Research Article

The Effect of Freezing Temperatures on *Microdochium majus* and *M. nivale* Seedling Blight of Winter Wheat

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Exposure to pre-emergent freezing temperatures significantly delayed the rate of seedling emergence ($P < 0.05$) from an infected and a non-infected winter wheat cv. Equinox seed lot, but significant effects for timing of freezing and duration of freezing on final emergence were only seen for the *Microdochium*-infested seed lot. Freezing temperatures of -5°C at post-emergence caused most disease on emerged seedlings. Duration of freezing (12 hours or 24 hours) had little effect on disease index but exposure to pre-emergent freezing for 24 hours significantly delayed rate of seedling emergence and reduced final emergence from the infected seed lot. In plate experiments, the calculated base temperature for growth of *M. nivale* and *M. majus* was -6.3°C and -2.2°C , respectively. These are the first set of experiments to demonstrate the effects of pre-emergent and post-emergent freezing on the severity of *Microdochium* seedling blight.

1. Introduction

Microdochium nivale (Fr.) Samuels and Hallett (teleomorph *Monographella nivalis* (Schaffnit) E. Müller) and *Microdochium majus* (Wollenw.) Glynn and S.G. Edwards (teleomorph *Monographella*) can cause seedling blight of cereals in the UK. *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale* were reclassified as species by Glynn et al. [1]. Before this, mention of *M. nivale* refers to both subspecies unless stated. *Microdochium spp.* may be soil or seed borne; however, seed-borne inoculum is considered to be the predominant cause of seedling blight in the UK [2]. *Microdochium* seedling blight can cause death of cereal plants at the pre-emergent and post-emergence stages of development and surviving seedlings exhibit brown lesions on the coleoptile and roots [3]. Seedling death can result in significant yield losses when surviving plants cannot compensate for large reductions in establishment [4]. In addition, *M. majus* and *M. nivale* inoculum from coleoptile and root lesions has been demonstrated to be able to cause foot rot disease and stem colonisation in glasshouse experiments [5].

Microdochium seedling blight is more severe at cold temperatures and low soil moisture contents [6]. Hare et al. [7] described a strong correlation between the rate of seedling emergence from a wheat seed lot naturally infected with 72% *M. nivale*-infection and final emergence over a range of temperatures and soil moisture contents. In many situations, winter wheat seedlings are likely to be exposed to air temperatures below 0°C . However, the only published work at near freezing temperatures is that by Bateman [8] who reported that maintaining newly emerged wheat seedlings from *M. nivale*-infected seeds at 0 – 1°C for several weeks increased disease severity.

Despite the lack of evidence for the effect of temperatures below 0°C on the severity of seedling blight from naturally infected wheat seeds, freezing has been observed to increase both the incidence and severity of *Microdochium* seedling blight on oats and barley from surface-inoculated seeds and soil-borne *M. nivale* inoculum. Rawlinson and Colhoun [9] described 4-hour freezing (-6°C) on 4 occasions at weekly intervals beginning 1 month after planting increased the incidence of isolation of *M. nivale* from oat seedling

mesocotyls and roots grown from untreated seeds in *M. nivale*-infected soil.

When surface inoculated barley seeds with conidia of *M. nivale* (1×10^6 conidia mL⁻¹) were frozen 10 days after planting at -2°C for 48 hours, coleoptiles lesion index increased to 100% compared to 5% on seedlings exposed to 2°C , and 39% on seedlings maintained at 10°C [10]. It is possible that temperatures below 0°C stop winter wheat seedling growth [11] giving *M. majus* and *M. nivale* increased opportunity for infection. However, there is a lack of information for the effects of temperatures below 0°C on the development of seedling blight from seed-borne *Microdochium* spp. in wheat and on *M. majus* and *M. nivale* growth.

A series of controlled environment experiments were designed to test the following hypotheses: (i) timing, duration, and severity of freezing does not affect seedling blight from seeds naturally infected with *M. majus* and *M. nivale*; (ii) timing, duration, and severity of freezing does not affect seedling emergence from non-infected seeds; (iii) *in vitro* growth of *M. majus* and *M. nivale* does not occur below 0°C .

2. Materials and Methods

2.1. In Vitro Growth of *Microdochium majus* and *M. nivale*. Five *M. majus* and 5 *M. nivale* isolates from the Harper Adams culture collection were cultured on potato dextrose agar (PDA) at 15°C for 8 days. Plugs of 5 mm diameter from the edges of actively growing colonies were transferred to Petri dishes containing 20 mL wheat flour agar (5% (w/w) winter wheat cv. Equinox flour; 2% (w/w) No. 1 agar (Oxoid Ltd, Basingtoke, UK)). Four dishes of each isolate were incubated in darkness at 5, 10, 15, and 20°C . Fungus colony diameters were measured in 2 directions at 90° angles at 2 day intervals and fungus growth rates (mm day⁻¹) calculated. Base temperatures for growth of *M. majus* and *M. nivale* were calculated by extrapolation following simple regression of the growth rate of each isolate. Data was analysed using *t*-test.

2.2. Effect of Freezing on the Rate of Seedling Emergence, Final Seedling Emergence, and Severity of *Microdochium* Seedling Blight. Two winter wheat seed lots cv. Equinox (88% *Microdochium* infection; 95% germination potential (infected seed lot) and 0% *Microdochium* infection; 98% germination potential (non-infected seed lot)) were used in this experiment. Due to a lack of incubator space, experiments for each seed lot were conducted separately. Experiments were conducted testing exposure to temperatures of 0°C or -5°C for 12 hours or 24 hours. Each seed lot was surface-sterilised by immersion in 10% NaOCl solution (1% available chlorine) for 3 minutes, rinsed 3 times in sterile distilled water, placed on sterile filter paper, and dried in a flow of sterile air. The severity of *Microdochium* spp. infection was determined by plating 200 surface-sterilised seeds of each seed lot onto PDA amended with $130 \mu\text{g mL}^{-1}$ streptomycin sulfate (Sigma-Aldrich Company Ltd., Dorset, UK) and $25 \mu\text{g mL}^{-1}$ Bavistin DF (carbendazim 50% w/w; BASF, Bury St. Edmunds, UK).

The germination potential of each seed lot was assessed by the tetrazolium biochemical test [12]. PCR analysis [13] confirmed both *M. majus* and *M. nivale* to be present in the infected seed lot and not present in the non-infected seed lot.

John Innes No. 2 compost was passed through a 5 mm sieve and autoclaved (121°C ; 1.08 bar) for 1 hour on 3 consecutive days and adjusted to 40% w/w soil water content. For each seed lot, 100 seeds were planted crease-down 20 mm deep in 45 seed trays. Trays were watered every 3 days to maintain constant 40% w/w soil water content. Trays were placed in an incubator set at 12 hours light (11°C) and 12 hours darkness (7°C) according to a fully randomised design and re-randomised daily. Freezing (12 hours or 24 hours at 0°C or -5°C) was applied 7 days (pre-emergent) or 28 days (post-emergent) after planting to 5 trays in a separate incubator. After freezing, trays were returned to their original incubator. Seedlings not exposed to freezing were used as controls.

Rate of seedling emergence (seedlings days⁻¹) was calculated from daily plant counts [14]. Final emergence and disease severity were measured at GS 12. A disease index on emerged seedlings was calculated (1), where *a* is the number of seedlings with category 0 symptoms (no symptoms), *b* is the number of seedlings with category 1 symptoms (≤ 2 lesions on coleoptile), *c* is the number with category 2 symptoms (> 2 lesions on coleoptile), *d* is the number with category 3 symptoms (total necrosis of coleoptile), *e* is the number with category 4 symptoms (total necrosis of coleoptile and deformed seedling growth) and *N* is the number of seedlings assessed [15],

$$\frac{(a \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4)}{N \times 4} \times 100. \quad (1)$$

Diseased cotyledons were surface sterilised and plated onto PDA amended with $130 \mu\text{g mL}^{-1}$ streptomycin sulfate to confirm *Microdochium* spp. were the causal agents of disease.

Each experiment was repeated twice and the data combined prior to analysis. Analysis of each seed lot was conducted separately. A factorial analysis of variance was conducted with rate of seedling emergence, final emergence and disease index as variables, and exposure to freezing, timing, severity and duration of freezing as factors using Genstat 5.0 (Rothamsted Experimental Station, Hertfordshire, UK). Significant probabilities are given as $P < 0.05$. Data for the infected and non-infected seed lots were square-root transformed prior to analysis to ensure normality. Disease index values for the infected seed lot could not be transformed to a normal distribution, therefore standard error values are presented.

3. Results

3.1. In Vitro Growth of *Microdochium majus* and *M. nivale*. There were no significant differences between growth rates of the 5 *M. nivale* and 5 *M. majus* isolates so data was pooled for each species. The calculated base temperature for growth of *M. nivale* was significantly lower than *M. majus*. The growth rate for *M. majus* was significantly faster than *M. nivale* (Figure 1).

FIGURE 1: *In vitro* growth of 5 *Microdochium majus* and 5 *Microdochium nivale* isolates on winter wheat cv. Equinox flour agar.

3.2. Effect of Freezing on the Rate of Seedling Emergence and Final Seedling Emergence. For the infected and non-infected seed lots, timing and duration of freezing had a significant effect on rate of seedling emergence. Only pre-emergent freezing and exposure to freezing for 24 hours significantly delayed the rate of seedling emergence for the infected and non-infected seed lots (Table 1). The timing of freezing*duration of freezing interaction only significantly affected rate of seedling emergence from the non-infected seed lot. Exposure to pre-emergent freezing for 24 hours significantly delayed rate of seedling emergence (Table 1).

For the infected seed lot, exposure to freezing, duration and timing of freezing, and the timing of freezing*duration of freezing and freezing temperature*timing of freezing interactions had a significant effect on final seedling emergence. Exposure to freezing significantly reduced final seedling emergence compared to non-frozen seedlings. Only exposure to pre-emergent freezing for 24 hours and pre-emergent freezing to -5°C significantly reduced final seedling emergence (Table 2). Timing, duration, and severity of freezing had no significant effect on final seedling emergence from the non-infected seed lot (data not shown).

3.3. Effect of Freezing on the Severity of *Microdochium* Seedling Blight. Disease symptoms did not occur on seedlings grown from the non-infected seed lot. Isolations from diseased coleoptiles of seedlings grown from the infected seed lot confirmed *Microdochium spp.* were the causal agents of disease. Seedlings exposed to 0°C had significantly less disease than non-frozen seedlings. Seedlings exposed to -5°C generally had significantly more disease than seedlings frozen to 0°C , but only exposure to -5°C for 24 hours post-emergence significantly increased disease above non-frozen seedlings (Figure 2). Timing and duration of freezing had no significant effect on disease index when seedlings were exposed to 0°C or -5°C . Post-emergent freezing caused more severe seedling blight than pre-emergent freezing.

TABLE 1: Effect of timing (a) and duration (b) of freezing on rate of seedling emergence (seedlings day $^{-1}$) from a winter wheat cv. Equinox seed lot naturally infected (88%) with *Microdochium spp.* and a non-infected seed lot, and the timing of freezing*duration of freezing interaction (c) on rate of seedling emergence (seedlings day $^{-1}$) from a non-infected winter wheat cv. Equinox seed lot.

(a)		
Timing of freezing	Infected seedlot	Non-infected seedlot
None	0.055	0.253
Pre-emergent	0.046	0.221
Post-emergent	0.058	0.267
LSD ($P < 0.05$)	0.0267	0.0246
SEM	0.0120	0.0111
DF	79	80
CV (%)	16.6	7.1

Data are back transformed, statistical analysis performed on square root transformed data.

(b)		
Duration of freezing	Infected seedlot	Non-infected seedlot
None	0.055	0.253
12 hours	0.056	0.258
24 hours	0.048	0.229
LSD ($P < 0.05$)	0.0267	0.0246
SEM	0.0120	0.0111
DF	79	80
CV (%)	16.6	7.1

Data are back transformed, statistical analysis performed on square root transformed data.

(c)						
Timing of freezing	Duration of freezing		LSD	SED	DF	CV (%)
	12 hours	24 hours				
Pre-emergent	0.250	0.193	0.0269	0.0111	80	7.1
Post-emergent	0.267	0.267				

Rate of emergence of non-frozen seedlings 0.253 seedlings day $^{-1}$.

Data are back transformed, statistical analysis performed on square root transformed data.

4. Discussion

This is the first study to demonstrate that exposure to zero and sub-zero temperatures can affect the severity of *Microdochium* seedling blight on winter wheat from naturally infected seeds. For the infected and non-infected seed lots, freezing for 24 hours was required to significantly delay rate of seedling emergence. Freezing may increase the opportunity for seedling infection as *M. majus* and *M. nivale* could continue to grow *in vitro* at temperatures below the minimum air temperatures for growth of winter wheat seedlings [11]. Lowest emergence from the infected seed lot was caused by pre-emergent freezing (-5°C) for 24 hours 7 days after planting. This is in line with the results obtained

TABLE 2: Effect of exposure to freezing temperatures (a), timing and duration of freezing (b) and the timing of freezing*duration of freezing and freezing temperature*timing of freezing interactions (c) on final emergence (%) of seedlings from a winter wheat cv. Equinox seed lot naturally infected (88%) with *Microdochium* spp.

(a)	
Exposure to freezing	Final emergence (%)
Yes	71
No	56
LSD ($P < 0.05$)	0.9
SEM	0.4
DF	79
CV (%)	17.8

Data are back transformed, statistical analysis performed on square root transformed data.

(b)			
Timing of freezing	Final emergence (%)	Duration of freezing	Final emergence (%)
None	71	None	71
Pre-emergent	46	12 hours	64
Post-emergent	67	24 hours	48
LSD ($P < 0.05$)	1.0		1.0
SEM	0.4		0.4
DF	79		79
CV (%)	17.8		17.8

Data are back transformed, statistical analysis performed on square root transformed data.

(c)						
Timing of freezing			LSD	SED	DF	CV (%)
Duration of freezing	Pre-emergent	Post-emergent				
12 hours	65	60	0.9	0.4	79	17.8
24 hours	34	69				
Freezing temperature	Pre-emergent	Post-emergent	0.9	0.4	79	17.8
0° C	52	62				
−5° C	40	72				

Final emergence of non-frozen seedlings 71%.

Data are back transformed, statistical analysis performed on square root transformed data.

by Perry [10] when emergence was lowest from barley seeds surface-inoculated with *M. nivale* and exposed to -2°C for 48 hours 10 days after planting.

Post-emergent freezing (0°C and -5°C) increased the disease index compared to pre-emergent freezing. This is possibly because pre-emergent freezing resulted in heavily diseased seedlings not emerging. These results suggest that freezing increases the severity of *Microdochium* seedling blight rather than directly damaging the winter wheat seedlings as in line with previous observations [16, 17] no damage was seen on the coleoptiles of frozen seedlings from the non-infected seed lot. A similar trend for zero and sub-zero post-emergent temperatures increasing seedling blight

FIGURE 2: Disease index on seedlings grown from a winter wheat cv. Equinox seed lot naturally infected (88%) with *Microdochium* spp. exposed to different freezing temperatures at pre- and post-emergence. Vertical bars represent standard error.

severity has been reported for soil-borne *M. nivale* infecting ryegrass [18] and oats [9] and *Fusarium avenaceum* infecting barley from artificial soil inoculation [19].

Throughout this investigation no attempt was made to distinguish between *M. majus* and *M. nivale*. *Microdochium majus* had a faster *in vitro* growth rate than *M. nivale* which could confer a competitive advantage upon it but Glynn et al. [20] in *in vivo* experiments found no differences in pathogenicity. The effect of freezing temperatures on seedlings growing in a range of soil moisture conditions is a further avenue for research. The results of this investigation may be used to more accurately target the use of fungicide seed treatments for the control of *Microdochium* seedling blight to planting conditions where seedling blight is likely to occur.

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References

- [1] N. C. Glynn, M. C. Hare, D. W. Parry, and S. G. Edwards, "Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium nivale* leads to elevation of varieties *majus* and *nivale* to species status," *Mycological Research*, vol. 109, no. 8, pp. 872–880, 2005.
- [2] D. W. Parry, T. R. Pettitt, P. Jenkinson, and A. K. Lees, "The cereal *Fusarium* complex," in *Ecology of Plant Pathogens*, P. Blakeman and B. Williamson, Eds., pp. 301–320, CAB International, Wallingford, UK, 1993.
- [3] C. S. Millar and J. Colhoun, "*Fusarium* diseases of cereals—IV. Observations on *Fusarium nivale* on wheat," *Transactions of the British Mycological Society*, vol. 52, pp. 57–66, 1969.
- [4] J. Humphreys, B. M. Cooke, and T. Storey, "Effects of seed-borne *Microdochium nivale* on establishment and grain yield of winter-sown wheat," *Plant Varieties and Seeds*, vol. 8, no. 2, pp. 107–117, 1995.
- [5] I. M. Haigh, P. Jenkinson, and M. C. Hare, "The effect of a mixture of seed-borne *Microdochium nivale* var. *majus* and

- Microdochium nivale* var. *nivale* infection on *Fusarium* seedling blight severity and subsequent stem colonisation and growth of winter wheat in pot experiments,” *European Journal of Plant Pathology*, vol. 124, no. 1, pp. 65–73, 2009.
- [6] C. S. Millar and J. Colhoun, “*Fusarium* diseases of cereals—VI. Epidemiology of *Fusarium nivale* on wheat,” *Transactions of the British Mycological Society*, vol. 52, pp. 195–204, 1969.
 - [7] M. C. Hare, D. W. Parry, and R. A. Noon, “Towards the prediction of *Fusarium* seedling blight of wheat,” in *A Vital Role for Fungicides in Cereal Production*, Hewitt et al., Ed., pp. 211–220, Bios Scientific, Oxford, UK, 1995.
 - [8] G. L. Bateman, “Control of seed-borne *Fusarium nivale* on wheat and barley by organomercury seed treatment,” *Annals of Applied Biology*, vol. 83, pp. 245–250, 1976.
 - [9] C. J. Rawlinson and J. Colhoun, “The occurrence of *Fusarium nivale* in soil,” *Plant Pathology*, vol. 18, pp. 41–45, 1969.
 - [10] D. A. Perry, “Pathogenicity of *Monographella nivalis* to spring barley,” *Transactions of the British Mycological Society*, vol. 86, pp. 287–293, 1986.
 - [11] J. R. Porter and M. Gawith, “Temperatures and the growth and development of wheat: a review,” *European Journal of Agronomy*, vol. 10, no. 1, pp. 23–36, 1999.
 - [12] “International rules for seed testing,” *Seed Science & Technology*, vol. 13, pp. 464–480, 1985.
 - [13] N. C. Glynn, R. Ray, S. G. Edwards et al., “Quantitative *Fusarium* spp. and *Microdochium* spp. PCR assays to evaluate seed treatments for the control of *Fusarium* seedling blight of wheat,” *Journal of Applied Microbiology*, vol. 102, no. 6, pp. 1645–1653, 2007.
 - [14] E. M. Khah, R. H. Ellis, and E. H. Roberts, “Effects of laboratory germination, soil temperature and moisture content on the emergence of spring wheat,” *Journal of Agriculture and Science*, vol. 107, pp. 431–438, 1986.
 - [15] I. M. Haigh, *The effects of temperature and soil water on Fusarium seedling blight of winter wheat and its effective control by fungicide seed treatments*, Ph.D. thesis, Harper Adams University College, 2003.
 - [16] M. K. Pomeroy, C. J. Andrews, K. P. Stanley, and J. Y. Gao, “Physiological and metabolic responses of winter wheat to prolonged freezing stress,” *Plant Physiology*, vol. 78, pp. 207–210, 1985.
 - [17] C. W. Windt and P. R. Van Hasselt, “Development of frost tolerance in winter wheat as modulated by differential root and shoot temperature,” *Plant Biology*, vol. 1, no. 5, pp. 573–580, 1999.
 - [18] S. J. I. Holmes and A. G. Channon, “Glasshouse studies on the effect of low temperature on infection of perennial ryegrass seedlings by *Fusarium nivale*,” *Annals of Applied Biology*, vol. 79, pp. 43–48, 1975.
 - [19] M. N. Smith and C. R. Olien, “Pathological factors affecting survival of winter barley following controlled freeze tests,” *Phytopathology*, vol. 68, pp. 773–777, 1978.
 - [20] N. C. Glynn, M. C. Hare, and S. G. Edwards, “Fungicide seed treatment efficacy against *Microdochium nivale* and *M. majus* in vitro and in vivo,” *Pest Management Science*, vol. 64, no. 8, pp. 793–799, 2008.