Global Research Journal of Agricultural and Biological Sciences Vol.3(4) pp.351 –359 October 2012.
Available online http://www.globalresearchjournals.org/?a=journal&id=grjabs

Full Length Research.

The effect of seed-borne *Microdochium majus* and *M. nivale* infection on early winter wheat seedling growth.

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Accepted 20th March 2012.

***Microdochium majus* and *M. nivale* can cause seedling blight of cereals in maritime climates. Experiments were conducted to test whether seed-borne *M. majus* and *M. nivale* infection affects early winter wheat seedling growth. For 7 winter wheat seed lots, seed-borne *Microdochium* spp. did not consistently affect rate of imbibition or germination at 15 °C and 5 °C under water potentials of 0 MPa and -1 MPa, significant effects were observed at 5 °C. Seed-borne *Microdochium* spp. infection had no significant effect on rate of seedling emergence from soil at 10 °C but allowing seedlings to develop to GS 12 demonstrated that when present, more severely diseased seedlings emerged slower than slightly diseased or healthy seedlings. Increased disease severity significantly reduced the first leaf lengths for 4 of the 7 seed lots but healthy seedlings did not have significantly longer first leaves than slightly diseased seedlings. Although performed on a limited number of seed lots, this investigation suggests that seed-borne *M. majus* and *M. nivale* can affect all aspects of early seedling growth, particular effects are probably due to the location of inoculum within individual seeds.**

Key words: seedling blight, *Microdochium*, temperature, water potential, winter wheat.

Introduction

Microdochium majus (Wollenw.) Glynn and S.G. Edwards and *Microdochium nivale* (Fr.) Samuels and Hallett can cause Fusarium seedling blight of winter wheat in the United Kingdom (U.K.). Previously these pathogens were known as *Microdochium nivale* var. *majus* and *Microdochium nivale* var. *nivale* but were reclassified as separate species by Glynn *et al.* (2005). References to work on *M. nivale* published before Glynn *et al.,* (2005) and cited in this paper refer to both var. *majus* and var. *nivale* or *M. majus* and *M. nivale* respectively, unless stated otherwise. Seedling blight can be caused by seed or soil-borne *Microdochium* spp., although seed-borne inoculum is believed to be the most important source of infection in the U.K. (Parry *et al.*, 1993). Fusarium seedling blight symptoms are typically pre- and post-emergent seedling death causing reduced plant populations. Disease symptoms can vary in severity from localised lesions on the coleoptile and roots to extensive coleoptile necrosis, abnormal radicle and/or plumule development (Parry *et al.*, 1993).

The most severe seedling blight caused by *Microdochium* spp. occurs when seedling emergence is slow. Most severe disease in glasshouse experiments occurred at low temperatures and dry soils between 6.1 and 16.4 °C at 8.8 to 24.5 % w/w soil moisture for winter wheat in which the seed had been surface-inoculated with 5000 *M. nivale* spores seed^{-1} (Millar and Colhoun, 1969). Lowest final emergence in pot trials using a winter wheat seed lot with 72 % *M. nivale* infection occurred in cold dry soil between 6 and 12 °C and water potentials of -5 to -10.8 KPa (Hare *et al.*, 1995).

However, the effects of *Microdochium* spp. on early seedling growth are not well documented. Rennie *et al.* (1990) reported a significant negative relationship between the incidence of seed-borne *M. nivale* infection and percentage germination of 40 winter wheat seed lots in 1987, but not for 50 seed lots in 1988. For 8 wheat seed lots infected with *M. nivale*, more seeds germinated under a 12 h:25 °C; 12 h:15 °C cycle than a 6 h:15 °C; 18 h:10 °C cycle (Cristani, 1992). Seed infection by *Microdochium* spp. can adversely affect early cereal seedling growth. Hare *et al.* (1995) demonstrated a significant relationship between rate of seedling emergence and final emergence for a winter wheat seed lot with 72 % *M. nivale* infection grown under conditions of 6 and 12 °C and water potentials of -5 to -10.8 KPa. The reason for increased seedling blight caused by *Microdochium* spp. in cold dry soils is not known but increased seedling blight incidence and severity may arise when slower seedling growth increases the opportunity for infection and colonization. In the absence of *Microdochium* spp., cold and/or dry soils delayed wheat germination but final germination was unaffected (Hampson and Simpson, 1990; Kamaha and Maguire, 1992). Similar effects of temperature and water potential on the rate of seedling emergence from non-infected seeds have also been demonstrated by DeJong and Best (1979) and Khah *et al.* (1986).

The aims of this paper were to investigate the effect of (i) seed-borne *Microdochium* spp. infection on the rate of imbibition and germination of winter wheat at 2 temperatures and 2 water potentials, (ii) seed-borne *Microdochium* spp. infection on rate of seedling

emergence and (iii) rate of seedling emergence on seedling blight severity and first leaf lengths in a series of controlled environment studies at 10 °C in soil at 60% maximum water holding capacity which are typical of conditions during winter wheat seedling emergence in the U.K. and conducive to seedling blight disease development.

Materials and Methods

Seven winter wheat seed lots were used in this investigation (Table 1). PCR analysis (Glynn *et al*., 2005) confirmed *M. majus* and *M. nivale* were present in all seed lots. *Microdochium* spp. infestation of seeds was determined by surface-sterilising for 3 min in 10% NaOCl, rinsing 3 times in sterile distilled water, drying in a flow of sterile air and plating onto PDA amended with 130 µg ml^{-1} streptomycin sulfate (Sigma-Aldrich Company Ltd, Dorset, UK) and 25 µg ml^{-1} BAVISTIN DF (50 % w/w carbendazim; BASF, Bury St. Edmunds, UK). Dishes were incubated at 10 °C for 10 days and seeds determined as infected or non-infected by examining the fungal mycelium colonies (Booth, 1971).

Table 1: Extent of seed-borne *Microdochium* spp. infection of 7 winter wheat seed lots

seed lot	cultivar	% infection
1	Cadenza	29
2	Equinox	45
3	Equinox	88
4	Hereward	55
5	Riband	30
6	Riband	73
7	Unknown	36

Microdochium spp. infection was determined by plating surface sterilised seeds onto PDA amended with 130 µg ml^{-1} streptomycin sulfate and 25 µg ml^{-1} BAVISTIN DF. Fungal colonies were examined 10 days after incubation at 10°C.

Experiment 1 Effect of seed-borne *Microdochium* spp. infection on rate of imbibition

Water potentials of polyethylene glycol (PEG) 4000 (BDH Chemicals Ltd, Poole, UK) solutions at 15 °C and 5 °C were determined using a HR-33T Dewpoint Microvoltmeter (Wescor Inc., Utah, USA) following the method of Michel and Kaufmann (1971).

Forty surface-sterilised seeds for each seed lot were placed crease down on 2 sheets of autoclaved Whatman no. 1 filter paper in a 9 cm Petri dish. Five ml of sterile distilled water (0 MPa) or a PEG solution (-1 MPa) were added to each dish and the dish sealed with PARAFILM (Pechiney Plastic Packaging Inc., Neenah, USA). Dishes were incubated in darkness at 15 °C or 5 °C in a randomised design. There were four Petri dishes of seed from each seed lot for each water potential (0 and -1 MPa) at each temperature. Dishes were examined daily and imbibed seeds (radicle visible through the seed coat) removed, surface-sterilised and plated onto amended PDA to determine whether they were infected with *Microdochium* spp. Imbibition was classified as complete when no seeds imbibed within a dish for 5 consecutive days. For each dish, rate of imbibition was calculated for *Microdochium* spp. infected and non-infected seeds (Equation 1).

Equation 1 $$\text{mean seedling imbibition time (day)} = \frac{\Sigma (D * n_i)}{\Sigma n_i}$$

Where n_i is the number of seedlings which imbibed on day D (number of days after experiment start). Mean rate of imbition (seeds day^{-1}) is the reciprocal of the mean seed imbibition time.

Data were analysed separately for each seed lot using factorial ANOVA with seed infection, water potential and temperature as factors.

Experiment 2 Effect of seed-borne *Microdochium* spp. infection on rate of germination

Twenty-five surface-sterilised seeds from each seed lot were placed crease down on 2 sheets of autoclaved Whatman no. 1 filter paper in a 9 cm Petri dish. Five ml of sterile distilled water (0 MPa) or a PEG solution (-1 MPa) were added to each dish and the dish sealed with PARAFILM. Dishes were incubated in darkness at 15 °C or 5 °C in a randomised design. There were four Petri dishes of seed from each seed lot for each water potential (0 and -1 MPa) at each temperature. Dishes were examined daily and germinated seeds (coleoptile $\geq$0.5 cm and 3 roots $\geq$0.5 cm, or the total root length $\geq$3 cm) removed, surface-sterilised and plated onto amended PDA to determine whether they were infected with *Microdochium* spp. Germination experiments were terminated after 20 days at 15 °C and 60 days at 5 °C. For each dish, rate of germination was calculated for *Microdochium* spp. infected and non-infected seeds (Equation 2). Data were analysed separately for each seed lot using factorial ANOVA with seed infection, water potential and temperature as factors.

Equation 2 $$\text{mean seedling germination time (day)} = \frac{\Sigma (D*n_g)}{\Sigma n_g}$$

Where n_g is the number of seedlings which germinated on day D (number of days after experiment start). Mean rate of germination (seeds day^{-1}) is the reciprocal of the mean seed germination time.

Data were analysed separately for each seed lot using factorial ANOVA with seed infection, water potential and temperature as factors.

Experiment 3 Effect of seed-borne *Microdochium* spp. infection on rate of seedling emergence

Fifty surface-sterilised seeds from each seed lot were planted crease down 20 mm deep in sterilised John Innes No. 2 compost into 4 seed trays (21.5 cm x 15.5 cm x 5 cm). Trays were placed in a randomised design in a dark controlled environment cabinet (Controlled Environments Ltd, Manitoba, Canada) at 10 °C. Trays were watered every 2 days using sterile distilled water to keep the soil at 60% maximum water holding capacity. Due to limited incubator space, only seedling emergence from one soil water content could be examined. The temperature and soil water content were chosen to replicate typical conditions during seedling emergence in the U.K. which are conducive to seedling blight development. Trays were examined daily and emerged seedlings (GS 10) removed, seeds from emerged seedlings were surface-sterilised and plated onto amended PDA. Dishes were incubated and seed infection with *Microdochium* spp. determined. For each tray, rate of seedling emergence for *Microdochium* spp. infected and non-infected seeds was calculated (Equation 3).

Equation 3 $$\text{mean seedling emergence time (day)} = \frac{\Sigma (D*n_e)}{\Sigma n_e}$$

Where n_e is the number of seedlings which emerged on day D (number of days after planting). Mean rate of emergence (seedlings day^{-1}) is the reciprocal of the mean seedling emergence time. For each seed lot, rates of seedling emergence from infected and non-infected seeds were analysed by t-test.

Experiment 4 Effect of disease severity from seed-borne *Microdochium* spp. infection on rate of seedling emergence and first leaf length

Fifty surface-sterilised seeds from each seed lot were planted crease down 20 mm deep in sterilised John Innes No. 2 compost into each of 4 seed trays. Trays were placed into a controlled environment cabinet set at 10 °C with 12 h light. Trays were watered every 2 days using sterile distilled water to keep the soil at 60% of its maximum water holding capacity. Due to limited incubator space with lighting available, only seedling emergence from one soil water content could be examined. The temperature and soil water content were chosen to replicate typical conditions during seedling emergence in the U.K. which are conducive to seedling blight development. Trays were examined daily and seedling emergence (GS 10) recorded. After 25 days, seedlings (GS 12) were assessed for seedling blight severity and classed as: healthy (no symptoms), slightly diseased (lesions on the coleoptile) or heavily diseased (total necrosis of the coleoptile and deformed seedling growth). First leaf lengths were measured. For each tray, rate of emergence for healthy, slightly diseased and heavily diseased seedlings was calculated (Equation 3). For each seed lot, rate of seedling emergence and first leaf length were analysed using ANOVA with seedling blight severity (healthy, slightly diseased and heavily diseased) as factors.

Results

Experiment 1 Effect of seed-borne *Microdochium* spp.infection on rate of imbibition

The rate of imbibition was significantly faster at 15 °C than 5 °C and at 0 MPa than -1 MPa for all seed lots (Table 2). Non-infected seeds imbibed significantly faster than infected seeds for seed lots 1, 3 and 7 but not for seed lots 2, 4, 5 and 6. There was a significant 3 way interaction between seed-borne *Microdochium* spp. infection, temperature and water potential for seed lots 4, 5 and 6. Results were inconsistent at 15 °C. At 5 °C, non-infected seeds imbibed faster than infected seeds, with differences being more pronounced at 0 MPa than -1 MPa (Table 3). There were, in addition, significant 2 way interactions between temperature x water potential for seed lots 1, 2, 3, 4 and 6 (Table 4). For all seed lots, rate of imbibition was significantly slower at -1 MPa than 0 MPa at both temperatures with smaller differences at 5 °C. Only for seed lot 4 was there a significant 2 way interaction between seed-borne *Microdochium* spp. infection and temperature; infected seeds (0.219 seeds day^{-1}) imbibed significantly faster than non-infected seeds at 5 °C (0.167 seeds day^{-1}; LSD = 0.0368, SED = 0.0179, DF = 24).

Experiment 2 Effect of seed-borne *Microdochium* spp. infection on rate of germination

There was no significant 3 way interaction between seed-borne disease x temperature x water potential interaction or 2 way interaction between seed-borne disease x water potential for any of the seed lots. Only for seed lot 2 was there a significant 2 way interaction between seed-borne *Microdochium* spp. infection x temperature; the rate of germination of non-infected seeds was significantly faster than infected seeds at 5 °C (Table 5). There was a significant 2 way interaction between temperature and water potential for seed lots 1, 2, 3, 4, 5 and 7 (Table 6). For seed lots 1, 4 and 5, rate of imbibition was significantly slower at -1 MPa than 0 MPa only at 15 °C. The opposite occurred for seed lot 7. For seed lots 2 and 3, changing the water potential from 0 MPa to -1 MPa caused much greater reductions

in rate of germination at 15 °C. Overall rate of germination was significantly faster at 15 °C than 5 °C for all seed lots. The rate of germination was faster for all seed lots at 0 MPa than -1 MPa but this was only significant for seed lots 1, 2, 3 and 5 (Table 7). Seed-borne *Microdochium* spp. infection had an inconsistent effect on rate of germination and only for seed lots 1 and 5 did non-infected seeds germinate significantly faster than infected seeds (Table 7).

Table 2: Effect of temperature, water potential and seed infection on the mean rate of imbibition (seeds day^{-1}) for 7 winter wheat seed lots infected with *Microdochium* spp.

	Seed lot						
	1*	2*	3*	4*	5*	6*	7
Temperature							
15 °C	0.484a (35)	0.552a (35)	0.479a (38)	0.382a (35)	0.405a (33)	0.487a (38)	0.361a (37)
5 °C	0.177b (37)	0.204b (36)	0.185b (38)	0.192b (36)	0.169b (32)	0.184b (38)	0.158b (37)
Water potential							
0 MPa	0.382a (36)	0.448a (36)	0.373a (39)	0.350a (35)	0.353a (31)	0.337a (38)	0.304a (37)
-1 MPa	0.248b (36)	0.276b (36)	0.261b (38)	0.216b (36)	0.203b (33)	0.261b (39)	0.200b (37)
Seed infection							
Infected seed	0.297a (14)	0.353a (20)	0.307a (33)	0.294a (19)	0.279a (10)	0.306a (25)	0.237a (15)
Non-infected seed	0.327b (22)	0.360a (16)	0.322b (6)	0.265a (16)	0.267a (22)	0.290a (14)	0.261b (22)
LSD (P<0.05)	0.0211	0.0216	0.0216	0.0260	0.0168	0.0140	0.0203
SED	0.0102	0.0105	0.0105	0.0126	0.0082	0.0068	0.0098
DF	24	24	24	24	24	24	24
%cv	5.2	5.0	5.3	6.8	4.4	3.5	5.6

Each seed lot was analysed separately.
For each seed lot, means with the same letter are not significantly different (P>0.05).
Values in parenthesis represent the average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.
* Seed lots have significant 2 and 3 way interactions between temperature, water potential and seed infection (Table 3 and Table 4).

Table 3: Effect of seed infection, temperature and water potential on the mean rate of imbibition (seeds day^{-1}) for the 3 winter wheat seed lots where there was a significant 3 way seed infection x temperature x water potential interaction

Seed lot	Temperature (°C)	seed infected	water potential (MPa)		LSD (P<0.05)	SED	DF	%cv
			0	-1				
4	15	yes	0.412ab (18)	0.350b (19)	0.0521	0.0252	24	6.8
	15	no	0.443a (16)	0.330b (17)				
	5	yes	0.209ab (20)	0.112d (19)				
	5	no	0.361c (16)	0.130d (16)				
5	15	yes	0.496a (9)	0.323b (10)	0.0336	0.0163	24	4.4
	15	no	0.482a (22)	0.323b (24)				
	5	yes	0.193b (11)	0.090e (12)				
	5	no	0.291c (20)	0.133d (21)				
6	15	yes	0.493a (25)	0.419bc (25)	0.0281	0.0136	24	3.5
	15	no	0.452b (13)	0.394c (13)				
	5	yes	0.205d (24)	0.136f (25)				
	5	no	0.246d (13)	0.160e (14)				

Each seed lot was analysed separately.
For each seed lot, means with the same letter are not significantly different (P>0.05).
Values in parenthesis represent average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.

Table 4: Effect of temperature and water potential on the mean rate of imbibition (seeds day^{-1}) for the 5 winter wheat seed lots where there was a significant 2 way temperature x water potential interaction

Seed lot	temperature (°C)	water potential (MPa) 0	water potential (MPa) -1	LSD (*P*<0.05)	SED	DF	%cv
1	15	0.632a (36)	0.356b (35)	0.0298	0.0144	24	5.2
	5	0.195c (36)	0.160d (38)				
2	15	0.700a (35)	0.421b (35)	0.0306	0.0148	24	5.0
	5	0.251c (37)	0.162d (36)				
3	15	0.583a (38)	0.385b (39)	0.0305	0.0148	24	5.3
	5	0.209c (39)	0.162d (37)				
4	15	0.427a (34)	0.340b (35)	0.0368	0.0179	24	6.8
	5	0.280c (36)	0.121d (36)				
6	15	0.472a (38)	0.407b (38)	0.0199	0.0096	24	3.5
	5	0.225c (38)	0.148d (39)				

Each seed lot was analysed separately.
For each seed lot, means with the same letter are not significantly different (*P*>0.05).
Values in parenthesis represent average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.

Table 5: Effect of seed infection and temperature on the mean rate of germination (seeds day^{-1}) for the winter wheat seed lot where there was a significant 2 way seed infection x temperature interaction

Seed lot	temperature (°C)	seed-borne disease Yes	seed-borne disease No	LSD (*P*<0.05)	SED	DF	%cv
2	15	0.260a (13)	0.254a (10)	0.0141	0.0068	24	3.5
	5	0.067b (13)	0.075b (10)				

Means with the same letter are not significantly different (*P*>0.05).
Values in parenthesis represent average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.

Table 6: Effect of temperature and water potential on the mean rate of germination (seeds day^{-1}) for the 6 winter wheat seed lots where there was a significant 2 way temperature x water potential interaction.

Seed lot	temperature (°C)	water potential (MPa) 0	water potential (MPa) -1	LSD (*P*<0.05)	SED	DF	%cv
1	15	0.249a (22)	0.180b (21)	0.0265	0.0129	24	7.1
	5	0.071c (22)	0.072c (23)				
2	15	0.298a (23)	0.219b (23)	0.0141	0.0068	24	3.5
	5	0.080c (23)	0.062d (24)				
3	15	0.261a (23)	0.186b (23)	0.0256	0.0124	22	7.0
	5	0.061c (24)	0.046d (24)				
4	15	0.174a (23)	0.144b (23)	0.0249	0.0121	24	8.2
	5	0.034c (24)	0.038c (23)				
5	15	0.172a (22)	0.117b (22)	0.0316	0.0153	24	10.1
	5	0.050c (23)	0.054c (23)				
7	15	0.129a (23)	0.150a (22)	0.0299	0.0145	24	9.7
	5	0.060b (23)	0.041c (23)				

Each seed lot was analysed separately.
For each seed lot, means with the same letter are not significantly different (*P*>0.05).
Values in parenthesis represent average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.

Experiment 3 Effect of seed-borne *Microdochium* spp. infection on rate of seedling emergence

Diseased seedlings were produced from all seed lots. *Microdochium* spp. infection had no consistent effect on rate of seedling emergence (Table 8). Infected seeds in seed lots with significant differences (3 and 6) appeared to emerge quicker than non-infected seeds.

Experiment 4 Effect of seedling blight disease severity from seed-borne *Microdochium* spp. infection on rate of seedling emergence and first leaf lengths

Heavily diseased seedlings did not occur in seed lots 4, 5 and 6 (Table 9). Only for seed lot 6 did healthy seedlings emerge significantly faster than slightly diseased seedlings.Heavily diseased seedlings emerged significantly slower than healthy and slightly diseased seedlings for seed lots 1 and 2 but not for seed

lots 3 and 7. No significant differences were observed between rate of seedling emergence for healthy, slightly diseased and heavily diseased seedlings for seed lots 3 and 7.

There was no significant difference between the first leaf lengths of healthy and slightly diseased seedlings for all 7 seed lots (Table 10). When heavily diseased seedlings did occur (seed lots 1, 2, 3 and 7), they had significantly reduced first leaf lengths compared with both healthy and slightly diseased seedlings; the reduction in length was generally 50% or more (Table 10).

Table 7: Effect of seed infection, temperature and water potential on rate of germination (seeds day^{-1}) for 7 winter wheat seed lots infected with *Microdochium* spp.

	Seed lot						
Temperature	1*	2*	3*	4*	5*	6	7*
15 ^{o}C	0.213a (21)	0.257a (23)	0.222a (23)	0.159a (23)	0.144a (22)	0.181a (23)	0.139a (22)
5 ^{o}C	0.072b (23)	0.071b (23)	0.054b (24)	0.036b (24)	0.052b (23)	0.068b (23)	0.051b (23)
Water potential							
0 MPa	0.147a (22)	0.172a (23)	0.144a (24)	0.090a (23)	0.103a (23)	0.124a (23)	0.092a (23)
-1 MPa	0.120b (22)	0.129b (23)	0.104b (23)	0.083a (23)	0.082b (22)	0.110a (23)	0.087a (22)
Seed infection							
Infected seeds	0.119a (8)	0.148a (13)	0.126a (18)	0.084a (13)	0.082a (7)	0.126a (15)	0.083a (8)
Non-infected seeds	0.147b (14)	0.151a (10)	0.121a (5)	0.089a (10)	0.104b (16)	0.109b (8)	0.096a (14)
LSD (P<0.05)	0.0188	0.0100	0.0181	0.0176	0.0223	0.0223	0.0211
SED	0.0091	0.0048	0.0087	0.0085	0.0108	0.1080	0.0102
DF	24	24	24	24	24	24	24
%cv	7.1	3.5	7.0	8.2	10.1	8.9	9.7

Each seed lot was analysed separately.
For each seed lot, means with the same letter are not significantly different (P>0.05).
Values in parenthesis represent average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.
* Seed lots have significant 2 and 3 way interactions between temperature, water potential and seed infection (Table 5 and Table 6).

Table 8: Effect of seed-borne *Microdochium* spp. infection on rate of seedling emergence (seedlings day^{-1}) for 7 winter wheat seed lots at 10 ^{o}C

Seed lot	infected seeds	non-infected seeds	t-test analysis
1	0.082 (18)	0.086 (30)	0.458
2	0.087 (20)	0.091 (25)	0.155
3	0.074 (38)	0.066 (10)	0.050
4	0.058 (22)	0.062 (25)	0.320
5	0.065 (13)	0.058 (33)	0.327
6	0.079 (36)	0.067 (10)	0.035
7	0.073 (13)	0.069 (36)	0.276

Values in parenthesis represent average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.

Table 9: Effect of disease severity at GS 12 on the mean rate of seedling emergence (seedlings day^{-1}) from 7 winter wheat seed lots naturally infected with *Microdochium* spp. infection at 10 °C.

	Seed lot						
Disease category	1	2	3	4	5	6	7
healthy	0.070a (28)	0.107a (27)	0.071a (13)	0.082a (25)	0.099a (30)	0.089a (12)	0.102a (36)
slightly diseased	0.073a (10)	0.100a (8)	0.074a (17)	0.072a (21)	0.084a (14)	0.065b (32)	0.083a (10)
heavily diseased	0.055b (9)	0.074b (10)	0.058a (17)	*	*	*	0.080a (4)
LSD (*P*<0.05)	0.0281	0.0316	0.0447	0.0558	0.0361	0.0245	0.0391
SED	0.0124	0.0140	0.0197	0.0228	0.0147	0.0100	0.0173
DF	9	9	9	6	6	6	9
%cv	6.8	6.5	10.7	11.6	6.9	5.1	8.2

* no seedlings.

For each seed lot, means with the same letter are not significantly different (*P*>0.05).
Values in parenthesis represent average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.

Table 10: Effect of disease severity at GS 12 from seed-borne *Microdochium* spp. infection on first leaf length (mm) for 7 winter wheat seed lots at 10 °C.

	Seed lot						
Disease category	1	2	3	4	5	6	7
healthy	110a (28)	99a (27)	90a (13)	84a (25)	109a (30)	94a (12)	98a (36)
slightly diseased	96a (10)	87a (8)	82a (17)	53a (21)	88a (14)	80a (32)	81a (10)
heavily diseased	42b (9)	39b (10)	46b (17)	*	*	*	25b (4)
LSD (*P*<0.05)	1.1	1.0	1.4	2.0	1.7	1.5	1.4
SED	0.5	0.4	0.6	0.8	0.7	0.6	0.6
DF	9	9	9	6	6	6	9
%cv	8.0	7.0	10.1	13.8	9.9	9.2	10.9

* no seedlings.

For each seed lot, means with the same letter are not significantly different (*P*>0.05).
Values in parenthesis represent average number of seeds.
Analyses were performed on square root transformed data. Means shown are back-transformed data but statistics refer to the transformed data.

Discussion

Seed-borne *Microdochium* spp. infection did not consistently adversely affect the growth of emerged winter wheat seedlings before GS 12 for the 7 seed lots under the experimental conditions used. Hare (1997) reported good linear relationships between mean disease severity and mean rate of seedling emergence for winter wheat seed lots naturally infected with *M. nivale* in pot trials. Investigations have demonstrated slower seedling growth from non-infected seeds in cold dry soils without reducing final emergence (DeJong and Best, 1979; Khah *et al*., 1986). This implies that soil conditions are probably determining seedling blight severity by influencing seedling growth, rather than increased *Microdochium* spp. pathogenicity at lower temperatures and/or reduced soil water potentials, although Haigh and Hare (2012) demonstrated that *M. majus* and *M. nivale* were able to grow *in vitro* at temperatures where winter wheat growth stops. Therefore, it follows that slower seedling emergence increases the opportunity for the transfer of seed-borne infection to the seedling but seed-borne *Microdochium* spp. infection in this study did not consistently negatively affect seedling growth. The trend in Experiment 4 that more heavily diseased seedlings emerged slowest appears to support this hypothesis. Further work is required at additional temperatures and soil water contents. Indirect evidence also suggests that faster seedling emergence reduces the opportunity for infection. Winter wheat seeds soaked in water for 1 to 12 h before *Fusarium culmorum* inoculation and planting had a faster rate of seedling emergence and lower pre-emergent death and disease severity compared to unsoaked seeds, whilst seedlings from seeds soaked for 3 days had increased disease severity (Malalasekera and Colhoun, 1968). It is probable that soaking seeds for 3 days depressed seedling vigour, increasing the opportunity for infection.

Seed-borne *Microdochium* spp. infection did not consistently significantly delay the rate of imbibition (3 seed lots) and germination (2 seed lots) in this investigation. Culture filtrates of 12 *M. nivale* isolates exhibited phytotoxicity towards wheat seeds and inhibited germination and seedling growth at 20 ^{o}C (Manka and Chelkowski, 1985). It is likely that *Microdochium* spp. as mycelium or culture filtrates have different effects on germinating wheat seeds to infection situated within seeds. Seed-borne *Microdochium* spp. infection did not significantly delay the rate of seedling emergence (GS 10) for any of the seed lots used and for only 3 of the 7 seed lots (seed lots 1, 2 and 6) tested was the severity of disease at GS12 significantly affected by rate of emergence. It is not possible to determine whether disease severity is affected by rate of emergence or the rate of emergence is affected by the severity of seedling infection. In addition, heavily diseased seedlings had significantly reduced first leaf lengths compared with healthy seedlings: potentially reducing the chances of seedling recovery. Plants growing from more severely diseased seedlings have been shown to be less productive (Haigh *et al.*, 2009). This investigation appears to suggest that seed-borne *M. majus* and *M. nivale* do not consistently affect a certain phase of early winter wheat seedling growth; rather they can negatively affect all aspects of early seedling growth. No attempt was made to distinguish between *M. majus* and *M. nivale* in this study. Following reclassification by Glynn *et al.* (2005), further investigations are warranted to determine whether the 2 *Microdochium* spp. affect early seedling growth in different ways.

Millar and Colhoun (1969) and Hare *et al.* (1995) have demonstrated that seedling blight caused by *M. nivale* is more severe under cold temperatures and low soil water potentials. Whilst rate of germination in this experiment followed similar trends to those reported for disease-free seeds (Hampson and Simpson, 1990; Kamaha and Maguire, 1992), *Microdochium* spp. did not consistently adversely affect rates of imbibition or germination at reduced temperatures or water potentials. It is possible that lower water potentials than those used in this experiment may be required for this to occur. It is probable that the amount of seed-borne inoculum within individual seeds will affect early seedling growth. In addition, fungal inoculum may have been situated in a more critical location within seeds giving rise to heavily diseased seedlings. *Microdochium nivale* may be present in all cereal seed components but is most abundant in the inner pericarp/outer epidermis (Bateman, 1983; Chelkowski *et al.*, 1990; Cristani, 1992). Bateman (1983) proposed that inoculum in these seed components was predominantly responsible for causing seedling blight. *Microdochium nivale* might only be expected to reduce the rate of seedling emergence and directly affect seedling growth once disease has manifested itself on the coleoptile. This probably explains why effects were only seen at GS12 for all seed lots.

Differences in winter wheat cultivars and their vigour and an interaction with the experimental conditions used in this study may also have influenced the results observed between *Microdochium* spp. and early winter wheat seedling growth. Although generally similar trends occurred across the seed lots, differences were observed. It is possible that faster growing cultivars or more vigorous seed lots may be able to outgrow seedling blight. Differential responses of wheat cultivars to temperature have been recorded (Porter and Gawith, 1999) and differences in winter wheat cultivar susceptibility to *Fusarium* spp. present on the seed surface (Arseniuk *et al.*, 1993) have been demonstrated. Subsequent investigations into the interactions between seed size, degree and location of *Microdochium* spp. infection, soil temperature and soil water potential may result in the more targeted use of fungicide seed treatments against Fusarium seedling blight.

Acknowledgements

The authors thank Harper Adams University College and Chemtura Europe Ltd for financial support.

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