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THE IMPACT OF *FUSARIUM GRAMINEARUM* INFECTION ON DIFFERENT PLANT SEEDS

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Abstract
Healthy seeds are essential for the optimal plant population and yield, but seed-borne pathogens, such as *Fusarium* spp., may reduce seed germination, quality and cause damping-off of the seedlings. *Fusarium graminearum* is a dominant pathogen of cereal crops and can cause significant losses of grain yield and quality. It is important to evaluate the role of alternative inoculum source in crop rotation. The aim of this study was to assess the impact of *F. graminearum* infection on different plant seed germination and seed infestation. The research was conducted at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, in 2017. Visually healthy seeds of bean (*Vicia faba* L.), pea (*Pisum sativum* L.), lupine (*Lupinus angustifolius* L.), soybean (*Glycine max.* (L.) Merr.), lucerne (*Medicago sativa* L.), white (*Trifolium repens* L.) and red (*Trifolium pratense* L.) clover were inoculated with 10 mL of *F. graminearum* suspension, adjusted to 1×10^6 conidia per mL. Seed infection was counted 2 and 6 days after inoculation (DAI), seed germination energy and reduction rate – after 3 DAI and germination index – 6 DAI. Results showed that all inoculated seeds were covered with typical to *F. graminearum* red-purple mycelium. The results of inoculated seeds with *F. graminearum* showed red-purple mycelium growth on the seeds (infection from 21.25 up to 100%). The results showed that germination energy decreased on pea (2.56%) and lupine (7.79%) seeds. Our results suggest that various plant seeds differently react to *F. graminearum* infection. The highest infection of *F. graminearum* was obtained on pea, lupine seeds and the least on red clover.
Key words: germination, infection, inoculation.

Introduction

One of the important inputs for crop production is seeds. Healthy seeds are essential for the optimal plant population and yield. Numerous diseases are associated with seeds. Diseased seed often looks discoloured or shrunken, but there may be exceptions. Conventionally, the cereal seeds are treated with fungicides regardless of seed health and expected disease. Some of the diseases are controlled by sorting the seeds by size and seed density during seed cleaning. Seed-borne disease cause at least 10% of yield losses. The seed-borne pathogens may reduce seed germination, quality and cause damping-off of the seedlings. Seed-borne diseases are associated with a lack of adequate amount of fungicides to control diseases. The understanding of the seed-borne pathogens may be helpful in reducing losses and improving yields (Borgen, 2005; Leslie & Summerell, 2006; Cram & Fraedrich, 2010; Farrag & Moharam 2012; Yang *et al.*, 2013; Purahong *et al.*, 2014).

Fusarium spp. associated pathogens are important to cereal crops causing significant losses of grain yield and quality. *Fusarium* spp. infected seeds could look healthy but has fungus inside. *Fusarium graminearum* Schw. cause Fusarium head blight (FHB) of small grain cereals. The mycotoxin contamination and yield reduction make FHB primarily cereal disease. There are several *Fusarium* species which cause FHB, but *F. graminearum* is the most frequent species in many cereal-growing regions. The primary host plants *F. graminearum* are wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), oats (*Avena sativa* L.), triticale (x Triticosecale Wittm.), rye (*Secale cereale* L.), maize (*Zea mays* L.). There are several reports stating that this fungus causes symptoms on some non-graminaceous crops. *Fusarium* spp. also is associated with seed-borne diseases. The infected seed inoculum is a source of Fusarium foot rot (Ali *et al.*, 2005; Leslie & Summerell, 2006; Broders *et al.*, 2007; Burlakoti *et al.*, 2008; Xu *et al.*, 2008; Cram & Fraedrich, 2010; Yang *et al.*, 2013; Mourelos *et al.*, 2014; Purahong *et al.*, 2014). Seed-borne pathogens can be present on seeds without evident signs. Many factors are related to seed-borne diseases (Cram & Fraedrich, 2010). The detection of seed-borne diseases is an important aspect of its management. Therefore, it is important to use healthy seed material to have optimal and good quality yield. The increased spread of *F. graminearum* was recently noticed in Lithuanian cereals (Suproniene *et al.*, 2015). That could lead to an increased inoculum of *F. graminearum* in the primary source of infection – host plant residues remaining in the soil. Therefore, it is important to know the possible influence of this fungus on the other crop rotation plants, which could be susceptible to the pathogen. The aim of this study was to assess the impact of *Fusarium graminearum* infection on different plant seed germination and seed infestation.

Materials and Methods

The research was carried out at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, in Central Lithuania (54º53'32.4 N 23º50'16.2 E) in 2017. The infection of *Fusarium graminearum* was assessed on different legume seeds:

bean (*Vicia faba* L.), pea (*Pisum sativum* L.), lupine (*Lupinus angustifolius* L.), soybean (*Glycine max.* (L.) Merr.), lucerne (*Medicago sativa* L.), white (*Trifolium repens* L.) and red (*Trifolium pratense* L.) clover.

F. graminearum 4vkv4 was isolated from spring wheat (FHB-infected head) in 2016. The isolate was identified morphologically as *F. graminearum* and verified by species-specific PCR, using the protocol and primer pairs suggested by Demeke *et al.* (2005). Before the experiments, *F. graminearum* isolates were cultured on Potato dextrose agar (PDA) at 25 °C for 7 days in the dark. Then isolate was transferred onto Spezieller Nährstoffarmer Agar (SNA) (Nirenberg, 1976), incubated at 25 ± 2 °C for 14 days, until the formation of a macroconidial mass.

The seed inoculation procedure was made based on a modified Petri-dish test suggested by Purahong *et al.* (2012) with some modifications. Each tested plant seed was inoculated with *F. graminearum* 4vkv4 isolate, in four replicates. One replicate consisted of 20 visually healthy seeds, inoculated with 10 ml of *F. graminearum* suspension. The *F. graminearum* macroconidial concentration was adjusted to 1×10^6 conidia per ml. All tested plant seeds before inoculation were surface sterilised in 2% sodium hypochlorite for 3 min and then rinsed 6 times with sterile distilled water. The control treatment was soaked only in sterile distilled water. Various plant seeds were laid out in 15 cm diameter glass Petri plates with sterile double-layer filter paper. To increase relative humidity, each replicate of Petri was put in a clear polyethylene bag and then incubated at 22 °C in the dark.

The infection of different plant seeds was counted 2 and 6 days after inoculation (DAI), germination energy after 3 DAI and germination index – 6 DAI. Seed germination was evaluated according to Aswathaiah *et al.* (1993). Germination reduction rate was calculated after 3 DAI according to Purahong *et al.* (2012). Germination index was calculated after 6 DAI according to Opio & Photchanachai (2016). Germination index was calculated after the seeds with normal germination were classified and recorded. The seeds were considered germinated when they had root and shoot. *F. graminearum* infected plant seeds were identified visually and/or by mycelium completely covering the seeds (Figure 1). The experiment was repeated. After the experiment, re-isolation of mycelium was done and morphologically identified.

Data analysis was performed using ANOVA, from the package SELEKCIJA. Duncan's multiple range test was used to determine the significant difference between different treatments ($P<0.05$) (Raudonius, 2017).

Results and Discussion

This study was carried out to evaluate the impact of *F. graminearum* on various plant seed germinations and infestations. The data was obtained by *in vitro* tests. In general, *F. graminearum* is an important pathogen causing Fusarium head blight (FHB) of small grain cereals. However, it causes diseases on other plants (Purahong *et al.*, 2012; Barros *et al.*, 2014; Suproniene *et al.*, 2016). Broders *et al.* (2007)

Bean Pea White clover

Figure 1. Symptoms on seeds inoculated with *F. graminearum* (4vkv4) compared with control (water) at 4 DAI. Figure shows inoculated bean, pea and white clover seeds. Each seed is presented on four (upper) inoculated and two (bottom) control plates.

Table 1

The germination of various plant seeds inoculated with *F. graminearum* 4vkv4

Seeds	Energy, %		Reduction, %	Index
	3 DAI		3 DAI	6 DAI
Bean	36	b	39	0.2
Lupine	8	ab	77	0.0
Soybean	85	c	78	1.3
Pea	3	a	78	0.0
Lucerne	94	cde	76	5.5
White clover	96	e	79	2.8
Red clover	90	cde	79	5.5

Note. Values with different letters in the columns are significantly different according to Duncan's test at P<0.05.

found out that *F. graminearum* is an important seed and seedling pathogen of maize and soybean.

The seeds were inoculated with a macroconidial suspension of *F. graminearum* to test for the probability to cause infection. The symptoms of *F. graminearum* infection showed up after 2 DAI and the germination index of bean, pea, lupine, soybean, lucerne, white and red clover seeds scored after 6 DAI (Figure 1). In an examination of inoculated seeds with *F. graminearum,* red-purple mycelium growth was detected on the seeds (infection from 21.25 up to 100%). The results show that the percent of seed infection and seedling germination depends on the plant species (Figure 2). Broders *et al.* (2007) evaluated 105 isolates of *F. graminearum* and found out the moderate and high level of pathogenicity on both maize and soybean.

The seed germination energy, after inoculation of different plant seeds with *F. graminearum,* showed variation between plant species (Table 1). The germination energy in inoculated seeds varied from 2.56% in pea to 96.25% in white clover. The results showed that germination energy decreased on pea (2.56%) and lupine (7.79%) seeds. Very low germination energy shows high susceptibility to infection. Our results of nearly all tested seeds showed that germination reduction was observed by almost 80%. This study showed that germination index varied within different plant seeds. Purahong *et al.* (2012) found out that germinated wheat seeds after 2 DAI and healthy seedling inoculated with *F. graminearum* decreased compared with control. Farrag & Moharam (2012) reported that *F. oxysporum* and *F. solani* inoculated cucumber (*Cucumis sativus* L.) seed germination decreased compared with control.

The results of *F. graminearum* contamination on various plant seeds are provided in Figure 2. The symptoms of *F. graminearum* infection on seeds were observed on 2 and 6 DAI. The inoculated seeds with *F. graminearum* were accompanied by characteristic red–purple mycelium completely covering different plant seeds (Figure 1). The highest infection of *F.*

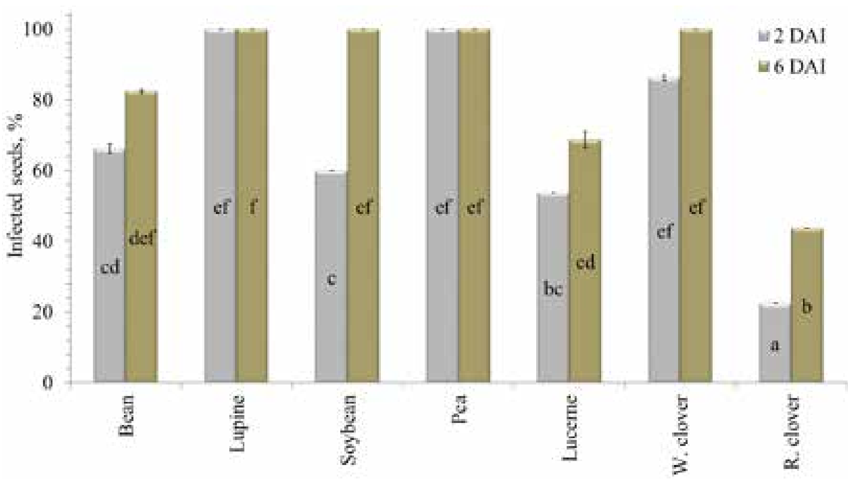

Figure 2. The infection of various seeds inoculated with *F. graminearum* isolate 4vkv4. Values with different letters in the columns are significantly different according to Duncan's test at P<0.05.

graminearum was obtained on pea and lupine seeds (100%). It was established that white clover (86%) seeds after 2 DAI were infected similarly as lupine and pea. Moderate infection after 2 DAI was on lucerne (53%), soybean (60%) and bean (66%). The lowest infection was seen on red clover at 2 DAI – 22%, as well as 6 DAI – 43%. This study shows that after 6 DAI a similar tendency of infection was observed as after 2 DAI. The results show that the highest susceptibility to *F. graminearum* had pea and lupine seeds and the least red clover. Purahong *et al.* (2012) found out different aggressiveness of *F. graminearum* isolates on wheat seeds. Farrag & Moharam (2012) reported dominance of *Fusarium* spp. (32%) pathogens followed by *Rhizoctonia* spp. (12%). This study demonstrated the *F. graminearum* capability to infect various plant seeds. Our data indicate that red clover is more suitable for cereal crop rotation, because of less susceptibility to *F. graminearum.* On the other hand, peas and lupines grown in cereal crop rotation could serve as an alternative host and source of inoculum, though leaving *F. graminearum* infected residues in the field. Therefore, further studies and monitoring are still required.

Conclusions

In our case, bean, pea, lupine, soybean, lucerne, white and red clover seeds showed susceptibility to *F. graminearum*. The results obtained in this study suggest that various seeds differently react to *F. graminearum* infection. As present research shows, red clover is less susceptible to *F. graminearum.* It was found that the highest susceptibility to *F. graminearum* had pea and lupine seeds. However, further studies and monitoring is still required.

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