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# Greenhouse and field experiments with winter oilseed rape cultivars resistant to *Plasmodiophora brassicae* Wor.

Veronika Řičařová [a, *], Jan Kazda [a], Petr Baranyk [b], Pavel Ryšánek [a]

[a] Department of Plant Protection, Czech University of Life Sciences Prague, Kamýcká 165, Prague 6, Czech Republic
[b] Union of Oilseed Growers and Processors, Czech Republic

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ABSTRACT

Clubroot disease, caused by *Plasmodiophora brassicae* (Wor.), has been affecting on winter oilseed rape (*Brassica napus* L.) in the Czech Republic over the past 4 years. Therefore, research on *P. brassicae* in the Czech Republic is important for developing effective strategies to manage clubroot under Czech environmental conditions. Experiments with *P. brassicae*-resistant cultivars of winter oilseed rape were conducted in an infested field and greenhouse. In the greenhouse, six resistant cultivars were grown in the infested soil collected from various fields in the Czech Republic and were assessed for index of disease (ID %). The best results achieved by cultivar Mentor (2 ± 0.7%) closely followed by cultivar SY Alister (5 ± 1.1%), the worst one was cultivar CHW 241 (30 ± 3.8%). In the field experiments seven resistant cultivars were grown, and disease development was monitored monthly. The lowest index of disease brought cultivar Andromeda (3 ± 0.8%) and PT 235 (4 ± 1.5%), the highest ID has cultivar CWH 241 (46 ± 6.5%) in the first season and in the second season any cultivar achieved 25% ID. Yields were measured at the end of the cropping season. The highest yield was achieved by cultivar SY Alister (6.1 t/ha) in the first season and cultivar PT 242 (5.03 t/ha) in the second season. The inoculum level was measured across the field by (qPCR), and an infestation map was created. The highest spore concentration was found on the field entrance. Collectively, the information obtained on the effectiveness of host resistance and pathogenic diversity of *P. brassicae* populations from the Czech Republic may help to more effectively manage clubroot in this country.



## 1. Introduction

Winter oilseed rape is the second most important crop in the Czech Republic (CSO – Czech Statistical Office, 2015 http://www.czso.cz). Clubroot caused by *Plasmodiophora brassicae*, can infect all members of the cruciferous family (*Brassicaceae*; Dixon, 2009a). The pathogen induces the formation of galls on infected roots that affect growth and water and nutrient uptake (Dixon, 2009a), leading to wilting or even death of the infected plants and loss of yield (Dixon, 2009b; Wallenhammar, 1996, 1999; Strelkov et al., 2006). The pathogen life cycle comprises a primary part and a secondary part (Ayers, 1944; Ingram and Tommerup, 1972). The resting spores germinate, and the primary zoospores are released to the soil and seek root hairs, where primary infection occurs (Ayers, 1944). The primary phase of infection occurs in both susceptible and resistant cultivars of oilseed rape (Hwang et al., 2012). After the primary plasmodia are formed, they develop into zoosporangia, and then secondary zoospores are then released into the soil again. The secondary zoospores penetrate to the root cortex of the host. Secondary plasmodia are formed in the cortex. The pathogen stimulates the hypertrophic growth of the root tissues, resulting in the formation of galls. The secondary plasmodia are transformed into resting spores, and the root galls disintegrate, thereby releasing resting spores into the soil (Ayers, 1944).

Currently, the pathogen also infects winter oilseed rape in the Czech Republic (Kazda et al., 2013). Infected stands were reported throughout the country in fall 2011. Serious infestations were found mainly in the northern and northeastern region of the country, and since then, the pathogen has continued to spread (Řičařová et al., 2016a). However, its appearance in the agricultural areas depends on appropriate weather conditions in the sowing period and during the autumn (Rod, 1996; Kazda et al., 2013). The damages to oilseed rape are not yet very high at the national scale (UOGR – Union of Oilseed Growers and Processors, unpublished data). Nevertheless,

* Corresponding author.
*E-mail address:* ricarova@af.czu.cz (V. Řičařová).

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some fields in the country are already heavily infested (UOGR). The situation is more serious due to the lack of economic and ecological way of protecting against this pathogen (Dixon, 2009b; Hwang et al., 2012). The problem could be suppressed using resistant cultivars of winter oilseed rape along with the appropriate field crop rotation (Diederichsen et al., 2009; Peng et al., 2014). For assessing the potential of the pathogen, it is also necessary to know the amount of inoculum, that is, the spore load (Rennie et al., 2011). The molecular detection technique quantitative PCR (qPCR) can be successfully used for estimating of the amount of inoculum in the soil (Rennie et al., 2011; Hwang et al., 2012).

The main objectives of this study was to test the resistant cultivars of winter oilseed rape. The resistance of cultivars is pathotype specific, and all cultivars might not be functioning on each *P. brassicae*-infested field (Diederichsen et al., 2009, 2014). Therefore, one part of this study was greenhouse testing of resistant cultivars in *P. brassicae*-infested soils from different locations of the Czech Republic to verify whether the cultivars maintain the resistance regardless the locality and different *P. brassicae* isolate. The same resistant cultivars were tested in a field experiment on an infested field by using a standard agricultural approach and machinery to prove the resistance and check the yielding parameters in the field conditions. The *P. brassicae*-infested field was also screened with qPCR for estimating of the inoculum level across the field. A field infestation map was then created. These experiments will provide information on the cultivation of resistant cultivars in agricultural practice and recommendations for the farmers cultivating oilseed rape to manage clubroot on their fields.

## 2. Materials and methods

### 2.1. Greenhouse experiments

#### 2.1.1. Soil samples

The soil samples were collected from 15 fields clubroot – infested oilseed rape fields (Fig. 1). The samples comprised three random subsamples from an infested patch with severe clubroot symptoms. The subsamples were collected to depth of 20 cm. The (GPS) coordinates of the patches were recorded. The sampling process was performed twice, once for each year of testing, every time in summer.

#### 2.1.2. Resistant cultivars

The greenhouse experiment used cultivars with proven clubroot resistance – SY Alister, CWH 241, Mendel, Mendelson, Mentor, PT 235 and susceptible variety of oilseed rape as a control, which were supplied from seed distributing companies thanks to Union of Oilseed Growers and Processors. The name of control wasn't mentioned, because of marketing purposes. This susceptible oilseed rape cultivar is a widely grown and its association with high susceptibility to *P. brassicae* could endanger its sales. In the second year of tests, Chinese cabbage cv. Granaat, a universal host of *P. brassicae*, was added.

#### 2.1.3. Experimental design

The experiment was conducted twice during 2013–2014. Resistant cultivars were sown in the pots filled with infested soils separately for each locality. Thirty plants of each cultivar were sown. The pots were placed in a technical insulator and regularly watered and fertilized. The temperature was maintained in a range at 20–22 °C, which is optimal for infection (Dixon, 2009a). The plants were harvested after 6 weeks. They were removed from the soil and the roots were washed with tap water.

#### 2.1.4. Disease assessment

Each plant was assessed at harvest for the severity of clubroot symptoms. Disease severity was assessed on a 0–3 scale, where 0 represents no galls, 1 represents a few small galls, 2 represents moderate galling, and 3 represents severe galling (Kuginuki et al., 1999). The severity ratings for each experimental unit were converted to an index of disease (ID) using the following formula (Strelkov et al., 2006):

$$\mathrm{ID}(\%) = \frac{\sum(n \times 0 + n \times 1 + n \times 2 + n \times 3)}{N \times 3} \times 100\%,$$

where $n$ is the number of plants in each class; $N$ is the total number of plants, and 0, 1, 2, and 3 are the symptom severity classes (scores).

#### 2.1.5. Data analysis

Data were analyzed using Statistica 12 (StatSoft CR, Ltd., Prague, Czech Republic). The normal distribution was tested with the Shapiro–Wilk test and, on the basis of this test result, the nonparametric Kruskal–Wallis test was chosen for the data from

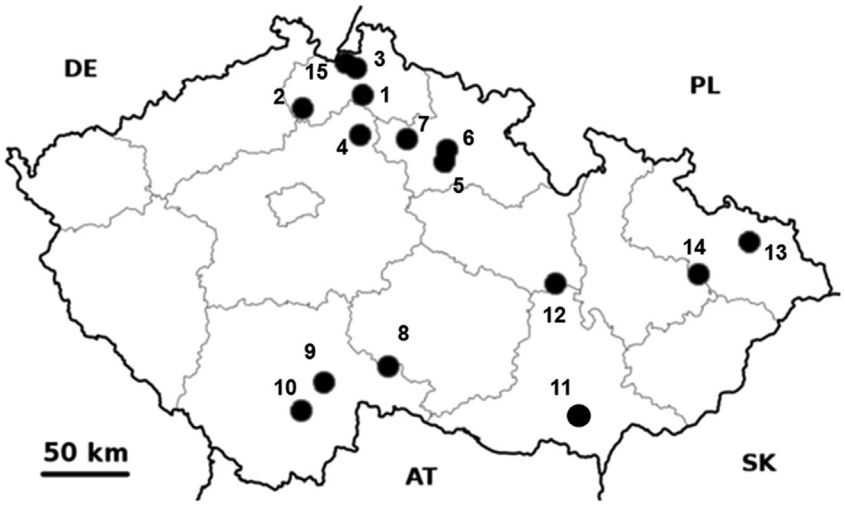


**Fig. 1.** Localities with clubroot used for soil sampling in greenhouse experiment. 1. Modlibohov, 2. Holany, 3. Bílý Kostel nad Nisou, 4. Horka u Bakova, 5. Třebnouševes, 6. Miletín, 7. Kbelnice, 8. Žirovnice, 9. Horusice, 10. Hrdějovice Ves, 11. Velké Bílovice, 12. Pohledy, 13. Kozmice, 14. Klokočov, and 15. Hrádek nad Nisou.