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MONITORING OF *GLOBODERA* SPP. IN LITHUANIA USING DIAGNOSTIC MORPHOMETRIC ANALYSIS AND POLYMERASE CHAIN REACTION

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Abstract. A total of 11,406 soil samples from 2,742 ha were collected in 10 administrative regions of Lithuania during the year 2006. A total of 672 cysts were selected and species were morphologically identified as *Globodera rostochiensis*. Of these cysts, 117 from 5 administrative regions of Lithuania were identified using polymerase chain reaction (PCR) analysis. Two pairs of species-specific primers were used to distinguish *G. rostochiensis* from *G. pallida*. This is the first application of a PCR method for identification of *Globodera* species in Lithuania.
Key words: *Globodera rostochiensis*, *Globodera pallida*, polymerase chain reaction, PCR, cyst nematodes, quarantine pests, occurrence

Introduction

Two polymorphic species *Globodera rostochiensis* and *G. pallida* are quarantine pests in Europe that attack potato. They formerly belonged to *Heterodera rostochiensis* until Stone (1973) described *G. pallida* as separate species, now recognised as valid species. *G. pallida* is very similar to *G. rostochiensis,* but differs from it only by a few morphological characters (Brzeski 1998). Studies on *G. rostochiensis* cysts in Lithuania have been performed since 1955 (Mastauskis 1955), but only morphological methods have been used. *G. pallida* has not been identified in Lithuania yet. However, Lithuania imports plant products from different countries, such as Germany, Netherlands and Sweden (Lukošiūtė 2005), where *G. pallida* is present (CABI 1997). Therefore, *G. pallida* is a potential threat to Lithuania. The aim of the present study was to analyse the cyst nematode species *Globodera* that occurred in potato fields in Lithuania using PCR analysis.

Material and methods

A total of 11,406 samples of soil from a 2,742 ha field were collected and cysts were recovered by a flotation method with application of Schuling's centrifuge (Stanelis 2004). A total of 672 cysts were collected from 10 Lithuanian administrative regions and species were identified morphologically (Stanelis 2002). Of all selected cysts, 117 from 5 administrative regions of Lithuania (Vilnius, Kaunas, Šiauliai, Telšiai and Utena) were identified by PCR analysis following the method of Fullaondo *et al*. (1999) with some changes indicated below. PCR analyses were performed in the State Plant Protection Service, Phytosanitary Research Laboratory.

Prior to DNA extraction, single cysts were soaked in distillate water for 24 hours. After that, they were transferred to 1.5 ml tubes and crushed using plastic micro-pestles (Eppendorf) with 180 µl of lysis solution supplied in the genomic DNA extraction kit NucleoSpin Tissue (Macherey-Nagel, Germany). DNA was extracted following the manufacturer's instructions. DNA samples were stored at -20°C.

Two pairs of species-specific primers were used to distinguish *G. rostochiensis* from *G. pallida*: 5' GCAAGCCCAGCGTCAGCAAC 3', 5' GAACATCAA CCTCCTATCGG 3' and 5' TGTCCATTCCTCTCCAC CAG 3', 5' CCGCTTCCCCATTGCTTTCG 3', respectively. The sequences of primers and the modified conditions of PCR were those as described by Fullaondo *et al*. (1999).

DNA amplification was carried out in a 25 µl final volume of reaction mixture containing 1 × Taq buffer with KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPmix, 1 U recombinant Taq DNA polymerase (Fermentas, Lithuania),

0.6 µM forward and reverse primers (Biopolymer Factory, Germany), 5 µl (about 20 ng) genomic DNA and DEPC-treated water (Roth, Germany) up to 25 µl. A negative control with no template DNA and positive control with DNA of *G. rostochiensis* or *G. pallida* were used. Cysts of nematodes for positive control were obtained from the National Plant Protection Laboratory of the Nematology Division, France.

The amplification was performed in a thermocycler (Mastercycler Personal 5332, Eppendorf, Germany) under the following conditions: initial denaturation for 4 min at 94°C, 40 cycles of denaturation for 1 min at 94°C, primers annealing for 1 min at 60°C, extension for 2 min at 72°C followed by a final extension for 10 min at 72°C.

DNA amplification products were visualised by horizontal electrophoresis in 2% agarose gel with ethidium bromide (0.5 µg/ml) in 1 × TAE buffer at 82 V (6 V/cm). DNA were visualized in UV light. The sizes of DNA fragments were estimated using the DNA marker GeneRuler™ 100 bp DNA Ladder (Fermentas, Lithuania).

Results and discussion

All 672 potato nematode cysts from 10 administrative regions of Lithuania were identified morphologically as *G. rostochiensis*. Analysis of different field areas and the previous crop showed that the majority of cysts were found in small fields with various plant species. *G. rostochiensis* infested 116 fields out of 437 with an area less than 1 ha (94.91% of a total of 672 cysts), 23 fields out of 220 with an area from 1 to 5 ha, 26 fields out of 39 with an area from 5 to 10 ha, whereas only one infested field was recorded among the fields with an area over 10 ha. The most intensively infested areas were those located near homesteads.

PCR analysis of all 117 cysts revealed that the lengths of all amplified DNA bands were specific to *G. rostochiensis* (315 bp) (Fig. 1). No DNA extracts produced the amplification product specific to *G. pallida* (798 bp).

Only 35.04% of DNA extracts produced PCR products specific to *G. rostochiensis*. The absence of any species-specific DNA amplification bands in some samples, in our opinion, could be due to a low amount of extracted DNA. Fleming *et al*. (1998) revealed a positive correlation between the yield of DNA extracted from *G. rostochiensis* cysts and the number of viable nematode eggs. In our investigation, cysts were collected from their natural environment (field soil samples), which allows the assumption that crop rotation, unfavourable environmental conditions and a long period of dormancy can decrease the viability of cysts, as well as the DNA extraction yield. Besides, it is known that *G. pallida* is less common than *G. rostochiensis* in most countries of Europe (Pylypenko *et al*. 2005; Smith *et al*. 1997). In conclusion, both methods used in the present study confirmed the presence of *G. rostochiensis* and the absence of *G. pallida* nematodes in Lithuania. The absence of the invasive *G. pallida* species might be explained by good phytosanitary conditions in Lithuania. It is necessary to continue studies and the monitoring of potato cyst nematodes with application of a sensitive PCR method to avoid the occurrence of *G. pallida* from imported pants, as well as to control the population of *G. rostochiensis*.

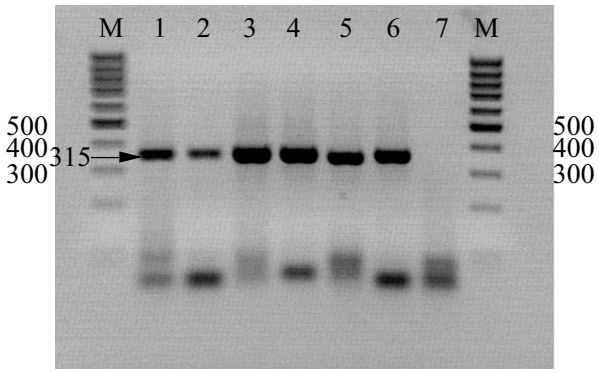

Figure 1. PCR products of *G. rostochiensis* collected in 5 regions of Lithuania: 1 – Panevėžys, 2 – Kaunas, 3 – Vilnius, 4 – Telšiai, 5 – Utena. Lanes 6 and 7 are positive and negative controls of *G. rostochiensis*, respectively, M – DNA markers. The arrow shows sizes of DNA amplification products.

Conclusions

Only *G. rostochiensis* was found in Lithuania, whereas *G. pallida* was not recorded until 2007 (using morphological analysis and a sensitive PCR method based on DNA analysis).

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Globodera spp. monitoringas Lietuvoje taikant morfometrinę analizę ir polimerazinės grandininės reakcijos metodą

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Santrauka

Ištyrus 11406 dirvožemio pavyzdžius, paimtus iš 2742 ha plotų, esančių 10 administracinių rajonų, ir rastus bulvių cistinius nematodus, apibūdinus pagal morfologinius požymius, rasti tik *G. rostochiensis* nematodai. Morfologinio tyrimo rezultatai buvo tikrinami PGR metodu, naudojant dvi poras *G. rostochiensis* ir *G. pallida* rūšims specifinių pradmenų. Nustatyta, kad Lietuvoje paplitusi tik viena karantininė nematodų rūšis – *G. rostochiensis* – tuo tarpu jai artima kita karantininė rūšis – *G. pallida* – šalyje neregistruota (iki 2006 m. imtinai).

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