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# *Microdochium nivale* and *M. majus* as causative agents of seedling blight in spring cereals

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## Abstract

Seedling blight, mostly caused by *Fusarium* spp. and *Bipolaris sorokiniana,* is a common disease in spring cereals. This research confirmed the presence of *Microdochium* fungi in a complex of seedling blight causative agents. Pathogens *Microdochium nivale* and *M. majus* in the seed and in stem base of seedlings of spring barley, spring wheat, spring triticale and spring oats were identified and quantified using a quantitative real-time polymerase chain reaction (qPCR) in 2013–2015. Both species were detected in the seed of all spring cereals tested. The amount of *M. nivale* and *M. majus* DNA was the highest in seeds of barley. Both *Microdochium* pathogens were present in the stem base of seedlings of all spring cereals tested; however, a high variation between cereal species and years was established. In most cases, the quantity of *M. nivale* DNA was the lowest in the seedlings of oats, while that of *M. majus* – in the seedlings of barley compared to the other cereal species tested. Higher contents of *M. majus* and *M. nivale* DNA were identified in the stem base of the seedlings emerged from untreated seeds compared with the seedlings emerged from the fludioxonil-treated seeds. However, the effect of tebuconazole on the reduction of *M. nivale* and *M. majus* DNA was inconsistent. Our findings suggest that *M. nivale* and *M. majus* occur in the seed of spring cereals and cause seedling blight, therefore research on these pathogens needs to be extended.

Key words: fludioxonil, *Microdochium majus*, *M. nivale*, seed infection, seedling blight, tebuconazole.

## Introduction

Formerly *Microdochium* (syn. *Lanosa nivalis* (Fr.), *Fusarium nivale* (Fr.)) were initially identified as *Fusarium* species on the morphological basis (Wollenweber, Reinking, 1935), but later studies differentiated the genus *Microdochium* from *Fusarium* (Samuels, Hallett, 1983). Glynn et al. (2005) described *M. nivale* and *M. majus* as separate species: *Microdochium nivale* (Fr.) Samuels & I.C. Hallett and *Microdochium majus* (Wollenw.) Glynn & S.G. Edwards. Both pathogens are important nontoxigenic fungal pathogens of many cereal crops causing seedling blight, foot rot and also belong to the Fusarium head blight fungal complex (Amein et al., 2007; Walker et al., 2009; Nielsen et al., 2011; Jørgensen et al., 2012). *Microdochium* fungi are associated with retardation of seed germination (Hudec, Muchova, 2010) and significant yield losses (Humphreys et al., 1995; Amein et al., 2007).

*M. nivale* causes seedling blight and brown foot rot and tend to occur under cooler conditions and have trivial meaning in warmer localities (Doohan et al., 2003; Roháčik, Hudec, 2005). Brown foot rot on winter wheat stem base is present in significant amounts with *M. nivale* and *M. majus* (Bateman et al., 2000). *M. nivale* was more common on winter barley than on winter wheat (Dawson, Bateman, 2001). In another study winter wheat was more susceptible to *Fusarium* species and *M. nivale* than winter barley (Hudec, 2007). Usually *Microdochium* species is present as one or both (Nicholson et al., 2002). The Ren et al. (2015) study established that *M. nivale* was a more aggressive Fusarium seedling blight pathogen causing 30% higher disease severity on wheats than *M. majus*. According to Matusinsky et al. (2008), *M. nivale* was found on the stem base most frequently on winter wheat and the statistical association was confirmed between *M. nivale* and *M. majus*. Out of these, *M. nivale* tended to decrease in the summer season.

*M. nivale* (*Fusarium nivale*) was identified on winter wheat and rye stem base and *M. majus* was found on rye in 1970 in Lithuania (Špokauskienė, 1991), but there is no information about *Microdochium* spp. causal agent on stem base of spring cereals. Cockerell et al. (2009), based on the limited data sets in Scotland, suggested that spring wheat and oats are at risk from high levels of *Microdochium* infection, and spring barley is also at risk but at levels exceeding 30% seed infection. It is known that *Microdochium* affects seedlings at temperatures as low as 3°C (Haigh et al., 2009). In an inoculation experiment at 10°C, Simpson et al. (2000) found that both *Microdochium* species were pathogenic to wheat and rye but only *M. nivale* caused significant disease in oats. These studies showed that *Microdochium* species can be important pathogens in spring cereals also. *M. nivale* and *M. majus* as seedling blight pathogens in spring cereals in Lithuania have not been described before. The present study was undertaken to determine whether *M. nivale* and *M. majus* affect seed and stem

base of spring cereals and estimate the susceptibility of different spring cereal species to both pathogens.

## Materials and methods

*Field experiment.* Field experiments involving spring barley cv. 'NFC Tipple', spring wheat cv. 'Tybalt', spring triticale cv. 'Nilex' and spring oats cv. 'Vendela' were carried out at Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry during 2013–2015. Experiments were set up in three treatments using untreated seeds, fludioxonil (25 g $l^{-1}$, commercial product Maxim 025 FS (Syngenta))-treated seeds at a dose of 2.0 l $t^{-1}$ and tebuconazole (60 g $l^{-1}$, commercial product Chambel 6 FS (Makhteshim Chemical Works Ltd.)) at a dose of 0.5 l $t^{-1}$. Seeds were treated using a liquid seed dresser Hege 11 (Wintersteiger, Austria). The experiments were laid out in a randomized complete block design in four replications with 15 $m^2$ plot size. In all experimental years, cereals were pre-crops.

*Sampling and DNA extraction.* Before sowing, a seed sample of 10 g per species was taken from naturally infected seeds and homogenized with a mixer mill MM400 (Retsch, Germany). Thirty plants (BBCH 13) with disease symptoms were collected from each treatment from three replications and 1 cm long stem base segments were powdered in liquid nitrogen. A total of 100 mg per each seed and plant sample were taken for the DNA extraction with a NucleoSpin Plant II (Machery-Nagel, Germany) according to the manufacturer's instructions.

*Quantitative real-time polymerase chain reaction (qPCR) analysis.* qPCR was done according to the protocol of Nielsen et al. (2013) with the following modifications: total volume of 15 µl consisting of 7.5 µl Power SYBR Green PCR Master Mix (Applied Biosystems, USA), 300 nM of each primer, 0.5 µg $µl^{-1}$ BSA (Thermo Fisher Scientific, Lithuania) and 2.5 µl template DNA. Genomic DNA extracted from seeds and plant stem bases was diluted 1:20 and PCR reactions were performed in duplicate on all samples. PCR was run in a 7900HT Fast Real-time PCR System (Applied Biosystems, USA) using Nielsen et al. (2013) cycling protocol. A list of specific primers used is provided in Table 1.

***Table 1.*** A list of sequences and species specific primers used for analysis

| Species detected | Primer | Sequence (5′-3′) | Source |
|---|---|---|---|
| *Microdochium nivale* | Mniv1f | TTGGCTTGCACAAACAATACTTTTT | Nielsen et al., 2013 |
| | Mniv1r | AGCACAACAGGCGTGGATAAG | |
| *Microdochium majus* | Mmajus1f | AACCCCTCCCGGGTCAG | Nielsen et al., 2013 |
| | Mmajus1r | GGATAAACGACACTTGAAGACAGAAAA | |
| Plant EF1α | Hor1f | TCTCTGGGTTTGAGGGTGAC | Nicolaisen et al., 2009 |
| | Hor2r | GGCCCTTGTACCAGTCAAGGT | |

*M. nivale* and *M. majus* pure culture originated from Leibniz-Institut DSMZ, Germany. DNA concentrations and quality of fungi (obtained from pure culture) for standard curves were measured by a biophotometer (Eppendorf, Germany). Six-fold dilution sets starting from 1:10 DNA were used (Suproniene et al., 2010; Nielsen et al., 2013).

*Meteorological conditions.* Cool weather is favourable for *Microdochium* fungi (Doohan et al., 2003). It has been noted that sowing seeds in cold soil increases seed germination time and this can lead to infection of seedlings with soil pathogens (Hwang et al., 2000). During the spring cereal growing seasons, the amount of rainfall was the highest (52 mm) in 2015 and the lowest (30–32 mm) in 2013 and 2014 (Table 2). Average air temperature and the average soil surface temperature during spring cereal growing season was lowest in 2014 and 2015 while highest in 2013.

***Table 2.*** Meteorological observations

| Time | Average air temperature °C | | | Amount of rainfall mm | | | Average soil surface temperature °C | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 | 2013 | 2014 | 2015 |
| Week before sowing | 8.5 | 11.9 | 6.1 | 2.9 | 0.7 | 5.7 | 4.5 | 3.1 | 1.4 |
| Sowing time | 10.8 | 14.7 | 9.3 | – | – | – | 0.3 | 5.8 | 2.7 |
| Week after sowing | 12.4 | 12.3 | 11.5 | 0.0 | 0.0 | 18.8 | 4.7 | 2.8 | 5.5 |
| Week after plant emergence | 16.1 | 6.6 | 10.9 | 8.0 | 3.9 | 13.5 | 10.4 | 2.0 | 6.3 |
| Plant emergence-sampling time | 24.3 | 11.8 | 15.1 | 19.4 | 27.2 | 13.5 | 16.1 | 5.2 | 8.3 |

*Statistical analysis.* Relationships between amounts of pathogen DNA in seeds and stem bases or seedling blight severity were determined by the correlation analyses ($P \leq 0.05$) using *SAS Enterprise Guide 7.1* (SAS Institute Inc.). Standard deviation of the set of data values was calculated. Real-time PCR values are presented as the amount of fungal DNA per amount of plant DNA.

## Results and discussion

Naturally infected seeds were analyzed for *Microdochium nivale* and *M. majus* presence by qPCR. The results showed that *M. nivale* and *M. majus* were present in all seed samples of spring barley, spring wheat, spring triticale and spring oats (Table 3). Nielsen et al. (2011) also detected *M. nivale* and M. *majus* at significant amounts in almost all winter wheat, spring barley, oats, rye and triticale grain samples. Both fungal species were detected in all Danish cereal species, but *M. majus* prevailed against *M. nivale* in most years in all cereal species except rye, in which *M. nivale* represented a larger proportion of the biomass and was more prevalent than *M. majus* (Nielsen et al., 2013). In our experiments, *M. nivale* and *M. majus* in seeds determined equally while biomass of *M. nivale* in some cases was higher.

***Table 3.*** Quantity of *Microdochium nivale* and *M. majus* (fungal DNA pg per plant DNA ng) on the seed of spring cereals, expressed as mean values and standard deviation

| Crop | 2013 | 2014 | 2015 |
|---|---|---|---|
| | *M. nivale* | | |
| Spring barley | 5.38 ± 0.43 | 1.86 ± 0.32 | 4.49 ± 0.56 |
| Spring wheat | 0.31 ± 0.02 | 0.21 ± 0.02 | 0.26 ± 0.00 |
| Spring triticale | 2.29 ± 0.79 | 0.07 ± 0.00 | 0.26 ± 0.24 |
| Spring oats | 1.74 ± 0.37 | 0.43 ± 0.01 | 0.94 ± 0.39 |
| | *M. majus* | | |
| Spring barley | 2.13 ± 0.27 | 0.46 ± 0.13 | 2.65 ± 0.54 |
| Spring wheat | 0.76 ± 0.07 | 0.02 ± 0.01 | 0.29 ± 0.01 |
| Spring triticale | 2.35 ± 0.02 | 0.01 ± 0.00 | 0.08 ± 0.01 |
| Spring oats | 1.35 ± 0.57 | 0.07 ± 0.03 | 0.61 ± 0.06 |

*M. nivale* was observed in different amounts in all spring cereal seeds and investigation years. A smaller quantity of *M. nivale* DNA was identified in spring wheat seeds compared to other cereal species. According to DNA quantities in seeds, spring barley was the most susceptible to *M. nivale*. Host plant response to pathogen infection was shown in all experimental years. Our results agree with those of Nielsen et al. (2011) who found that the amounts of *M. nivale* and *M. majus* in winter wheat were generally lower than in other cereal grains.

*M. majus* also prevailed in spring barley seeds and higher DNA quantities were found in 2013 and 2015. The year 2014 was characterised by small quantities of both *Microdochium* species in the seed of spring cereals and in 2013 by large quantities. Significant interactions between cereal genotypes and fungal species have been established suggesting that resistance/tolerance mechanisms and genes may influence the disease caused by individual *Microdochium* species (Ren et al., 2015). Our study suggests that barley might be most susceptible to both *Microdochium* species compared to the other spring crops, while wheat might be most resistant; however, investigations of different genotypes are essential to get more valid results.

In the field trials, visual seedling blight incidence and severity index varied between crops and investigation years, but symptoms of the disease were observed in all investigated crops (data not shown). In all experimental years, spring oats were the least damaged by seedling blight of all the species tested. Haigh and Hare (2012) have reported that disease symptoms did not occur on seedlings grown from the non-infected seed lot *in vitro* and the presence of *Microdochium* spp. was confirmed on seedlings from the infected seed lot. In our study, all seed lots were infected with both *Microdochium* species and therefore we expected to detect them in disease-affected stem bases.

Analyses of qPCR for identification of causal pathogens in stem base of the seedlings of different spring cereals confirmed the presence of both pathogens *M. nivale* and *M. majus* (Table 4). The amount of their DNA varied between host plants and years. The quantity of *M. nivale* DNA in most cases was the lowest in the seedlings of oats, while *M. majus* – in barley seedlings compared to the other tested species McNeil et al. (2012) found a clear difference in host (spring barley and spring oats) preference of *Microdochium* species. The current study suggests the advantage of *M. majus* over *M. nivale* on spring wheat, spring triticale and spring oats, whereas spring barley was more sensitive to *M. nivale*. Simpson et al. (2000) in the mixed inoculation trial found that *M. nivale* var. *majus* showed a selective advantage on winter wheat and winter oats seedlings and *M. nivale* var. *nivale* showed a strong selective advantage on winter rye seedlings.

***Table 4.*** The presence of *Microdochium nivale* and *M. majus* (fungal DNA pg per plant DNA μg) in naturally infected spring cereal seedling stem bases at BBCH 13 in 2013–2015

| Crop | *M. nivale* | | | | *M. majus* | | | |
|---|---|---|---|---|---|---|---|---|
| | min | max | average | SD | min | max | average | SD |
| Spring barley | 3.47 | 92.97 | 34.3 | 50.8 | 0 | 14.36 | 6.3 | 7.4 |
| Spring wheat | 0.71 | 89.27 | 30.6 | 50.8 | 9.36 | 370.20 | 132.0 | 206.3 |
| Spring triticale | 0.6 | 22.70 | 10.3 | 11.3 | 4.78 | 88.28 | 40.9 | 42.9 |
| Spring oats | 0 | 1.06 | 0.4 | 0.6 | 0.34 | 30.48 | 11.0 | 16.9 |

SD – standard deviation

Stem base of seedlings from untreated seeds contained higher amount of *M. majus* and *M. nivale* DNA than seedlings from fludioxonil-treated seeds (Table 5). Glynn et al. (2008) found that *M. nivale* and *M. majus* were highly sensitive to fludioxonil. There was a significant interaction between species and fungicide, with *M. majus* being proportionally more sensitive to fludioxonil than *M. nivale*. Walker et al. (2009) also reported that fludioxonil used for seed treatments was effective against *Microdochium* spp. Simpson et al. (2001) reported that tebuconazole showed little control of *M. nivale*. Other studies showed that tebuconazole has intermediate values for *Microdochium* fungi and no differences were found between the *Microdochium* species sensitivity (Walker et al., 2009). In the present study, the seed treatment fungicide fludioxonil tended to decrease the DNA amounts of both *Microdochium* in all crops and tested years. The other seed treatment fungicide tebuconazole showed variable results of biomass reduction of *M. nivale* and *M. majus*.

***Table 5.*** Amounts of *Microdochium nivale* and *M. majus* (fungal DNA pg per plant DNA μg) in the stem bases of spring cereals at BBCH 13, expressed as mean values and standard deviation

| Treatment | 2013 | | 2014 | | 2015 | |
|---|---|---|---|---|---|---|
| | *M. nivale* | *M. majus* | *M. nivale* | *M. majus* | *M. nivale* | *M. majus* |
| Spring barley | | | | | | |
| Untreated | 3.47 ± 0.44 | 4.41 ± 0.12 | 6.45 ± 0.45 | 0 | 92.97 ± 7.56 | 14.36 ± 0.36 |
| Fludioxonil | 0.18 ± 0.02 | 0 | 0 | 0 | 1.20 ± 0.20 | 0 |
| Tebuconazole | 1.46 ± 0.21 | 4.03 ± 0.87 | 1.75 ± 0.18 | 0 | 86.02 ± 11.81 | 2.20 ± 0.20 |
| Spring wheat | | | | | | |
| Untreated | 1.90 ± 0.61 | 9.36 ± 0.25 | 2.50 ± 0.44 | 16.45 ± 0.93 | 89.27 ± 10.73 | 370.20 ± 77.08 |
| Fludioxonil | 1.07 ± 0.11 | 0.72 ± 0.12 | 0.43 ± 0.33 | 0 | 0 | 0.71 ± 0.01 |
| Tebuconazole | 1.21 ± 0.06 | 9.50 ± 0.97 | 0.71 ± 0.12 | 0.15 ± 0.00 | 1.29 ± 0.29 | 106.69 ± 7.96 |
| Spring triticale | | | | | | |
| Untreated | 22.70 ± 5.65 | 88.28 ± 5.18 | 0.60 ± 0.10 | 29.75 ± 0.97 | 7.55 ± 0.59 | 4.78 ± 0.55 |
| Fludioxonil | 7.47 ± 1.04 | 37.35 ± 3.08 | 0 | 0 | 0 | 0.12 ± 0.02 |
| Tebuconazole | 14.90 ± 3.39 | 76.71 ± 5.57 | 0 | 0 | 0.42 ± 0.02 | 0.59 ± 0.09 |
| Spring oats | | | | | | |
| Untreated | 1.06 ± 0.06 | 30.48 ± 0.48 | 0 | 0.34 ± 0.04 | 0 | 2.22 ± 0.22 |
| Fludioxonil | 0.32 ± 0.02 | 0 | 0 | 0 | 0 | 0.51 ± 0.01 |
| Tebuconazole | 1.46 ± 0.46 | 0 | 0 | 0.10 ± 0.00 | 0 | 0.59 ± 0.09 |

In our experiments, some correlation between quantity of *M. nivale* and *M. majus* DNA in seed and in stem base was found. Strong and significant correlation with both fungi species was determined in spring triticale and spring oats (Table 6). In spring wheat and spring triticale, seedling blight severity index strongly correlated with the DNA quantity of both pathogens in stem base. The relationship between all parameters tested in spring barley was insignificant and correlation varied from medium to low. According to Turner et al. (2001), visual and PCR analyses on stems varied in relation to disease incidence or severity of symptoms and to the amount of pathogen DNA. Ramanauskienė et al. (2014) have previously reported that stem base and foot rot disease

***Table 6.*** Correlation between quantity of *Microdochium nivale* and *M. majus* DNA in seeds and in stem bases and seedling blight severity index in different spring cereals in 2013–2015

| | Quantity of *M. nivale* in stem bases | Quantity of *M. majus* in stem bases |
|---|---|---|
| Spring barley | | |
| Quantity of *M. nivale* in seeds | 0.685 | |
| Quantity of *M. majus* in seeds | | 0.246 |
| Seedling blight severity index | 0.285 | 0.520 |
| Spring wheat | | |
| Quantity of *M. nivale* in seeds | −0.214 | |
| Quantity of *M. majus* in seeds | | −0.261 |
| Seedling blight severity index | 0.743* | 0.760* |
| Spring triticale | | |
| Quantity of *M. nivale* in seeds | 0.968** | |
| Quantity of *M. majus* in seeds | | 0.947** |
| Seedling blight severity index | 0.877** | 0.751* |
| Spring oats | | |
| Quantity of *M. nivale* in seeds | 0.844* | |
| Quantity of *M. majus* in seeds | | 0.857* |
| Seedling blight severity index | −0.605 | −0.176 |

* – significant at the 0.05 level (two-tailed), ** – significant at the 0.01 level (two-tailed)

incidence did not correlate with fungal DNA amounts from diseased stems. Other authors indicate significant relationship between visual infection symptoms and the incidence of *M. nivale* and *M. majus* on winter wheat (Matusinsky et al., 2008).

Results of our research showed that *M. nivale* and *M. majus* occur in spring cereals (wheat, barley, triticale and oats) and cause seedling blight. Control of seedling blight should be focused on complex pathogens and both *Microdochium* species as well.

# Conclusions

1. Pathogens *Microdochium nivale* and *M. majus* were identified and quantified in the seed of spring barley, spring wheat, spring triticale and spring oats using a quantitative real-time polymerase chain reaction. The amount of *M. nivale* and *M. majus* DNA was the highest in the seeds of barley.

2. Real-time PCR confirmed the presence of both pathogens *M. nivale* and *M. majus* in the stem bases of seedlings of all cereal species tested; however,

high variation between cereal species and years was established. In most cases, the quantity of *M. nivale* DNA was the lowest in the seedlings of oats, while that of *M. majus* – in barley seedlings compared to the other tested cereal species.

3. Stem base of the seedlings from the untreated seeds contained significantly higher amount of *M. majus* and *M. nivale* DNA compared to those of the seedlings from the fludioxonil-treated seeds, while tebuconazole showed inconsistent influence on the reduction of DNA amount of *M. nivale* and *M. majus*.

4. Positive correlation between quantity of *Microdochium nivale* and *M. majus* DNA in seed and in stem base of spring triticale and spring oats was determined. Seedling blight severity index significantly correlated with the quantity of DNA of both pathogens in stem base of spring wheat and spring triticale.

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# *Microdochium nivale* ir *M. majus* – vasarinių javų daigų puvinių sukėlėjai

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## Santrauka

Vasarinių javų daigų puvinius dažniausiai sukelia *Fusarium* ssp. ir *Bipolaris sorokiniana*. Šis tyrimas patvirtino, kad ir *Microdochium* grybai, sudarę kompleksą su kitais patogenais, sukelia daigų puvinius. *Microdochium nivale* ir *M. majus* patogenai vasarinių miežių, vasarinių kviečių, vasarinių kvietrugių bei avižų sėklose ir daigų apatinės stiebo dalies pažaidose 2013–2015 m. buvo nustatyti taikant kiekybinę ir kokybinę realaus laiko polimerazės grandininę reakciją (*qPGR*). Abi rūšys buvo aptiktos visų vasarinių augalų sėklose. Didžiausias kiekis *M. nivale* ir *M. majus* DNR buvo vasarinių miežių sėklose. Abu *Microdochium* patogenai nustatyti ant visų tirtų vasarinių javų daigų stiebo apatinės dalies, tačiau rasti kiekiai varijavo tarp augalų ir tyrimo metų. *M. nivale* DNR kiekis daugeliu atvejų buvo mažiausias avižų daiguose, o *M. majus* – miežių daiguose, lyginant su kitų tirtų rūšių augalais. Neapdorotų sėklų daigų stiebo apatinėje dalyje buvo nustatyti didesni kiekiai *M. majus* ir *M. nivale* DNR nei beicuotų fludioksonilu, o tebukonazolo įtaka mažinant *M. nivale* ir *M. majus* DNR kiekį buvo nenuosekli. Tyrimo duomenys parodė, kad *M. nivale* ir *M. majus* pažeidžia vasarinių javų sėklas ir sukelia daigų puvinius, todėl šių patogenų tyrimus būtina plėsti.

Reikšminiai žodžiai: daigų puviniai, fludijoksonilas, *Microdochium majus*, *M. nivale*, sėklų infekcija, tebukonazolas.

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