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Management of PCN (*Globodera* spp.) populations under Norwegian conditions

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Summary

In 1955 the potato cyst nematode (PCN) was recorded for the first time in Norway. This detection immediately triggered extensive surveys which continued until the 1990s and revealed the occurrence of PCN in 6406 properties. In 2009 a new national survey was initiated of ware potato fields. Certified seed potato production areas have been sampled intensively since the first detection of PCN. The distribution of species and pathotypes is of crucial importance for potato production. The yellow species *Globodera rostochiensis* (yPCN) occurs in the pathotypes Ro1, Ro2, and Ro3, while the white species *G. pallida* (wPCN) has been detected in the pathotypes Pa1, Pa2 and Pa3. The most common pathotype is Ro1 forming 98% of total finds. The detections of wPCN and yPCN Ro3 in commercial fields have increased in the last 5 years. Commercial chemical fumigants, organophosphates or carbamate nematicides have not been used in Norway since the early 1970s. Most Norwegian potato cultivars have the resistance genes, Gro-1 (H1) from *Solanum tuberosum* ssp. Andigena, which is effective only against yPCN Ro1. Management of yPCN Ro1 relies on 4 years crop rotation. Certified seed potatoes are used in combination with crop rotations using non-host crops, alternating susceptible and andigena-resistant potato cultivars. Finds of wPCN and virulent yPCN result in a ban on growing potato for 40 years. The possible use of early potato and *Solanum sisymbriifolium* as trap crops, and the importance micro-organisms anagonistic to PCN is considered in current research. Norwegian statutory regulations have been sensitive to new information on PCN, and without doubt contributed in preventing PCN infestations in the seed potato areas, and probably also prevented further spreading of wPCN and virulent yPCN as each the find has been placed under quarantine. A better prognosis of rates of decline in PCN numbers and infectivity in field soil could allow for a reduction in the quarantine period, which would have immediate positive economic effects for farmers and for the local enterprises.

Key words: *G. rostochiensis*, *G. pallida*, pathotypes, quarantine regulations, Norway

Introduction

Potato cyst nematodes (PCN) *Globodera* spp. are thought to have originated in the Andean region of South America, and have been introduced into Europe after 1850. Subsequently, in Nordic region PCN were detected in Sweden 1922, Denmark 1928, Finland 1946, Faroe Island 1951, Island 1953, and Norway 1955 (Videgård, 1969; Øydvin, 1975).

After the detection of PCN in Norway extensive surveys were carried out and regulations were implemented. The first statutory regulation for PCN dates from 1956, and has later been amended several times. The latest amendment was made in April 2010 (Anon., 2010). All regulations prohibit the introduction and spread of PCN with soil and plant materials. Early control strategies included the use of chemical fumigants and resistant potato cultivars in infested fields. The taxonomic separation of the yellow *Globodera rostochiensis* (yPCN) and the white species *G. pallida* (wPCN), together with emerging information on the existence of pathotypes caused a change in the strategy involving a controlled use of resistant cultivars to avoid the increase of resistant breaking pathotypes. Today both species are regarded as quarantine pests.

In the preceding decades great emphasis has been placed on documenting freedom from PCN in the production of certified seed potatoes, and on the detection of infested fields and their placement under effective quarantine regulations. In the early 1960s import and movement of all kinds of potato seed was prohibited, as a measure to prevent the introduction of new PCN populations, and to prevent contamination of uninfested land. In addition proper crop rotation and the use of cultivars with resistance have been enhanced.

Commercial chemical fumigants, organophosphates or carbamate nematicides have not been used in Norway since the early 1970s. Today, non-virulent (yPCN) is managed by crop rotation, while infestations by (wPCN) or virulent (yPCN) results in 40-years ban on growing potato. Most Norwegian potato cultivars have the resistance genes, Gro-1 (H1) from *Solanum tuberosum* ssp. andigena. Crop rotations using non-host crops, alternating susceptible and resistant cultivars are important control measures, but not easy to implement in Norway due to restricted acreage suitable for long rotations. Hence, the use of resistant potato cultivars becomes important, but requires a better knowledge on the species and pathotypes present. An overview of the PCN management strategies and studies done since PCN were detected in Norway are presented in this paper.

Materials and Methods

The history of regulations has been compiled by reviewing official documents. Information on distribution and biology of PCN has been collected from survey reports, current compilations and published experimental data. With regard to survey data the current sampling system is shown in Table1.

Table 1. Type of soil sampling to estimate PCN occurrence and density for statutory and advisory purpose

Type of Sample	Number of cores pr. Sample.	Total bulked Sample	Area to provide one bulk sample	Patterns of cores grid (Core size 25.0x2.5 Cm.)
Routine	50	250ml	0.5 Ha	Line distance 10m
Certified potato	50	250ml	0.25 Ha	Line distance 7m
Quarantine proposes	9	250ml	$100m^2$	Line distance 3m
Statutory tests	25	1000ml	$100m^2$	Line distance 2m
Suspicion of PCN infection	-	500ml	-	Soil and plant samples
In stored Packing houses of ware potatoes		250ml	min 30 ton potato	Min. 1 sample pr. Consignment

PCN Management

Occurrence of PCN in surveys of areas for potato production

The detection of PCN in Agder-County in 1955 immediately triggered an extensive survey activity of both production fields and home gardens. This activity continued until the end of the 1990s,

and provided a significant increase in our knowledge of the distribution of PCN. In 1974 PCN had been detected in all counties south of the Dovre Mountains with the exception of Hedmark. In 1985 PCN was recorded in the counties of Møre and Romsdal, Hedmark and Sør Trøndelag. In 1993 the nematode was registered in the county of Nord Trøndelag in the municipalities of Frosta, and Stjørdal in 2004. In the northernmost counties of Finmark, Nordland and Troms old and empty cysts without juveniles or eggs were detected, but subsequent monitoring has failed in revealing new infections (Table 2).

The intensive surveys carried out from 1955 until 2000 demonstrated PCN to occur in 6406 properties, and 47% of these observations were made in home gardens (Table 2). PCN was recorded in 5% the total 89162 samples analysed. However, in these surveys many samples were collected in the county of Hedmark, an area with low infection and high sampling activity due to the large production of certified seed potato. In order to update the information on the distribution of PCN a new national survey of ware potato fields was started in 2009. This first year 2375 samples were analysed from the county of Rogaland and PCN was discovered in a frequency of 14%. This survey will continue until all major potato areas have been covered.

Table 2. Occurrence of PCN in Norway by counties, on properties (farms and home gardens), mumber of analysed samples with and without PCN, during the extensive survey period 1955–2000.

County	Number of property with PCN				Analysed samples		
	Home gardens (%)*	Farms	Without information	Total	with PCN (%)**	without PCN	Total
Østfold	213 (55)	173	1	387	509 (7)	6280	6789
Akershus	361 (73)	131	1	493	163 (3)	4688	4851
Hedmark	126 (67)	6	57	189	166 (0.5)	28439	28605
Oppland	202 (88)	17	10	229	866 (7)	11700	12566
Buskerud	421 (91)	42	1	464	127 (5)	2425	2552
Vestfold	140 (41)	161	44	345	566 (10)	5262	5828
Telemark	120 (73)	32	12	164	97 (2)	4392	4489
Aust-Agder	383 (56)	152	145	680	381 (30)	862	1243
Vest-Agder	519 (26)	381	1060	1960	292 (30)	654	946
Rogaland	326 (46)	306	70	702	432 (14)	2482	2914
Hordaland	24 (12)	78	99	201	57 (14)	343	400
Sogn of Fjorane	132 (25)	271	130	533	801 (13)	5157	5958
Møre og Romsdal	6 (55)	5	0	11	14 (2)	610	624
Sør-Trøndelag	4 (25)	9	3	16	18 (0.5)	3020	3038
Nord -Trøndelag	2 (10)	19	0	21	57 (0.8)	6820	6877
Nordland	0	1	0	1	1 (0.1)	675	676
Troms	0	1	0	1	3 (0.6)	446	449
Finmark	0	0	0	1	1 (0.8)	114	115
Not information on county of origin	0	0	0	8	3 (1)	239	242
Total	2979 (47)	1785	1642	6406	4554 (5)	84 608	89 162

* % in relation to the total proprieties with PCN (farms + home gardens)
** % in relation to the total of samples analysed.

PCN and certified seed potato

Official controls of certified seed potatoes started in 1939. At that time the regulations were aimed at controlling Synchytrium endobioticum, Potato Mosaic Virus, and preventing the entry of Septinontarsa decemlineata. So at this time there was no prevention against the importation of PCN in seed potato (Jørstad, 1951). In 1957, however, *Heterodera rostochiensis* f. solani was incorporated in the regulations on certified seed potatoes (Larsen, 1951). Surveying areas with production of certified seed potatoes for PCN started already in 1956, soon after the first detections of PCN in Norway. By now, areas with seed potato production have been under constant monitoring for more than 50 years. Each year about 3000 soil samples are taken (normally after lifting) and analysed for PCN to clear areas for certified seed potato production. These areas are so far free of PCN.

Nematode virulence

The diversity of pathotypes was first recognised when the reproductive ability of PCN populations was tested on *Solanum* clones containing PCN resistance genes, e.g. gene H1 from *Solanum tuberosum* ssp. andigena. These resistance genes were identified in collections of wild or cultivated Andean potatoes, and were recognised as a potential tool for PCN control. 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 species (Videgård, 1969; Bumulucz & Øydvin, 1976).

PCN species and pathotypes found in Norway

The correct identification to species and pathotype is of crucial importance for the kind management and regulations to be imposed. Studies on the identity of species and pathotypes have been carried out since the middle of the 1970s until present time. This is done in accordance with the EPPO diagnostic protocol PM 7/40(2) (EPPO, 2009) and includes morphology, iso-electric focusing and molecular methods. The initial studies on pathotypes were made according to Kort et al. (1977). Under Norwegian conditions all pathotypes of wPCN and most pathotypes of yPCN except Ro1 are considered virulent on andigena-resistant potatoes. Hence, in the 1980s a simpler method was introduced using resistant potato cv. Saturna (resistant to Ro1, Ro4) with the susceptible variety Kerrs Pink as control. Populations developing on cv. Saturna are classified as resistance breakers. In Norway occurs yPCN Ro1, Ro2 and Ro3, and wPCN Pa1, Pa2 and Pa3, Pathotype Ro1 is the predominant and represents 98% of the infections.

The earlier surveys indicated that yPCN had a moderate distribution and occurred mainly in the southern part of the country, while the distribution of wPCN was much restricted and limited mainly to home gardens. However in the last 5 years we have detected infections by wPCN and resistant breaking yPCN (Ro3) in a small number of commercial fields.

In recent times we observed large morphological variability in PCN regarding shape and length of stylet, tail, and characters of the perineal pattern. Studies including DNA-based diagnostics of these populations are at present underway.

Crop rotation

Since 1956 crop rotation on a 4 year basis was included in the statutory regulation. In this early stage resistance was not recognised. In 1970 the use of resistant potato cultivars was officially recommended in Norway. For Norwegian farmers today the best long-term policy to manage yPCN Ro1 is still crop rotation using non-host crops, and alternating between susceptible and resistant cultivars every 4 years. This recommendation includes the use of certified seed potatoes. The 4 years rotations, however, are complicated by restricted acreage.

Potential for using trap crops

The use of early potatoes as a trap crop is recommended for reducing PCN populations. A recommendation for Norwegian farmers with early potato production is to plant and grow for sufficient time to permit nematode infection, and lift before the start of nematode reproduction.

The establishment of *Solanum sisymbriifolium* in field trials using the commercial varieties Pion and White Star are in progress. So far studies in 2009 (Holgado et al., 2010), in the counties of Nord Trøndelag and Rogaland, using White Star with two sowing times have been completed. Sowing in early May showed White Star incapable of competing with weeds. Sowing in July after early potatoes using rimsulfuron to control weeds was more promising. In Nord Trøndelag, White Star continued to grow until the first frost period in October. In Rogaland manual weeding was made on several occasions to enhance the establishment of White Star. The preliminary studies indicated that S. sisymbriifolium could established successfully if due attention was paid to proper weed management and sowing time.

Occurrence and pathogenicity of microbial antagonists parasitic to PCN

Soil from fields with PCN was collected and bait used for antagonistic fungi, as previously described by Holgado & Crump (2003). The soil samples were collected in the counties of Nord Trøndelag (six localities) and Rogaland (five localities). Four growing chambers were filled with test soils. For each species two chambers were inoculated with twenty cysts. Each chamber was planted with two potato chits of susceptible potato cultivars Beate or Desirée. Females developing on the root surface were examined monthly. The females were counted and infected specimens were removed from the roots and placed onto moist filter paper in a Petri dish and assessed for infection. Fungi that sporulated, and could be identified were transferred on nutrient agar. The following nematophagous fungi were found: *Pochonia chlamydiosporia*, *Paecilomyces lilacinus* and *Catenaria* spp.

Historical review of regulation and management of PCN in Norway

The management of PCN has been based mainly on regulatory restrictions. The first regulation on PCN specifically is dated 31 May 1956 and prescribed potato or tomato cultivation every 4 years, and prohibited soil or infected plants to be moved (Anon., 1956). In 1962 the regulation was amended by incorporating a ban on the movement of equipment and machinery used in the infected area, unless it was cleaned, inspected and found free of PCN (Anon., 1962). In 1964 the Ministry of Agriculture reviewed the general statutory rules from 1916 (Anon., 1916), and launched a new law dealing with prevention and control of plant pests (Anon., 1964). This resulted in a revision of the PCN regulation, which now included the use of PCN resistant potato cultivars (Anon., 1970). For using resistant cultivars a surveillance and official control of the infected areas was recommended, and the control strategies now included the use of chemical fumigants in highly infested fields. The survey activity detected new infestations, and infested fields were placed under quarantine regulations (Anon., 1976). In 1977 a new regulation appeared were the taxonomic separation of yPCN and wPCN, together with emerging information on the existence of pathotypes was taken into consideration. The regulation of 1977 recomended a controlled use of resistant cultivars, to avoid the increase of resistant breaking pathotypes (Anon., 1977). In 1990 a new regulation incorporated a prohibition of growing potatoes in areas with wPCN or virulent yPCN, and included further the possibility of imposing a ban on growing potato in areas close to the farms, or on farms sharing equipment or machinery with infested farms. (Anon., 1990).

In 1998 the Ministry of Agriculture delegated the responsibility for maintaining good plant health to the Norwegian Plant Inspection Service. A new regulation for PCN was produced, and included measures for potato manufacturing facilities; if PCN was found, sanitary measures are compulsory to the houses committed to packing potatoes. For farmers the movement of their own seed potatoes from packing houses was prohibited. Also the possibility of economic compensation for farms affected with a ban for growing potatoes is mentioned (Anon., 1998). In 2000 a new

PCN regulation stipulated that all potato producers and manufacturing facilities must be officiallylisted and have an internal control system for PCN management, including preventive measures against its dissemination. The regulation mentioned the possibility of a long quarantine period for resistance breaking PCN populations, in practice more than 40 years due to the long persistence of PCN in the absence of host plants (Anon., 2000).

Today the Norwegian Food Safety Authority is in charge of import and export inspections for plants and plant products, and surveillance and official control programs for regulated pests. Recently in April 2010 a new regulation was introduced (Anon., 2010) prescribing surveillance and official control of PCN-infected and non-infected areas where potatoes are produced. This new strategy implies the demarking and regulation of the area actually infested.

Discussion

During the last 55 years our knowledge on the occurrence of PCN in Norway has grown considerably, and PCN is today considered as one of the most noxious pests in Norwegian potato production. The recognition of PCN as an important potato pest has increased, and it has become apparent that continuous cropping of susceptible cultivars on land heavily infested with yPCN (Ro1) may easily result in an average yield loss exceeding 50%. Both yPCN and wPCN are quarantine pests subjected to national regulations. An infection by wPCN and virulent yPCN adds a serious aspect to the situation because of the strict regulation involving a minimum of 40-years ban on growing potatoes. The duration of this ban is based on studies from Northern Ireland indicating a persistence of eggs and juveniles for 42 years in soil without host plants (Turner, 1996).

With this in mind, the first priority of management is to keep farms free of PCN, and access to clean seed potatoes is fundamental. Great emphasis has been placed on documenting freedom from PCN in the certified production. The domestic production of seed potato has been kept free of PCN by frequent inspections and analyses for more than 50 years. The fact that farmers are not allowed to import seed potatoes adds to the level of security.

Although the survey activities steadily increase our knowledge of the PCN situation, our experience indicates that it is necessary to have better information on the distribution of virulent populations. As continuous cropping of cultivars with H1 resistance gene on land infested with the yPCN Ro1 decimates the nematode population fairly rapidly, there is a temptation to use resistant cultivars too often. The selection for resistance-breaking pathotypes has been demonstrated in a long-term field trial where an infestation of yPCN Rol was shown to contain initially low frequencies of andigena resistance-breaking genes. The continuous cropping of Ro1 resistant potato cultivars for 10 years resulted in Ro3 overcoming Ro1 (Bomulucz & Øydvin, 1976). Complementary laboratory studies indicated that continuous growing of H1 resistant cv. Saturna in soil with Ro1/Ro3 mixtures, showed that an initial Ro3 frequency as low as 0.1% in 5 years time resulted in high populations of Ro3 (Magnusson et al., 1999). This is in line with experience of other European countries where intensive cropping of potato cultivars with the H1 resistance gene has allowed wPCN to increase (Minnis et al., 2000). These observations suggest that routine-testing PCN populations for virulence would be important.

Correct identification to species and pathotype is of crucial importance for management and regulations to be imposed. It is likely that each introduction of PCN into Norway represents only a sub-set of the original population gene pool. Consequently, each introduction is likely to have been genetically distinct with a limited variability. Our morphological studies indicate a degree of variation that needs to be analysed further by a combination of morphological studies, and molecular techniques focussing on our domestic populations.

The use of traps crops as early potato cultivars is aimed at reducing PCN populations. The effectiveness of this method is highly dependent on the time frame for hatching and reproduction

of the PCN population. However cropping potatoes as trap crop requires careful timing to avoid increasing rather than reducing PCN populations. Hence, trap-cropping systems with early potatoes need to be closely adapted to local conditions, which also will be covered in future studies. The inclusion of *S. sisymbriifolium* as trap crop in Norwegian management systems is not without agronomic complications and will be investigated further.

The presence of natural enemies to cyst nematodes indicates that there is reason to suspect that plant parasitic nematodes in Norwegian fields are living under various degrees of natural control. However, it will be necessary to screen these fungal isolates to select the best isolates for testing in long term field trials, which will be an important task in our further studies.

Norwegian statutory regulations have been sensitive to new information on PCN. The regulations have without doubt contributed in preventing PCN infestations in the seed potato areas, and probably also prevented further spreading of wPCN and virulent yPCN as each the find has been placed under quarantine. Permanent grass as a statutory regulation in home garden plots may have contributed to reduce the spread of wPCN to commercial fields. The regulations have most probably made possible the early reduction in use of chemical fumigants, organophosphates or carbamate nematicides. These chemicals have not been used since the early 1970s.

However, to further alleviate the present situation for Norwegian farmers it is necessary to provide new information for a better prognosis of rates of decline in PCN 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, and will contribute to the sustainable development of potato production.

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