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Use of petal test in early-flowering varieties of oilseed rape (*Brassica napus* L.) for predicting the infection pressure of *Sclerotinia sclerotiorum* (Lib.) de Bary

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ABSTRACT

Sclerotinia sclerotiorum is one of the most important pathogens of winter oilseed rape plants. It causes the white mould disease of rape, thus significantly reducing the yield. The aim of our research was to use a spring oilseed rape variety sown in autumn or an early-flowering variety of winter oilseed rape to predict the infection pressure of *S. sclerotiorum* in a given year. Field experiments were conducted in 2008–2012 at the experimental station of the Czech University of Life Sciences Prague at Červený Újezd, 20 km west of Prague. In the experiment, we used one variety of spring oilseed rape (Lužnice, LU) and one early-flowering variety of winter oilseed rape (Californium, CA). The methodology of the petal test involved isolating pathogenic fungi from fallen petals on a nutrient medium (potato dextrose agar) in Petri dishes. The first term of collection was the beginning of petal fall (BBCH 61) and the last term was the end of flowering (BBCH 69). The dishes with petals were visually analysed after 1 week. The results were compared with the actual occurrence of white mould of rape in the stand. The occurrence of white mould of rape was strongly influenced by the progress of weather conditions over the given year. Infected petals and stems with symptoms of white mould of rape were found to be moderately correlated ($r = 0.80$). Spread of white mould spores was mostly observed in two terms (BBCH 62 and BBCH 65). Statistically significant differences were not observed in the infection of petals of spring (LU) and winter (CA) oilseed rape. Spring oilseed rape (LU) and early-flowering varieties of winter oilseed rape (CA) can be used to determine the strength of infection pressure of *S. sclerotiorum* in the stand in a given year, thereby improving protection against white mould of rape.

1. Introduction

Oilseed rape (*Brassica napus* L.) is the most important oil crop in the European Union and the third most important in the world, with a production of 20.9 and 70.1 million tonnes of seeds, respectively (USDA, 2014). This is the most important oilseed crop in the Czech Republic. In 2013, it was cultivated across 418.8 thousand hectares, representing 16.9% of arable land (CSO, 2014). In the Czech Republic, rapeseed is grown as a winter crop and is sown in August. Only about 5–7 thousand hectares are sown in spring (March–April).

Oilseed rape is a potential host plant for >71 species of micro-organisms (viruses, bacteria and fungi). Of these, only 10 are highly pathogenic for the rapeseed (Prokinová, 2000). The most important pathogenic diseases are blackleg of rape (*Leptosphaeria maculans*), white mould of rape (*Sclerotinia sclerotiorum*), Verticillium wilt of rape (*Verticillium dahliae*), grey mould of rape (*Botrytis cinerea*), downy mildew of cabbage (*Peronospora brassicae*), crucifer black spot (*Alternaria brassicae*), light leaf spot of rape (*Cylindrosporium concentricum*) and powdery mildew of rape (*Erysiphe cruciferarum*).

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Clubroot of rape (*Plasmodiophora brassicae*) has spread in some areas as well. In the Czech Republic, an average of 10–100% of oilseed rape stands are damaged by blackleg of rape (*L. maculans*) and white mould of rape (*S. sclerotiorum*) (Prokinová, 2000).

S. sclerotiorum (Lib.) de Bary is a necrotrophic fungal pathogen affecting a wide range of plants. It infects >400 species of plants worldwide, including important crops and numerous weeds (Bolton et al., 2006). *S. sclerotiorum* is one of the most important pathogens of winter oilseed rape. It causes white mould of rape, thus significantly reducing yield. White mould of rape is observed in all areas where oilseed rape is grown. Bradley et al. (2006) reported that white mould of rape is a major disease of the oilseed rape plant in the northern United States. In Minnesota and North Dakota, it causes annual losses of tens of millions of dollars.

The fungus produces on average 1.5–3-cm large sclerotia, which can survive in the soil ≥10 years (Prokinová, 2000). The germination of sclerotia depends on soil moisture and temperature. Sclerotia can germinate carpogenically at temperatures of 7–24 °C and high soil humidity persisting for at least 10 days without drying (Hao et al., 2003). Sclerotia germinate only on the surface of the soil or within a depth of a maximum of 5 cm. Small, light brown mushrooms called apothecia grow from sclerotia, which contain asci that release ascospores. Under optimal conditions (e.g. fallen petals on the leaves), these spores germinate and infect the plant (Paul, 2003). Petals play a significant role in the infection of rape plants, because the infection sites are located under the petals, through which the mycelium of the pathogen penetrates into the leaf tissues. Ascospores lying directly on the leaf surface do not germinate (Jamaux and Spire, 1999; Lamarque, 1983; Mc Lean, 1958).

The first visible symptoms of white mould on oilseed rape appear at the end of and after flowering. Elongated and watery spots are found on the stem, which quickly turn grey often with a silvery tint causing the plant epidermis to tear and peel. The infection site is often inside the stem padded with white mycelium, which forms the sclerotia (Prokinová, 2000). After these sclerotia drop to the surface of the soil, the fungal life cycle is completed. Apart from infection via spores, *S. sclerotiorum* can primarily infect the host plant through mycelium as well, which grows directly into the base of plants from the soil (Paul, 2003).

Therefore, among others, this research focuses on developing reliable forecasting programs. Some are based on the evaluation of environmental conditions (humidity, temperature, presence of disease in recent years, etc.). Others apply the sum of effective temperatures and precipitation for the period from the end of butonization (the phase of flower bud appearance) to the beginning of flowering in the ScleroPro system (Koch et al., 2007). The petal test, that is, detecting pathogens in the stand by its isolation from the petals, is a prediction method frequently used in other countries (Turkington et al., 1991).

Many authors (Baillet et al., 2013; Turkington et al., 1991) state that the petal test method can predict the occurrence of white mould of rape. Unfortunately, due to the time consuming Petal test (one week for cultivation), it is not possible to spray fungicides based on the prognosis in agricultural praxis.

Our aim was to modify (speed up) this method, so that it can be used for decision whether to use a fungicide. In contrast to previous studies, we used rapeseed, which has an earlier onset of flowering. We used the early-flowering variety of winter rape (Californium, CA) and spring oilseed rape (Lužnice, LU) sown in autumn. Approximately 60–80% of spring rape sown in autumn overwinters in the conditions of the Czech Republic, with a flowering period of about 5–7 days earlier than winter rape. The aim was to determine the number of infected petals of early-flowering oilseed rape by ascospores of *S. sclerotiorum*. By evaluating the disease occurrence, the number of plants with symptoms of white mould of rape was determined, as was the relationship between the number of infected petals and the number of infected plants of oilseed rape. From the obtained results, the use of spring rape variety sown in autumn or the early-flowering variety of winter oilseed rape was assessed to predict the infection pressure of *S. sclerotiorum* in a given year.

2. Material and methods

Small-plot field experiments were conducted at the experimental station of the Czech University of Life Sciences Prague (CULS) in Červený Újezd from 2008 to 2012. The experimental areas span from 50° 04′ N latitude to 14° 10′ E longitude, at an altitude of 405 m above sea level. The representative genetic soil is brown soil with a loess layer. The soil reaction is neutral, with medium humus content. The average annual air temperature is 6.9 °C and the average annual rainfall is 549 mm. The duration of the growing season of winter oilseed rape is 330–340 days. The experiments were repeated four times, with land plots of 15-m^2 area each. The oilseed rape was sown on the following dates: 26 August 2007, 26 August 2008, 23 August 2009, 25 August 2010 and 25 August 2011. Fifty seeds were sown per square metre, with 40 seeds germinating in 2007, 39 seeds in 2008, 38 seeds in 2009, 36 seeds in 2010 and 43 seeds per square metre in 2011. The harvest was held on 28 July 2008, 7 July 2009, 28 July 2010, 27 July 2011 and 23 July 2012. One variety of winter oilseed rape (CA) and one variety of spring rape (LU) were sown. The methodology was based on the isolation of pathogenic fungi from falling petals of both rapeseed varieties on a culture medium (potato dextrose agar, PDA) in Petri dishes. PDA is a general–purpose medium used to cultivate yeasts. PDA is composed of dehydrated potato infusion and dextrose, which encourage abundant fungal growth (4 g of potato infusion, 20 g of dextrose and 15 g of agar per litre, pH: 5.6 ± 0.2 at 25 °C). Agar is added as the solidifying agent.

PDA was poured into sterile Petri dishes after autoclaving at about 50–70 °C under sterile conditions. We collected fallen petals that remained on the leaves. The petals were transferred with sterile tweezers into a Petri dish with breeding medium (agar). The tweezers were sterilized with Desident Cavicide® (15–18% isopropyl alcohol, <0.03% sodium hydroxide, 1–5% 2-butoxyethanol and <0.3% benzethonium chloride). The first term of sampling was conducted in the beginning of the first petal fall (BBCH 61) and the last sampling by the end of flowering (BBCH 69).

Overall, we collected fallen petals six times at weekly intervals (Table 1) in ten Petri dishes from each plot. We placed five fallen petals in each Petri dish. After sampling, the Petri dishes were stored for 7 days in a dark room at 20 °C. After a week, the Petri dishes with petals were visually analysed at the Department of Plant Protection at the CULS. The percentage of infected petals with spores of *S. sclerotiorum* was determined. *S. sclerotiorum* forms white mycelia and later black sclerotia on the Petri dishes. We compared the results of infected petals by regression and correlation analysis to the actual infected stems with symptoms of white mould of rape in the stand about a week before harvest.

We observed an average daily temperature (degrees Celsius) and precipitation (millimetre) during April and May. The monthly values were compared with the long-term average (Table 2).

Statistical analyses were conducted using Statgraphic Centurion XV software by one-way ANOVA (analysis of variance; multiple range tests), Tukey's test ($P = 0.05$) and simple regression.

3. Results and discussion

Each experimental year (2008–2012) showed a different