The effect of temperature on the *in vitro* growth rate of sclerotinia homoeocarpa isolates of different origin

Kate Entwistle, Tatsiana Espevig, Jo Anne Crouch, Karin Normann and Marina Usoltseva

Introduction

Although *Sclerotinia homoeocarpa* is the name generally ascribed to the fungus that causes Dollar Spot disease on cool season turfgrasses, recent research has proposed a revised name for the fungus that causes Dollar Spot disease as it occurs on cool season turf in the USA[1]. From a maintenance point of view, Dollar Spot is regarded as one of the most important turfgrass diseases, especially in the USA, but it is a newly recorded disease on turfgrasses across Scandinavia. Having initially been documented in Norway in 2013[2] and in Sweden in 2014[3], it is now known to exist on more than 20 Nordic golf courses and has caused up to 70-80% dead turf on affected greens and fairways. To enable effective management of the potential symptoms, it is important to clearly understand the parameters that enable the fungus to actively cause disease. In the USA, disease development is encouraged by an air temperature of up to 30 °C and high relative humidity[4,5] but no information is currently available on the environmental conditions that favour Dollar Spot disease in Scandinavia. The results obtained from previous research in Sweden on the in vitro growth requirements of *S. homoeocarpa* isolates collected from across Scandinavia were inconclusive[6], but they proposed lower cardinal temperatures compared to those documented for *S. homoeocarpa* isolated from Dollar Spot infected turf elsewhere around the world. As part of the current research programme, this study aims to identify whether there are differences in the temperature growth characteristics of *S. homoeocarpa* isolates collected from turfgrasses in Scandinavia, the USA and the UK.

Materials and methods

Of the ten isolates used in this study (Table 1), three were received from the USA as pure cultures and seven (from Denmark, Norway, Sweden and UK) were isolated directly from infected turfgrass samples that were received in to NIBIO (Landvik). To obtain fungal isolates from the infected turfgrass samples, pieces of symptomatic leaf tissue were washed in cold running tap water for 30-60 min, surface-sterilised in 70% ethanol for 60s, blotted dry on sterile filter paper and placed in 90-mm diameter Petri plates containing water agar (20 g/L agar of media). After 4-d incubation at room temperature and 16-h light, water agar plugs containing the target fungus were transferred to 50% potato dextrose agar (PDA, 19.5 g of Fluka Analytical PDA (Sigma-Aldrich, St. Louis, MO) and 7.5 g/L agar). Pure cultures of all ten fungal isolates were maintained at room temperature in Petri plates on 50% PDA and in long-term storage at -80°C in 1.5ml Eppendorf tubes.

Isolate no.	Country of origin	Received in NIBIO (mo/yr)	Host grasses	GenBank number
14.10	Denmark	7/14	*Poa annua*	–
14.12	Norway	7/14	*Agrostis stolonifera*	KJ775860
14.15	Sweden	9/14	*Festuca rubra spp.*	–
14.16	Sweden	9/14	*Festuca rubra spp.*	–
14.112	Sweden	10/14	*Poa annua*	–
MB01	USA	12/14	*Agrostis stolonifera*	KF545290
SH44	USA	12/14	*Agrostis stolonifera*	KF545299
RB19	USA	12/14	*Cynodon dactylon x transvaalensis*	KF545306
17.11	UK	8/17	*Festuca rubra spp.*	–
17.12	UK	8/17	*Poa pratensis*	–

Tab. 1: Isolates of Sclerotinia homoeocarpa and GenBank accession numbers of DNA sequences used in this study

Each of the 10 isolates of *S. homoeocarpa* was grown on 50% PDA in Petri plates at 0, 4, 8, 16, 24, 32 and 40 °C for 17 days or until the fungal growth reached the edge of the Petri plate. Four Petri plates for each of the *isolate* x *temperature* combinations were centrally inoculated with 5 mm plugs taken from the leading edge of actively growing 3-day-old cultures, sealed with Parafilm and incubated in the dark. The increase in colony diameter was recorded daily (mm). The Petri plates that were incubated at 0 °C and 40 °C were maintained at their respective temperatures for 3 more days after which time they were transferred to 16 °C for three further days incubation, to enable assessment of fungal regrowth. The *in vitro* daily growth rates were determined by dividing of the recorded colony diameter (mm) (minus 5 mm of original plugs) by the corresponding number of days of the growth. The reduction in the growth rate at 16 °C after being at 0 °C and 40 °C for 3 weeks was calculated as percentage of daily growth rate at 16 °C (data not shown). The data were analysed by the SAS procedure proc ANOVA using the statements for 1-factorial completely randomized design either among the isolates within each temperature or among the temperatures within each isolate (SAS Institute, version 9.4). Fisher LSD at 5% probability level identified significant differences among the treatments.

Isolate	0 °C	4 °C	8 °C	16 °C	24 °C	32 °C	40 °C
	---------- Daily growth rate, mm day^{-1}----------						
14.10	0.1 b	2.1 bc	7.1 a	18.8 ab	29.8 ab	14.9 a	0.04 ab
14.12	0.4 a	3.4 a	7.0 ab	19.2 a	29.6 ab	11.8 b	0.04 ab
14.15	0.1 b	2.0 cd	6.8 abc	19.6 a	32.2 a	5.6 c	0.06 a
14.16	0.1 b	2.4 b	6.1 d	14.6 de	23.1 d	13.0 b	0.01 bc
14.112	0.1 b	2.3 bc	7.0 ab	17.8 abc	27.2 bc	13.2 b	0.03 bc
MB01	0.1 b	2.5 b	6.4 bcd	16.0 cde	16.3 e	2.3 d	0.04 ab
SH44	0.4 a	3.1 a	5.9 d	14.0 e	12.6 f	4.3 c	0.00 c
RB19	0.1 b	2.2 bc	7.0 ab	17.2 abcd	26.8 bc	14.8 a	0.02 bc
17.11	0 .0 b	2.2 bc	6.6 abcd	17.1 abcd	26.9 bc	12.3 b	0.03 ab
17.12	0.1 b	1.7 d	6.2 d	16.6 bcd	24.6 cd	5.8 c	0.02 bc
P (ANOVA)	<.0001	*<.0001*	*0.010*	*0.001*	*<.0001*	*<.0001*	*0.017*

* The same letter indicates no differences among the isolates within the same temperature based on Fisher LSD at 5% probability level.

Tab. 2: The effect of temperature from 0 °C to 40 °C on growth rates of 10 isolates of Sclerotinia homoeocarpa of different origin.

Results and discussion

With regard to the seven Scandinavian and UK isolates, the maximum growth rate was recorded at 24 °C. There were significant differences in growth rate among the isolates (Table 2) but curiously, the three isolates from Sweden all produced significantly different growth rates when incubated at 24 °C. The two USA isolates from cool-season grasses produced significantly lower growth rates at 24 °C than did all Scandinavian and UK isolates. Moreover, the growth rate of MB01 and SH44 at 24 °C did not differ significantly from that at 16 °C (results from ANOVA among the temperatures within each isolate not shown).

All ten isolates resumed growth when incubated at 16 °C after having been maintained at 0 °C for 3 weeks (data not shown). However, the growth rates of isolates from Denmark, Sweden and UK were reduced by an average of 28% when compared to their growth at 16 °C prior to chilling. No reduction in growth rate was recorded for either the USA isolates collected from cool-season grasses (MB01 and SH44) or the Norwegian isolate 14.12 (data not shown). Thus, it appears that the minimum temperature for mycelial growth of *S. homoeocarpa* in this study was in accordance with Bennett[7] who reported 0-2 °C. Moreover, this apparent lack of inhibition in growth for MB01, SH44 and 14.12 could indicate a potential for enhanced survival during cold winter conditions but further work would be necessary to confirm this.

For all isolates except 14.15 from Sweden, incubation at 16 °C after incubation at 40 °C for three weeks, resulted in a reduction in growth rate of >98% (vs. 83% for 14.15) when compared to the isolate growth rates at 16 °C without the high temperature exposure (data not shown). Thus, the maximum growth temperature for most isolates in this study was between 32 and 40C, higher than previously reported[7].

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Authors:

Kate Entwistle
The Turf Disease Centre (UK)

Tatsiana Espevig
NIBIO – Norwegian Institute of Bioeconomy Research

Jo Anne Crouch
Systematic Mycology & Microbiology Lab, Department of Agriculture (USA)

Karin Normann
Asbjørn Nyholt ApS (Denmark)

Marina Usoltseva
Botanisk Analysgrupp (Sweden)

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