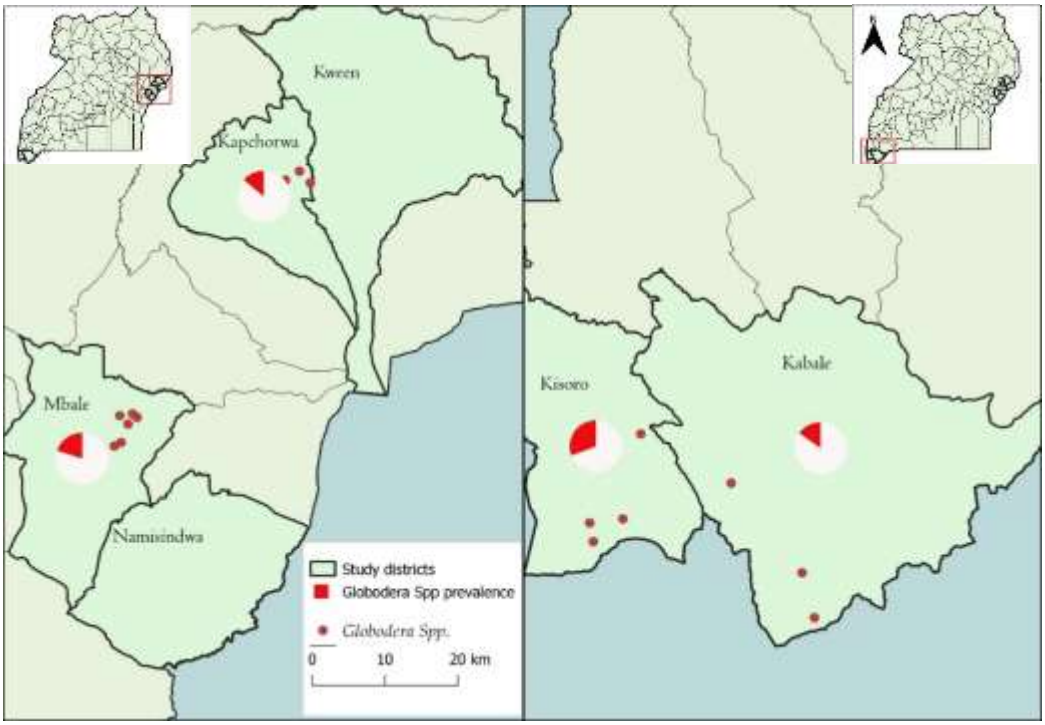


Cysts (of potato cyst nematodes –*Globodera spp.*) were recovered from 17 out of 132 fields (12.8%) sampled, with Kisoro district recording the highest number of fields infested with potato cyst nematodes at 30.7% prevalence (Figure 5). All fields sampled in Kween and Namisindwa districts were free of potato cyst nematodes. **Koch postulates, following molecular and morphologic characterization of the cyst isolated in Uganda were conducted to confirm the species; our results indicate that the cyst belong to a population of *Globodera rostochiensis*, and this represents the first confirmed report of the presence of this species in Uganda**. Potato cyst nematodes were more prevalent in the south western region at 21% as opposed to 10% in the north eastern region. Specific data regarding location (GPS), owner of the farm, inoculum levels and potato cultivar grown in the fields that were found to be positive for PCN are available in Annex 1. The results related to PCN identification have been sent as a First Disease Note to Plant Disease Journal, and this paper has been accepted for publication (see Annex 1).

**Figure 4.** (Left) Comparison of two root systems of potato recovered from a field with a healthy plant (left) and a RKN-infected plant (right); (right) Sampling process.

In addition to the identification of *G. rostochiensis*, another cyst nematode species has been isolated. *Globodera artemisiae* has been identified molecularly in one field in Mbale (MBL04); this species can be frequently confused with *G. rostochiensis*, a species that infects Artemisia. Further molecular, morphological and pot tests studies are currently being conducted to confirm this report and explore the potential implications of the presence of this species in potato growing areas of Uganda.

**Figure 5.** Distribution of potato cyst nematodes (*Globodera rostochiensis*) in SW and NE Uganda.




Overall, **81% of all fields recorded nematode infestation**, considering both soil and plant samples. Presence of these nematode species in potato roots is evident to the wide host-range preference of these organisms, particularly RKN, and prevailing susceptibility of underlying potato cultivars grown by respective farmers.

## Results from potato disease analyses

***Prevalence of bacterial wilt (Ralstonia solanacaerum):*** On Kelman's Tetrazolium medium, red-pinkish colonies were observed (Figure 6) and, on 2% Peptone agar medium, white mucoid colonies were observed (Kinyua et al., 2012). **All farms surveyed in Mbale had bacterial wilt infection while Kapchorwa had 92% of farms infected**. Namisindwa had 33% farm infection and Kween 86.4%. These observations were confirmed with NCM-ELISA tests (Figure 7); DAS-ELISA scores are summarized in Table 3.

**Table 3:** Sum of scored for DAS-ELISA detection *Ralstonia solanacaerum* recorded for each district

| Districts | Sum of scores |
|---|---|
| Kapchorwa | 10 |
| Kween | 11 |
| Mbale | 4 |
| Namisindwa | 5 |

***Prevalence of Phytopthora infestans, Fusarium* spp.*, Rhizoctonia solani,* and *Alternaria* spp.*:*** Microscopic examination of cultures on PDA medium detected *Fusarium spp* which had a purplish color with micro and macro conidia (Figure 8). **Fusarium infections were generally low**, with Kween having 3 infected farms, Namisindwa two and Mbale one farm only. ***Phytopthora infestans, Rhizoctonia solani*, and *Alternaria* spp. were not observed on any culture and therefore not present on any of the farms sampled.**

**Figure 6**: Visualization of *Ralstonia solanacearum* colonies on Kelman's Tetrazolium media.

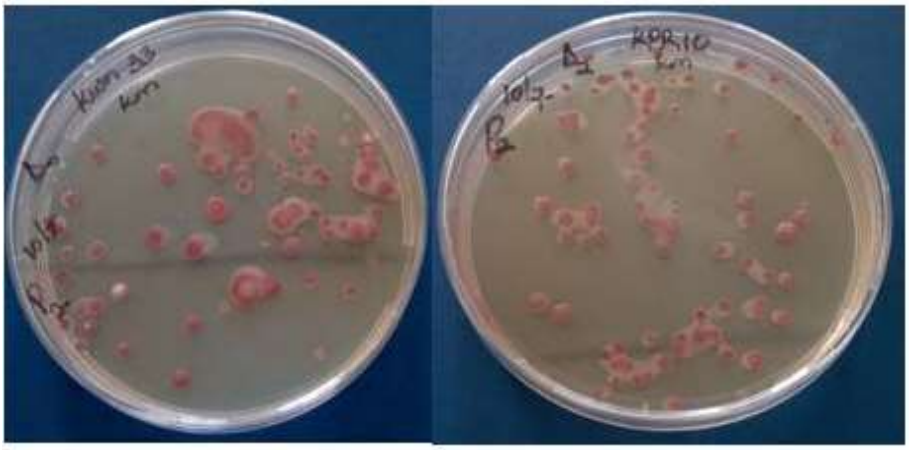


**Figure 7:** Nitrocellulose membranes of NCM-ELISA showing both positive and negative *Ralstonia solanacearum* infections in tubers.

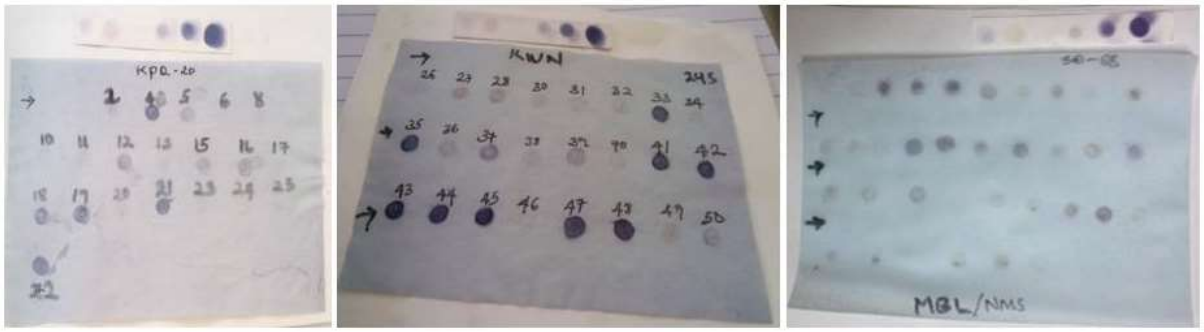


**Figure 8.** Visualization of *Fusarium* spp. colonies on Potato Dextrose Agar (PDA).


***Prevalence of Potato virus diseases:*** All districts were positive for all the potato viruses tested PVX, PVS, PVM, PVY, PVA and PVRV (Figure 2). Related to regions, the highest incidence of viral diseases was found in Kween (19.9 % of the fields) while Namisindwa had the lowest (7.6%). Related to viruses, the most prevalent was PVS (15.9%) while the least prevalent was PVA (1.5%); See detailed information per district in Table 4.

**Figure 2**. ELISA plates for the different viral diseases detected in potato leaves

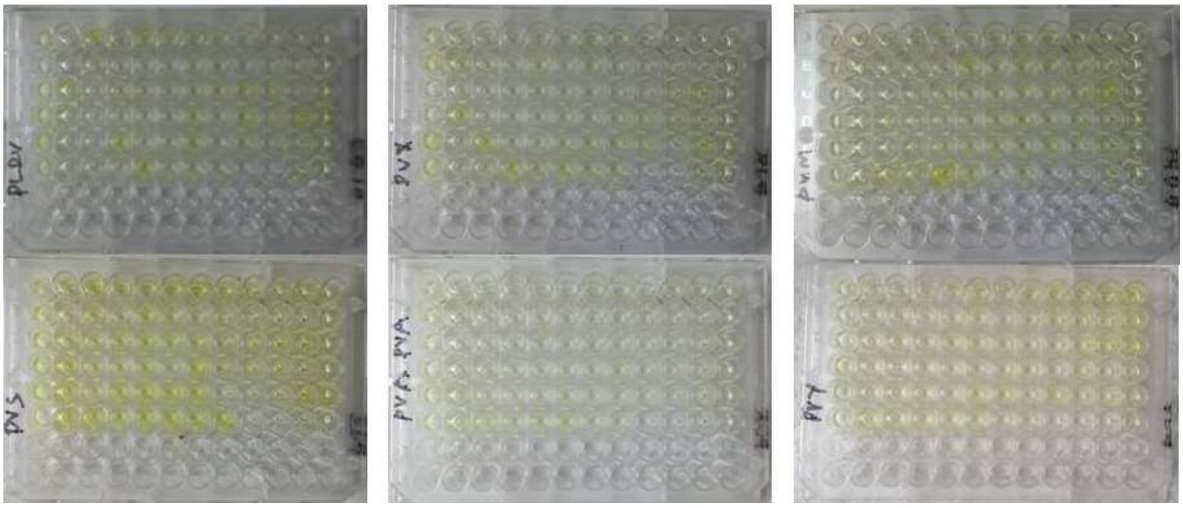


**Table 4.** Sum of scored for NCM-ELISA detection of potato viruses recorded for each district

| District | PVX | PVM | PVY | PVS | PVA | PLRV |
|---|---|---|---|---|---|---|
| Kapchorwa | 7 | 20 | 12 | 25 | 0 | 15 |
| Kween | 13 | 23 | 5 | 25 | 0 | 15 |
| Mbale | 8 | 9 | 6 | 9 | 6 | 3 |
| Namisindwa | 7 | 9 | 6 | 6 | 0 | 3 |
| Total count | 35 | 61 | 29 | 65 | 6 | 36 |

## Results from potato insect pest analyses

**Potato tuber moth (PTM) damage:** infested potato fields as absent in one (Namisindwa) of the four districts sampled (Figure 10). PTM prevalence rates was highest in Kapchorwa and Kween districts (92n%) and lowest in Mbale district. Despite the high prevalence rates in the two districts, incidence levels were relatively low i.e. < 35 % (Figure 11). Severity levels (PTM foliar defoliation rates) were also very low (<1%) in all the three districts (Table 5).

**Leaf miner flies (LMF) foliar damage:** LMF damage was seen in less than half of the sampled fields and only in Kapchorwa, Kween and Mbale districts (Figure 10). The number of plants showing LMF damage per field were also relatively low in the three districts (3-34 %). The severity of LMF damage on leaves was equally very low in the three districts ranging from <1 to about 2% (Table 5).

**Aphid abundance:** Aphids occurred in all the four districts sampled and in all farmers' potato fields Namisindwa district (Figure 10). However, prevalence rates in the other three districts was below 50%. Except for fields in Namisidwa district, less than a quarter of the potato plants within a field were infested by aphids in the other three districts; the average number of aphids per plant was highest in Namisidwa at 172.1 and lowest in Kapchwora (< 1). Aphid infestation was generally low ($\leq$ 2 aphid per plant) in Kapchorwa, Kween and Mbales districts.

**Whitefly abundance:** White fly infestation was absent in Kapchwora and Kween districts and generally low (< 40%) in Mbale and Namisindwa districts (Figure 10). The infestation rates and number of whiteflies in the two districts were very low ($\leq$ 1 whitefly per plant) (Figure 11; Table 5).

**Figure 3**. Prevalence of insect pests in farmers' potato fields in Uganda (June 2019 survey).

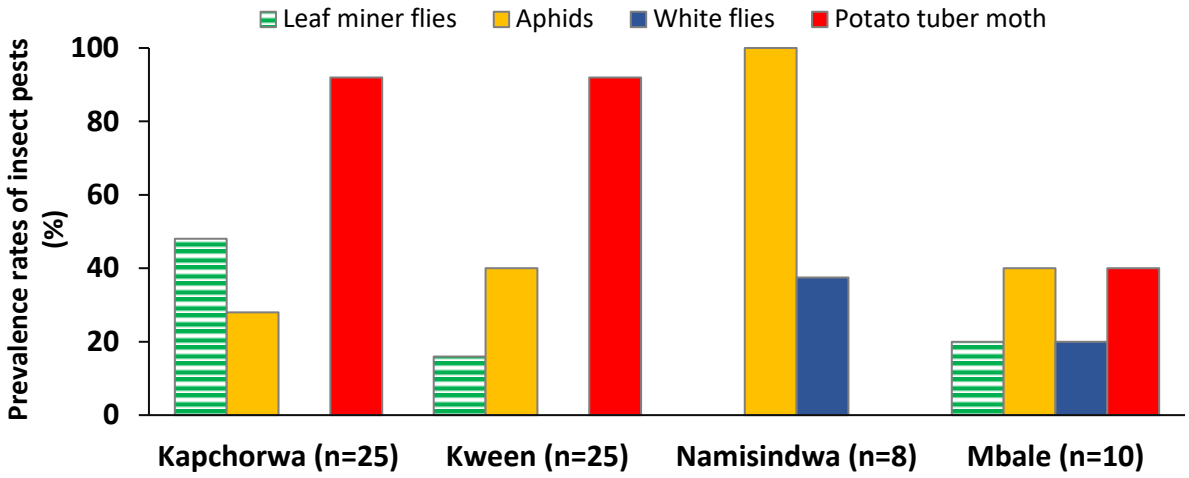



**Figure 4**. Incidence of insect pests in farmers' potato fields in Uganda (June 2019 survey).

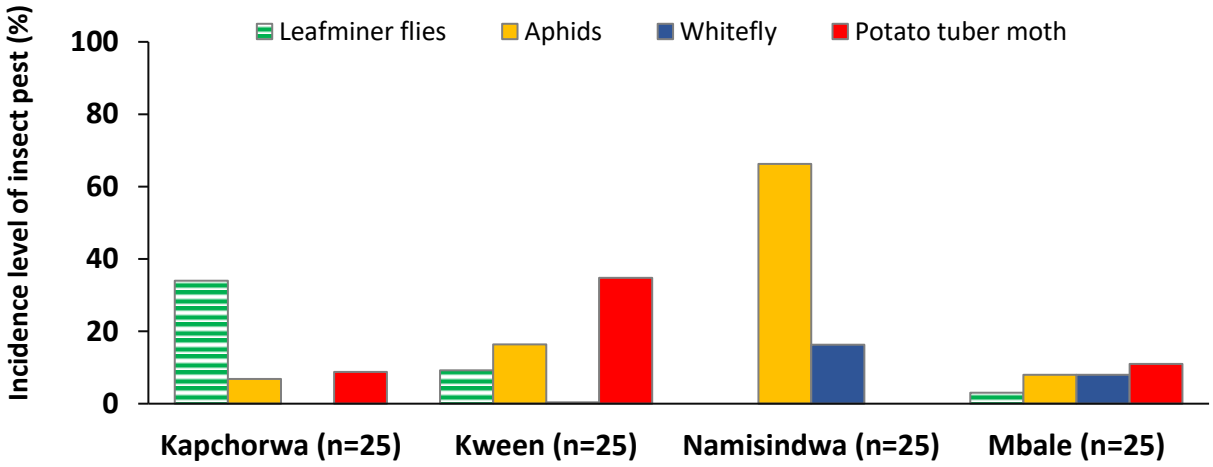



**Table 5.** Infestation rates (severity) of insect pests in farmers' potato fields in Uganda (June 2019 survey)

| | Kapchorwa (n=25) | Kween (n=25) | Mbale (n=10) | Namisindwa (n=8) | Overall (n=68) |
|---|---|---|---|---|---|
| Leaf area damaged by leaf miner fly (%) | 1.7 ± 1.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0 | 0.7 ± 0.4 |
| Number of aphids per plant | 0.4 ± 0.2 | 2.2 ± 1.2 | 0.9 ± 0.5 | 172.1 ± 63.7 | 21.3 ± 9.8 |
| Number of white flies per plant | 0 | 0 | 0.1 ± 0.1 | 1.0 ± 0.8 | 0.1 ± 0.1 |
| Number of leaves affected by PTM larvae | 0.3 ± 0.1 | 0.9 ± 0.2 | 0.2 ± 0.2 | 0 | 0.5 ± 0.1 |
| Number of PTM larvae seen | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.1 ± 0.1 | 0 | 0.2 ± 0.1 |
| Proportion of leaves damaged by PTM (%) | 0.3 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0 | 0.2 ± 0.1 |
| Number of PTM adults seen | 0.5 ± 0.1 | 0.6 ± 0.1 | 0 | 0 | 0.4 ± 0.1 |

## Conclusions and recommendations

The most relevant findings of the surveys that were undertaken from November 2018 until October 2019 is the confirmation, for the first time ever, of the presence of PCN (*Globodera rostochiensis*) in seventeen potato fields (both in NE and SW areas) in Uganda. In most of the cases, farmers are growing the same potato variety (cv Rangume or Kingini), which is quite likely to be susceptible to PCN. Hence, the involvement of CIP and private potato seed companies for the provision of PCN-resistant varieties that are suitable to the local agronomic needs and to farmers' preferences is paramount for the future of the potato industry in Uganda. Whenever possible, movement of contaminated seeds and soil from the farms that became positive for PCN infestation to neighbouring fields should be avoided and farmers should be made aware of the risks liked to this pest. PCN is a pest that is nearly virtually impossible to eradicate, once established in a field, so preventive measures to avoid spread of PCN must be prioritized. The incidence levels PCN detected (see Table 1, Annex 1) are low compared to the incidence of PCN observed in other neighbouring countries (Coyne and Cortada, personal communication); thus, at this stage, the implementation of contingency measures and the awareness creation among farmers and extension service providers from national programs (NARO and MAAIF) is paramount to minimize the spread of the pest across the country. Nevertheless, PCN is usually detected once a minimum level of inoculum (cyst / 200 cc soil) has been built in the soil and therefore, it cannot be discarded that the presence of PCN could be further spread, but it is still under the detection level thresholds. In addition, the preliminary molecular detection of *G. artemisiae* (still unconfirmed) also shades light into the possibility that other species, including the PCN species *G pallida*, could also be present in the country. Further prospections in a near future may be needed, in order to monitor the evolution of the populations and the potential increase of the inoculum levels of PCN in the soil. Capacity building of national stakeholders (farmers, extension officers, national programs) will be fundamental to manage PCN in the country, in a near future. Finally, the confirmation of the presence of *G. rostochiensis* in Uganda corroborates that PCN is a quarantine invasive species now spread across EA (Kenya, Uganda and Rwanda) and hence, there is an urgent need to assess the presence of this pest in other neighbouring countries (Tanzania, Malawi, Ethiopia, DRC). Having an understanding on what is the prevalence and incidence of PCN in the region is key for the potato

sector and a fundamental step for the implementation of a regional strategy for contention and mitigation of PCN.

Based on the results obtained in this survey, bacterial wilt caused by *Ralstonia solonacearum* is also a widespread threat to potato producers, as it featured as the most prevalent potato disease that was detected across all the farms surveyed. This is a quarantine pest that seriously hampers potato production, and its impact is aggravated in fields where PPN are present, as bacteria in the soil have easier access to the vascular cylinder of the plants through the wounds infringed in the roots by the infective nematodes. Noteworthily, the prevalence levels of other pathogenic fungi from the soil were very low, suggesting that most of the soil health problems could be derived from the bacterial wilt- nematode combination. Another concern is the silent but fast spreading of several virus diseases, as was evidenced by the visual observations in the fields, but also from the results obtained in the laboratory using molecular techniques. The high prevalence levels of bacterial wilt and viruses could be as a result of low-quality potato seed, usually recycled over a few seasons, being distributed in the sub region, coupled with lack of information on the potential importance of such plant health threats. Efforts should be made to further survey more areas for purposes of characterizing the bacterial wilt strains and viral diseases of potato.

Beyond RKN, that were widely present in the survey regions as expected, the lesion and spiral nematodes may not have potato as their preferential host, however, these use potato as a transition host until such a time when their preferred hosts are re-cultivated. These conditions may in part be responsible for the recurrent prevalence of not only these species of nematodes but also several other species among farmer fields. Except for the high aphid numbers recorded in Namisindwa district, insect infestation intensity levels observed in the current study were very low. This could be due to routine application of insecticides. All farmers interviewed indicated that they use insecticides (several different products) and that they use them alone or in combination and, mostly, on a calendar basis, usually with more than 4 applications per cropping season. Farmers in Mbale and Kapchorwa have previously reported using tank mixtures containing both insecticides and fungicides on a routine basis in their potato fields (Okonya and Kroschel, 2015). The high number of aphids recorded in Namisindwa could be because this is a major potato producing area and only few farmers had planted potato during the survey period. The elevation

of Namisindwa district was also lower than that of the other three districts meaning that temperatures here are higher and hence favour faster insect pest development.

Farmers in the Elgon and southwestern region (considering Uganda districts neighbouring Rwanda) overly depend on potato for their livelihood. Consequent, to this overdependence is the mono-cropping of potato what presents an annual breeding ground for potato nematodes and other biotic constraints observed in this study. In north eastern Uganda, particularly in districts such as Mbale, Namisindwa, Kapchorwa, and Kween, most potato fields are male owned. In these fields, males took the highest of responsibilities including choice of planted crop, potato variety planted and pest control. Rwangume, a potato variety registered the highest preference among male and female owned potato fields. Understanding the roles and responsibilities of men and women is important in order to increase awareness and build up farmers knowledge to implement effective and durable management systems, and target each of the two groups with specific activities, based on the type of interventions needed (i.e. introduction of new potato varieties, improve pest and disease scouting, implementation of rotation systems). Environmentally friendly pest control approaches should be encouraged, as much as possible, to achieve an integrated pest and disease control program to keep nematodes, insect pests, and observed diseases in check, which will eventually benefit farmers and conserve the environment.

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## Annex 1: PLANT DISEASE NOTES

NOTE: This paper has been submitted o Plant Disease and has been accepted with minor revisions; this is pending re-submission.

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**First report of potato cyst nematode *Globodera rostochiensis* (Wollenweber, 1923) infecting potato (*Solanum tuberosum* L.) in Uganda.**

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Potato cyst nematodes (PCN; *Globodera rostochiensis*, *G. pallida*) are quarantine pests and have recently been reported from Kenya and Rwanda (Mwangi *et al*. 2015; Mburu *et al*. 2018; Niragire *et al*. 2019). In East Africa, potato is an important staple food crop for millions of people, although yields are currently far below potential. Field surveys were conducted in Uganda to assess the incidence of PCN in farmers' potato fields. Soil samples were collected from 124 farms in areas neighboring Kenya and Rwanda (November 2018 - April 2019). Within each field, a ≈2 Kg composite soil sample from 20 sample points was constituted. Soil was thoroughly mixed, air-dried and sieved (1 mm mesh). Nematode cysts were extracted from three sub-samples of 200 cc

per field sample, using a Fenwick can (EPPO, 2013). Cysts were recovered from 17 samples (13.7%) with counts $\bar{\chi}$ = 2.6 cyst/ 200 cc soil. One cyst from two randomly selected fields (MBL 03, MBL 07; Table 1) was dissected under a stereo-microscope: 20 eggs/ cyst were separately inoculated onto potato (*Solanum tuberosum*) cv. 'Shangi' plants in pots containing steam sterilized loam soil. Plants remained in the greenhouse and harvested after three months. A mean $\bar{\chi}$ = 12 cyst/ plant were recovered and 15 females and 31 second-stage juveniles (J2s) used for morphometric analyses. Female length (L) ranged from 280.5 – 446.3 µm ($\bar{\chi}$ = 365.1 ± 45.0 µm), width (W) from 200.3 – 440.5 µm ($\bar{\chi}$ = 319.3 ± 63.4 µm) and the L/W ratio was 1.2 ± 0.2; Granek's ratio (*n* = 8) varied from 1.57 – 3.52 µm, ($\bar{\chi}$ = 2.7 ± 0.6 µm) and the distance from anus to vulval basin was 26.31 – 62.73 µm ($\bar{\chi}$ = 50.1 ± 11.7 µm). The J2 stylet length ranged from 14.93 – 22.59 µm ($\bar{\chi}$ = 19.37 ± 1.86) with round-shape stylet knobs. Length for the hyaline tail was 12.64 – 27.63 µm ($\bar{\chi}$ = 23.05 ± 3.80) and the true tail ranged from 30.06 – 54.48 µm ($\bar{\chi}$ = 43.33 ± 4.87 µm); body length varied from 332.02 – 427.29 µm ($\bar{\chi}$ = 394.00 ± 19.30); all morphometric parameters matched those described for *G. rostochiensis* (Subbotin & Franco, 2012). DNA was extracted (Qiagen DNeasy® Blood & Tissue kit; Qiagen Group; USA) and amplified using candidate ITSF/R primers targeting the ITS1-5.8S-ITS2 regions (modified from Mburu *et al.* 2018). One PCR reaction contained 0.5 µM of each primer (forward and reverse), 5 µl of 5X GoTaq® Buffer (Promega), 2 mM $MgCl_2$, 200 µM DNTPs, 0.125 µl GoTaq® DNA polymerase (5 u/ µl), 1 µl DNA template (final volume = 25 µl). PCR cycling included 2 min initial denaturation phase and 40 PCR-cycles. The PCR amplicons (500 bp) were sequenced and edited using BioEdit Sequence Alignment Editor. DNA sequences were analyzed using the NCBI-BLAST tool: sequences MN450308 and MN450309 showed similarity to the 5.8S (*E*= $4e^{-140}$; 97.64% identity) and 18S (*E*= $5e^{-139}$; 98.61% identity) ribosomal RNA gene of non-African *G. rostochiensis* isolates. A phylogenetic analysis showed that Ugandan populations cluster with Kenyan *G. rostochiensis* isolates but are less closely related to Rwandan populations or other *Globodera* species. Our findings highlight the need to conduct a comprehensive epidemiologic survey for developing a regional PCN-management strategy.

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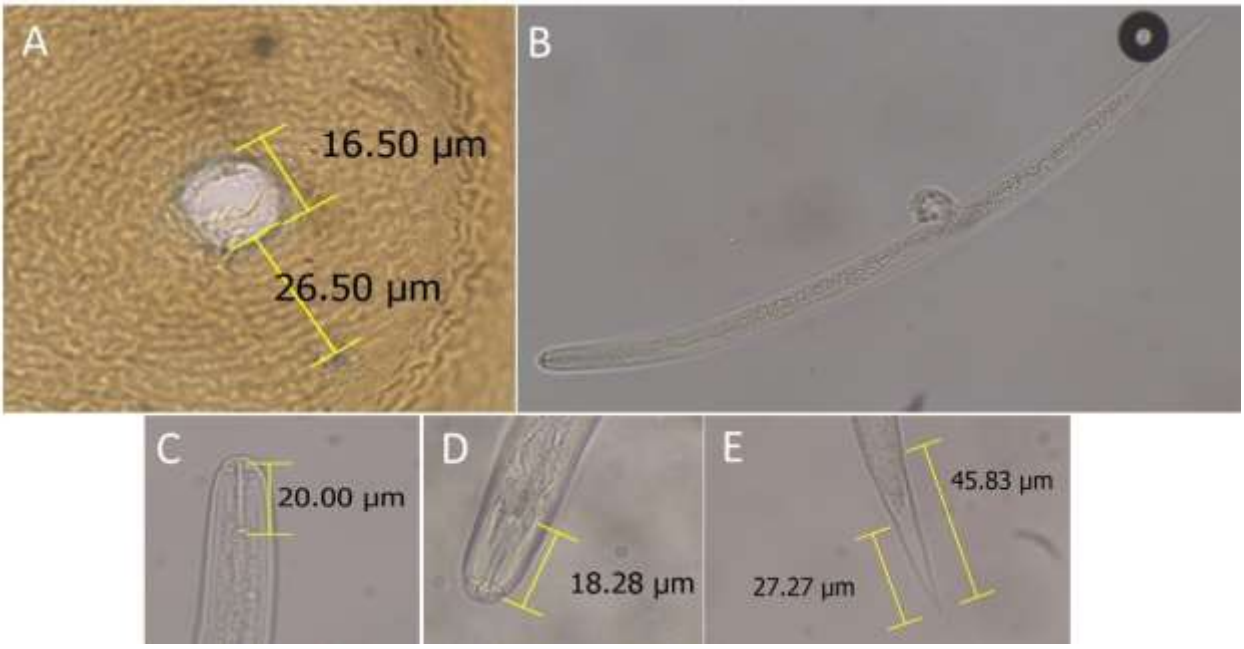


**Figure 5.** Morphometric analysis of *Globodera rostochiensis* isolate from Uganda. **(A)** females' vulval cones showing the measurement of the vulva diameter (16.50 µm) and the distance from the vulva to the anus (26.50 µm); **(B)** Body habitus of a J2; **(C & D)** Pharyngeal region of a second-stage juvenile (J2), presenting stylet measurements from the base of the stylet to the tip and displaying rounded stylet-knobs **(D)**; **(E)** J2's true tail (47.82 µm) and hyaline tail (25.07µm).

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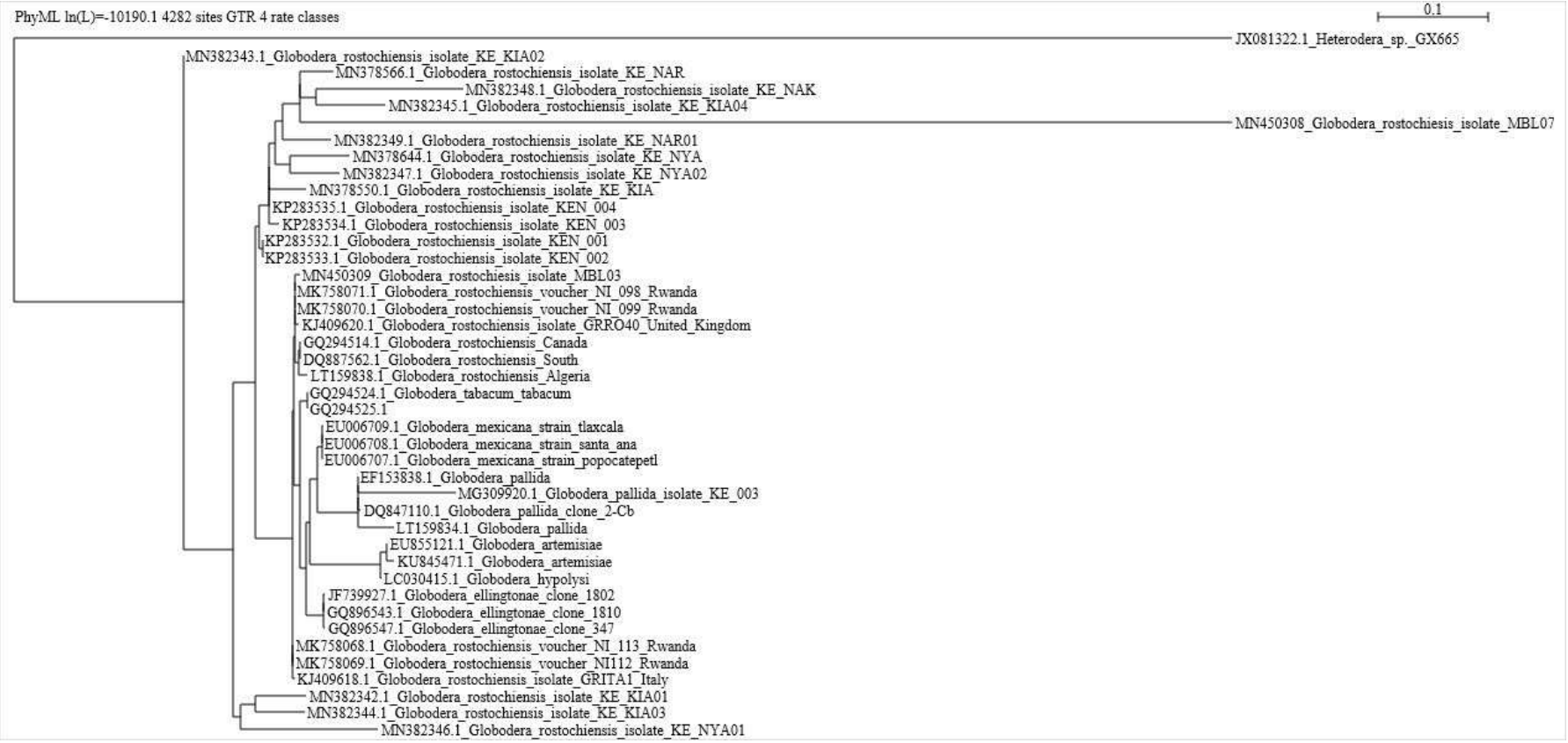


**Figure 6.** Phylogenetic tree presenting the *Globodera rostochiensis* isolates MN450308 and MN450309 aligned with isolates of seven *Globodera spp*., including isolates from Kenya and Rwanda. Phylogenetic tree was done using Tree Figure Drawing Tool Version 1.4.3 to Edit the Tree. 2006-2016, Andrew Rambaut Institute of Evolutionary Biology, University of Edinburgh.

**Table 3.** Field in Uganda situated in north-east and south-western regions where cyst nematodes were isolated, including information on altitude of the fields (m.a.s.l.- meters above sea level), GPS coordinates, symptoms observed in the potato crops whenever these were observed in the field, potato variety planted in the field at the time the sampling took place and the number of cysts extracted from 200 cc of soil.

| Sample ID | District | Regions | Altitude (m.a.s.l) | GPS North | GPS East | Generalized wilting of potato crop | Patchiness observed in the crop | Stunted growth observed in potato crop | Potato variety planted | Cysts per 200 cc soil |
|---|---|---|---|---|---|---|---|---|---|---|
| KPR 04 | Kapchorwa | North East | 2048 | 01$^0$20.304’ | 034$^0$24.519’ | Yes | Yes | Yes | Rwangume | 1 |
| KPR 19 | Kapchorwa | North East | 2113 | 01$^0$04.290’ | 034$^0$15.997’ | Yes | Yes | No | Unknown | 1 |
| KPR 26 | Kapchorwa | North East | 2072 | 01$^0$22.865’ | 034$^0$28.038’ | No crop | No crop | No crop | N/A | 1 |
| KWN 51 | Kween | North East | 2222 | 01$^0$22.180’ | 034$^0$26.996’ | No crop | No crop | No crop | N/A | 1 |
| MBL 03 | Mbale | North East | 1966 | 01$^0$02.128’ | 034$^0$14.307’ | Yes | No | No | Rwangume | 4 |
| MBL 04 | Mbale | North East | 1894 | 01$^0$02.395’ | 034$^0$14.810’ | Yes | Yes | Yes | Rwangume | 6 |
| MBL 07 | Mbale | North East | 2037 | 01$^0$04.565’ | 034$^0$15.652’ | Yes | Yes | No | Rwangume | 5 |
| MBL 10 | Mbale | North East | 1929 | 01$^0$04.415’ | 034$^0$14.719’ | No | Yes | Yes | Rwangume | 4 |
| MBL 13 | Mbale | North East | 1952 | 01$^0$03.780’ | 034$^0$15.322’ | Yes | No | Yes | Rwangume | 2 |
| MBL 62 | Mbale | North East | 2078 | 01$^0$21.981’ | 034$^0$28.841’ | No | No | No | Rwangume | 2 |
| KBL 28 | Kabale | South West | 2332 | 01$^0$23.130’ | 029$^0$56.908’ | No crop | No crop | No crop | N/A | 1 |
| KBL 33 | Kabale | South West | 2241 | 01$^0$26.646’ | 029$^0$57.864’ | No crop | No crop | No crop | N/A | 1 |
| KSR 01 | Kisoro | South West | 2028 | 01$^0$19.226’ | 029$^0$40.556’ | No | Yes | Yes | Kinigi | 2 |
| KSR 11 | Kisoro | South West | 2246 | 01$^0$12.260’ | 029$^0$44.490’ | No | Yes | Yes | Kinigi | 1 |
| KSR 03 | Kisoro | South West | 2219 | 01$^0$20.692’ | 029$^0$40.835’ | No | Yes | Yes | Rangume | 10 |
| KSR 08 | Kisoro | South West | 1996 | 01$^0$18.912’ | 029$^0$43.111’ | No crop | No crop | No crop | N/A | 1 |
| RBD 20 | Rubanda | South West | 2019 | 01$^0$16.101’ | 029$^0$51.450’ | No crop | No crop | No crop | N/A | 2 |