CLUBROOT – AN EMERGING DISEASE FACED BY CZECH OILSEED RAPE GROWERS*

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Clubroot is caused by the pathogen *Plasmodiophora brassicae* Woronin and has become a serious problem in oilseed rape production in the Czech Republic. The disease was previously widespread in commercial vegetable production and in hobby gardens. Whereas previously restricted only to the NE, since 2010 oilseed rape clubroot has been spreading across the whole country. A five-year monitoring of clubroot occurrence based on disease symptoms detection on oilseed rape fields was accomplished by the Union of Oilseed Growers and Processors. The presence of *P. brassicae* and clubroot symptoms was reported in all regions of the Czech Republic, except the Ústecký region, and in 31 out of 76 districts. At present, at least 130 fields are known to be infested by the pathogen, but this number is very likely underestimated. Some soil samples were also tested using the conventional polymerase chain reaction (PCR) method to evaluate the soil usability. All 14 suspected samples tested positive.

Plasmodiophora brassicae, occurrence, Czech Republic, *Brassica napus* subsp. *napus*, PCR

DE GRUYTER OPEN

doi: 10.1515/sab-2016-0015
Received for publication on December 9, 2015
Accepted for publication on April 27, 2016

INTRODUCTION

Oilseed rape, in the Czech Republic especially winter oilseed rape (*Brassica napus* subsp. *napus*), has become one of the most important sources of edible oil. Within the last 25 years, its area of cultivation in the CR tripled (Czech Statistical Office – https://www.czso.cz/csu/czso/zem_ts.). Together with increased cultivation of winter oilseed rape, problems with pests and pathogens have steadily grown. One of its most significant pathogens is *Plasmodiophora brassicae* Woronin (Kazda et al., 2013).

The soilborne disease is able to infect all members of the cruciferous family (Brassicaceae*)* (Dixon, 2009b). The pathogen induces the formation of galls on infected roots, which affects growth as well as water and nutrient uptake (Dixon, 2009b), leading to wilting or even death of the infected plants and therefore yield losses (Wallenhammar, 1999; Strelkov et al., 2006). Because the typical symptoms occur on roots, the disease is called clubroot. The life cycle of the pathogen is divided into a primary and secondary phase. The resting spores germinate and release moving zoospores, which seek host root hairs to penetrate and begin the primary phase of infection (Ayers, 1944). Inside the roots, the pathogen produces a multinucleate formation, called a paraplasmodium, containing secondary zoospores, which are released back into the soil, and the secondary phase of infection begins. Zoospores penetrate to the root cortex of the host, where the paraplasmodium is created again and induces the formation of the clubs containing the resting spores. When the plant dies, the clubs rot, and the spores are released into the soil (Ayers, 1944;

* Supported by.the Ministry of Agriculture of the Czech Republic, Project No. QJ1310227, and by the Internal Grant Agency of the Czech University of Life Sciences Prague (CIGA), Project No. 20142031.

Ingram, Tommerup, 1972). The weight of the mature clubs can reach up to 1 kg (Rod, 1996). Every infected plant has the potential to introduce up to 8×10^8 resting spores into the soil (Hwang et al., 2011). The average number of oilseed rape plants per 1 m^2 is approximately 40–60, depending on the cultivar (Baranyk et al., 2007); therefore, the infection potential of *P. brassicae* is enormous. The resting spores of *P. brassicae* are extremely resistant to adverse external factors and can survive in soil for 20 years or more (Dixon, 2009b). The pathogen can spread by spores at an extremely high rate (Howard et al., 2010; Hwang et al., 2014). Any transport of soil particles containing resting spores participates in this dissemination. Dissemination involves, for example, water and wind erosion, transport of soil on machinery or harvested products, and final spreading on a field by soil cultivation as the most common routes (Hwang et al., 2014). Fifty spores in 1 g of soil are enough for infection in unfavourable conditions, whereas in optimal conditions, just 1 spore per 10 g of soil is sufficient (Rod, 1996).

In the last 10–15 years, *P. brassicae* began to rapidly spread in oilseed rape growing regions in more than 60 countries including Canada, Germany, Poland, Great Britain, and France where it caused 10–15% yield losses (Dixon, 2009a). In Canada, the pathogen dispersed very quickly. In Alberta province (Canada) near Edmonton, While in 2003 only 12 infested fields were detected, in 2007 it was as much as 250 fields (Strelkov et al., 2014), and even more than 1000 infested fields were detected in 2012 (Strelkov et al., 2012). Moreover, the pathogen also spread to the neighbouring province of Manitoba (Cao et al., 2009).

The clubroot outbreak in the United Kingdom is associated with the extension of oilseed rape growing areas; the biggest losses have recently been recorded in Scotland and Wales (Dixon, 2009a). Significant infection has also been observed in areas of spring oilseed rape in Sweden (Wallenhammar et al., 2014). In Germany, clubroot on oilseed rape was diagnosed in 2005 and since then it has been spreading through oilseed rape growing areas (Diederichsen et al., 2014). In Poland, *P. brassicae* is present in all provinces (Jedryczka et al., 2014). Because *P. brassicae* lives in the soil, its identification and monitoring may be rather challenging because the symptoms do not occur at a sufficient level every year, especially if the weather is not favourable and the pathogen can survive unnoticed on fields for a long time (Strelkov et al., 2012).

In the Czech Republic, *P. brassicae* is traditionally found on fields where cruciferous vegetables have been grown for long term (Chytilová, Dušek, 2007). Previously, studies carried out in the Czech Republic were conducted only on fields where clubroot symptoms were observed on cruciferous vegetable plants. In 1984–1986, monitoring of clubroot occurrence and losses caused by clubroot was conducted (Rod, 1986, 1988, 1996). This monitoring effort revealed clubroot occurrence throughout the country. Only two areas showed no incidences – Central Bohemia (districts of Benešov, Kolín, Beroun, and Praha-east) and South Moravia (Břeclav, Hodonín, Uherské Hradiště districts). The highest infestation level was found in the regions with high vegetable production (Mělník, Litoměřice, Pardubice, Hradec Králové, Olomouc, Prostějov districts, and in Brno-city). The highest occurrences were recorded for cauliflower, cabbage, savoy cabbage, and kohlrabi, whereas rather moderate or low occurrences were found in oilseed rape. Overall, clubroot was found on 3–4 thousand ha every year, and the total infested area was estimated at 15–20 thousand ha, which represented 0.3–0.4% of total arable land in the CR at the time of this study. In 45% of the cases, clubroot occurred in patches; in 41% of cases, clubroot spread across entire fields; and just in 14% of cases the occurrence was isolated. Another hidden continuous source of clubroot infection are hobby gardens. The first infested loci on fields are frequently discovered in sections that are adjacent to private gardens because the gardeners deposit remains of infected plants onto fields to be plowed.

The highest losses shown in the aforementioned study (Rod, 1986) were recorded for savoy cabbage (35%), cauliflower, cabbage, and oilseed rape (29%). According to information provided by vegetable growers (1984–1986), clubroot occurred in fields in the previous decade, and one-third of farmers stated that clubroot appeared for the first time in their fields during the past five years. Nevertheless, there were also localities with clubroot appearing for more than 50 years. More than 40% of the respondents confirmed that clubroot was still spreading.

In recent years, the growers (Union of Oilseed Growers and Processors (UOPG), pers. comm.) have reported increasing incidence of clubroot on winter oilseed rape. There have been no scientific reports on clubroot spreading over the Czech Republic since the time of the above-mentioned study conducted in the 1980s. Therefore, the aim of the present research was to describe the recent spread of clubroot into areas of winter oilseed rape cultivation and to verify the use of PCR methods for detecting the spores of *P. brassicae* resting in soil.

MATERIAL AND METHODS

Distribution of *P. brassicae* in the Czech Republic

The monitoring of *P. brassicae* was performed with the help of agronomists from the Union of Oilseed Growers and Processors (UOGP) in 2011–2015. Agronomists together with oilseed growers were moni-

Table 1. Numbers of clubroot infected fields (*n*) and of newly infested localities in 2015, total counts and percentage representation

Region	District	*n*	New records in 2015	Total	%
Jihočeský	České Budějovice	3	0	5	4
	Tábor	2			
Jihomoravský	Břeclav	1	0	1	1
Karlovarský	Cheb	2	0	3	2
	Karlovy Vary	1			
Královéhradecký	Hradec Králové	3	5	19	15
	Jičín	13			
	Náchod	1			
	Trutnov	2			
Liberecký	Česká Lípa	11	9	38	29
	Jablonec nad Nisou	1			
	Liberec	21			
	Semily	5			
Moravskoslezský	Frýdek-Místek	6	5	41	31
	Nový Jičín	8			
	Opava	17			
	Ostrava-město	10			
Olomoucký	Šumperk	1	0	2	2
	Jeseník	1			
Pardubický	Svitavy	2		2	2
Plzeňský	Domažlice	1	1	3	2
	Plzeň-sever	2			
Středočeský	Benešov	2	4	9	7
	Mladá Boleslav	3			
	Mělník	2			
	Příbram	2			
Vysočina	Havlíčkův Brod	1	1	6	5
	Jihlava	3			
	Pelhřimov	1			
	Třebíč	1			
Zlínský	Uherské Hradiště	1	0	2	2
	Vsetín	1			
Total		130	25	130	100

toring selected fields where oilseed rape was cultivated in autumn. Based on growers' knowledge of each field, the sites of the most probable *P. brassicae* occurrence were identified, and the plants growing there were visually checked. The presence of *P. brassicae* was detected based on the presence of typical symptoms on roots. Some fields were inspected in spring as well as during any problems incidence. Based on agronomists' reports, a map of clubroot occurrence on the territory of the Czech Republic was created.

PCR detection of *P. brassicae* from soil

Soil sampling was carried out in randomly chosen locations (Fig. 1.) suspected of being infested by *P. brassicae*. The samples contained 5 random subsamples from the infested patch. Subsamples were collected to a 20 cm depth. The collected soil samples were air dried for two weeks, thoroughly homogenized, sieved (mesh 2 mm), and used for DNA extraction and testing with a conventional PCR. The total DNA was extracted from 500 mg of soil with a EurX Gene Matrix Soil DNA Purification Kit (EurX Ltd., Gdansk, Poland) according to the manufacturer's instruction. The concentration and purity of the DNA were determined spectrophotometrically by measuring the absorbance at 260 and 280 nm.

Infestation by the clubroot pathogen was confirmed using conventional PCR, using the *P. brassicae*-specific primers TC2F (5´-

Table 2. Precipitation, average temperatures (August–November), and numbers of newly clubroot infested localities – a comparison between selected regions

Year	Region	Precipitation (mm) + mean temperature (°C)										New infestations (*n*)
		VIII		IX		X		XI		VIII–XI mean		
2011	Czech Republic	69	*18*	48	*14.6*	44	*7.8*	1	*2.5*	41	*10.7*	24
	Liberecký r.	102	*16.9*	56	*13.5*	61	*7.6*	1	*2.9*	55	*10.2*	0
	Moravskoslezský r.	81	*15.9*	26	*12.5*	45	*8*	0	*2.7*	38	*9.8*	23
2012	Czech Republic	75	*18.2*	49	*13.3*	56	*7.4*	39	*4.8*	55	*10.9*	54
	Liberecký r.	103	*17*	33	*12.2*	37	*6.7*	70	*4.5*	61	*10.1*	17
	Moravskoslezský r.	63	*18.1*	75	*13.5*	102	*7.9*	36	*5.7*	69	*11.3*	6
2013	Czech Republic	85	*17.7*	74	*11.8*	44	*9*	36	*4.1*	60	*10.7*	15
	Liberecký r.	78	*16.8*	95	*11.2*	58	*9.2*	60	*3.8*	73	*10.3*	7
	Moravskoslezský r.	58	*17.8*	110	*11*	31	*9.5*	42	*4.4*	60	*10.7*	0
2014	Czech Republic	91	*15.7*	96	*14.1*	49	*10*	23	*6*	65	*11.5*	12
	Liberecký r.	69	*15.2*	81	*13.6*	55	*9.9*	14	*6*	55	*11.2*	5
	Moravskoslezský r.	113	*15.9*	105	*12.5*	55	*8*	35	*2.7*	77	*9.8*	2
2015	Czech Republic	67	*21.3*	33	*13.1*	52	*7.8*	76	*5.7*	57	*9.4*	25
	Liberecký r.	71	*20.5*	36	*12.2*	56	*7.5*	71	*5.2*	59	*8.7*	9
	Moravskoslezský r.	47	*15.9*	43	*12.5*	38	*8*	58	*5.6*	47	*7.7*	5
2005–2015	Czech Republic	88	*17.5*	58	*13.3*	41	*8.2*	44	*4.3*	58	*10.8*	
	Liberecký r.	114	*16.6*	66	*12.6*	56	*7.9*	63	*4.1*	74	*10.3*	
	Moravskoslezský r.	91	*16.9*	78	*12.7*	47	*8.3*	49	*4.2*	66	*10.5*	

AAACAACGAGTCAGCTTGAATGCTAGTGTG-3′) and TC2R (5′- CTTTAGTTGTGTTTCGGCTA GGATGGTTCG-3′)(Cao et al., 2007). PCR amplifications were conducted using a thermocycler (Bio Rad DNA Engine Peltier Thermal Cycler , Bio-Rad Laboratories, Hercules, USA) in a 25 µl volume containing 18.05 µl of double distilled sterile water, 0.2 µl (1 U) of DreamTaq™ DNA Polymerase (ThermoFisher Scientific, Waltham, USA), 2 µl of $MgCl_2$ (2mM), 2.5 µl of 10 × DreamTaq™ Buffer (ThermoFisher Scientific, Waltham, USA), 0.5 µl of both forward and reverse primer (10µM), 0.25 µl of each dNTPs (0.2mM), and 1 µl of total DNA extracted from the soil. A positive control containing all of the reaction components and 10 ng of *P. brassicae* DNA, along with a negative control consisting of all the components except a DNA template, were included in each PCR assay to ensure proper reaction conditions and the absence of DNA contaminants in the reaction mixtures. The cycling parameters were as follows:

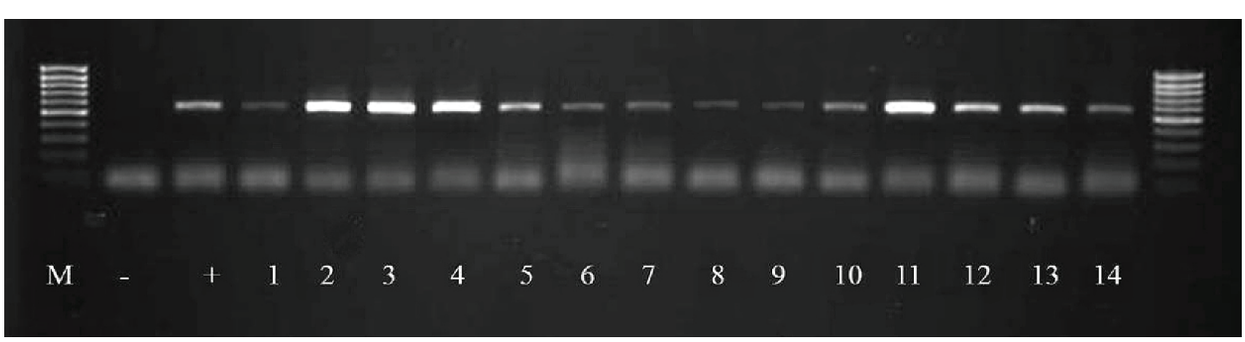

Fig. 1. PCR detection of P. brassicae in soil samples using species-specific PCR primers TC2F and TC2R (Cao et al., 2007)

M – marker, (–) – negative control, (+) – positive control, 1 – Modlibohov (Liberecký region), 2 – Holany (Liberecký r.), 3 – Bílý Kostel (Liberecký r.), 4 – Horka u Bakova (Středočeský r.), 5 – Třebnouševes (Královéhradecký r.), 6 – Miletín (Královéhradecký r.), 7 – Kbelnice (Královéhradecký r.), 8 – Žirovnice (Vysočina r.), 9 – Horusice (Jihočeský r.), 10 – Hrdějovice (Jihočeský r.), 11 – Bílovice (Jihomoravský r.), 12 – Pohledy (Pardubický r.), 13 – Kozmice (Moravskoslezský r.), 14 – Klokočov (Moravskoslezský r.)

Fig. 2. Clubroot infested localities reported in the Czech Republic by the years 2011–2015

an initial heat denaturation step at 94ºC for 2 min, 35 cycles of 94°C for 45 s, annealing at 65°C for 45 s and extension at 72°C for 45 s, with a final extension at 72°C for 7 min. The amplification products (10 µl of reaction mixture per loading well) were resolved on 1% agarose gels stained with ethidium bromide and visualized under UV light.10

RESULTS

Clubroot occurrence

Clubroot began to appear to a greater extent in different regions in autumn 2011. Disease incidence was detected on 23 farms in 5 different districts, mainly located in North and South Moravia. The incidence in autumn 2012 was even higher at 55 newly infested localities reported. They were found mainly in North Bohemia and in isolated locations in North Moravia and East Bohemia. Clubroot occurrence in 2013 was weaker, with just 15 newly infested localities reported. In autumn 2014, the disease appeared again, probably due to wet and warm weather conditions in autumn. New spots were reported in West and North Bohemia, for a total of 12 localities. In spring 2015, some heavily infected fields even had to be plowed under. In North Moravia, which has the longest history of oilseed rape clubroot occurrence, 25 newly infested localities were reported. Based on this monitoring, a map of the overall situation was compiled (Fig. 2 and Table 1), indicating the regions that require control measures to prevent further spread of the pathogen (Figs. 3, 4). Table 2

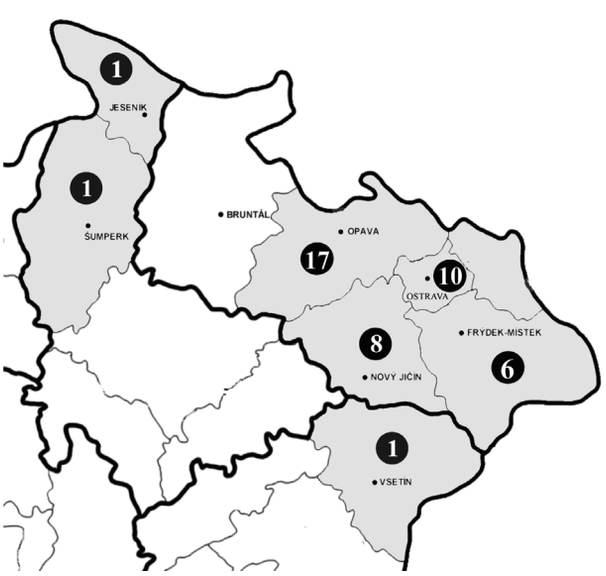

Fig. 3. Total numbers of localities infested by P. brassicae in the Moravskoslezský, Olomoucký, and Zlínský regions, North Moravia by spring 2015

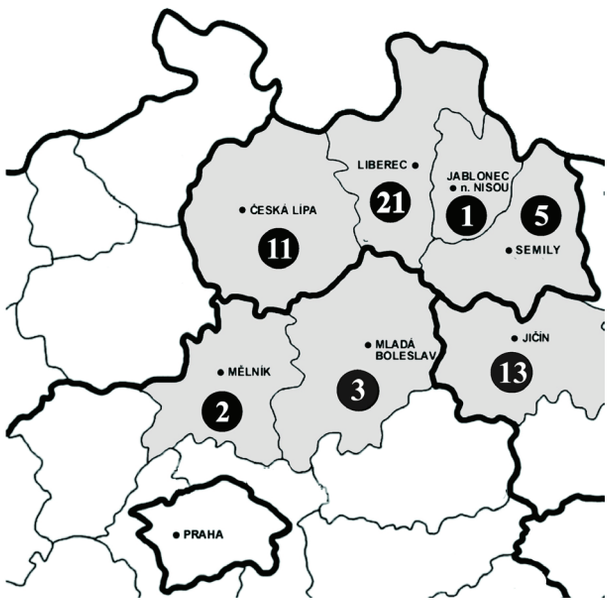

Fig. 4. Total numbers of localities infested by P. brassicae in the Liberecký, Středočeský, and Královéhradecký regions, Bohemia by spring 2015

shows the precipitation and temperature trends within 2011–2015. Nevertheless, there is seemingly no direct relationship between the weather and the number of newly infestated localities (Table 2).

Currently, the most clubroot contaminated region is Moravskoslezský, with 41 infested fields in four different districts (Frýdek-Místek, Nový Jičín, Opava, and Ostrava-city) (Fig. 2). The second most contaminated region is Liberecký, with 38 infested fields in four districts (Česká Lípa, Jablonec n. Nisou, Liberec, and Semily) (Fig. 3), and the third most contaminated is the neighbouring region Královéhradecký, with 19 infested fields (districts Hradec Králové, Jičín, Náchod, and Trutnov). The subsequent infested regions are Středočeský (9 fields), Vysočina (6 fields), Jihočeský (5 fields), Karlovarský and Plzeňský (3 fields each), Pardubický, Olomoucký and Zlínský (2 fields each), and Jihomoravský (1 field) (Table 1). The Ústecký region in the NW Bohemia is the only region without oilseed rape clubroot incidence. Summing up, there were at least 130 clubroot-infested fields in the Czech Republic by 2015.

PCR detection of *P. brassicae* from soil

The presence of *P. brassicae* was confirmed by conventional PCR analysis in all 14 tested soil samples (Fig. 1).

DISCUSSION

This study aimed to identify potential infestation by *P. brassicae* in field soils used for oilseed rape cultivation and to raise awareness of the scale of this phenomenon in the Czech Republic. Based on data of the Czech Statistical Office (https://www.czso.cz/csu/czso/zem_ts.), the area of vegetable production has significantly decreased over the last twenty-five years, from 33 134 ha in 1989 to 12 615 ha in 2014 (when 1911 ha were covered by cruciferous vegetables). On the other hand, the area of oilseed rape production exhibited massive growth, from 102 376 ha in 1989 to 418 808 ha in 2013 (winter and spring oilseed rape). A great deal of vegetable production has been replaced by regular oilseed rape cultivation; therefore, transmission of *Plasmodiophora brassicae* from infected vegetables was much easier (UOGP, pers. comm.). This paper reports on clubroot infestation in oilseed rape. Before 2011, clubroot in winter oilseed rape had been frequently reported, mainly in areas near Opava and Šumperk (North Moravia). These districts are currently in the most infested region – Moravskoslezský has 36% of infested fields in four different districts. The Liberecký region has 27% of infested areas. The above-mentioned regions were the first where clubroot disease reportedly caused yield losses on oilseed rape fields. The regions belonging to foothill areas traditionally cultivate cruciferous vegetables as one of the most important food sources. For example, at all localities in the Opava district (Moravskoslezský region), there has been a strong tradition of growing cabbage, and localities with clubroot problems such as Darkovice, Hlučín or Kravaře have been famous for cabbage cultivation (Nošovické zelí) (K o p e c k ý , D u š e k , 2012). The same is the situation in the Liberecký region, in the Semily district (with well-known specially shaped cabbage called Vysocké) (K o p e c k ý , D u š e k , 2012). The long-lasting practice in cabbage cultivation is also in the Jihočeský region (Tábor district) around Veselí n. Lužnicí (with a cabbage called Veselské) (C h y t i l o v á , D u š e k , 2007), where the fields have been heavily infested, too. Nevertheless, the current problem of clubroot infestation on winter oilseed rape plants cannot only be attributed to the simple replacement of cole vegetable by winter oilseed rape because the areas of former vegetable cultivation are not at all comparable with those of the present winter oilseed rape cultivation (see above). There is further evidence of *P. brassicae* spreading onto fields where vegetables have never been grown. Its establishment on these fields is supported by tight crop rotations, as winter oilseed rape is usually grown on the same field every fourth or even third year. On the other hand, despite previous problems with clubroot infestation in vegetable crops (R o d , 1996), the low occurrence of clubroot on winter oilseed rape has been reported in the Jihomoravský region (South Moravia). This part of the Czech Republic is the warmest and driest, and conditions are unfavourable not only for the pathogen but also for winter oilseed rape. Winter oilseed rape is grown here to some extent, but crop rotation is usually not as tight as in other regions. Compared to cruciferous vegetables, it is grown without irrigation. Moreover, local soils are mostly neutral or slightly alkaline and thus are probably not as conducive to the pathogen. Another region without any reported clubroot – Ústecký, is also relatively dry because it lies in the rain shadow of the Ore Mountains. Soils here also have higher pH on average than in other regions.

Despite the fact that in field trials, both the number of infected plants and the disease severity correspond to climatic characteristics, on a large scale in this survey, the number of new reports of infested fields seems not to have a direct relationship with weather on regional scale (Table 2). Table 2 shows that the years 2013 and 2014 in September slightly differed from the rest of years monitored, because the sums of precipitation were rather above-average. The temperature in 2014 was higher than in 2013, which resulted in the increase of infested localities in the next year 2015. Finally, the number of infested localities may be partly attributed to fluctuation in the annual activities of both growers and UOGP agronomists, which can vary in different years. This activity was probably higher in 2012 when the serious problems with clubroot began. Later, some

growers might reconcile with the presence of *P. brassicae* and start to grow resistant varieties, hampering easy visual diagnostics. This stresses the importance of using molecular methods for direct soil testing. Some growers also believe clubroot is quarantined, and thus they may not realize its presence.

Based on our monitoring, it can be concluded that *P. brassicae* currently endangers almost all arable land in the Czech Republic. Our detection method proved to be applicable in practice for testing soil samples. The results of PCR tests confirmed the screening results made by symptomatic diagnosis. It is probable that molecular techniques, such as conventional PCR (Cao et al., 2007), will confirm that the spread of *P. brassicae* is even higher than presented in this study. The real extent of clubroot infested areas is probably much larger because lots of clubroot patches were not found or recorded, especially in large fields of more than 100 ha. Furthermore, agronomists from the UOGP primarily only inspected the fields of growers belonging to the UOGP, which means that only about half of the total area of winter oilseed rape cultivation was monitored. Thus, our data on the area and number of infested fields are very likely underestimated.

Polish (Jedryczka et al., 2014) and Canadian studies (Strelkov, Hwang, 2014) have shown that the pathogen exists in a large number of soils, even in those not under oilseed rape cultivation. One of the causes may be the cultivation of oilseed rape in the past and the maintenance of the pathogen on weeds, which are susceptible to *P. brassicae* (Dixon 2009b). The most infested regions in the CR, Liberecký and Moravskoslezský, neighbour the areas in Poland, where massive infestation of fields has also been observed (Jedryczka et al., 2013); therefore, there is speculation about pathogen dispersal from one side or the other. Areas on both sides of the border are not only historically and culturally interconnected, but also have similar climates with relatively high amounts of precipitation supporting the pathogen.

A conventional PCR method can provide information about pathogen presence or absence, which is useful for the initial screening of fields that are suspected of infestation. Seeding resistant cultivars in a field suspected from infection could be economically adverse, because resistant cultivars on non-infested field provide lower yield and the seed is more expensive. Therefore, to avoid losses caused by clubroot or wrongly chosen oilseed rape cultivar, testing soil for clubroot infestation by the PCR diagnostic method is recommended.

CONCLUSION

In conclusion, *P. brassicae* is currently very widespread in oilseed rape in the Czech Republic and its incidence in the country will surely further increase (Kazda et al., 2013). Therefore, preventive measures (liming, growing resistant varieties) should be undertaken by oilseed rape growers to reduce further spread of this dangerous pathogen and the consequent losses. The PCR method was found to be sufficient for clubroot detection in soil and next steps in clubroot mitigation.

ACKNOWLEDGEMENT

Out thanks are due to all who patiently helped with the manuscript revision and figures editing, and to UOGP agronomists Jaromír Dědek and Karel Sypták.

REFERENCES

Ayers GW (1944): Studies on the life history of the club root organism, *Plasmodiophora brassicae*. Canadian Journal of Research, 23, 143–149.

Baranyk P, Balík J, Kazda J, Kuchtová P, Soukup J, Škeřík J, Volf M (2007): Oilseed rape – cultivation, utilisation, and economy. Profi Press s.r.o., Prague.

Cao T, Tewari JP, Strelkov SE (2007): Molecular detection of *Plasmodiophora brassicae*, causal agent of clubroot of crucifers, in plant and soil. Plant Disease, 91, 80–87.

Cao T, Manolii VP, Hwang SF, Howard RJ, Strelkov SE (2009): Virulence and spread of *Plasmodiophora brassicae* [clubroot] in Alberta, Canada. Canadian Journal of Plant Pathology, 31, 321–329.

Chytilová V, Dušek K (2007): Methodology of cruciferous plant testing for resistance against clubroot. Crop Research Institute, Prague. (in Czech)

Diederichsen E, Frauen M, Ludwig-Müller J (2014): Clubroot disease management challenges from a German perspective. Canadian Journal of Plant Pathology, 36, 85–98. doi: 10.1080/07060661.2013.861871.

Dixon GR (2009a): The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. Journal of Plant Growth Regulation, 28, 194–202.

Dixon GR (2009b): *Plasmodiophora brassicae* in its environment. Journal of Plant Growth Regulation, 28, 212–228.

Howard RJ, Strelkov SE, Harding MW (2010): Clubroot of cruciferous crops – new perspectives on an old disease. Canadian Journal of Plant Pathology, 32, 43–57.

Hwang SF, Ahmed HU, Strelkov SE, Gossen BD, Turnbull GD, Peng G, Howard RJ (2011): Seedling age and inoculum density affect clubroot severity and seed yield in canola. Canadian Journal of Plant Science, 91, 183–190.

Hwang SF, Howard RJ, Strelkov SE, Gossen BD, Peng G (2014): Management of clubroot (*Plasmodiophora brassicae*) on canola (*Brassica napus*) in western Canada. Canadian Journal of Plant Pathology, 36, 49–65.

Ingram DS, Tommerup IC (1972): The life history of *Plasmodiophora brassicae* Woron. Proceedings of the Royal Society B: Biological Sciences, 180, 103–112.

Jedryczka M, Korbas M, Jajor E, Danielewicz J, Kaczmarek J (2013): The occurrence of *Plasmodiophora brassicae* in agricultural soils in Wielkopolska region, in 2011–2012. Progress in Plant Protection, 53, 775–778. (in Polish)

Jedryczka M, Kasprzyk I, Korbas M, Jajor E, Kaczmarek J (2014): Infestation of Polish agricultural soils by *Plasmodiophora brassicae* along the Polish-Ukrainian border. Journal of Plant Protection Research, 54, 238–241.

Kazda J, Řičařová V, Prokinová E, Grimová L, Baranyk P (2013): Clubroot (pathogen *Plamodiophora brassicae*) threatens oilseed rape in the Czech Republic. Úroda, 61, 6, 28–32. (in Czech)

Kopecký P, Dušek K (2012): Screening the *Brassicas* Czech national collection for sources of clubroot resistance. Acta Horticulturae, 960, 147–150. doi: 10.17660/ActaHortic.2012.960.20.

Rod J. (1986): Preliminary data on the occurrence of clubroot (*Plasmodiophora brassicae* Wor) in Czechoslovakia. In: Proc. 10th Plant Pathology Conference, Brno, Czech Republic, 103–104.(in Czech)

Rod J. (1988): Occurrence and noxiousness of clubroot of crucifers in Czechoslovakia. Záhradnictvo, ١٣, 556–557. (in Czech)

Rod J. (1996): Reports – Agent of clubroot of crucifer. Central Institute for Supervising and Testing in Agriculture(ÚKZÚZ), Brno, 37 (Special Issue), 1–45. (in Czech)

Strelkov SE, Hwang SF (2014): Clubroot in the Canadian canola crop: 10 years into the rutbreak. Canadian Journal of Plant Pathology, 36, 27–36.

Strelkov SE, Manolii VP, Harding MW, Hwang SF, Fei W, Rong S, Burke DA, Pugh CA, Nielsen JM, Barnes A, JacobsenC, Vadnais M, Feindel D (2014): The occurrence of clubroot on canola in Alberta in 2014, Clubroot Survey Report 2014.

Strelkov SE, Tewari JP, Smith-Degenhardt E (2006): Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. Canadian Journal of Plant Pathology, 28, 467–474.

Strelkov SE, Manolii VP, Jurke C, Rennie DC, Orchard D, Hwang SF, Laflamme P (2012): The occurrence of clubroot on canola in Alberta in 2011. Canadian Plant Disease Survey, 92, 122.

Wallenhammar AC (1999): Monitoring and control of *Plasmodiophora brassicae* in spring oilseed brassica crops. Swedish Univerity of Agricultural Sciences, Uppsala.

Wallenhammar AC, Almquist C, Schwelm A, Roos J, Marzec-Schmidt K, Jonsson A, Dixelius C (2014): Clubroot, a persistent threat to Swedish oilseed rape production. Canadian Journal of Plant Pathology, 36 (Suppl. 1), 135–141.

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