# Infestation of Polish agricultural soils by *Plasmodiophora brassicae* along the Polish-Ukrainian border

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**Abstract:** There has been a rapid, worldwide increase in oilseed rape production that has resulted in enormous intensification of oilseed rape cultivation, leading to tight rotations. This in turn, has caused an accumulation of pests as well as foliar and soil-borne diseases. Recently, clubroot has become one of the biggest concerns of oilseed rape growers. Clubroot is caused by the soil-borne protist *Plasmodiophora brassicae* Woronin. The pathogen may be present in groundwater, lakes, and irrigation water used in sprinkling systems. It can be easily transmitted from one field to another not only by water, but also by soil particles and dust transmitted by wind and on machinery. The aim of our overall study was to check for *P. brassicae* infestation of Polish agricultural soils. This paper presents the 2012 results of a study performed along the Polish-Ukrainian border in two provinces: Lublin (Lubelskie Voivodeship) and the Carpathian Foothills (Podkarpackie Voivodeship), in south-east Poland. Monitoring was done in 11 counties, including nine rural and two municipal ones. In total, 40 samples were collected, out of which 36 were collected from fields located in rural areas and four from municipal areas, with two per municipal region. Each sample was collected at 8–10 sites per field, using a soil auger. The biotest to detect the presence of *P. brassicae* was done under greenhouse conditions using seedlings of the susceptible Brassicas: *B. rapa* ssp. *pekinensis* and the Polish variety of oilseed rape *B. napus* cv. Monolit. Susceptible plants grown in heavily infested soils produced galls on their roots. A county was regarded as free from the pathogen, if none of the bait plants became infected. The pathogen was found in three out of 40 fields monitored (7.5%) in the Carpathian Foothill region. The fields were located in two rural counties. The pathogen was not found in Lublin province, and was also not detected in any of the municipal counties. The detection with a biotest was fully confirmed by PCR-based molecular detection of *P. brassicae* DNA in soil samples.

**Key words:** clubroot, oilseed rape, *Plasmodiophora brassicae*, resting spores, soil infestation

## Introduction

Clubroot, caused by the protozoan *Plasmodiophora brassicae* Woronin, is a damaging and commonly occurring pathogen of cruciferous plants in Europe and North America (Agrios 1988; Diederichsen *et al*. 2009; Strelkov and Hwang 2014). The pathogen includes many pathotypes, differing in virulence on different hosts (Lüders *et al*. 2011). For the last few years, the pathogen has posed a serious challenge to growers of oilseed rape in Poland (Korbas *et al.* 2009). The disease was reported by farmers from various regions of the country. According to recent reports the pathogen is present in more than one-third of the area under cultivation of oilseed rape. The further expansion of the pathogen is still being observed (Konieczny 2012).

Resting spores of *P. brassicae* can survive in soil for 20 years or more and maintain infectivity (Dixon 2009a). It was also demonstrated that resting spores can be present in pond water sediment (Datnoff *et al*. 1984). The disease can spread at an extremely high rate. It was found, that after three years from the onset of disease symptoms on a small number of plants, the pathogen can infest the whole field in favorable conditions. This situation appears when no crop rotation is used, which may happen in regions with intensive cultivation of oilseed rape. Research carried out in the north and north-east of Germany and in some parts of France, England, and Scandinavia showed that the presence of *P. brassicae* on fields of this crop is associated with plant cultivation intensity (Rimmer *et al*. 2007). In Alberta, Canada, spring oilseed rape fields, referred to as canola, showed similar situations with many fields of no more than a one-year break between the cultivation of canola (Gossen *et al*. 2013).

Research on clubroot in Poland mostly concentrated on vegetable Brassicas, where clubroot has caused considerable problems (Robak 1991). The first studies on winter oilseed rape were performed by Kurowski *et al.* (2008), who demonstrated the usefulness of fluazinam in decreasing the severity of disease symptoms, similarly to the activity of this compound in vegetable Brassicas (Robak 2001). The studies of Korbas *et al*. (2009) covered West Pomerania, Pomerania, Varmia and Mazuria, as well as

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parts of Kujavia-Pomerania, Lower Silesia, Carpathian Foothills, and Lubuskie as the most endangered regions of Poland. The occurrence of *P. brassicae* was demonstrated in plants and soils obtained from fields, where clubroot was found on crops or weeds belonging to the Brassicaceae family. Recent studies by Jedryczka *et al*. (2013) showed that the disease was also present in regions regarded as free from this pathogen, e.g. in Wielkopolska (Great Poland). This result was obtained based on thorough monitoring of soil samples taken at random from agricultural fields. The aim of this paper was to examine the incidence of *P. brassicae* in the agricultural soils of Poland along the border with the Ukraine, to check how far east in Poland and the European Union, the pathogen occurs in soils. We wanted to recognise the scale of the problem in terms of the incidence and severity of clubroot, since the disease has a great impact on yield of oilseed rape (Pageau *et al*. 2006), which is commonly grown in Central Europe.

## Materials and Methods

### Experiment location

Soil samples were collected randomly from fields with agricultural crops. The monitoring was done in 2012, in counties located in two provinces along the Polish-Ukrainian border: Lublin province (Lubelskie Voivodeship) and the Carpathian Foothills (Podkarpackie Voivodeship). In total, 11 counties were monitored, with nine rural and two municipal ones (Fig. 1). In Lublin province the following rural counties were studied: Chelmski (6 fields), Hrubieszowski (6), Tomaszowski (5), and Wlodawski (3). There was also one municipal county Chelm (2) studied in this province. In the Carpatian Foothills, the counties were as follows: Jaroslawski (5 fields), Przemyski (4), Lubaczowski (3), Bieszczadzki (2), and Leski (2) as well as one municipal county Przemysl (2).

### Soil sampling

Soil sampling was done using a soil auger (Spychalski and Kosiada, model 117084) with a container in the shape of an inverted truncated cone (Agroekspert Polska). The soil was sampled from the 0–20 cm layer. Depending on field size, a sample was taken from 8 to 10 locations per field, with 200 g of soil per location. In total, *ca.* 1.6 to 2 kg of soil were collected per field. For further studies, the sub-samples from a field were mixed well, and lumps of soil were crushed to obtain a uniform soil substrate.

### Plant biotest

Part of each soil sample (500 g) was mixed with peat (pH 3.5) at a ratio of two parts of the test soil to one part of peat. Both components were thoroughly mixed and then put into four pots (7 × 7 cm) and placed on small trays.

The biotest to detect the presence of *P. brassicae* was done under greenhouse conditions using seedlings of *Brassica rapa* ssp. *pekinensis* Pe-Tsai 'One Kilo SB' F1 and a Polish variety of oilseed rape (*B. napus* cv. Monolit). The chosen susceptible plant species were used as bait plants. After six weeks of cultivation at 14 h light, 20°C/10 h darkness, 16°C, the plants were removed from the soil and the roots were rinsed in water, for ease of observation. The result was defined as positive if clubs of *P. brassicae* were present on roots. The assessment was done using a 0 to 4 scale, where 0 was a healthy plant with fully developed roots and 4 was a small plant with roots changed to a club. The county was regarded as free from serious occurrence of the pathogen when none of the studied samples contained plants with infected roots.

### Molecular detection of *P. brassicae* in soil samples

The total DNA from the soil samples was extracted from 10 g of soil using the FavorPrep Soil DNA Isolation Midi Kit (Favorgen, USA) according to the manufacturer's instructions. The concentration and purity of DNA were determined spectrophotometrically by measuring the absorbance at 260 and 280 nm. Working solutions were adjusted to 20 ng/µl with Tris-ethylenediaminetetraacetic acid (TE) buffer (pH 8.0). DNA samples were placed at –20°C for long-term storage and at 4°C for immediate processing. The PCR reaction was done in Eppendorf tubes using 2 µl of target DNA, 0.7 U of Dream Taq polymerase (Thermo Scientific), PCR DreamTaq buffer, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, and 1 µM concentrations of TC2F and TC2R primers (Cao *et al.* 2007). To preclude negative detections being regarded as arising from PCR inhibition, duplex reactions were done in all cases along with the detection of the DNA of *Alternaria brassicicola* – another rapeseed pathogen. To obtain this product 1 µl of 1300 × diluted ITS fragment of this fungus (Polish iso-


**Fig. 1.** Strong signals of the detection of *P. brassicae* obtained from soil samples with the use of species-specific PCR primers TC2F and TC2R designed by Cao *et al.* (2007). Duplex PCR reaction was done using ABRA1 and ABRA2 primers and the DNA extracted from *A. brassicicola*, based on the method by Iacomi-Vasiliescu *et al.* (2001): 1 – Jaroslaw, JAR; 2 – Radymno, JAR; 3 – Korczowa, JAR; 4 – Laszki, JAR; 5 – Torki, PRZ; 6 – Hucisko, PRZ; 7 – Babice, PRZ; 8 – Ruszczelczyce, PRZ; 9 – Oleszyce, LUB. *Explanations*: JAR – Jaroslawski County, PRZ – Przemyski County, LUB – Lubaczowski County. Asterisks on figure 1 point out the samples with positive detection of *P. brassicae*. The detection of *A. brassicicola* was positive in all samples

late ATW 41-1) was amplified using ABRA1 and ABRA2 primers (0.1 μM), described by Iacomi-Vasiliescu *et al*. (2001). Sterilised water was added to the total volume of 16 μl. Twenty μl of mineral oil was used to cover the PCR mixture. To perform the PCR reaction, the following protocol was used: 2 min at 94°C, followed by 45 cycles of 30 sec at 94°C, 20 sec at 65°C, and 20 sec at 72°C, with a final extension of 5 min at 72°C. Separation of the PCR products was done on a 2.0% agarose gel, stained with ethidium bromide and visualised with UV light.

## Results

The random study of Polish agricultural soils from the Polish-Ukrainian border detected 37 samples negative to clubroot (92.5%) and three samples positive to clubroot (7.5%). Soil infested with *P. brassicae* was found in two out of nine rural counties; both of them were located in Podkarpackie Voivodeship). In Jaroslawski county, two out of the five monitored fields were infested, whereas at Przemyski county, it was one field out of the four studied. None of the fields located in municipal counties were infested by *P. brassicae.* In two cases (samples from Przemyski and Jaroslawski counties), the disease severity was numerically higher on *B. rapa*, whereas in one case (Radymno, Jaroslawski county) the symptoms on oilseed rape plants were more severe than those on the susceptible standard *B. rapa* ssp. *pekinensis*. The remaining samples were negative for clubroot. The detection of *P. brassicae* with a biotest was confirmed by PCR-based molecular detection of *P. brassicae* DNA in soil samples.

Based on this monitoring, a map has been drawn, which points out the region that needs special care to avoid further spread of the disease (Fig. 2).

## Discussion

Previously, studies carried out in Poland were done only on fields where clubroot symptoms were observed on rapeseed plants. It is not surprising, therefore, that in soil samples taken from infested soils, *P. brassicae* was identified. This study determined the severity of the occurrence of *P. brassicae* in agricultural soils taken from crop fields other than winter or spring oilseed rape. Until now, random monitoring at farmers' fields, in respect to the occurrence of this dangerous pathogen in soils, has been reported for the Wielkopolska region only (Jedryczka *et al*. 2013). In most cases of that study, the soil samples were free from pathogen quantities which would be sufficient enough to cause symptoms of clubroot on susceptible cruciferous plants. Nevertheless, the pathogen was found in eight rural counties (25.8%).

This study aimed at recognising potential infestation by *P. brassicae* of field soils used for agricultural purposes, and to be aware of the scale of this phenomenon in the eastern part of Poland, being at the same time the east border of the European Union. The surveillance done in fields located on the Polish-Ukrainian border showed that in some regions the pathogen is present, in spite of a less intensive cultivation of oilseed rape. Even in the absence of oilseed rape as a host plant, the pathogen was present in 7.5% of the soil samples. Agricultural soils in the Ukraine, adjacent to the Polish-Ukrainian border, also contained moderate amounts of *P. brassicae*, as reported by the Ukrainian specialists Andriy Bilan and Yarina Sheremet (personal communications). Similar to the situation in Wielkopolska (Jedryczka *et al*. 2013), none of the fields located in the municipal counties were infested by *P. brassicae*. However, fields with soil infested by this pathogen were found in close proximity to an international highway. It is, therefore, of utmost importance to perform a deeper local search in this region. It is also very important to implement high standards of cleanliness as concerns farm machinery. Both of these necessary conditions should be carried out as Canada has done, through recently introduced regulations (Strelkov and Hwang 2014).

Prior to our study, clubroot surveillance activities have been initiated in Canada (Strelkov *et al.* 2009; Dokken *et al*. 2010). A survey in south and central Alberta in 2012, concerning 390 commercial canola crops, revealed 64 new records of clubroot. Moreover, independent surveys by county personnel revealed another 169 new records, for

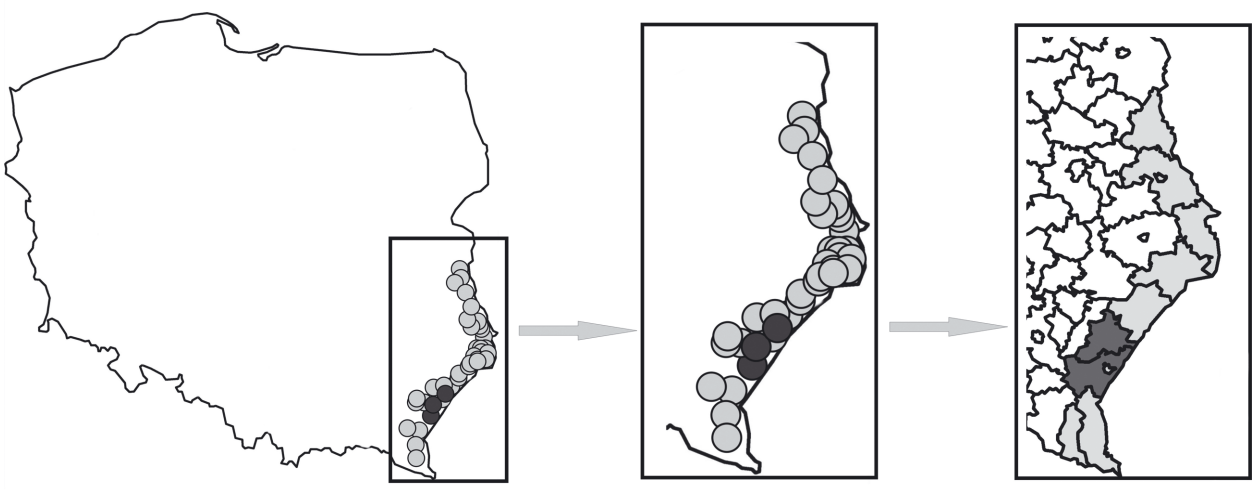

**Fig. 2.** Infestation by *P. brassicae* of randomly collected agricultural soils in counties on the Polish-Ukrainian border: dark grey – infested by *P. brassicae*; light grey – not infested; white – not studied

a grand total of 233 clubroot-infested fields (Strelkov *et al*. 2013). The disease severity was mostly low, but moderate to high levels occurred in 25% of the fields. Testing of soil samples in Saskatchewan and Manitoba in 2013, in the absence of clubroot symptoms, covered over 200 fields of these two provinces and yielded three positive samples (1.5%) (Strelkov and Hwang 2014). Both Polish and Canadian studies, although differing in their incidence of *P. brassicae*, showed that the pathogen exists in numerous soils, even without oilseed rape cultivation. One of the reasons may be the cultivation of oilseed rape in the past and maintaining of the pathogen on weeds, that are susceptible to clubroot (Dixon 2009b).

Based on our monitoring, it can be concluded that *P. brassicae* is currently present in south-east Poland. It is probable that molecular techniques, such as conventional PCR (Cao *et al*. 2007) or quantitative PCR (Rennie *et al*. 2011), that are of higher sensitivity than biotests, will show that the presence of *P. brassicae* is even higher than that demonstrated in this study. Preventive action, such as liming and growing resistant varieties, should be taken to reduce the further spread of this dangerous pathogen.

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