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EVALUATION OF DOWNY MILDEW (*HYALOPERONOSPORA BRASSICAE*) INFECTION SEVERITY ON DIFFERENT CRUCIFEROUS OILSEED CROPS

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Diseases constitute an important economic problem in oilseed rape (*Brassica napus*) cultivation. Although downy mildew has been counted so far as a minor disease, under intensive cultivation system and short rotation interval, the impact of diseases could increase in the future, especially under predicted more humid northern climatic conditions. This research study is the first report about the severity of downy mildew infection on cruciferous crops in Estonia. During two years (2010 – 2011), downy mildew infection severity was assessed in six different cruciferous crops (*B. napus*, *B. juncea*, *B. nigra*, *Sinapis alba*, *Raphanus sativus*, *Eruca sativa*) in field trials located in Eerika, Tartu County, Estonia. On both study years, four disease assessments were done. Downy mildew infection started a week earlier in 2010, but the overall disease pressure was lower compared to 2011. Based on our field trial results, *S. alba*, *E. sativa* and *R. sativus* plants were significantly more resistant to downy mildew compared to other tested crops. Furthermore, in 2010 downy mildew symptoms were not recorded on two cruciferous species *E. sativa* and *R. sativus*. *Brassica nigra* and *B. napus* plants were the most susceptible to downy mildew, with especially severe infection in 2011. Based on our two years data, downy mildew can damage foliage on spring oilseed rape (*B. napus*) and black mustard (*B. nigra*) in a notable extent. We conclude that downy mildew is an important pathogen, which should be monitored on different oilseed cruciferous crops in Estonia.

Keywords: *Brassica napus*, *B. juncea*, *B. nigra*, *Sinapis alba*, *Raphanus sativus*, *Eruca sativa*, downy mildew

INTRODUCTION

Cruciferous oil seed crops such as oilseed rape (*Brassica napus* L.) and turnip rape (*Brassica rapa* L.) have been important cash crops for farmers in Estonia since the end of 1990s. Since then, the cultivation area has increased tremendously and culminated in 2010 at 100 000 ha that comprised approximately 26% of total cultivation area of cereal-dominated crop rotations (Järvan, 2016; Statistics Estonia, 2019). However, the cultivation area has now stabilized at 75 000 ha (16%) (Statistics Estonia, 2019). Growth in extensive monocultures and short rotation has caused serious problems for oilseed rape and turnip rape with several insects like pollen beetle (*Brassicoglyphus aeneus* Fabr.) (Metspalu *et al.*, 2015), flea beetles (*Phyllotreta* spp.) (Metspalu *et al.*, 2014), etc. and several soilborne diseases such as Sclerotinia stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) (Ilumäe *et al.*, 2009) and clubroot (*Plasmodiophora brassicae* Woronin) (Järvan, 2016), which are difficult to control. Heavy input of synthetic pesticides for disease and insect control puts considerable economic pressure on farmers (Ilumäe, 2013) and causes resistance to widespread pesticides (Brandes & Heimbach, 2018; Kovacs *et al.*, 2015).

The cruciferous pathogen *H. brassicae* is an obligate oomycete. Although the downy mildew (*Hyaloperonospora brassicae* (Gaum)) has been considered a minor disease of oilseed rape in Australia (Van de Wouw *et al.*, 2016), in the Baltic region (Latvia and Lithuania), UK (Hardwick *et al.*, 1991) and elsewhere (Bankina *et al.*, 2012; Petraitiene, 2006), still lately has reported by Mohammed *et al.* (2017) severe epidemics in Western Australia. The pathogen *H. brassicae* is widespread and harmful for cruciferous oilseed crops and vegetables (Mohammed *et al.*, 2018a; Coelho & Monteiro,

2018; Coelho & Montiro, 2003). Symptoms appear as white fungal growth that develops on the underside of leaves and yellow/brown irregular lesions that develop on the upper leaf side. Although downy mildew can infect the host plant during growing period, it is still most severe on oilseed rape at the seedling stage when the pathogen can cause seedling death (Oxley & Evans, 2009). Damage is worse in wet conditions at approximately 15°C (Saharam *et al.*, 2018) and in case of infection on pods (Hardwick *et al.*, 1991). Furthermore, under favourable conditions and in intensive cultivation with short crop rotation, downy mildew could become an acute problem in cruciferous oilseed crops in the northern Baltic region.

Different cruciferous crops like winter oilseed rape, white mustard (*Sinapis alba* L.) and oilseed radish (*Raphanus sativus* L.) have been used quite successfully as green manure and cover crops to suppress soilborne pathogens and reduce foliar disease progression (Larkin *et al.*, 2010; Larkin & Halloran, 2014; Larkin, 2017; Olanya *et al.*, 2014; Runno-Paurson *et al.*, 2014, 2015, 2019). Use of cover crops could be a promising alternative disease control strategy for environmentally friendly producers. Oilseed radish, rucola (*Eruca sativa* (Mill)), white mustard and mustard green (brown mustard) (*Brassica juncea* L.) have been previously reported as effective trap plants for flea beetles, pollen beetles and cabbage seedpod weevil (*Ceutorhynchus obstrictus* (Marsham) (Veromann *et al.*, 2012; Metsalu *et al.*, 2014; Kovacs *et al.* 2017). However, the pathogen *H. brassicae* persists as oospores on infected plant debris in the soil (George, 2014). Thus, it is very important that cover crop plants are not susceptible to downy mildew and pathogen does not accumulate in the soil.

Behind the high diversity of Baltic potato late blight pathogen (*Phytophthora infestans*) populations is the result of sexual reproduction, which benefits the survival, adaptability and diversity of the pathogen in the climate of North-Eastern Europe (Kiiiker *et al.*, 2018, 2019). Furthermore the presence of sexual reproduction changes the epidemiology (Mayton *et al.*, 2000) and increases the adaptability of the pathogen, thus changes the way in which disease control must be approached (Yuen & Andersson 2013). There are reports from higher pathotypic variation on *B. oleracea* (Coelho *et al.*, 2012). Thus under the changed climate conditions (Sepp *et al.*, 2018), in case of *P. infestans*, oomycete *H. brassicae* could happen similarly scenario that through sexual reproduction the genetic diversity and control problems increases in northern Europe.

By now, the knowledge about downy mildew infection severity on oilseed rape, including other cruciferous oilseed crops, has been missing in the northern Baltic region. Therefore, the main aim of this study was to evaluate downy mildew development and severity on spring oilseed rape, and on five alternative cruciferous oil seed crops (*B. juncea*, *B. nigra*, *S. alba*, *R. sativus*, *Eruca sativa*) with the purpose to find species that are less susceptible to disease for possible application as cover crop, in trap cropping or seed production. The study was conducted in climatically two different years allowing for characterization of the variability of downy mildew infection severity.

MATERIALS AND METHODS

Field experiment

Downy mildew (*H. brassicae*) occurrence was evaluated in 2010 and 2011 in six different cruciferous plant species in the Eerika experimental field of the Estonian University of Life Sciences in 2010 and 2011, Tartu County, Estonia (58°21'N, 26°39'E). The plants were grown in a randomized complete block design with three replicates of each of the following species: spring oilseed rape (*Brassica napus* (L.) cv. 'Maskot'), mustard green (brown mustard) (*B. juncea* (L.) Czern.) cv. 'Jadrjonaja', black mustard (*B. nigra* (L.) W. D. J. Koch), rucola/arugula (*Eruca sativa* subsp. *sativa* (Mill.) Thell.) (= *E. sativa*) cv. 'Poker'), oilseed radish (*Raphanus sativus* (L.) var. *oleiformis* (Pers.) = *R. sativus*), cv. 'Bille') and white mustard (*Sinapis alba* (L.), cv. 'Branco'). In both years, seeds were obtained from the seed collection of the Estonian University of Life Sciences. In both study years, the size of each plot was 1 m × 5 m as recommended in other studies for cruciferous crops (Veromann *et al.*, 2012, Kalischuk & Dossdall 2004, Kovacs *et al.*, 2017). To minimize inter-plot interactions, there was an 1 m wide buffer zone of bare soil around each plot. The whole experimental field was surrounded by a hay meadow. Plots were sown on the 12th of May in 2010 and 9th of May in 2011, at 250 seeds per m² (seeds obtained from the seed collection of the Estonian University of Life Sciences). The germination of seed was similar for different species in different years, and no differences were noticed in plant density (on average 200 plants per plot) between plots. In both years, the same standard crop management practices were used in all trial plots. The fertilizers and pesticides were not used. The plant growth stage (BBCH) was assessed using the decimal code system of Lancashire *et al.* (1991).

Weather conditions

In 2010, the air temperature (Eerika Weather Station present at the site) in May and June were similar to the long-term (42-year) average, but in July, the temperature was 4.1°C higher than the long-term average (Table 1). The rainfall in 2010 was similar to the long-term average in June, but in July, it was lower with only 38.4 mm (70.6 mm for 42-year average). In 2011, the temperature in May was similar to the long-term average, but June was hotter by 1.9°C and July hotter by 2.4°C (Table 1). The rainfall in 2011 May was similar to the long-term average, but much lower in June and July (Table 1). In the third ten-day period of July, the warm conditions (with temperatures over 30°C for more than 10 days) were suppressive/unfavourable to downy mildew infection.

Downy mildew assessment

Downy mildew occurrence was monitored from June 23 to July 12 2010 (four observations) and from 1st of July to 22nd of July 2011 (four observations), at GS 31 – 35 to GS 75 – 77 (Table 2). Disease assessments were made under natural infection conditions as a percentage of total foliage once each week, until the culmination of the disease. In all species in each of the replications, ten randomly selected plants per plot were assessed for downy mildew infection. Downy mildew infection on test plants was assessed according to the 0 – 100% scale (EPPO Bulletin, 2003). The area under the disease progress curve (AUDPC) was calculated from the date of first occurrence of downy mildew until the

last observation of the disease in the trial according to Shaner & Finney (1977) by using the following formula: $\Sigma R = n[(R_{i+1} + R_i)/2] (t_{i+1} - t_i)$, where R_i is the disease severity (percentage leaf surface blighted) for the previous (i th observation), and R_{i+1} the severity for the current observation and t_i and t_{i+1} are the corresponding times of measurements (day of year), and n is the total number of observations.

Table 1. Average monthly temperatures (°C) and precipitation (mm) in Eerika experimental field during the vegetation and the corresponding long-term averages (1969 – 2011).

Month	Ten-day period	Average temperature (°C)			Sum of rainfall (mm)		
		2010	2011	1969 – 2011	2010	2011	1969 – 2011 (average)
May	I	7.3	8.0	9.7	39.2	0.2	12.9
	II	16.7	11.8	11.4	44.6	46.6	20.9
	III	12.4	13.0	12.8	13.6	11.6	22.8
June	I	13.3	19.7	14.9	41.4	0.0	20.9
	II	13.6	15.9	15.1	17.8	24.8	26.9
	III	15.9	16.2	16.2	38.8	10.4	28.0
July	I	19.9	20.0	17.3	11.6	9.2	19.3
	II	22.9	18.6	17.4	3.0	30.4	24.8
	III	22.4	21.0	17.8	23.8	8.6	26.5
May–July	I–III	16.0	16.0	14.7	233.8	141.8	203.0

Data analysis

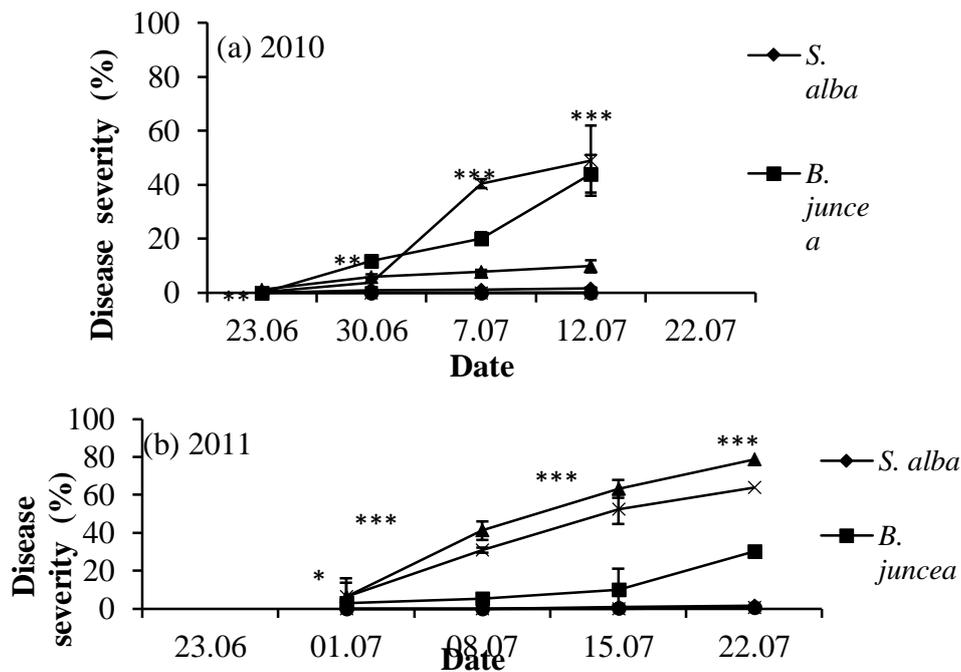
Statistical analysis of collected data was performed with Statistica 13 (Quest Software Inc, Aliso Viejo, CA, USA). Differences in the severity of downy mildew infection in dependence of year, cruciferous crop species and their interaction were tested by ANOVA. Both factors “year” and “species” were treated as fixed categorical variables. Tukey HSD post-hoc tests ($P = 0.05$) were applied to separate the differences among the means between years and cruciferous crop species. The level of statistical significance for all effects was $P = 0.05$ except when noted.

Table 2. Growth stages (GS) of studied cruciferous species at each observation date in 2010 and 2011.

Species	Disease evaluation date					
	2010	23 June	30 June	7 July	12 July	–
	2011	23 June	1 July	8 July	15 July	22 July
	Year					
<i>B. napus</i>	2010	55–60	61–63	65–68	68–71	
	2011	60–62	65–68	68–70	71–72	72–74
<i>B. nigra</i>	2010	58–60	65–67	68–70	71–72	
	2011	63–65	67–69	71–72	72–74	75–77
<i>B. juncea</i>	2010	55–60	65–67	69–70	71–72	
	2011	63–65	68–69	71–72	73–74	75–77
<i>E. sativa</i>	2010	31–35	55–57	57–63	63–66	
	2011	55–62	61–63	65–67	68–69	71–72
<i>R. sativus</i>	2010	31–34	57–59	55–63	58–65	
	2011	55–62	63–65	67–68	71–73	73–75
<i>S. alba</i>	2010	62–64	68–70	72–74	72–74	
	2011	65–67	71–72	73–74	73–74	75–77

RESULTS AND DISCUSSION

In 2010, first occurrence of downy mildew infection was found on the 23 June with slight infection on *S. nigra* and *B. napus* plants ($F_{5,12} = 5.85$, $p < 0.01$) (Figure 1a). On the 30 June, the downy mildew infection on *B. juncea* plants reached 11.7% (average \pm SE of replicate plots 0.50%), which was significantly higher than that on other test plant species ($F_{5,12} = 13.52$, $p < 0.001$). At that date, the plants of *S. alba* were only slightly infected (Figure 1a). Significantly higher downy mildew infection was recorded a week later on the 7 July; at that date, the infection reached 40.4% (\pm SE 11.0%) on *B. nigra* and 20.1% (\pm SE 2.6%) on *B. juncea* (Figure 1a). Hereafter the disease progression diminished and culminated at 48.8% (\pm SE 13.0%) infection on *B. nigra* plants and 44% (\pm SE 7.0%) infection on *B. juncea* plants (Figure 1a). Downy mildew infection was not found on *E. sativa* and *R. sativus* plants during the 2010 growing season (Figure 1a).



* Error bars are standard error of the means. The asterisks demonstrate the significance of the species effect. Significant at $P < 0.05$; ** Significant at $P < 0.01$; ***Significant at $P < 0.001$

Figure 1. Development of downy mildew (*H. brassicae*) on six different cruciferous oilseed crops in 2010 (a) and 2011 (b).

In 2011, first downy mildew symptoms were found on the 1 July on *B. napus*, *B. nigra* and *B. juncea* plants (Figure 1b). First disease symptoms were recorded on *S. alba* two weeks later on the 15 July (Figure 1b) and disease development culminated at very low level with 1.6% (\pm SE 0.9%) on the 22 July (Figure 1b). Downy mildew developed significantly faster on *B. napus* and *B. nigra* plants from the 8 July onwards compared to other cruciferous species ($F_{5,12} = 13.74$, $p < 0.001$). On *B. juncea*, downy mildew development progressed from the 15 July and culminated on the 22 July at 30% (\pm SE 11.2%). *Eruca sativa* and *R. sativus* plants were infected only slightly by the end of observation period ($< 1\%$). By the end of the observation period, 80% (\pm SE 4.8%) of foliage was infected by downy mildew on the *B. napus* plots (BBCH 72 – 74) and 64% (\pm SE 7.8%) on *B. nigra* plots (BBCH 75 – 77) (Figure 1a).

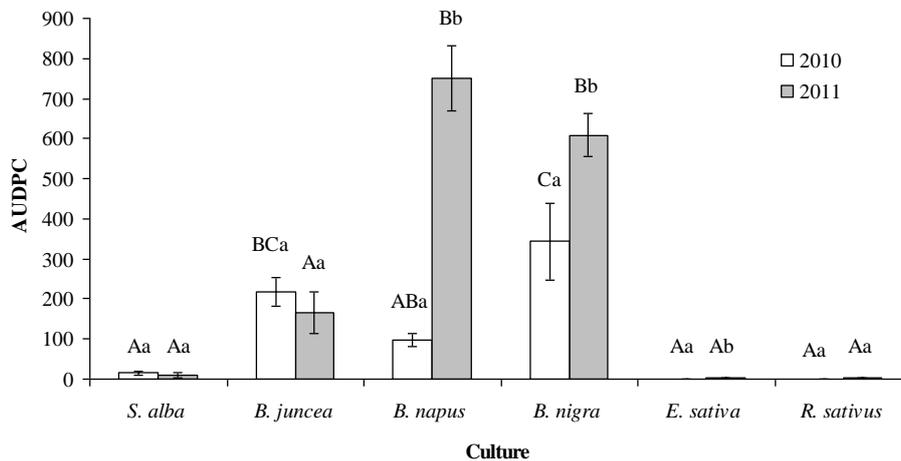
In this study, we assessed the development of downy mildew on *B. napus* (the main crop) and compared it with the disease development in five alternative oilseed crops. In both growing seasons, the weather conditions were such that downy mildew assessments were possible, but the development of the disease was somewhat different. June 2010 was wet and cool, thus excellent for infection and spread of downy mildew. In 2010, the disease infection started a week earlier, but the infection pressure was lower, and downy mildew spread stopped also earlier compared to the growing season 2011. Overall, the growing season 2011 turned out more favourable for downy mildew development. However, conidia of *H. brassicae* can germinate in temperatures ranging widely from 5 to 30°C, and the optimum for germination is 15 – 25°C (Achar, 1998). Downy mildew pathogen conidia germinate when exposed to free water, and once germinated the most rapid development of downy mildew occurs at temperatures between 20 – 24°C (Larka, 2001).

Based on AUDPC values, downy mildew pressure differed significantly between the two observation years ($F_{1,24} = 32.35$, $P < 0.001$) and test species ($F_{5,24} = 50.25$, $P < 0.001$). Although the disease pressure in 2010 was lower, there were still significant differences in AUDPC values between six studied crops ($F_{5,12} = 11.03$, $P < 0.001$). In 2010, two crops, *E. sativa* and *R. sativus*, had no infection at all, and *S. alba* had a very low AUDPC value of 14.8 (Figure 2). In this year, the highest value of 344 was observed in *B. nigra*. Similarly, in 2011, significant differences between six test crops were found ($F_{5,12} = 9.08$, $P < 0.001$). AUDPC values were very low in three crops: < 2.0 in *E. sativa* and *R. sativus* and 8.9 on *S. alba* (Figure 2). The highest AUDPC values were 751 in *B. napus* and 608 in *B. nigra* plants (Figure 2). Combined two-year data showed a significant interaction effect of year and species on the AUDPC value for downy mildew ($F_{5,24} = 19.67$, $P < 0.001$), indicating that *B. napus* and *B. nigra* were significantly more susceptible in 2011 than 2010.

Quantitative disease resistance assessment on downy mildew with AUDPC showed significantly lower infection values in the 2010 growing season than in 2011. Downy mildew caused diseases are polycyclic. Each step of the life cycle, including conidial germination, infection, latency and sporulation, is influenced by weather conditions (Kofet & Fink, 2007). In addition, significant differences in foliar resistance to downy mildew were found between six tested cruciferous crops. *Eruca sativa* (arugula) cultivar ‘Poker’ and *R. sativus* cultivar ‘Bille’ plants were not infected in 2010, and even under the very favourable conditions in 2011, the infection started at the end of the observation period and stayed at a very low level. In contrast, in more humid conditions, e.g. in Korea Yongin region downy mildew poses a serious threat to arugula production in plastic greenhouses (Choi *et al.*, 2010). On *S. alba* plants, the downy mildew infection rates in both growing seasons were also less than 2% and *B. juncea* was moderately infected. Similarly, in the study of Mohammed *et al.* (2018b), *B. juncea* cultivar ‘Dune’ showed the greatest resistance, particularly the older plants

(40 day-old-plants). At the same time, two cruciferous species, *B. nigra* and *B. napus* were susceptible to downy mildew. Similarly, Van de Wouw et al. (2016) observed significantly less downy mildew on a *B. juncea* breeding line, compared to *B. napus* cultivars. Resistance to *H. brassicae* in diverse cruciferous host range offers new disease management approaches (Mohammed *et al.*, 2019). Given that downy mildew poses a considerable threat to oilseed crops, resistant species and resistant cultivars available for several crops should be harnessed, especially in organic management.

On both growing seasons, the temperature was warmer than long-term average. Our results showed strong interaction between year and species to the downy mildew susceptibility. There are several reports of severe attacks of *H. brassicae* on cruciferous oilseed crops or vegetables (Mohammed *et al.*, 2017; Coelho *et al.*, 2002; Carlsson *et al.*, 2004). Thus, in more favourable conditions pathogen *H. brassicae* is able to cause even higher damage as in this study found in northern Baltics. Therefore more resistant crops and cultivars is necessary to seek out and cultivate.



* Error bars denote the standard error of the means. Means followed by different capital letters above each bar indicate a significant influence ($P < 0.05$) of cruciferous oilseed crops in different years (Tukey HSD test). Means followed by different small letters above each bar indicate a significant influence ($P < 0.05$) of year (Tukey HSD test).

Figure 2. Mean area under the disease progress curve (AUDPC) values of downy mildew (*H. brassicae*) infection on different cruciferous oilseed crops.

CONCLUSIONS

These results provide useful suggestions in environmentally sustainable plant cultivation (green manure and cover crops) and plant protection (trap crops) strategies, which are based on multitrophic interactions. In both growing seasons downy mildew (*H. brassicae*) infection was registered on most test crop species. The two growing seasons were different, but the results clearly highlighted a consistent variability in the susceptibility to disease among studied species. Based on our field trial results, *S. alba*, *E. sativa* and *R. sativus* had significantly less downy mildew infection compared to other tested crops. Furthermore, in 2010 downy mildew on *E. sativa* and *R. sativus* was not recorded. Besides oilseed rape (*B. napus*), also black mustard (*B. nigra*) was susceptible to downy mildew. This must be considered especially in organic cultivation where synthetic fungicides are not allowed for disease control. The results of our study demonstrated that downy mildew is for spring oilseed rape and black mustard in Estonian field conditions a considerable, rather than minor disease. Given that wetter conditions are expected under global climate change in Estonia (Sepp *et al.*, 2018; Climate Change Adaptation Development Plan until 2030), that can particularly favour polycyclic diseases, we argue that downy mildew can become an even more significant pathogen in Estonia in future climates. Thus, more attention should be paid to cultivar resistance of spring oilseed rape and other cruciferous oilseed crops and resistance to downy mildew should be included in local resistance breeding programmes.

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