Project description
Title: Studies on the biology of potato cyst nematodes (*Globodera* spp.) under Nordic conditions for improving management and regulation in Norway.

PART 1: The KMB project application to: TheFoundation for Research Levy on Agricultural Products and the Agricultural agreement

Potato cyst nematodes (PCN) are worldwide potato pests. The yellow species (yPCN) *G. rostochiensis*, and the white species (wPCN) *G. pallida* are quarantine pests subjected to national regulations. For farmers, PCN infestations result in costly production systems and loss in sales value of farms. Societal consequences by far exceed yield losses. The occurrence of populations (all wPCN and virulent yPCN pathotypes) capable of breaking the resistance in potato cultivars in current use, and the long persistence of PCN in soil without host plants complicates the management. Effective management requires reliable information on virulence, decline rates in population densities and infectivity in soil. This kind of information is insufficient for Norwegian conditions, as the existing information comes from other Western European countries, and relative few studies has been done in Nordic area in recent years. It is also crucial to know what conditions or practices increase these decline rates. Today, non-virulent yPCN is managed by crop rotation, while infestations by wPCN or virulent yPCN results in a 40-years ban on growing potato. Knowledge on nematode virulence and population density decline rates in soil is needed for decisions on control measures. In this project, biotests will identify the pathotypes, species will be confirmed by morphological and molecular methods. In field studies, decline rates in the absence of host plants will be monitored. Factors that contribute to population density decline will be identified. The occurrence, pathogenicity and control efficacy of microbial antagonists, and the use of molecular tools for their identification and quantification will be topics of a PhD study. The potential of including an early potato crop or *Solanum sisymbriifolium* as trap crops for reducing PCN numbers will be determined in field trials. Resistance test of most commercial potato cultivars on the Norwegian market will provide potato producers and advisers with updated information on nematode resistance, which will improve the management of PCN. The results of this project will improve the current management systems. The generated information may allow for a shorter quarantine periods and make Norwegian potato production more efficient and sustainable. Julius Kühn-Institut, Germany, Rothamsted research,UK, Universidad Autonoma de Madrid Spain will be important research collaborators. A project reference group represented by farmers, advisers, industry and authorities will take part in discussions and evaluation of the project progress.

1. **Objectives**

<u>Main objective:</u>

To increase the scientific basis for amending the management system for PCN, *Globodera* spp under Nordic conditions.

<u>Sub-objectives</u>:

(1) To determine the virulence occurring in selected PCN populations.
(2) To establish the decline rate in PCN numbers and infectivity in field soils.
(3) To determine the occurrence and role of microorganisms antagonistic to PCN.
(4) To evaluate the decline of PCN numbers in field trials with an early potato crop or *Solanum sisymbriifolium*

(5) To characterize the degree of resistance of selected potato varieties available in Norwegian market
(6) Exploratory studies on the PCN- Potato- Pathosystem

2. **Frontiers of knowledge and technology**

Potato cyst nematodes

Potato cyst nematodes (PCN) have worldwide economic significance as parasites of potato (Marks & Brodie, 1998, Plantard *et al*., 2008), and cause losses of about 9% of the potato production in Europe (Evans & Rowe, 1998). PCN are thought to have co-evolved with their host plants in the family *Solanaceae* in the Andean region of South America, and have been introduced into Europe after 1850 (Evans *et al*., 1975). It is likely that each nematode introduction represents a sub-set of the original population gene pool. Consequently, each introduction is likely to have been genetically distinct with a limited variability. This may have become further fragmented and restricted during subsequent spreading within Europe and the Nordic area (Turner & Evans, 1998). This introduction of distinct populations and genome constitutions of introduced PCN is reflected by todays' variability within the genus. Evidently, PCN has been present in Norway since 1955 (Magnusson & Hammeraas, 1994) and has since then become one of the most important pests of potato. PCN has two species: the yellow species (yPCN) *Globodera rostochiensis* and the white species (wPCN) *G. pallida*. In addition to potato, PCN can infect aubergines, tomato and some other plants in the family *Solanaceae*.

The life cycle of PCN is typical for cyst nematodes (Fig. 1). Juveniles hatch from eggs in the presence of host plant roots, invade roots where they become sedentary while feeding from an induced nutritional tissue complex, *i.e.* syncytium induced in the root in response to specific chemical signals from the nematode. The nematode female feed continuously throughout its development, while the nematode male stops feeding after the premature stages and becomes a vermiform adult. The males mate with several females and die shortly afterwards. After fertilization, the female produces eggs that are filled with the infective juvenile stages. After cessation of egg production, the female dies and forms the hard (Ø=0.5 mm) nematode cyst, which protects eggs and juveniles from desiccation, predation, and agro-chemicals. Following a mandatory chilling period, juveniles hatch when stimulated by hatching factors exuded from nearby roots of host plants.

The introduction of few PCN cysts into an area will usually go unnoticed for many years, during which infested soil can be spread unknowingly to nearby farms. The time required for PCN symptoms to appear depends on the frequency of potato cultivation, and may occur after five to six potato crops, corresponding to 20 years if potatoes are grown in a 4-years rotation (Evans & Brodie, 1980). Depending on the potato cultivar and the nematode population density, crop losses caused by PCN can be up to 70% (Greco, 1988).
Both PCN species are quarantine pests regulated in the national plant health legislation (Anon. 2000). Strict local regulations are imposed on detection of species and pathotypes virulent on resistant cultivars. and because of the long persistence of PCN in soil. Detection of the most common type of yPCN (to which resistant cultivars are available on the Norwegian market) results in the official requirement of controlled production including the choice of cultivar, length of rotation and equipment/field hygiene. There will be a ban on growing of plants for planting and movement of soil to adjacent farmland. The farmer will be required to clean machinery and to wash root crops free of soil. The presence of wPCN and resistance-breaking populations of yPCN is even more serious and will result in a 40-years ban on

growing potato or other host plants, the preclusion of movement of soil and sharing of machinery with other farms.

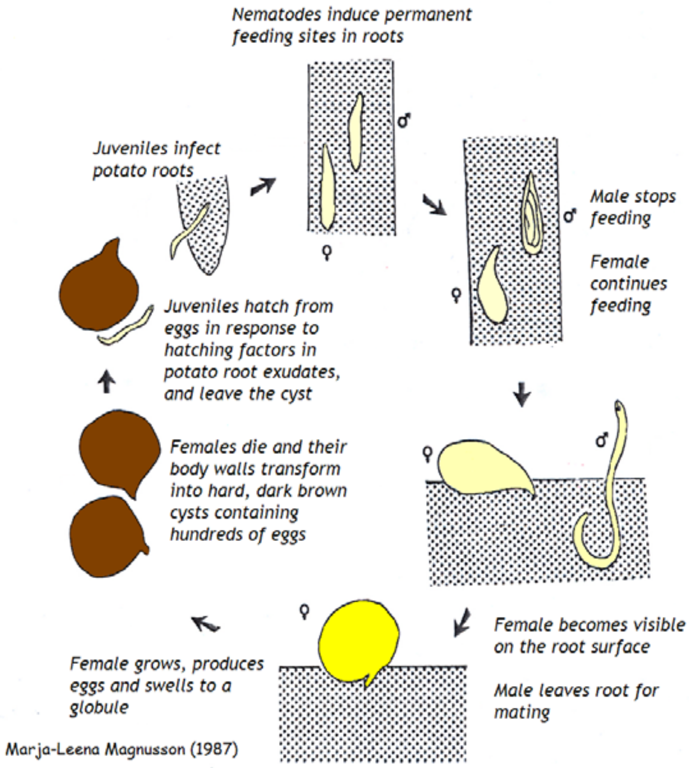

Figure 1. Life cycle of yPCN *Globodera rostochiensis* (from Magnusson M-L., 1987).

Potato production and economic importance of PCN in Norway

To assess the importance of PCN control, the importance of the potato crop to Norwegian agriculture and to rural economies, must be considered. In Norway, potatoes are grown on 13 787 ha with an annual production of 120 000 tons for industrial use, 70 000 tons for fresh consumption, 65 000 tons for starch and spirits, 35 000 tons for seed potato and the rest for animal feed or from production from home use in private gardens.

In Norway today, there is no estimate of the monetary damage caused by PCN. However, the societal consequences of PCN infestations and the associated regulations take proportions far exceeding the yield losses *per se*. For many Norwegian farms potato is the prime cash crop, they are specialized in potato production and have made considerable investmenmts in specialized buildings and machinery. Infestations of PCN greatly increase the costs of production, and thus reduce profitability in gross return and market value of the farm. Because many rural communities and their processing industries depend on ample and easily accessible supply of potato, the rural economies will suffer because of the reduction in profitability. In view of the strict regulations imposed, a new understanding of aspects of PCN virulence and its persistence in soil as well as information on agricultural measures for fostering natural population density decline is urgently needed.

Nematode virulence

The term virulence refers to the ability of a nematode to reproduce on a plant resistant to that specific nematode (Roberts, 2002). The diversity of pathotypes was first recognised when the reproductive ability of PCN populations was tested on *Solanum* clones that contained PCN resistance genes, *e.g.* gene Gro-1 (H_1) from *Solanum tuberosum* ssp. *andigena* (Howard &

Fuller, 1971.). These resistance genes were identified in collections of wild or cultivated Andean potatoes and their introgression into commercial cultivars were recognised as a potential tool for the control of PCN. However, it became evident that many PCN populations could reproduce on *Solanum* clones despite the presence of these resistance genes. This led to the recognition of a series of virulent "resistance-breaking" pathotypes within both PCN species (Fleming & Powers, 1998).

Attempts have been made to classify PCN pathotypes (Kort *et al.*, 1977; Canto-Saenz & de Scurrah, 1977). In this classification European yPCN occurs in the pathotypes Ro1, Ro2, Ro3, Ro4 and Ro5, while the European wPCN has the pathotypes Pa1, Pa2 and Pa3. Under Norwegian conditions, the pathotypes Ro2, Ro3 of yPCN and all pathotypes of wPCN are considered virulent on resistant potato cultivars in current use. Knowing the characteristics of pathotypes thus is most important to plant breeders. Since reliable molecular identification methods for pathotypes are lacking, and the fact that different populations of the same pathotype may show a range of virulence on the same cultivar makes biotests according to the current pathotyping system the only practical option for clarifying the pathotype spectra.

Management PCN with Gro-1 (H_1) gene for resistance

In Western Europe, populations of yPCN (Ro1) have been largely managed through the use of potato cultivars with the Gro-1 (H_1) resistance gene. No cultivars carrying the Gro-1 (H_1) gene have resistance to wPCN or to virulent yPCN. Hence, the use of such cultivars can decrease population densities of yPCN (Ro1) up to 80%, whilst at the same time allowing virulent populations to reproduce unhindered (Withehead, 1998). The repeated growing of resistant cultivars allowed *G. pallida* to become the dominant species in most of England and Wales (Minnis *et al.*, 2000), and similar changes in dominance are likely to occur also in other parts of Europe, where the intensive use of cultivars resistant only to Ro1 is practised (Dale & de Scurrah, 1998). There are currently no potato cultivars available in Europe with full resistance to virulent yPCN or to any wPCN pathotypes (Blok *et al.*, 2000). This is because the genetic diversity of wPCN introduced into Western Europe appears to be greater than that of yPCN (Blok & Phillips, 1995; Grenier *et al.*, 2001, Plantard *et al.*, 2008).

An unrestricted use of cultivars with partial resistance to *G. pallida* could result in an increase in resistance-breaking pathotypes because such cultivars will allow a proportion of juveniles to reach maturity and hence select for virulent populations. Therefore, proper management programmes, which alternate non hosts, resistant varieties with susceptible are essential if potato production is to continue on land infested with PCN.

Nematode decline rates

In the absence of host stimulation only a small portion of the eggs hatch from the cyst each year, and in wPCN this occurs at a lower rate than in yPCN. Data from Northern Ireland indicate that up to 30 years without host plants are required to render PCN incapable of reproduction (Turner, 1996). In Norway, the decline of yPCN in fallow soil may reach 40-70% of the cyst content already in the first year following potato (Bioforsk unpubl); reduction levels similar or higher have been reported from Sweden (Andersson, 1987) and Finland (Tiilikkala, 1991a). Various factors such as climate, crop plants and nematode antagonists have been suggested to cause these high levels of PCN mortality and decline rates. Nematode fitness may depend on the level of neutral lipids present in the bodies of the infective juveniles, and determines both the inoculum potential (*i.e.* infectivity) and the rate of survival in field soils. Lower levels of lipids result in lower infection potential and reduced survival (Robinson *et al.*, 1987). It has also been suggested that the low temperatures at the higher latitudes of the Nordic area restrict the build-up of lipid reserves of PCN juveniles and reduce infectivity and survival (Tiilikkala, 1991b). It is likely that also the composition of nematode lipid reserves will be important for fitness.

Host Range

Both species of PCN have host ranges restricted to the family *Solanaceae* (Sullivan *et al*., 2007), and potato is the most important crop within this family grown in temperate regions. Apart from potato, tomato and aubergines are hosts. Some *solanaceous* weeds can support PCN, and species like *S. nigrum* and *S. alatum* are host plants of wPCN, while *S. dulcamara* allows both species to reproduce (Stelter & Engel, 1975).

Nematode antagonists

The importance of microorganisms as antagonists of nematodes is well-known (Stirling, 1991; Davies, 1998), and the role of nematode parasitic fungi like *Nematophthora gynophila* and *Pochonia chlamydosporia* in the natural suppression of the cereal cyst nematode *Heterodera avenae* has been reported from the UK and elsewhere (Kerry 1982, Davies 1998). Norwegian surveys have revealed that infections of PCN by nematode parasitic fungi and bacteria are common (Bioforsk unpubl.), and in the Trøndelag region the fungus *Paecilomyces lilacinus* was reported from females of yPCN (Holgado & Crump, 2003). Since this fungus does not attack the contents of cysts it will not be effective in a non-host situation. The egg-parasite, *P. chlamydosporia*, is expected to be more important in such a situation. However, successful control by antagonists relies on the understanding of how these parasites survive in soil and establish in the rhizosphere, and their ability to parasitize species of PCN. This information is lacking in Norway. Molecular techniques like real time PCR, RFLP, qrt-PCR for monitoring the occurrence, abundance and activity of *P. chlamydosporia* and *P. lilacinus* have been developed (Atkins *et al.*, 2003, 2005).

Trap cropping

The use of an early crop of potato is aimed at reducing PCN populations. In this method, the crop is planted and grown for sufficient time to permit nematode infection, but it is destroyed before the start of nematode reproduction. The effectiveness of this method is highly dependent on the time frame for hatching and reproduction of the PCN population. Trap cropping with potatoes requires careful timing to avoid increasing rather than reducing PCN populations. If susceptible plants are destroyed after 4 weeks few juveniles are trapped, whereas destroying the crop after 8 weeks may result in nematode population increase (Whitehead, 1977). Hence, trap-cropping systems with early potatoes need to be closely adapted to local conditions.

Some non-tuber-forming *Solanum* spp., which are grown from seed can also induce hatch of PCN (Scholte, 2000). *Solanum sisymbriifolium*, which is fully resistant to PCN, stimulates hatch but would not allow the nematodes to complete its life cycle. *S. sisymbriifolium* has been studied in The Netherlands for many years, and the risk of establishment as a weed is considered not to exist under Dutch conditions. The inclusion of this species in cropping systems has not caused major problems as *S. sisymbriifolium* is not a host plant for important pests and diseases (Scholte & Vos, 2000). Under Norwegian conditions the reliability of this trapping method needs to be confirmed.

Relationships within the pathosystem

The PCN-Potato-Pathosystem contains a multitude of compatible and non-compatible interactions between the pathogens and the soil ecosystem. In host defence, pathogen invasion is recognized by proteins encoded by plant resistant genes (Van Loon, 1977; Somssich & Hahlbrock, 1998). A better understanding of the basic cellular and molecular mechanisms that control the outcome of the PCN-host plant interaction, and the identification of pathogen-derived molecules (elicitors), which determine early pathogen recognition may be essential in conferring susceptibility or resistance to the pathogen (Kombrink *et al*., 1993).

Successful parasitism by PCN involves on the nematode part (Fig.1) the recognition of hatching factors, host finding, infection of the root, tissue selection, feeding site initiation, feeding site maintenance, mating and reproduction. Environmental biotic and abiotic factors and plant responses may all be expected to interfere with each link in this chain, resulting in different levels of compatibility within the pathosystem. This will affect nematode fitness, and may be reflected in nematode lipid levels and composition. Increasing the understanding of this pathosystem will be important for the future management of PCN.

3. **Research tasks**

To improve future management routines for PCN we need to increase the knowledge of PCN under Nordic conditions. We need to expand the knowledge on the decline rates of nematode field population densities and the reduction in the infection potential over time. More information is needed on the role of nematode parasitic fungi and their potential as components of future integrated pest management of PCN. It is also very important to define the safe use of early potato as a practical control method, and the potential for using *S. sisymbriifolium* as a trap crop in Norway. We need also to explore components of the PCN-Potato-Pathosystem. The occurrence and pathogenicity of microbial antagonistic parasitic to PCN will be a subject for a PhD-study contained within this project.

4. **Research approach, methods**

(1) To determine the virulence in selected populations.

Virulent populations, *i.e.* isolates capable of multiplying on the resistant potato cultivars Saturna, will be classified to pathotype at Julius Kühn-Institut (JKI) in Braunschweig, Germany. The biotests for identifying pathotypes will include clones and hybrids of *Solanum tuberosum*, *S. kurtzianum*, *S. verne*i and *S. multidissectum* (Kort *et al.*, 1977). These studies will be complemented by documenting the morphology at Bioforsk Plant Health in Ås by using interference contrast microscopy supported by the ”Leica Image Processing and Analysis System”. The work will concentrate on second stage juveniles and cysts of the selected *Globodera*-populations, which will be compared to standard populations of *G. rostochiensis* (*i.e*. Great Britain Ro1 “Ecosse” and German Ro5 “Harmerz”) and *G. pallida* (Duddingston Pa1 and Switzerland Pa2/3 “Chavornay”), *G. tabacum*, *G. artemisiae* and *G. achilleae*. All standard populations will be reared in special cultivation units kept in a growth room at Bioforsk in Ås. Selected populations will be subjected to further studies by molecular techniques, at Bioforsk Plant Health in in close collaboration with JKI.

(2) To establish the decline rate in PCN numbers and infectivity in field soils.

Population density decline of the nematode over time will be studied in the field. In Norway, the earliest incidences of PCN-infections date back to the 1950ties. Our records contain information on PCN infections in about 20 municipalities belonging to 9 counties. Especially the counties Aust-Agder, Trøndelag and Rogaland have many cases where farms have been subjected to permanent quarantine. Such fields will be sampled in 2010-2012. Most of these fields have been free from PCN host plants for 20-50 years. PCN cysts will be tested for hatch of their contents by submersion in root exudates from 3-week-old potato plants. The ability of hatched juveniles to infect and reproduce will be tested on susceptible potato (cv. Kerr’s Pink) grown in 500 ml pots in the green house, and inoculated by a known number of juveniles. The experiments are conducted in growth cabinets at Bioforsk Ås at 20^oC and 18 hrs light period. After 3 months, the shoots will be severed and the soil air-dried. Nematode cysts will be extracted by flotation using the Fenwick can. The number of new females/cysts developed on the roots will index the infection potential.

<u>(3) To study the occurrence and role of microorganisms as antagonists of PCN.</u>
The occurrence of microbes, including nematode-parasitic fungi, within the nematode cysts will be determined in recently infested fields or in fields subjected to varying durations of quarantine regulations. This will be done as a PhD project covering the occurrence, pathogenicity and control efficacy of fungi parasitic on PCN. Soils with nematode cysts will be collected from already identified fields in the counties of Nord-Trøndelag, Sør-Trøndelag, Agder, Vestfold, Sogn og Fjordane and Rogaland and assayed for parasitic fungi. Molecular methods for quantifying *P. chlamydosporia* and *P. lilacinus* in soil will be used. The range of soils with different histories of PCN infestations will be used for greenhouse assays to examine their potentially varying degrees of suppressiveness. Portions of each test soil will be amended to standard greenhouse potting mix, infested with a standard number of PCN. According to population density development within one growth period of potato, soils will be classified for degrees of suppressiveness in comparison to a non-amended, infested greenhouse potting mix (Westphal, 2005). Application of the method will be critical to predict whether active microbial suppression of PCN is important for the emipirically recognized population density decline in fields. In addition, this method will provide fresh nematode material for fungal isolation experiments.

To screen for *P. chlamydosporia*, eggs from cyst will be collected on a 20µ filter, washed in sterile distilled water and transferred to 9 cm Petri dishes with water agar amended with streptomycin sulfate, chloramphenicol and chlorotetracyclin hydrochloride, and incubated at 19^oC for 24-48 hours. Hyphal tips of fungi growning from infected eggs and juveniles will be transferred to Potato dextrose agar and kept at 25^o C for further growth and examination of the colonies and other morphological properties. Screening for *P. lilacinus* will be made in a 14 cm Petri dishes as growing chambers with susceptible potato (cv. Kerr's Pink). These containers, inoculated with fresh PCN juveniles will be incubated in growth cabinets at 20^oC and 18 hrs light period. After 10 weeks, the frequency of the parasite infection will be determined by extracting cysts and recording the rate of infection in 50 specimens. Detected infections will be isolated and identified at Bioforsk Ås according to standard methods (Holgado & Crump, 2003).

Antagonist will be studied for their pathogenicity to wPCN and yPCN, using the growth chamber method described above. For these tests, inoculum of selected fungal isolates for field trials will be produced in the laboratory. For example, *P. lilacinus* needs to be reared on wheat grain substrate. *P. chlamydosporia* will be grown on a wheat brand/powered grain to obtain chlamydospores, *i.e.* amended corn meal agar, (Kerry, 1991).

To evaluate the control potential two field trials with susceptible potato 'Ostara' will be conducted using approximately 10 litre pots inserted into the ground and serving as microplots. In 2011 all plots will be filled with sterile sand, planted with potato and the respective treatments inoculated with PCN. The pots will be harvested in autumn. In spring 2012 plots will be sampled for nematodes, planted with potato and inoculated with the nematophagous fungi. Both trials will have seven treatments with 5 replications: (a) potato, non-infested (b) potato + PCN; (c) potato + PCN + *P. chlamydosporia* (d) potato + PCN + *P.*

liliacinus (e) *Solanum sisymbriifolium* + PCN (f) *S. sisymbriifolium*+ PCN + *P. chlamydosporia*, and (g) *S. sisymbriifolium*+ PCN + *P. liliacinus*
In Nord Trøndelag and Jæren the experiment will be laid out on land infested with *G. pallida*, while in Østfold and Vestfold the nematode species will be *G. rostochiensis*. Grain or other media colonized with *P. lilacinus* will be applied at a rate of 0.4 t/ha. The application of *P. chlamydosporia* will be at a density of 5000 chlamydospores g^{-1} soil. These experiments will be continued in 2012 and the dynamics of PCN (cyst and egg counting) and fungi (qrt-PCR, DGGE fingerprinting) will be followed by spring- and autumn samplings, and related to the yield of potato. The nematode infectivity will be tested on the potato cultivar Ostara in the greenhouse.

The participation of the PhD student will be as Short Term Scientific Missions that consist on short visits to a laboratory in participating countries for the purposes of training or collaborative research.

<u>(4) To investigate the decline in PCN numbers in field trials with early potato and *S. sisymbriifolium.*</u>

The changes in population density of PCN will be studied in field trials in Jæren (Rogaland), Larvik (Vestfold), Rygge (Østfold) and in Frosta (Nørd Trøndelag). In these experimental fields 4 early potato cultivars resistant to yPCN (Ro1) *e.g* Rutt, Aksel, Juno and Halmet, as well as *S. sisymbriifolium* will be used with susceptible potato (cv. Ostara) serving as control. The experimental plots measure 4.5 x 3 m with sampling plots measuring 3.5 x 2 m. The treatments include two different planting and harvesting dates, so at each locality there will be 24 treatments each with 5 repetitions. The trials will be laid out in a fully randomised complete block design. The dynamics of yPCN will be studied using spring and autumn samplings. Spring samples will give information on the initial population density (Pi), while autumn samples will give information on the final population density (Pf). The ratio Pf/Pi is the multiplication factor. The smaller ratio, the lower reproduction, and hence the greater is the decrease in the numbers of yPCN.

<u>(5) To characterize the degree of resistance of selected potato varieties available in Norwegian market</u>
The degree of susceptibility of potatoes to PCN will be quantified according to the EPPO standard 3/68 (Anom. 2006) “Testing of potato varieties to assess resistance to *G. rostochiensis* and *G. pallida*” Resistance scoring notation of the tests will provide with a degree of resistance for the most common Norwegian potato varieties. The testing will be performed during 2010 to 2013, as results of the tests shall be confirmed by at least one other trial performed in another year. The arithmetic mean of the relative susceptibility in the 2 years shall be used to derive the score according to the standard scoring notation.

<u>(6). Exploring components of the PCN-Potato Pathosystem</u>
Genetic and biochemical studies will be conducted in order to explore important components of the host-parasite relationship, the activation of PR protein genes will be studied in plants of a resistant and susceptible potato cultivar. Roots and foliage from PCN-infected and non infected plants, grown in the green house, will be used for these analyses.

5. **Project organisation and management**
The administrative responsibility is with Dr. Ellen Merethe Magnus, Director of Bioforsk Plant Health and Plant Protection Division in Ås. Most of the research work, including sample analyses is made at the Bioforsk Plant Health and Plant Protection Division in Ås with Dr.

Ricardo Holgado as the project manager. Dr. Chister Magnusson and a PhD student will actively collaborate in the different research activities as needed. A PhD student will be registered at The Norwegian University of Life Sciences, and Bioforsk Ås will be the working place. The local collaborators in each region will be in charge of conducting the field experiments Tor Anton Guren (Østfold), Arne Vagle (Rogaland), Miriam Himberg (Vestfold) and Jon Olav Forbord (Nord Trøndelag).

The kind of user involvement on the regional plane includes farmers, local enterprises and authorities of the county administration:

- Norwegian Farmer's Union (Bondelaget).
- The Norwegian Horticultural Growers Association (Gartnerforbundet).
- The Norwegian Food Safety Authority
- Maarud, Hoff
- The Norwegian Seed Potato Association (Settepotet dyrkernes landslag)
- The Norwegian Agricultural Extension Service (Stjørdal og omegn forsøksring, Norsk Landbruksrågiving Rogaland, Vestfold forsøksring, Forsøksringen Sør Øst), Potato Forum with Dr. Borhild Glorvigen
- Farmers

Each of the listed organizations will be represented in the project's reference group (attached list), which actively participates in project discussions and evaluates the progress of the project work. Members of the reference group will assist in disseminating the project results to their respective members.

6. International co-operation

Dr. Björn Niere at Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health, Braunschweig, Germany, will be actively involved in the project by conducting the testing of PCN pathotypes.
Dr. Holger Heuer at JKI- Institute Epidemiology and Pathogen Diagnostics, Braunschweig Germany, will be involved in the project using molecular techniques for differentiation and characterization of *Globodera* spp. populations, development and application of qPCR for quantification of antagonistic to PCN in soils, Dr. Heur will be involved in the training of the PhD student in different molecular techniques needed to perform the studies.
Dr. Andreas Westphal at JKI Institute for Epidemiology and Pathogen Diagnostics, Münster, Germany will be involved in the soil suppressiveness/biocontrol aspects of this project. Dr. Westphal will also be involve in the initial training of the student and will assist in the specific design of the greenhouse bioassays. Dr. Westphal has long-term experience in suppressive soils towards different cyst nematodes. The laboratories of Dr. Westphal and Dr, Heuer will be part of the Short Term Scientific Missions of the Ph.D. student.
Dr. Kieth Davies Rothamsted Research UK. will be a important discussion partner in the project as he has been active in biological control for many years and has a good experience in this area. Dr. Davies has excellent expertise in the biology of the bacteria *Pasteuria* sp.
Prof. Dr. Francisca del Campo and Dr. Soledad Sanz-Alféres, Universidad Autonoma de Madrid Spain, will be involved in the initial characterization of components of the host-parasite relatiomnships between PCN and Potato. Plant-pathogen responses are the area of expertise of Prof. del Campo and Dr. Sanz-Alférez.

7. Progress plan - milestones

The time table of the project activities is presented in Table 1. The project requires a 4-year full time study. At an initial workshop, a detailed presentation of the project will be made and the reference advisory group of the project will be constituted. Selection (together with the Norwegian Food Safety Authority) of old quarantine fields for sampling is also an initial project item. Most work on nematode identity will be carried out in 2010-2012. Pathotype testing and DNA-analysis with morphological support will be carried out in all project years. This is also the case for the field studies involving early potato and *S. sisymbriifolium*. PCN decline in quarantine fields will be studied 2010-2012, with investigations on infection potential extending into 2013. The PhD-project makes its progress from survey activities over identification to pathogenicity and control potential. The final project year 2013 will mainly be devoted to finalising the thesis and preparing its defence. Most manuscripts are planned for 2012 and 2013. Regular project meetings with the reference group will serve as check points for the project milestones and will allow for feed-back from the advisory group, potentially allowing for some adjustments.

Table 1. Project activities and milestones for the period 2010-2013.

ACTIVITY /MILESTONES		**2010**				**2011**				**2012**				**2013**			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Reference PCN group	The PCN reference group is constituted	X															
	Reference group meetings	X			X	X			X	X			X	X			X
Virulence in selected populations	Resistance breaking test		X	X	X	X	X	X	X	X	X	X	X				
	Pathotype tests JKI Braunschweig (B. Niere)			X	X	X	X	X	X	X	X	X	X	X	X		
	Morphology microscopy	X	X	X	X	X	X	X	X	X	X	X					
	DNA-techniques (Ås-Braunschweig (H. Heuer)			X	X	X	X	X	X	X	X	X	X	X	X		
Decline in PCN numbers and infectivity in field soils	Site selection	X	X			X				X							
	Sampling				X				X				X				
	Bio test (laboratory studies)					X	X			X	X			X	X		
Occurrence and role of nematode-parasitic fungi a PhD. Student´s Project	Occurrence		X	X	X	X	X	X	X								
	Frequency of infection			X	X	X	X	X	X	X							
	Isolation of fungi			X	X	X	X	X	X	X	X	X					
	Identification incl. DNA studies.			X	X	X	X	X	X	X	X	X	X	X			
	Short Term Scientific JKI Braunschweig (H. Heur)			X	X	X		X			X		X				
	Pathogenicity					X	X	X	X	X	X	X	X				
	Inoculum production						X	X	X	X	X	X	X	X			
	Field trial on control									X	X	X	X	X	X	X	
	Short Term Scientific Missions JKI Münster/ Braunschweig (A. Westphal)			X	X	X		X			X		X				
	Thesis and defence													X	X	X	X
Resistance tests	Characterization of resistance degree (EPPO Standard)		X	X	X		X	X	X		X	X	X		X	X	
Studies on the PCN-potato pathosystem	Exploratory studies UAM Spain (F. del Campo/Sanz-Alférez)					X	X	X				X					
Study of decline in PCN numbers under trap crops	Using early cultivars and *S. sisymbriifolium* in Rygge, Frosta, Jæren and Vestfold		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Evaluating quarantine elements	New suggestions on future management of PCN													X	X	X	X
	Progress reports			X				X			X				X		X

Reporting activity	PhD study reports		X		X		X		X		X		X		X		X
	Final report preparation															X	X
	Publication									X	X	X	X	X	X	X	X

8. **Costs incurred by each research performing partner**

The total project cost amounts to **9 557 000** NOK. The main item in the budget is the costs for personnel, which is **6 722 000** NOK. This includes the cost of a PhD student. Another major item is the purchase of R&D services from the Biotechnology laboratory and the Julius Kühn-Institut, Germany and Universidad Autonoma de Madrid Spain. This amounts to a total cost of **1 250 000** NOK, (Table 2).

Table 2. Specification of project costs (in 1000 NOK).

SPECIFICATION OF COSTS (in 1000 NOK)	**2010**	**2011**	**2011**	**2011**	**Total**	
Personnel and indirect costs	930.5	962.5	962.5	962.5	3818	
PhD Student	708	732	732	732	2904	
	1638,5	**1694,5**	**1694,5**	**1694,5**		**6722**
Purchase of scientific services						
Molecular analyses	100	100	100	50	350	
JKI, Germany	200	200	200	100	700	
UAM (Spain)		100	100		200	
	300	**400**	**400**	**150**		**1250**
Other expenses						
Consultative support (Mattilsynet)	15	15	15	15	60	
Equipment	10	10	10	5	35	
Consumables	25	30	30	25	110	
Travel expenses	50	50	50	50	200	
	100	**105**	**105**	**95**		**405**
Field charges/ Field work						
Trøndelag	60	60	60	60		
Vestfold	60	60	60	60		
Jæren	60	60	60	60		
Østfold	60	60	60	60		
	240	**240**	**240**	**240**		**960**
Charge for growth rooms	50	50	50	30	180	
Office costs	5	5	5	5	20	
Publication	5	5	5	5	20	
	60	**60**	**60**	**40**		**220**
TOTAL	**2338.5**	**2499.5**	**2499.5**	**2219.5**		**9557**

9. **Financial contribution by partner**

The major financing body of this project would be the Foundation for Research Levy on Agricultural Products and the Agricultural Agreement. Additional economic resources will be provided by the Norwegian Food Safety Authority, associated partners (Forsøksringen) and local enterprises, and the Potato Industry (Table 3). The support required from the Norwegian

Research Council is 7 584 000 NOK for the total project period. The contribution from associated partners involved in the project (KMB andel), is 1 973 000 NOK. (Table 3)

Table 3. Financial sources for the project (in 1000 NOK)

CONTRIBUTIONS FROM PARTNERS (in 1000 NOK)	**2010**	**2011**	**2012**	**2013**		**SUM**
Bioforsk Plantehelse (own financing) (PCN related projects)	227	227	227	227	**908**	
Norwegian Food Safety Authority (*)	15	15	15	15	**60**	
Maarud	15	15	15		**45**	
National Agricultural extension service (own financing) 4 groups 240 per year	240	240	240	240	**960**	
Contribution from associated partners (KMB andel)	**497**	**497**	**497**	**482**		**1973**
Fondet / avtalemidler /NFR (application)	1841.5	2002.5	2002,5	1737.5		**7584**
Total Project Cost	**2338.5**	**2499.5**	**2499.5**	**2219.5**		**9557**

(*) The support of the Norwegian Food Safety Authority (Mattilsynet) is estimated to about NOK. 60 000. This estimate is based on the consultative support required by the project, which is estimated as 5 to 6 man-weeks.

PART 2: Exploitation of results

10. **Relevance for knowledge-building areas**

Effective management of PCN requires reliable information on virulence, decline rates of population densities and infectivity in soil as well as information on measures of enhancing the reduction in nematode numbers by agricultural practices. The excisting information, which for the most part is generated under conditions of central Europe and the British Isles, is insufficient for Norwegian conditions. Few studies has been done in Nordic countries in recent years. Implementation of good practices will increase the sustainability of the potato production and be helpful in designing production systems capable of meeting the high standards of health, quality and ethical values recognised in Norwegian food production.

For nematologists, the information obtained in this project will contribute to the principles of persistence and virulence of PCN, maybe the most noxious pest of potato in temperate regions of the world. Gaining new information on the biology of PCN under Norwegian field conditions will strongly support national management programs.

The studies carried out by the PhD student will generate information for Norway, and Nordic countries on the occurrence, importance and applicability of nematode antagonists of PCN. This may prove to be a central point in PCN-management, and will be a new important topic in our student-training programmes. Hence, the project will bring forward novel tools for advisory decision making on the future PCN management.

11. **Importance to Norwegian industry**

The implementation of the project results will also strengthen the scientific basis for decision-making of the Norwegian Food Safety Authority on PCN quarantine issues. The new findings of this project may alleviate present regulatory restrictions by a better knowledge of the virulence of targeted PCN populations, together with a better prognosis of decline rates in numbers and infectivity in field soil. Any possible reduction in the quarantine period would have immediate positive economic effects for farmers and for the local enterprises.

The potato industry requires regular and predictable deliveries from its contractors. Production of potato for industrial purposes (i.e. starch, spirits, chips, French fries a.o.) often involves growing nematode resistant cultivars. Nematode resistant potato grown in close rotations poses a significant risk for the build-up of resistance breaking nematode pathotypes, which would result in a ban on potato production. The project finds will increase the domestic production stability and reduce the dependence of the industry on import material.

Infections by wPCN and resistant breaking pathotypes of yPCN are getting more common. According to the literature wPCN has proven more difficult to control than yPCN (Trudgill *et al.* 2003), and may require rotations twice as long as for yPCN. Should wPCN become the dominating species in Norway the annual production value in potato of approximately 600 millions NOK. (www.ssb.no) would be considerably reduced. It is obvious that this project will increase the level of awareness of both PCN species.

Norwegian consumers demand healthy, safe and high quality food items. To achieve this, food production systems must embrace methods which are more sustainable, more environmentally friendly and which have smaller needs for agrochemicals. An efficient way to achieve this involves the use of lower input farming systems based on integrated crop protection and organic agriculture. This will be facilitated by this project providing information for improving PCN control without the use of chemicals. In addition the development of management tools and advisory decision support tools will ensure that research data becomes available to the industry, and producers. Training of farmers, advisors and students in key areas of PCN management will further ensure a rapid dissemination of the new PCN management strategies.

12. **Relevance for call for proposals and programmes**

The project address several priorities mentioned in the call of the Foundation for Research Levy on Agricultural Products and the Agricultural Agreement. Knowledge from this project will increase the efficiency in agriculture by developing better production methods. The improved management of PCN will contribute to a sustainable development of potato production and increase the confidence in Norwegian food. Research on the management of PCN is a current priority of producers and the potato industry, and the project may add valuable information to the area of biological control of nematode pests.
The project fits into the frames and meets the ambitions of the Food Program (Matprogrammet) of the Norwegian Research Council. The results of the project contributes in making the Norwegian food production more competitive and ensures that the food authorities have access to research-based, up-to-date knowledge that provides a basis for their supervision of potato production and setting of national and international regulations.

The project also meets the priorities of the Norwegian Agricultural Authority regarding quality, safety and stability in food production. In particular it emphasises the use of integrated pest management (IPM) strategies, which also are compatible with organic production systems. This is of importance in view of the national ambition of increasing the organic production. The project is supported by the public and the industrial sector, as well as by several other organisations related to potato production (attached support letters).

Finally, an important national obligation and priority is the restriction of the spread of alien species in Norway (Anon. 2007). PCN qualifies as alien species, and the results of the project will contribute in limiting the nematodes further spread and damage on agricultural land.

13. **Environmental impact**

There are no negative environmental consequences of this project. On the contrary the results of the project will have a positive impact on the environment by the development of sustainable approaches, and environmental-friendly methods for the control of PCN. Good control of PCN means that the use of nematicides in Norway still can be avoided, ensuring healthy food for consumers, In other European countries *e.g*. UK, pesticide residues in fresh produce are of great concern as a result of the need to apply nematicides at a rate of more than four times higher than the total amount of pesticide application in most other crops.

14. **Information and dissemination of results**

The main practical results will be made available for the collaborating parties, authorities and farmers, at excursions, seminars and on the following web pages www.bioforsk.no, http://potet.no. The scientific results will be presented at international scientific conferences, seminars /workshops, open-days, and symposia. Publications in both reviewed scientific journals, extension leaf-lets and regional press articles (as appropriate). The PCN reference group will establish itself as a leading body for the development and implementation of durable PCN control strategies. It will therefore become recognised as the first point of national reference, for scientists, producer, legislators and other users. It will also provide a forum for nematologists and potato producers from other regions, to share their knowledge and advice.

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