

POTATO CYST NEMATODES (PCN) IN THE CZECH REPUBLIC

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ABSTRACT

The first occurrence of *Globodera rostochiensis* was recorded and confirmed in 1954. After this, the first surveys were organised. These surveys were done by visually observing the potato roots and/or by analyses of soil samples, when the flotation technique was used. Diagnostics were performed by morphometrical methods and by comparing the found material with standards. In the 1980s, an enormous number of new occurrences of PCN were found. In the Czech republic, PCN is currently present in more than 1.000 land registers, mostly in small areas on private plots, where serious yield losses have been observed. Fortunately, the occurrence of PCN in areas used for seed potatoes is very rare, which may be the result of producers' adherence to charged phytosanitary measures. The survey and diagnostics are carried out in the Regional Diagnostics Laboratories of The State Phytosanitary Administration. The development of diagnostics methods is done in cooperation with The Czech Agricultural University (e. g., the PCR method). The investigation for the occurrence of the new PCN pathotypes is still being performed. In late 1990s, the first occurrence of *Globodera pallida* in our country was discovered. Until this time, this nematode had been found only in a few samples of imported potatoes. Control measures consist of required crop rotations, the use of PCN-resistant varieties, and by plant-health legislation, including quarantine measures. Chemical control is not used. Control measures are relaxed only after at least 10 years have passed since the last positive finding of PCN, determined by flotation measurement techniques and after this negative result has been confirmed by biological methods.

IZVLEČEK

KROMPIRJEVE OGORČICE V REPUBLIKI ČEŠKI

Prvi pojav rumene krompirjeve ogorčice, *Globodera rostochiensis*, je bil na Češkem zabeležen in potrjen leta 1954. Po tem je bil organiziran sistematični nadzor, ki je temeljil na pregledovanju krompirjevih korenin in/ali laboratorijskih analizah talnih vzorcev s pomočjo flotacijske tehnike. Identifikacija vrste je temeljila na morfometrijskih značilnostih in primerjavi najdenih primerkov s standardnimi (referenčnimi) materiali. V osemdesetih letih smo na Češkem naleteli na izredno veliko število novih žarišč rumene krompirjeve ogorčice. Trenutno imamo registriranih več kot 1000, v glavnem manjših, nahajališč (njiv) obravnavanega škodljivca, ki so večinoma v lasti zasebnih pridelovalcev. Vrsta *G. rostochiensis* povzroča na teh zemljiščih občutne izgube pridelka krompirja. V območjih, kjer se na Češkem prideluje semenski krompir, je na srečo zastopanost krompirjevih ogorčic zelo redka, kar je verjetno posledica ozaveščenosti pridelovalcev, ki upoštevajo ustrezne fitosanitarne zahteve. Sistematični nadzor in identifikacija krompirjevih ogorčic poteka v okviru regijskih diag-

nostičnih laboratorijev, ki so organizirani v sklopu državne fitosanitarne službe. Razvoj diagnostičnih metod (npr. PCR) poteka v sodelovanju s Češko kmetijsko Univerzo - The Czech Agricultural University. V teku so raziskave o zastopanosti oziroma razširjenosti novih biotičnih ras krompirjevih ogorčic. V poznih devetdesetih smo pri nas ugotovili tudi belo krompirjevo ogorčico, *G. pallida*, na katero pa smo naleteli le v nekaj vzorcih uvoženega krompirja. Varstvo rastlin temelji na upoštevanju zahtevanega kolobarja, pridelovanju odpornih kultivarjev krompirja ter upoštevanju fitosanitarnih predpisov, ki vključujejo tudi karantenske ukrepe. Kemičnega zatiranja krompirjevih ogorčic ne uporabljamo. Predpisani varstveni ukrepi se omilijo šele potem, ko preteče najmanj deset let od trenutka, ko je bila krompirjeva ogorčica na neki njivi zadnjič najdena; ugotovitev, da tega škodljivca ni več, pa mora biti hkrati potrjena tudi z biotičnim testom.

The first report about PCN in Europe was made in Germany in 1913. The nematode was described as a potato race of the sugar beet nematode. Only near the end of the 1930s was this species officially recognized. It was named *Heterodera rostochiensis*, a name suggested by Wollenweber in 1923.

PCN was probably introduced into Czechoslovakia during the Second World War from Germany, together with infested potatoes. After the war, thanks to political pressure, official attention was focused on the Colorado potato beetle and no one paid much attention to PCN for some time. The first observation of PCN in Czechoslovakia was in 1954 in the borderland.

Immediately after the first observation, the first Phytosanitary measures against the PCN were adopted, including procedures aimed at restricting its growth. These measures included forced surveys of the plots where potatoes or tomatoes were grown, a ban of at least five years on growing potatoes and tomatoes on infested plots, their neighbourhoods, or the area of the whole land register. During the communist regime, it was not difficult for district committees to impose these measures on cooperative farms and owners of the private plots. Violators of approved practices were penalized, and plants were removed from infested plots. Stringent quarantine measures were applied to the infested plots, whereas less stringent measures were ordered for the neighbouring, uninfested plots and the other plots belonging to the same user. During the first survey organised in the Czech Republic, which ended in 1955, 30 land registers with the occurrence of PCN were found. An increase in PCN was seen in the 1960s, which was largely a result of various political decisions that enforced the import of uncontrolled seed and ware potatoes from countries with significant levels of PCN infestation (the Soviet Union, the German Democratic Republic, and Poland). As a consequence, the second Phytosanitary measures, which were instituted in 1967, prescribed more detailed rules to prevent the spread of PCN. These measures were the first to involve the same methodological instructions for both PCN and potato wart disease (PWD). A very modern factor was confirmed – the possibility of using PCN-resistant varieties. During this time, PCN was found not only in the borderland, but also in interior areas, especially in light sandy soils, as well as in the seed potato areas. In 1970, PCN was found in 135 land registers, whereas in 1980 it was present in more than 265 land registers. This number increased three fold in the next ten years. Since that time, however, as a result of powerful measures, the number of infested land registers has not increased. At this time, PCN occurs in all 36 districts and in 801 land registers of the land where potatoes are grown, mostly on small plots, while large production fields are largely free of it. Although serious yield losses (20-70%) have been observed on small private plots and on cooperative fields in areas where early potatoes are grown which are associated with frequent potato cultivation and a high level

of cyst population density, no important overall economic loss to the industry has so far been measured. The latest "Methodological instructions to protect against the spread of PCN and PWD" were accepted in 1999 and represent a significant advance in the suppression of PCN in the Czech republic. These instructions clearly defined the type and range of the surveys, quantified quarantine measures, and possibilities of farming in the quarantine area. The basic objective was to prevent the spread of PCN outside the quarantine area and decrease its population density. Great emphasis is placed on these preventive measures, especially on the use of certificated seed potatoes. More stringent measures apply to seed potatoes, while less stringent measures for ware potatoes have been accepted.

According to this methodology, there are various types of surveys to test for the presence of PCN. The most important of these are as follows:

- a) a survey of fields designated for propagation of seed potatoes;
 - b) a detection survey;
 - c) a detailed survey;
 - d) a survey in greenhouses;
- a) A survey of fields nominated for propagation of seed potato is carried out, pursuant to which seed potato growth shall be established only on those fields for which a survey on PCN demonstrated negative results since the last harvest of potatoes. This survey shall be performed via the sampling and analysing or biological testing of soil samples taken from the relevant fields. A negative result is only one of the conditions for certification of the seed growth. Fields that were planted with potatoes in the current year are also subjected to inspections of the growths (with the exception of those varieties resistant to the local pathotype). The survey is arranged by the grower at his own expense, including the delivery of samples to the laboratory. Samples are collected under the supervision of the State Phytosanitary Administration ("SPA"). SPA is completely funded by the Ministry of Agriculture from the government budget. There are over 4200 samples taken each year. Samples are analysed in laboratories that have been, at their own request and SPA's recommendation, authorised to perform these analyses by the Ministry of Agriculture. SPA recommends authorisation only of those facilities for which personnel and equipment background will guarantee a high quality of analyses and tests and an objective evaluation of the results, and which will eliminate the spread of PCN and PWD. All protocols and results of analyses and tests are registered and archived by relevant laboratories. In the past three years, only one positive sample was found.
- b) A detection survey is fulfilled, in five-year intervals, by SPA in land registers of seed potato areas that are used for the production of basic seed potatoes, and in those fields or buildings that are used at least 20% of the time to grow or store potatoes. Potatoes being put into circulation have priority. The survey consists of visual inspections of the plants in the field and/or sampling and analysis or biological testing of soil samples. A survey is performed by SPA in nurseries and other fields where plants are grown for replanting in other fields that have been designated for commercial production.
- c) A detailed survey is carried out by SPA immediately after an occurrence of potato nematode has been detected and before termination of quarantine. It applies to those fields both in and outside the quarantine area that are suspected of being infested. Again, the survey is done by collecting and analysing soil samples from the fields in which potatoes are grown, stored, graded or processed, or by subjecting these samples to biological testing.

d) A survey in greenhouses is performed by SPA in five-year intervals consisting of random visual inspection of potato and/or tomato plants, collection and analysis, or by biological testing of samples of soils in which these plants were commercially grown (including those soils used for potato breeding).

Examinations of the soil samples taken from potato sorters and especially from consignments of imported potatoes are performed using special methodological instructions.

A detailed description of visual field inspections, carried out about 8 weeks after planting time, and/or soil sampling is the subject of relevantly quoted methodological instruction from 1999. For surveys and/or the retesting of infested plot soil, sampling is done by taking soil samples, consisting of a number of sub-samples, systematically distributed over the field. For up to 20 ares of the field area, one mixture sample is taken, and for over 20 ares, two mixture samples per each 50 ares are taken. For example, one hectare plot will yield 4 samples. The total sample must be well-mixed, and a 200 g sub-sample of dried soil is washed, using the accepted flotation technique. For the extraction of cyst, we use a modified Fenwick's apparatus. After washing, round cysts are picked off under the binocular microscope and examined under the high-quality optical microscope. The determination is based on whether the sample has certain physical properties and is carried out by a comparison of measured dates with morphological standards.

If any live cysts of PCN are found, a technical investigation is completed by SPA to determine all necessary data for decision on what extraordinary phytosanitary measures should be taken (i.e., the extent of probable dissemination, the origin of the occurrence, the extent and purpose of growing the host plants in the infested area, etc.). Then, SPA or the District Office defines a quarantine area, which could range from a single infested field and its immediate surroundings to a continuous area covering several land registers. As described above, more stringent criteria are used in seed production areas and when aggressive pathotypes have been identified.

In newly infested areas, certain positive samples are analysed for species and/or pathotype (in most cases only 3 plots are tested); morphological, biological and/or biochemical techniques are used.

Extraordinary phytosanitary measures are taken in the determined area, which consist of the following steps:

- on infested plots: destruction of the infested plants and tubers under official supervision, identification and destruction of wild-growing host plants, a ban on the planting of host plants and plants grown for replanting to other fields, and a requirement that only those non-seed potatoes that are resistant to the local pathotype may be grown for control or research purposes, and these plants may not be grown more frequently than once every four years;
- on other fields in the quarantine area: only non-seed potatoes of varieties resistant to the local pathotype shall be planted for propagation purposes, and on the same field no more frequently than once every four years

Machinery, vehicles, tools, footwear and other items used

- on infested fields must be cleaned of stuck soil, and before being used on another field, must be washed in the quarantine area by water; if the field is infested with an aggressive pathotype, they must be disinfected in the manners specified by SPA;
- on the fields in a quarantine area - must be cleaned of stuck soil prior to being moved outside of this area.

Retesting for infestation of a field is performed by SPA:

- no earlier than 10 years after the last detection of PCN, or
- at the request of the field owner or other user no earlier than 5 years after the last

occurrence, if the occurrence was a mild one in conditions which are unfavourable for the development of PCN, or if the methods of suppressing PCN have been implemented on the field under the supervision of SPA.

If the results of retesting are negative, the field is still regarded as suspected of being infested, and the same measures will be ordered as for other fields within the quarantine area that are suspected of being infested.

A complete lifting of the quarantine measures for the infested field and the relevant quarantine area is possible only if a re-testing, conducted no sooner than 20 years after the last occurrence of PCN, is negative. It is generally known, once a field has been found infested, eradication of the pest is a hard job, if ever achievable.

Although PCN was found in the Czech Republic in 1954, it lasted until the mid-1960s, when suspicion of the presence of aggressive pathotypes was confirmed and a mixture of different races was found in one plot. 20 years later, the large experiment had been organised. From 1983-89, 1200 infested plots were investigated for the occurrence of new pathotypes of *G. rostochiensis* and *G. pallida*. Only 8 populations were suspected of containing a pathotype other than Ro1. Tests with differential potato varieties indicated that four of the nematode populations were likely mixtures, with pathotype Ro1 prevalent. Three populations were suspected of containing *G. pallida*, but morphological characters agreed only with *G. rostochiensis*. One population corresponded with pathotype Ro5. Mr. Potocek, the leader of the project, estimated the occurrence of pathotypes, or virulence groups, different from Ro1 at only 0,3-0,4% of infested plots in the Czech Republic.

In 1998, during a routine survey in a quarantine area, the first occurrence of *G. pallida* in the Czech Republic was found and later confirmed by PCR for one plot in the borderland. Until this time, *G. pallida* had only been intercepted on imported potato consignments.

Based on the results of the survey, in 1999 we began a special task of classifying 8 of the above describing populations that we suspect to be mixture populations. Until now, we have been testing in a biological fashion (which use varieties with known resistance against the certain pathotypes), and our results will be confirmed by the Czech Agricultural University in Prague by biochemical methods. This task is going to be finished this autumn.

There are various direct and indirect measures used in the Czech Republic against PCN: The indirect measures include special crop rotations, using the presence of non-host plants and plant-health legislation, including quarantine measures. Use of premature harvesting of a susceptible potato variety (trap cropping) was also found to be effective, but this method is not widely used. The basic measure to control PCN is the use of resistant varieties. Resistance breeding and the use of resistant potato varieties create the most important part of the strategy for PCN treatment. There are 102 varieties described on the List of registered potato varieties, 75 of them are resistant against at least the pathotype Ro1. Eight of them are Czech in origin.

Direct methods of suppression include the use of nematicides. Because of their cost, toxicology and/or other drawbacks, these methods are rarely used, although both soil fumigants (dazomet /in dose 30-60 g per m²/, metam sodium or 1,3 dichlorpropene) or non-fumigants (especially granular form of carbamates in dose 50 kg per ha) were effective. Depending on the sort of chemicals used, as well as the soil and climate conditions and the method of application of these chemicals, the efficacy was between 50-99%.

Urea proved to be highly effective. Doses of 0.5-1.5 kg per m² was found to destroy larvae and eggs; doses of 0.1-0.3 kg per m² were found to stimulate the hatching of larvae, which die in the soil where the host plants are absent.

Methods that reach 100% efficacy after a short exposure are used to decontaminate infested stores, machinery, tools, and laboratory instruments. A review of experimen-

tally verified methods reveals that the following minimal concentrations and times of exposure are necessary to achieve this degree of efficacy:

- formalin 15% - for 30 min
- cresol 5% - for 15 min
- hot water 65 C - for 15 min
- steam 80 C - for 30 min

The presence of PCN outside the area designated for propagation of seed potatoes does not present enormous dangers. The effect of quarantine measures applied to infested plots and/or their neighbourhood has not been as troublesome as had been expected, and has mostly been limited to a ban on the planting of host plants and a prohibition on moving infested soils outside this quarantine area. Direct and indirect measures leading to decreasing the occurrence of PCN have been applied. Special crop rotations, as well as the use of resistant varieties could keep the accidental spread of the pest under the level of detectability.

There are many other pests associated with potatoes - the relatively new identified species (*Meloidogyne chitwoodi*, *Nacobbus aberrans*) and the old ones (*Ditylenchus destructor* and of course PCN). Although the problems with PCN have mostly been worked out, this pest remains problematic because of its harmfulness, the difficulty in controlling it, and its longevity. It is therefore on the list of quarantine pests practically in all European, and most of the world's, countries. Let us hope that with world-wide cooperation we will be able to manage these problems once and for all.