Globodera rostochiensis (Woll.) Behrens (*Tylenchida, Heteroderidae*), the only potato cyst nematode species found in Finland

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About 10 000 soil samples, 519 thereof infected with potato cyst nematode (PCN), were studied during 1984-1988. Cysts from infected samples were tested by isoelectric focusing to identify PCN species. All the infected samples were also tested with H1-resistant (Saturna) and susceptible (Bintje) potato cultivars to separate resistance breaking populations. Cysts from the roots of Saturna were tested by two-dimensional electrophoresis.

The potato seed production area in Finland was found to be free of PCN of any kind. In other parts of Finland all tested samples revealed *G. rostochiensis* banding pattern, but no *G. pallida* was found. Except for the most common pathotype Ro1-Ro4, we only found Ro2.

Key words: potato cyst nematode, PCN, *Globodera rostochiensis, Globodera pallida*, isoelectric focusing, two-dimensional electrophoresis

Introduction

In Finland, potatoes are grown commercially on about 41 000 hectares. The cultivated area extends from the southern coast (60° 00'N) up to the north (69° 00'N). The growing season for potatoes extends from mid-May to the end of August in southern Finland. In the north the growing season is more than one month shorter (MUKULA and RANTANEN 1987).

Potato cyst nematode (PCN), *Globodera rostochiensis*, was found in Finland for the first time in 1946 (VAPPULA 1954), and the first noticeable damage on commercial potato farms appeared in the early 1970s (SARAKOSKI 1976a). Since the beginning of the 1970s, PCN has been the most harmful pest of potatoes in Finland.

MAGNUSSON (1987) and TIILIKKALA (1987, 1991) have studied the biological and physical factors affecting the success of *G. rostochiensis*. In Finland, the whole life cycle of *G. rostochiensis* requires over 600 day degrees above 4.4°C. This means that *G. rostochiensis* is well adapted to the low soil temperatures and is able to develop in the whole potato growing area up to the polar circle. The northernmost observation in Finland is a few kilometers north of the Polar circle (SARAKOSKI 1976b). Considering that *Globodera pallida* needs

fewer day degrees to reproduce than does *G. rostochiensis* (FOOT 1978, MUGNIERY 1978, FRANCO 1979), one would expect *G. pallida* to be an even more successful species in Finland. If *G. pallida* females develop faster and produce eggs sooner than *G. rostochiensis* at lower temperatures (WEBLEY and JONES 1981), this could lead to gradual replacement of *G. rostochiensis*. On the other hand, at 24°C *G. rostochiensis* produces more juveniles than does *G. pallida* (WEBLEY and JONES 1981).

Both species are common in Central Europe, and have been found in Sweden (OLSSON 1985 a, 1985 b), Norway (ÖYDVIN 1973, 1978), Iceland (SIGGEIRSSON and VAN RIEL 1975), and in Denmark (JAKOBSEN and HANSEN 1983). In Finland, MAGNUSSON (1979) pointed out that although some of her populations, tested with test plants in the greenhouse, did not fit into any pathotype group, no observations of *G. pallida* had been made till 1979.

Reliable identification of species is a key factor when control programs are evaluated. These testing procedures should also be applicable to routine plant protection practice. Testing methods for identification of species and pathotyping of potato cyst nematodes were studied to develop a system for the analysis of populations with low numbers of larvae in the cysts.

According to several authors (FLEMING and MARX 1982, 1983, OHMS and HEINICKE 1983, FOX and ATKINSON 1984, MARX and FLEMING 1985, FLEMING 1987), isoelectric focusing of general proteins is a useful method to identify potato cyst nematode species. Isoelectric focusing and staining of general proteins have been used successfully also to determine other plant parasitic species e.g. with *Meloidogyne spp.*, (DALMASSO and BERGE 1978, LAWSON et al. 1984), and with animal parasites e.g. several *Cestoda* species (DIXON and ARAI 1985, 1987). We concentrated on isoelectric focusing and on identification of species directly from rude soil samples. Some problems related to the identification of pathotype are also discussed.

The aims of this study were 1) to investigate the probable disribution of *Globodera pallida* in Finland, and 2) the establishment of H1 resistance breaking pathotypes.

Material and methods

Soil samples of about one litre per hectare (ca. 0.2 dl subsamples collected randomly from the area) were taken during routine farm inspection by the Plant Quarantine Office, or were sent by farmers in July-August during 1984-88. The total number of one litre samples was about 10 000. The sampling area covered the whole country from southernmost Finland to north of the polar circle. The sampling intensity differed somewhat from area to area, being more intense in western and southern Finland (Fig.1).

Samples were washed either in a Fenwick can, 250 grams dry soil per wash, or in a Schuiling centrifuge, 1 dl dry soil per wash. Cysts were picked manually from the filter paper. Cysts were stored dry for several months in a refrigerator before identification of species. The rest of the samples were stored at about 5°C, until used in plant tests.

Isoelectric focusing (FLEMING and MARX 1983), with slight modifications, was used in identification of species as follows. About 20 cysts per sample were soaked in 1% glycine for at least 24 hours in 1.5 ml microcentrifuge tubes. After soaking, cysts were crushed, homogenates fuged and 20 microliter samples were pipetted onto pieces of filter paper on the 1 mm thick polyacrylamide gel plates, pH range 3.5-9.5 (LKB, Bromma, Sweden). Separation of the proteins was performed for 70 minutes at 4°C. Gels were stained for general proteins with Goomassie Brilliant Blue.

The rest of the soil was used for testing the samples with H1-resistant (Saturna) and susceptible (Bintje) potato cultivars. Pots of 2 dl, submerged in peat and filled with sample soil, were planted with both cultivars. Four replicates at least were used. The pots were kept in greenhouses at 18°C for eight weeks. Cysts formed on the roots of Saturna were picked and stored in a refrigerator for

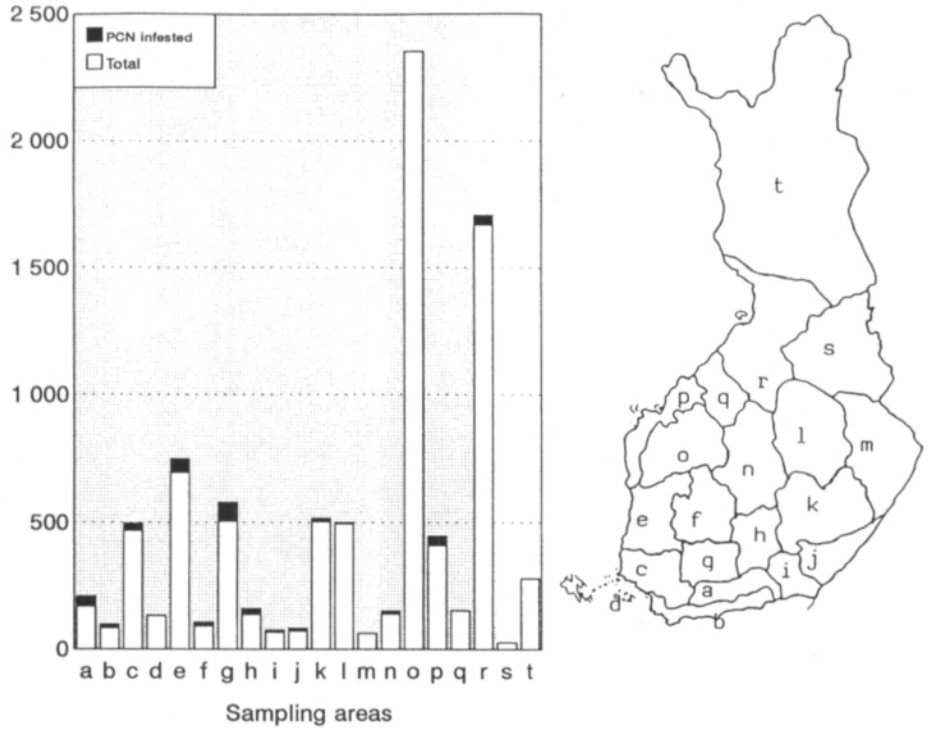

Fig. 1. Soil samples and sampling areas. Letters (a to t) indicate the sampling areas and white bars the total number of soil samples per area. Black bars present the numbers of PCN infested samples.

pathotype testing. Larvae from field samples were counted to get an estimate of initial densities for plant tests. Approximately ten cysts per sample, when available, were crushed and larvae were counted under a microscope without diluting.

Because isoelectric focusing separates species, but is not sensitive enough to identify pathotypes, 2-D electrophoresis (OHMS and HEINICKE 1985) was used for pathotype identification of cysts developed on the roots of the H1-resistant cultivar Saturna.

Results

All samples tested by isoelectric focusing revealed *G. rostochiensis* banding patterns. No *G. pallida* bands were seen.

Plant tests directly confirmed the results in all (519) but twelve samples tested with Bintje and Saturna. No cysts had developed on the roots of Saturna, except for twelve samples (Table 1). But, because the isoelectric banding pattern clearly showed them to be *G. rostochiensis*, we must be dealing with some other pathotypes of *G. rostochiensis* than Ro1 or Ro4. These "resistance breaking" populations were found in different localities and on areas of several Agricultural Advisory Centers, which means that they probably are independent of each other.

2D-electrophoresis revealed Ro1 or Ro2 pathotype banding patterns. In the gels it is impossible to separate these pathotypes from each other.

The small number of larvae in cysts extracted from soil samples from the fields where "resistance breaking" populations had been found (Table 1)

Table 1. Average number of cysts and larvae per cyst from Saturna tests.

Sample	Initial population		Saturna tests	
	Cysts/ 250 g soil	Larvae/ cyst	Cysts/ plant	Larvae/ cyst
53	170	98	34	11
112	286	38	35	40
121	70	97	18	37
135	19	50	3	5
146	245	66	263	61
204	29	54	3	33
246	128	68	25	63
258	280	87	51	82
284	145	42	23	35
322			26	19
348	145	18	1	26
460	75	75	48	23

was a remarkable and interesting point And so was the small number of cysts per plant, and especially the even smaller number of larvae per cyst extracted from roots of Saturna in most "resistance breaking" samples compared to the soil samples (Table 1). The difference was statistically signific-ant (paired t-test, p=0.0221) between the mean number of larvae per cyst from soil samples and that from potato roots.

Discussion

The total number of clearly visible bands in isoelectric focusing of general proteins of *G. rostochiensis* and *G. pallida* is highly dependent on the quality of the sample and the sample preparation method used. However, there are a couple of species specific and diagnostic major bands which are useful in identification of the species. Moreover, these diagnostic bands are not particularly sensitive either to the quality of the sample or the way a sample has been prepared, although the position of these bands may vary according to the preparation method. Our results are comparable with FLEMING and MARX (1982, 1983), and MARX and FLEMING (1985) because of the same methodology.

Our finding that *G. rostochiensis* seems to be the only PCN species in Finland is somewhat surprising. The other species, *G. pallida*, exists in all neighbouring countries. Moreover, climatic and other environmental factors in Finland rather favor *G. pallida* at the expense of *G. rostochiensis* than prevent its existence. Our suggestion is simply that *G. pallida* has not yet invaded Finland.

The potato seed production area along the west coast of the Baltic sea seems to be free of PCN of any kind. Commercial farmers often have contracts which provide the farmers to use seed potato of high quality. This may help to control the PCN problem on those areas.

The census procedure is directed to commercial potato farms, and hence leaves small-scale household farming out of control. Uncontrolled exchange of seed and machinery leaves at least theoretical possibilities that these small household gardens could function as a reservoir for PCN and *G. pallida* as well.

Species can be identified by isoelectric focusing of general proteins. Our results indicate that *G. rostochiensis* is the only potato cyst nematode species in Finland, but the pathotype question needs more attention. Application of the two-dimensional electrophoresis in pathotype identification has been used successfully elsewhere, but in Finland there have been some difficulties. The sample quality must be good, which in our situation is difficult to achieve. The places where "strange" pathotypes were found are few, indicating recent infection. Moreover, the small number of cysts, and especially the small number of larvae per cyst limits the applicability of the system for routine use, and hence the information needed and the purpose must be properly defined.

When comparing for example the Dutch (BAKER 1987) and German (HEINICKE pers. comm.) traditions in the use of 2D-electrophoresis in PCN identification, there is one obvious difference in their level of operation. BAKKER (1987) clearly emphasizes the importance of exact determination of alleles and hence the genetic structure of PCN popu-

lations. This, of course, preconceives optimalization of the procedure which, in turn, makes the whole system less applicable to routine work. In Germany the approach is different, more practically oriented. When certain spots are found in the 2D-gels, it automatically leads to the naming of certain pathotypes and to routine control procedures with resistant cultivars even though the exact pathotype and genetic background are unknown. In Finland the situation is easier because we have only one PCN species, and the number of populations where resistance breaking pathotypes have emerged is small. In this situation it is possible to handle all suspected cases individually by leaving them out of potato cultivation. This requires only the identification of abnormal cases which not necessarily require any complicated methodology. The problem which remains is the possibility some cysts to develop on the roots of resistant cultivars in the absence of resistance breaking pathotypes. In that case no or only few larvae will develop.

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SELOSTUS

Globodera rostochiensis Woll. (Behrens) on toistaiseksi ainoa Suomesta löydetty peruna-ankeroislaji

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Peruna-ankeroislajeja on kaksi: *Globodera rostochiensis* ja *G. pallida*, joilla molemmilla on useita patotyyppejä määriteltynä sen mukaan millä testiperunoilla ne lisääntyvät. *G. rostochiensis* on yleisempi laji Euroopassa, joskin *G. pallida* on lisääntynyt alueilla, joilla ankeroisenkestäviä perunalajikkeita on viljelty intensiivisesti. *G. pallida* -lajia esiintyy myös Tanskassa sekä Ruotsin ja Norjan eteläosissa. Ulkomaisten tutkimusten mukaan *G. pallida* on hyvin sopeutunut alhaisiin lämpötiloihin, joten se todennäköisesti myös menestyisi Suomessa paremmin ja olisi vahingollisempi kuin täällä jo oleva *G. rostochiensis*.

Tämän tutkimuksen tavoitteena oli selvittää: a) esiintyykö *G. pallida* Suomessa, b) onko Suomessa ankeroisenkestävillä perunalajikkeilla lisääntyviä *G. rostochiensis* -lajin patotyyppejä sekä c) miten bioteknisiä menetelmiä voidaan soveltaa peruna-ankeroisen lajimääritykseen. Tutkimus tehtiin pääosin Maa- ja metsätalousministeriön varoin MTTK:n ja kasvinsuojeluviranomaisten yhteistyönä. Tutkitut maanäytteet saatiin Maatilahallituksen ankeroiskartoituksen yhteydessä sekä MTTK:n neuvontaan tulleista lähetyksistä. Näytteitä oli yhteensä noin 10 000 ja niistä laji- ja patotyyppianalyyseihin sopivia elinvoimaisia ankeroisia oli 519 näytteessä. Näytteitä saatiin kaikkien maatalouskeskusten alueilta, joskin pääosa oli otettu Etelä-, Keski- ja Pohjois-Pohjanmaalta.

Puhtaiden näytteiden suuri määrä osoitti, että peruna-ankeroinen ei ole vielä yleistynyt Pohjanmaalla eikä se uhkaa välittömästi siemenperunatuotannon jatkumista Siemenperunakeskuksen toimialueella. Kaikki löydetyt ankeroiset kuuluivat *G. rostochiensis* -lajiin, joten *G. pallida* ei ole toistaiseksi levinnyt lainkaan maahamme tai se on erittäin harvinainen. Valtaosa löydetyistä ankeroisita oli tyyppiä Ro_1/Ro_4, joka ei lisäänny ankeroisenkestävillä (andigena-resistenteillä) lajikkeilla. Kahdentoista näytteen ankeroiset lisääntyivät testikasvina käytetyllä Saturnalla ja ne olivat alustavien tutkumusten mukaan tyyppiä Ro_2. Tämän uuden ankeroistyypin, eli ns. "resistenssin murtajan" todettiin esiintyvän piilevänä jo useiden maatalouskeskusten alueella, joten ankeroisenkestävien perunalajikkeiden (Saturna, Stina, Hertha, Aminca, Prevalent, Provita) jatkuva viljely samalla paikalla voi johtaa resistenssin murtajien valikoitumiseen vallitsevaksi missä tahansa Suomessa.

Tämän tutkimuksen mukaan lajin määritys voidaan tehdä luotettavasti ja nopeasti perunoiden juurista poimituista kystoista isoelektrisellä fokusoinnilla mikäli kystoissa on runsaasti toukkia. Lajin sisäisten erojen eli patotyyppien määritykseen tarvitaan testikasveja tai bioteknistä analytiikkaa, jonka soveltamisesta ja yhdenmukaisesta käytöstä ei ole päästy sopimukseen Euroopan ja Välimeren maiden kasvinsuojelujärjestössä (EPPO). Vuosittain löydettävien uusien resistenssinmurtajien määrä jäänee toistaiseksi niin vähäiseksi, että patotyyppien määrittäminen on helpointa tehdä kansainvälisenäyhteistyönä jonkun Keski-Eurooppalaisen laboratorion kanssa.