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A Survey of Free-Living Plant-Parasitic Nematodes Associated with Damage in Carrot (*Daucus carota* var. *sativus*) Fields in Norway

Marte Persdatter Tangvik
Plant Sciences

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Abstract

Nematodes cause yield reduction in agriculture worldwide. In research, the main focus has been on cyst nematodes and root-knot nematodes and not so much on free-living plant-parasitic nematodes. Thus, the importance of free-living plant-parasitic nematodes is likely underestimated. The aim of this survey was to get an overview of free-living plant-parasitic nematodes in carrot (*Daucus carota* var. *sativus*) production, and their association with damage. Soil samples were collected from 19 fields in the carrot-producing regions of Norway where nematode damage was suspected. The samples were analysed for free-living plant-parasitic nematodes. The analyses showed that free-living plant-parasitic nematodes occur frequently in carrot fields in Norway. Root lesion nematodes (*Pratylenchus* spp.) were the most frequently occurring genus and *P. crenatus* the most frequently occurring species. Symptoms of nematode damage were observed in all the samples where taproots were received for analysis. Symptoms observed in this survey include, but are not limited to, necrotic spots, forked taproots, split taproots, short taproots and taproots with galls. In fields with a high occurrence of *Longidorus* spp. and *Xiphinema*, growth of carrots seems to be severely disturbed. Geographical distribution of *Xiphinema* spp. seems to be limited to the south of Norway.

Keywords: Free-living plant-parasitic nematodes, carrot, distribution, symptoms, Norway

Preface

Ever since I first learned about nematodes back in 2014, I have been fascinated by these little creatures; the most abundant multicellular organism on earth, and yet they receive so little attention. Their mysterious world fascinated me to such a degree that I decided to my Master's thesis in the field of nematology. To increase my knowledge in nematology, as well as generally broaden my scientific horizon, I went to Wageningen University in The Netherlands to study for a year. I chose Wageningen because it is one of the top universities in the field of agriculture, and more specifically because they have a great research group in nematology. After a year of studying at Wageningen University, I was even more convinced that nematology was the right choice for me. I returned to Norway to begin the work for my Master's thesis.

This 60 ECTS Master's thesis is the end product of the Master's program in Plant Sciences at the Norwegian University of Life Sciences. The thesis was carried out as a part of the project FRITTNEMA - a collaboration between the Norwegian Institute for Bioeconomy Research (NIBIO) and the Norwegian Agricultural Extension Service (NLR).

It has been a year of learning, of ups and downs, of frustration but also of joy.

A deep thank you goes to my eminent supervisor Christer Magnusson for the patience, support and helpful advice while guiding me through this work.

I would like to thank BAMA and Gartnerhallen for the scholarship I received for this thesis. The scholarship made it possible for me to focus fully on the work that you can now see the result of.

I would like to thank Irene Rasmussen and Birgit Schaller for the elutriation, counting of nematodes, and the great company during my work at the lab.

A huge thank you goes to Stian for his invaluable assistance to typeset this thesis with LaTeX, for answering all my questions regardless of time of the day and for spending so much time to make this thesis look the way I wanted it to.

Thank you Anine, for being my companion from the very first day I moved to Ås. Astrid, thank you for always being my friend. To my mom and dad, thank you for the endless love and support. I am deeply grateful for being there for me, always, and especially through this last year. I know these past months have been hard for you too, but I finally made it.
My dear friend Eivind, you deserve the greatest thank you of them all. You never gave up on me, even in the darkest hours. You have supported me more than I could ever ask for and words cannot express how grateful I am.

Marte Persdatter Tangvik

11th of August, 2017

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Contents

1 Introduction

Carrot (*Dacus carota* var. *sativus*) is the largest vegetable crop in Norway, making up a third of the total vegetable production (SSB, 2017). In 2016, the production was 52,000 tonnes over an area of 1,600 ha (SSB, 2017). In carrot production, the taproot is the marketable product. The quality of the taproot is evaluated in accordance with the Norwegian standard for carrots (Standard Norge, 1999). The carrot is rejected if the quality does not fulfil the criteria in the standard. The Norwegian standard covers aspects such as size, shape and general appearance (e.g. no visible symptoms of damage from pests and diseases). Several biotic factors affect the quality of carrots, such as bacteria, fungi and plant-parasitic nematodes.

Plant-parasitic nematodes are tiny roundworms ranging from about 0.2 to 12 mm in size (Ravichandra, 2014), with the majority being smaller than 1 mm (Perry and Moens, 2011). Despite their small size and little consumption of plant tissue, they cause significant reductions in the marketable yield of carrots. Plant-parasitic nematodes feed on the root system; both the taproot, lateral roots and root hairs. Free-living plant-parasitic nematodes can either remain on the outside of the root while feeding (ectoparasites), or enter the root system (endoparasites) (Perry and Moens, 2011).

Filipjev (1934) uses 'parasitic nematodes' and 'free-living nematodes' as mutually exclusive terms. In this thesis, however, the term 'free-living plant-parasitic nematodes' is used as defined by Oostenbrink (1954); that is, as a contrast to the sedentary cyst- and root-knot nematodes. Thus, a group like root lesion nematodes (*Pratylenchus* spp.) is regarded as free-living plant-parasitic nematodes due to their migratory behaviour, despite the fact that these nematodes can complete their life cycle inside roots.

During nematode feeding and migration through the roots, the tissue is damaged, which results in impaired uptake of water and nutrients (Noling, 2016). As a consequence, plant growth is reduced and the taproot is underdeveloped. Plants can wilt and die off completely in cases of severe nematode damage. This is most likely to occur under dry conditions, as that is when consequences of insufficient water uptake is most severe due to short damaged roots and restricted availability of soil water.

In addition to restricting uptake of water and nutrients, nematodes can alter the morphology of the root. Examples of this includes branched roots (forking), excessive production of lateral roots, galls, swellings and twisted growth (Perry and Moens, 2011). These morphological changes are particularly crucial for a crop like carrot, where the root is the marketable product. Misshaped carrots are rejected by the Norwegian market, as they do not meet the criteria given in the Norwegian Standard. Nematodes can also create lesions (Castillo and Vovlas, 2007), that will reduce the aesthetic quality of the taproot. Lesions can be observed as brown, horizontal lines and cracks on the taproot. In addition to the reduced aesthetic quality, these lesions can serve as entrance ports for plant pathogens like bacteria and fungi (Agrios, 2005). Bacteria have no structure to directly penetrate the plant surface, and depend upon openings in the plant surface for infection (Agrios, 2005). Thus, nematodes might facilitate infection by bacteria in the root system.

In Norway, nematicides are banned due to their adverse effects for humans and in the environment. Thus, control of plant-parasitic nematodes relies on non-chemical methods such as crop rotation. Knowledge is the key when deciding on a crop rotation that will effectively reduce populations of plant-parasitic nematodes. Limited resources and a decline in the number of nematologists in the Nordic countries over the past decades (Webster et al., 2008), has resulted in a knowledge gap regarding the importance of free-living plant-parasitic nematodes. As this group of nematodes has not been extensively studied, their importance in agriculture is likely underestimated. The aim of this study is to increase the knowledge on free-living plant-parasitic nematodes associated with carrot production in Norway, with emphasis on geographical distribution and frequency of occurrence.

This is a Master´s Thesis within the study programme Plant Sciences at the Norwegian University of Life Sciences. The thesis has been a part of a project at the Norwegian Institute for

Bioeconomy Research, and funded by the Norwegian Agricultural Agency. This work was carried out in collaboration with the Norwegian Agricultural Extension service.

2 Methods

2.1 Soil sampling

Soil sampling was carried out by the Norwegian Agricultural Extension Service from July to October 2016. Samples were collected from fields with symptoms of nematode damage, both in fields under conventional and organic farming systems. Soil types sampled in this survey include sandy soils, sandy-loam soils, peat soils and moraine soil. Soil samples were collected in the whole circumference of the damaged area, in the transitional zone between healthy and damaged plants. Ten to 50 auger samples were collected, that made up a sample with a total weight of 1 to 2 kilograms (2000 ml). Samples represented a cross section of soil from the soil surface to a depth of approximately 20-30 cm to include the growth zone for plant roots. Soil was collected in plastic bags and put in a milk cartoon, to avoid desiccation. The samples were sent to the Norwegian Institute of Bioeconomy Research (NIBIO) where they were stored at 4°C until analysis.

Localities that were sampled are shown on municipality level in Fig. 2.1 and the number of fields sampled within each municipality is shown in Table 2.1. Set of basic map data is downloaded from Kartverket (Creative Commons Attribution ShareAlike 3.0). Climate data is downloaded from the Norwegian Metereological Institute.

Table 2.1: Number of fields sampled within each municipality

Municipality	Fields sampled
Arendal	4
Frosta	1
Horten	1
Hå	1
Klepp	1
Larvik	5
Smøla	1
Stokke	1
Ørland	2
Åsnes	2
Total number of samples	**19**

2.2 Plant sampling

Plants were collected from fields with symptoms of nematode damage. Dead plants were avoided, as nematodes often have migrated from these plants. Roots from 5 plants per field were sampled. A spade was used to ensure collection of the whole root system.

2.3 Extraction

Each soil sample was mixed thoroughly and a subsample of 250 ml soil was taken out. Extraction of nematodes from soil was done with a Seinhorst elutriator (Seinhorst, 1988). The Seinhorst elutriator is based on the principle of separating nematodes and soil in a rising water current in connected up-right glass columns. Due to their light weight and small size, the settling velocity of nematodes is less than the velocity of the upstream. Hence, nematodes will be collected in the upper part of the column whereas the heavier soil particles will settle further down. A tube is connected to the upper part of the elutriator to allow collection of the water in a separate container.

After elutriation, the water collected in the container is rinsed in three steps, in order to minimise the amount of soil particles in the sample. The first step is to filter the water through a bank of sieves. The sieve system consists of five sieves of different sizes that are stacked on top of each other. The sample is poured in the sieve and rinsed until the water that comes out on the bottom is clear. Subsequently, the solids that remain in the sieves are carefully washed into a new beaker.

In the next step, the sample is washed through a new system that consists of a funnel with a metal sieve supporting a 2601 Munktell filter paper (Munktell Filter AB, Grycksbo Sweden). The metal sieve with its three supporting feet is placed in a petri dish with enough water to cover the filter paper. The petri dish is left on a dark surface, illuminated, for a minimum of 24 hours. After 24 hours the water in the petri dish is collected in a 40 ml test tube. The test tube is labeled and stored in the fridge until counting.

Fig. 2.1: Overview of municipalities sampled

2.4 Counting

Water suspensions in test tubes containing the extracted nematodes were counted in the stereo microscope for each sample. Plant-parasitic nematodes were identified, counted and grouped into the following categories: Root lesion nematodes (*Pratylenchus* spp.), stunt nematodes (*Tylenchorhynchus* and *Merlinius* spp.), stubby-root nematodes (Fam. Trichodoridae), spiral nematodes (*Helicotylenchus* and *Rotylenchus* spp.), dagger nematodes (*Xiphinema* spp.), needle nematodes (*Longidorus* spp.), pin nematodes (*Paratylenchus* spp.), root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Globodera* and *Heterodera* spp.).

2.5 Mounting

After counting, each sample was transferred from the test tube to a counting dish. In the counting dish, specimens from the aforementioned nematode groups (subsection 2.4) were transferred into smaller dishes. Nematodes were subsequently transferred onto a microscope slide in a drop of water. The microscope slide was held over the flame from a spirit lamp long enough to heat relax the nematodes. A new microscope slide was cleaned with 70% ethanol and a drop of fixative was placed on the slide. The dead nematodes were transferred onto this microscope slide. Three strands of glass fibres were placed in a triangle around the nematodes to support the cover slip. A cover slip was placed on the slide and excess liquid around the cover glass was removed with a piece of filter paper whilst the slide was under the stereo microscope. After the removal of excess fixative, the cover-slip was sealed with finger nail polish and kept cold until identification.

Table 2.2: Literature used for morphological identification of nematodes

Nematode group	Taxon	Reference key
General		Mai (1996)
Stunt nematodes	*Tylenchorhynchus* *Merlinius*	Geraert (2011) Lab. handout
Root lesion nematodes	*Pratylenchus*	Loof (1978) Castillo and Vovlas (2007)
Spiral nematodes	*Helicotylenchus*	Sher (1966)
	Rotylenchus	Sher (1965) Castillo and Vovlas (2007)
Pin nematodes	*Paratylenchus*	Raski (1975*a*,*b*)
	Gracilacus	Raski (1976)
Stubby-root nematodes	Fam. Trichodoridae	Decraemer (1980) Lab. handout
Needle nematodes	*Longidorus*	Rothamstead Experimental Station (1973)
Dagger nematodes	*Xiphinema*	Rothamstead Experimental Station (1973)

2.6 Morphological identification

Nematodes were identified to species level in a Leica 6000B differential interference contrast microscope with the use of several identification keys and supporting literature. The literature used is listed in Table 2.2.

3 Results

Three main groups of results are presented in the following sections: relative occurrence of plant-parasitic nematodes in the soil samples, geographical distribution of free-living plant-parasitic nematodes, and descriptions and photographs of taproots showing variations in symptoms.

3.1 Relative occurrence

Free-living plant-parasitic nematodes were present in 18 out of 19 carrot fields at the time the soil samples were collected. Eleven genera and 18 species of plant-parasitic nematodes were identified. Eight of these genera were free-living plant-parasitic nematodes and two were sedentary nematodes (cyst- and root-knot nematodes) The relative occurrence of plant-parasitic nematodes is summarised in Fig. 3.1. Root lesion nematodes (*Pratylenchus* spp.) and stunt nematodes (*Tylenchorhynchus* and *Merlinius* spp.) were the most frequently occurring groups of plant-parasitic nematodes in this survey; they occcurred in 84% and 63% of the samples, respectively. Root-knot nematodes were detected in 37% of the samples and cyst nematodes were detected in 12% of the samples. The two latter groups will not be discussed in further detail, as the focus of this thesis is on free-living plant-parasitic nematodes and not sedentary nematodes.

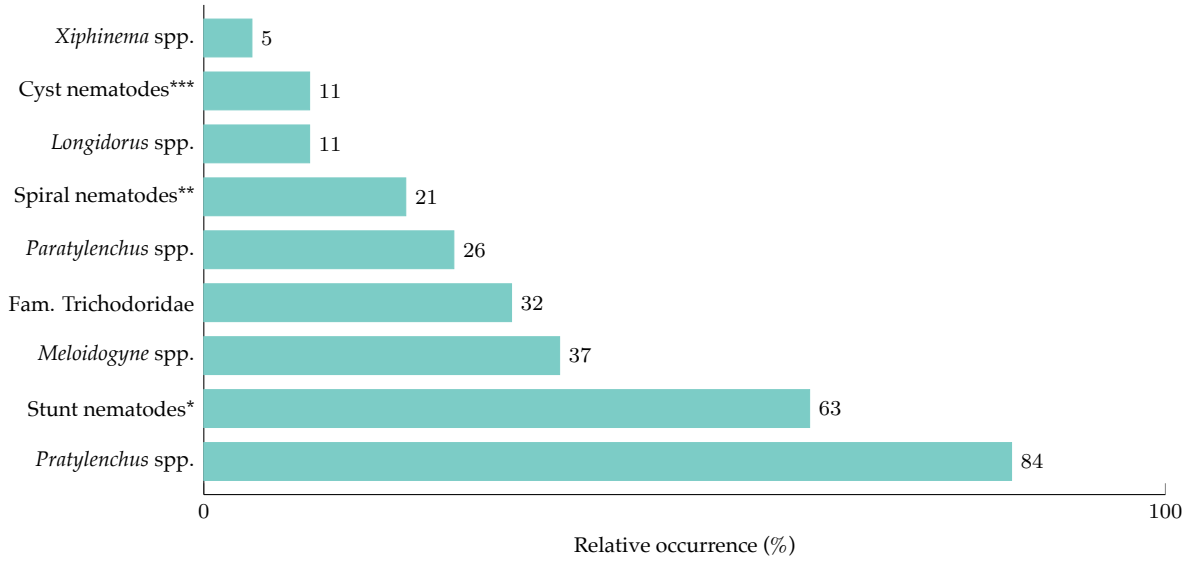


Fig. 3.1: Relative occurrence of plant-parasitic nematodes in carrot fields sampled (n=19) *Stunt nematodes=*Merlinius* spp. and *Tylenchorhynchus* spp., **Spiral nematodes=*Helicotylenchus* and *Rotylenchus* spp., ***Cyst nematodes=*Heterodera*

3.2 Geographical distribution

The geographical distribution is shown on municipality level, which means that some nematodes may occur in more than one sample per location. Maps are not presented for all species, but rather for the species considered to be of most interest. Thus, some maps are shown on genus level and others on species level.

Root lesion nematodes (*Pratylenchus* spp.) were identified in all samples (Fig. 3.2). *P. crenatus* was found in seven municipalities (Fig. 3.2a), in 13 of 19 samples. *P. pseudofallax* was found in two samples; one sample in Ørland and one in Stokke (Fig. 3.2b). *P. pseudopratensis* was occurred in a single sample from Arendal (Fig. 3.2c). In addition, root lesion nematodes were found in a sample from the municipality of Horten, but identification to species level was not possible due to a low number of specimens.

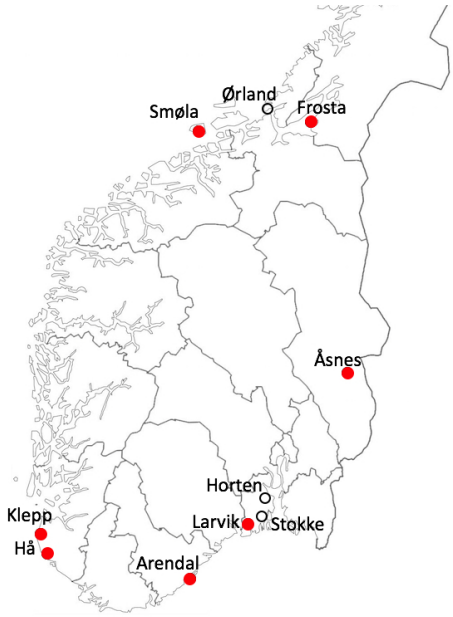
Pin nematodes (*Paratylenchus* spp.) were found in four locations in five samples (Fig. 3.2d). *Paratylenchus italiensis* was identified in one of the two samples from Ørland, whereas the specimens in the other samples were not identified to species level.

Stunt nematodes (*Tylenchorhynchus* and *Merlinius* spp.) were identified in 12 of the 19 samples, in eight of ten municipalities. *T. dubius* was found in five locations (Fig. 3.3a); in Klepp, Arendal, Larvik, Stokke and Åsnes. *T. maximus* was found in one sample from Klepp (Fig. 3.3b). *Merlinius* spp. was detected in three samples (Fig. 3.3c). *Merlinius brevidens* was found in Frosta, *M. nothus* was found in Hå, whereas the specimens of *Merlinius* spp. in the sample from Smøla was not identified to species level.

Nematodes belonging to Fam. Trichodoridae were detected in six samples, in five municipalities (Fig. 3.3d). *Trichodorus primitivus* was identified in a sample from Hå, together with *Paratrichodorus anemones* and *P. pachydermus*. *P. pachydermus* was also identified in a sample from Klepp. The Trichodorids in the four remaining samples were not identified to species level.

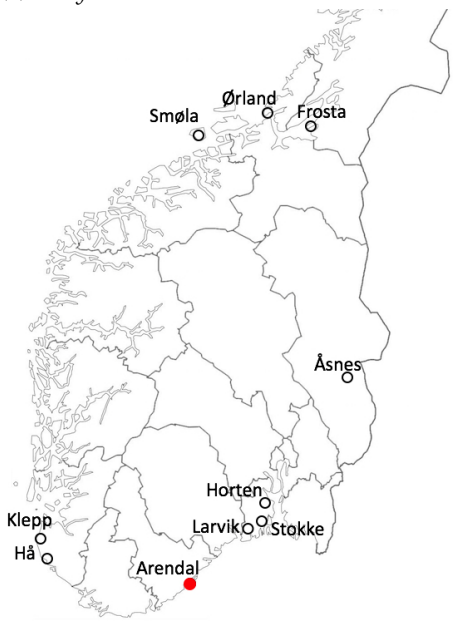
Spiral nematodes (*Helicotylenchus* and *Rotylenchus* spp.) were detected in samples from Arendal and Klepp. *H. digonicus* was found in a sample from Arendal (Fig. 3.4a); *H. lobus* and *H. pseudorobustus* were found in the same sample from Klepp (Fig. 3.4b and Fig. 3.4c). *Rotylenchus unisexus* occurred only in a single sample from Klepp (Fig. 3.4d).

The needle nematode *Longidorus elongatus* was found in two locations; in Klepp and in Arendal (Fig. 3.5a). The dagger nematode *Xiphinema diversicaudatum* was found in one sample from Arendal (Fig. 3.5b); the same sample that contained *L. elongatus*.

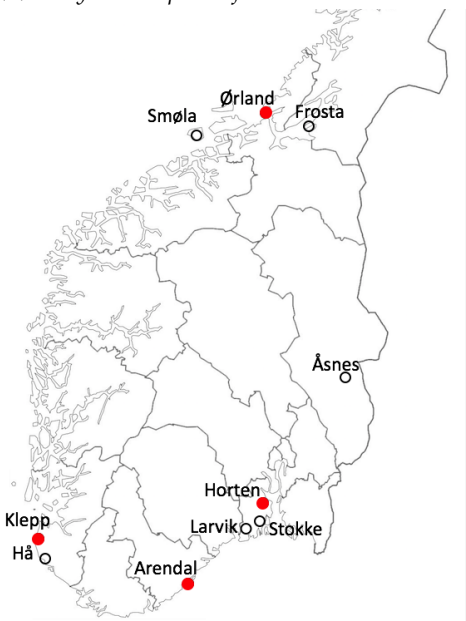


(a) *Pratylenchus crenatus*

(b) *Pratylenchus pesudofallax*

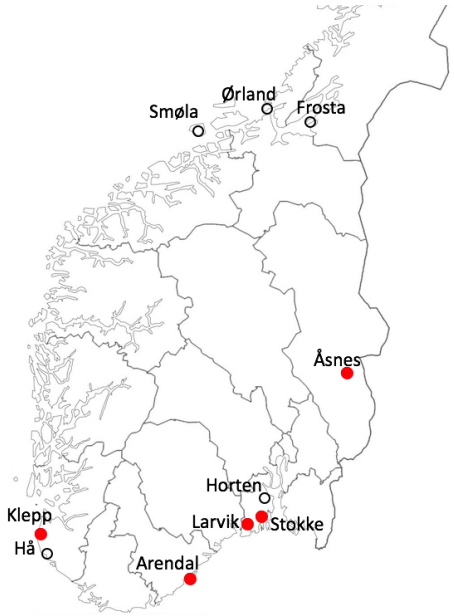


(c) *Pratylenchus pseudopratensis*



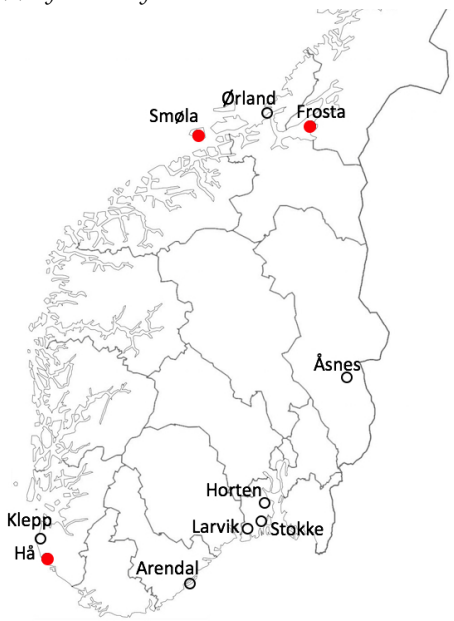
(d) *Paratylenchus* spp.

Fig. 3.2: (a), (b), (c): Root lesion nematodes and (d) Pin nematodes



(a) *Tylenchorhynchus dubius*

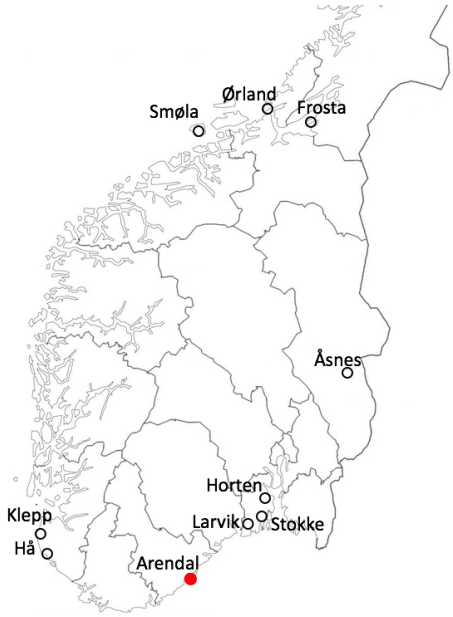
(b) *Tylenchorhynchus maximus*



(c) *Merlinius* spp.

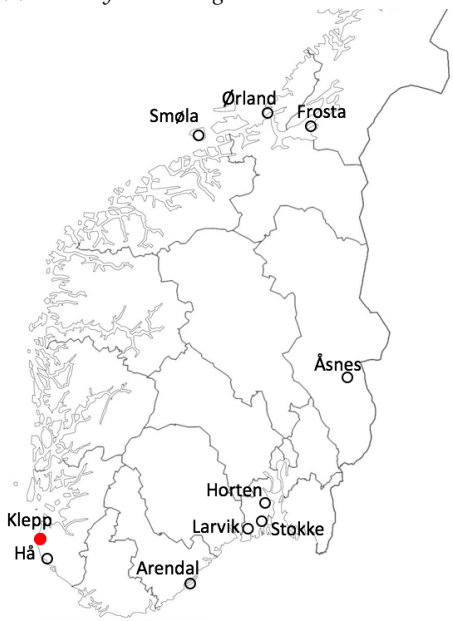
(d) Fam. Trichodoridae

Fig. 3.3: (a), (b), (c) Stunt nematodes and (d) Stubby-root nematodes



(a) *Helicotylenchus digonicus*

(b) *Helicotylenchus lobus*



(c) *Helicotylenchus pseudorobustus*

(d) *Rotylenchus unisexus*

Fig. 3.4: Spiral nematodes.

(a) *Longidorus elongatus* **(b)** *Xiphinema diversicaudatum*

Fig. 3.5: (a) Needle nematodes and (b) Dagger nematodes

3.3 Symptoms recorded

This section shows photographs of taproots from the sampled fields where taproots were sent to NIBIO for analysis. Various symptoms can often observed in the one taproot.

Symptoms observed in this survey includes

- Necrotic spots
- Elongated taproots
- Forked taproots
- Split and/or twisted taproots
- Galls caused by the bacterium *Agrobacterium tumefaciens*
- Galls caused by *Meloidogyne* spp.
- Swollen root tips
- Short taproots

Necrotic spots were observed frequently. Elongated taproots occurred in a sample from Arendal (Fig. 3.6a and Fig. 3.6d). In the same sample, forked taproots were observed (Fig. 3.6a, d). Additionally, galls caused by *Agrobacterium tumefaciens* were observed in this sample (Fig. 3.6b, c). A split and twisted taproot was observed in a sample from Frosta (Fig. 3.7a). Galls caused by *Meloidogyne* were observed in a sample from Klepp (Fig. 3.7c). This sample also shows necrotic spots, both on the taproots and on lateral roots (Fig. 3.7d).

In addition, symptoms of nematode damage on a larger scale, e.g. poor growth in patches the field, are described by the advisors from NLR that carried out the soil sampling.

(a) Forked, short taproot

(b) Taproot with galls produced by *Agrobacterium tumefaciens*

(c) Close-up of taproot with galls produced by *Agrobacterium tumefaciens*, in horizontal lines on taproot

(d) Eight carrots from the same sample. Elongated, forked, short and small taproots can be observed

Fig. 3.6: All taproots from the same sample from Arendal. Nematodes detected in this sample include *Helicotylenchus digonicus*, *Longidorus elongatus*, *Paratylenchus* spp., *Pratylenchus crenatus*, *Tylenchorhynchus dubius* and *Xiphinema diversicaudatum*

(a) Taproot split in two with twisted growth

(b) Close-up photograph of taproot with necrotic spots

(c) Severely disturbed growth. Two separate taproots intertwined. Large number of galls produced by *Meloidogyne* spp.

(d) Close-up of taproot in (c). Necrotic spots on lateral roots (to the left) and on taproot (to the right). Galls produced by *Meloidogyne* spp. observed on the lateral root in the bottom of the picture

Fig. 3.7: Taproots from three locations. **(a)** Taproot from a sample from Frosta. Nematodes detected in this sample include cyst nematodes (juveniles), *Merlinius brevidens*, *Pratylenchus crenatus* **(b)** Taproot from a field in Ørland. Nematodes detected in this sample include Fam. Trichodoridae, *Paratylenchus* and stunt nematodes **(c)** and **(d)** A sample from Klepp. Nematodes in this sample include cyst nematode juveniles, *Helicotylenchus lobus*, *Helicotylenchus pseudorobustus*, *Longidorus elongatus*, *Meloidogyne* spp. juveniles., *Paratrichodorus pachydermus*, *Paratylenchus* spp., *Pratylenchus crenatus*, *Rotylenchus unisexus*, *Tylenchorhynchus dubius*

4 Discussion

The aim of this thesis was to gain knowledge about free-living plant-parasitic nematodes in carrot production in Norway. More specifically, to get a preliminary overview of the geographical distribution, frequency if nematodes and their association with root symptoms. The damage potential of several species in this survey is not well-known, due to limited research on free-living plant-parasitic nematodes.

4.1 Root lesion nematodes (*Pratylenchus* spp.)

Pratylenchus spp. is present in virtually all climate zones (Potter and Olthof, 1993), and has a host range that includes approximately 400 host plants (Duncan and Moens, 2006). *Pratylenchus* spp. is ranked the third-most important group of plant-parasitic nematodes after cyst nematodes and root-knot nematodes by Jones et al. (2013). *Pratylenchus* spp. damages plants when they move through the tissue and damages cells, and when they enter and exit the root. The damage may be observed as horizontal, dark lesions or spots (Duncan and Moens, 2006, Loof, 1960). The lesions and spots turn dark because of phenolics that are released from the plant upon damage (Duncan and Moens, 2006). Cell damage can occur even without visible symptoms, but as it does not result in colour change in the root, it is complicated to observe the damage (Potter and Olthof, 1993).

In this survey, root lesion nematodes (*Pratylenchus* spp.) were found in all the sampled municipalities, occurring in 84% of the samples. The results from this survey is in line with a survey of carrot fields in Tasmania, Australia, where *Pratylenchus* spp. also was found in similar frequensis, with *P. crenatus* being the most common species (Hay and Pethybridge, 2005). In a survey of carrot fields in Scotland, *Pratylenchus* spp. occurred in 40% of the samples (Boag, 1979). This is significantly lower compared to our survey as well as the survey by Hay and Pethybridge (2005). A survey of organic vegetable crops in Germany showed that *Pratylenchus* spp. was present in 90% of the samples and *P. crenatus* was the most frequently-occurring species (Hallmann et al., 2007).

Townshend et al. (1978) found that in the United States, *P. crenatus* was distributed in regions with a mean annual temperature below 10°C and above 5°C, with an optimal temperature for reproduction at 10-15°C. In this survey *P. crenatus* was also found in locations with mean annual temperatures below 5°C. The high frequency of *P. crenatus* in this survey, as well as in the surveys from Tasmania and Germany, shows that *P. crenatus* is a common species in carrot and vegetable production. Loof (1960) stated that *P. crenatus* is extremely common in Europe, but has sometimes been confused with *P. penetrans*, as these two species often occur together.

In case of severe infection by *Pratylenchus*, uptake of water and nutrients from the soil is limited. In addition to direct damage to the plant, *Pratylenchus* can facilitate infection by other plant pathogens, such as fungi and bacteria. The lesions created by the nematodes can serve as openings for fungi and bacteria into the root and thus cause further damage to the plant. The interaction between nematodes and bacteria is particularly interesting, as bacteria can only enter a plant through natural openings (e.g. stomata) or wounds, such as those created by *Pratylenchus* spp. (Agrios, 2005). Studies have shown that bacteria can detect, and are attracted to, specific compounds released by plants upon damage (Antunez-Lamas et al., 2009). In this survey, a taproot from a field in Arendal was infected with *Agrobacterium tumefaciens* (Sletten, personal communication). The galls on the taproot produced by *A. tumefaciens* is distributed in horizontal lines, which resembles the size and shape of lesions that can be created by *Pratylenchus* spp. This could indicate that an interaction between *A. tumefaciens* and *P. crenatus* did occur in this case. Vrain and Copeman (1987) showed that *P. penetrans* increased the incidence and severity of galling by *A. tumefasciens* in red raspberry. Further research is needed to investigate the interaction between *Pratylenchus* spp. and *A. tumefaciens* on carrot and other crops.

According to the Horticultural Development Council (HDC), *P. crenatus* is associated with carrots, but it is not shown that carrot is a host plant for *P. crenatus* (HDC, 2002). However, a survey in Poland showed that poor growth in carrot fields associated with *P. crenatus* (Brzeski,

1970). HCD only lists the two *Pratylenchus* species *P. mediterraneus* and *P. penetrans* as causing damage in carrot crops (HDC, 2002, Bridge et al., 2005). Thus, the importance of other *Pratylenchus* species in carrot production needs to be further investigated.

4.2 Stunt nematodes (*Tylenchorhynchus* and *Merlinius* spp.)

Stunt nematodes include the two closely related genera *Tylenchorhynchus* and *Merlinius*. These nematodes feed as ectoparasites on the roots (Maggenti, 1981). This group of nematodes is not considered to be important pests in plant production HDC (2002). However, as both *Tylenchorhynchus* and *Merlinius* often occur in mixed populations with other plant-parasitic nematodes, it is challenging to determine their contribution to combined nematode damage in the field.

Two separate pot experiments by Sharma (1968, 1971) showed that reproduction of *T. dubius* in pots with carrots was poor. This is an indication that carrot is an unsuitable host for *T. dubius*. Barker and Davis (1996) states that *Tylenchorhynchus* spp. can modify tissues, but without visible symptoms on the root/plant. *Merlinius* spp. has been associated with crop damage, but primarily in grasses and cereals (HDC, 2002).

A greenhouse experiment in The Netherlands showed that reproduction *T. dubius* was highest at low temperatures (10°C) (Dao D, 1970). However, the behaviour of *T. dubius* at temperatures lower than 10°C remains unknown, as 10°C was the lowest temperature tested. Another greenhouse experiment showed population increase of *T. dubius* occurred in the temperature range between 10 and 25°C with 25°C being the optimum temperature for population increase (Malek, 1980). The other *Tylenchorhynchus* species, *T. maximum*, had a population increase in the range 15-25°C, with an optimum of 25°C. Populations of *Merlinius brevidens* increased at temperatures between 10 and 20°C, with an optimum temperature of 20°C. In this survey, *Tylenchorhynchus* spp. was not detected in the three northern-most municipalities (Smøla, Ørland and Frosta), whereas *Merlinius* spp. was found in both Smøla and Frosta. The results from the two greenhouse experiments, as well as the results from this survey, could indicate that *Tylenchorhynchus* spp. has a preference for warmer soils compared to *Merlinius* spp.

In the Netherlands, *T. dubius* is found almost exclusively on light sandy soils (Loof, 1959). As carrots are predominantly grown in light sandy soils, the frequency of *T. dubius* in our survey is consistent with the observations of Loof (1959).

4.3 Needle nematodes (*Longidorus* spp.) and dagger nematodes (*Xiphinema* spp.)

Longidorus and *Xiphinema* are so closely related that they can be discussed together in terms of plant parasitism. *Longidorus* spp. and *Xiphinema* spp. are large nematodes in comparison to other plant-parasitic nematodes; their normal size range is between two and eight mm, but they can be up to 12 mm. Other plant-parasitic genera are normally in the range of 0.5 to 1.5 mm. *Longidorus* spp. and *Xiphinema* spp. have long stylets, which means they can pierce into deeper cell layers without entering the root. The two genera feed on the root tips, which may cause swellings of the root tip that can be confused with galls produced by root-knot nematodes (Berg, 2009). Swellings/galls were observed on several samples that contained both *L. elongatus* and *X. diversicaudatum*. However, galls on one of the taproots were confirmed to be caused by the bacterium *Agrobacterium tumefaciens* (Sletten, personal communication). Hence, it is likely that the gall-like structures observed on the other taproots in the same sample is also caused by *A. tumefaciens* and not *L. elongatus* and/or *X. diversicaudatum*. Another symptom of *L. elongatus* is the growth of an abnormally long tap root (HDC, 2002). Abnormally long taproots were observed in the sample from Arendal that contained *L. elongatus* and *X. diversicaudatum*. It is unknown whether *X. diversicaudatum* can induce the growth of abnormally long taproots, or if that is a symptom only of *Longidorus*. In this case it is challenging to determine, as both species occurred in the same sample.

In addition to the direct damage they cause on the root through feeding, both genera can transmit plant viruses (Harrison and Cadman,

1959, Decraemer and Robbins, 2007). *Xiphinema* spp. can transmit Arabis mosaic virus, which has carrot as a host plant (ICTVdBManagement, 2007). Although carrot is listed as a host plant, it is not certain that the virus causes damage in carrot production.

A survey of *Longidorus* spp. in Norway showed that *L. elongatus* was distributed from the south-east part of Norway up to 69° North (Alphey, 1985). In this survey, however, *Longidorus* was found in two of the 19 samples (11%), only in the south of Norway. The number of fields sampled in this survey was low (n=19), and thus it is likely that the occurrence only in the south of Norway in this survey is random rather than an indication that *Longidorus* spp. is not distributed in other parts of Norway. In organic vegetable production in Germany, *Longidorus* spp. was found in only a single sample out of of 246 samples (Hallmann et al., 2007), whereas in a survey in Scotland, *Longidorus* spp. was found in 97% of the samples (Boag, 1979).

A pot experiment showed that no development of *L. elongatus* occurred below 8.3°C (Boag, 1985). None of the eggs hatched at 10°C whereas 80% hatched at 12.5°C. Most places along the coastline of Norway, where *Longidorus* is previously detected Alphey (1985), reach these temperatures during the summer months, which would allow for development of *L. elongatus*.

X. diversicaudatum seems to require a minimum temperature of 10°C for embryogeny through hatching of the juveniles (Dao D, 1970), and temperatures above 12.8°C for development (Neilson and Boag, 1996). These are requirements similar to those of *L. elongatus* with regard to hatching and development of juveniles. However, *X. diversicaudatum* seems to be restricted to the warmest parts of Norway, according to results from this survey as well as previous work by Støen (1975) and Brown and Taylor (1987). *L. elongatus*, on the other hand, seems to be widespread Alphey (1985).

In the field where *Longidorus* was found in Klepp, there had been a grassland for the six previous seasons. Red clover, common in grasslands, is a good host for *Longidorus*. Hence, this could explain the occurrence of *Longidorus* in that field. In 250 ml of soil, only one single specimens of *Longidorus* was found. This was the only field sampled in this survey with exclusively grass as a pre-crop. The difference in occurrence in this survey as compared to the survey by Alphey (1985) could be explained by the fact that Alphey (1985) surveyed all cultures and soil types, whereas this survey only surveyed carrot fields with suspected nematode damage.

In the sample from Arendal where *L. elongatus* and *X. diversicaudatum* were detected, carrot had been planted for the six previous seasons. This could indicate that planting carrot in the same field over several years increases the population of *Xiphinema* spp. and *Longidorus* spp. However, three other carrot fields on the same island in the municipality of Arendal (and the same farmer) did not contain *Xiphinema* spp. nor *Longidorus* spp., despite the fact that these fields also had been planted with carrot for the six previous seasons. Thus, factors other than plant species may be important for the occurrence of *Longidorus* and *Xiphinema*.

4.4 Pin nematodes (*Paratylenchus* spp.)

Paratylenchus is a genus of ectoparasitic migratory plant-parasitic nematodes. The normal size of species in this genus is <0.5mm. *Paratylenchus* spp. is considered a severe pest on carrot (Decker, 1989), but large numbers of this nematode if required to cause significant damage (Boag, 1979). 'Carrot sickness' caused by an unidentified *Paratylenchus* species was described by Oostenbrink (1954). A pot experiment on carrot with the same unidentified *Paratylenchus* species showed that the pots infested with >19.000 specimens caused a decrease of the weight of the underground plant parts up to 44% (Oostenbrink, 1954). In addition, the *Paratylenchus* population in one of the pots increased with a factor of 113.

Paratylenchus italiensis was the only pin nematode identified to species level. In other samples, identification to species level was not possible due to low numbers of specimens. Very little information is available on host range and pathogenicity of *P. italiensis P. bukowinensis* is the only species regarded to be of importance in carrot production. A symptom of this species is finger-shaped carrots (NIBIO, unpublished); a symptom that was observed in samples with *Paratylenchus* in this survey.. However, as the

population densities of *Paratylenchus* recorded in these samples were low, it is unlikely that *Paratylenchus* spp. caused these symptoms.

4.5 Spiral nematodes (*Helicotylenchus* and *Rotylenchus* spp.)

The group of spiral nematodes includes the two genera *Helicotylenchus* and *Rotylenchus*. Both *Rotylenchus* and *Helicotylenchus* spp. feed as ectoparasites on roots (Boag and Neilson, 1996). These genera do not induce specific symptoms in the plant, but rather causes general depression of growth in the field. Both the lack of specific symptoms caused by these nematodes, as well as the presence of other plant-parasitic nematodes, made it difficult to determine whether spiral nematodes were involved in the reduced growth that was observed in the fields in this survey.

Castillo et al. (1993) states that several *Rotylenchus* species are of economic importance. Reduced root weight in a pot experiment with carrot was observed in pots infested with *R. robustus* (Krall, 1990). This might be an indication that *R. robustus* is of potential importance. However, the host range and damage potential of the only *Rotylenchus* species found in this survey, *R. unisexus*, is unknown. *R. unisexus* is recorded from pastures an grasslands in Spain (Navas and Talavera, 2002) and from surrounding soybeans in South Africa (McDonald et al., 2001), but no symptoms of damage were observed. Of the *Helicotylenchus* species found in this survey, *H. pseudorobustus* is the only one considered to be damaging according to Ravichandra (2014).

Spiral nematodes were not found in three northern-most municipalities sampled in this survey (Frosta, Ørland and Smøla).

4.6 Stubby-root nematodes (Fam. Trichodoridae (*Trichodorus* spp. and *Paratrichodorus* spp.)

Stubby-root nematodes, which belong to the family Trichodoridae, are damaging to carrot crops worldwide (Decraemer, 1991, HDC, 2002). In this survey, *Trichodorus* and *Paratrichodorus* spp. were detected. These two genera are ectoparasites, which means that they remain outside the roots while feeding. Feeding by stubby-root nematodes mainly occur on cells in the root-elongation zone (HDC, 2002). When the cells in this zone are damaged, elongation of the root is disturbed, and the result is stubby roots (Decraemer, 1991).

P. pachydermus is known to cause so-called docking disorder in sugar beet in Great Britain, where they feed on seedlings and cause reduced growth of the plants in the early growth stages (Whitehead and Hooper, 1970). Symptoms of damage by stubby-roots nematodes were not observed in this survey. In addition to causing direct damage to the plant, stu are vectors for the viruses tobacco rattle virus and pea early browning virus.

Three species of stubby-root nematodes were detected in this survey: *Trichodorus primitivus*, *Paratrichodorus anemones* and *P. pachydermus*. The distribution of stubby-root nematodes in this survey is in line with the distribution reported by Alphey (1985), as it includes occurrence in municipalities both in the southern and middle part of Norway.

Members of this family are vulnerable to rough handling, such as when sampling the soil. Thus, the real occurrence of stubby-root might be higher than detected in this survey.

4.7 Challenges with nematode sampling from fields and population densities

A soil sampling of any field is a snapshot of the nematode population at a given time and a given place in the field. It should be noted that the samples in this survey were collected in fields where nematode damage was suspected. Thus, this is not a survey of carrot fields in general, but in carrot fields that are suspected to be damaged by nematodes.

A major flaw in this survey is that samples were collected from July until October; a time span of approximately four months. These four months covers almost the whole growing season in Norway. Hence, comparing population densities between samples is impossible, as nematode populations fluctuate throughout the growing season. For this reason, conclusions regarding population densities to be drawn from this survey is very limited. A suggestion for further research is be to collect samples throughout the whole

growing season, although this would be very time consuming and thus costly.

4.8 Challenges with morphological identification

Morphological identification can be challenging due to the limited morphological structures that are present in various nematode species. In addition, there are intra-specific variations in the morphology, which further complicates identification (Loof, 1991, Bert et al., 2011). Morphological identification is particularly challenging in samples where only very few specimens of a given genus is found. Generally, it is necessary to examine several specimens within each genera before it is identified to species level. As a consequence, not all nematodes in this survey were identified to species level.

4.9 Influence of soil types and cultivars on nematode occurrence

Samples were collected from fields with different soil types. It would have been interesting to investigate the impact of soil type on occurrence of free-living plant-parasitic nematodes. In this survey, however, too few samples were available from each soil type in order to draw any conclusions on the influence of soil types on nematode occurrence. In general, light soils, the soil type most commonly used for carrot production, are associated with higher occurrence of free-living plant-parasitic nematodes than other soil types (Gratwick, 1992).

Little research is done on the variation in resistance or tolerance of different carrot cultivars with regard to free-living plant-parasitic nematodes. The amount of data in this survey was considered to be insufficient to discuss the impact of cultivars on nematode damage.

4.10 Conclusions

- Free-living plant-parasitic nematodes occur frequently in carrot fields in Norway
- *Pratylenchus* is the most frequently-occurring genus and *P. crenatus* is the most frequently-occurring species
- In fields with a high occurrence of *Longidorus* spp. and *Xiphinema* spp., growth of carrots seems to be severely disturbed
- Geographical distribution of *Xiphinema* spp. seems to limited to the south of Norway

4.11 Further research

Further research is necessary to determine the importance of free-living plant-parasitic nematodes in agriculture, as well as to develop effective control mechanisms to reduce the yield losses due to nematodes in the future. A point of interest is to gain insight in the specific symptoms caused by different genera and species. As per now, this is a challenge, as many plant-parasitic nematodes are present in mixed populations. A complicating factor is that some genera, such as *Trichodorus* and *Paratrichodorus* do not thrive under greenhouse conditions, as they are very sensitive to fluctuating water levels. A suggestion is therefore to do experiments where clay pots (to allow water to pass) are placed in fields, inoculated with a single species of nematodes, and then observe symptoms on the plants.

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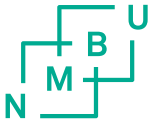

Norges miljø- og biovitenskapelig universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway