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Elucidating the impact of environmental factors on the growth of *Colletotrichum coccodes* strains isolated from potato tubers in Great Britain

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ABSTRACT

Black dot and silver scurf caused by *Colletotrichum coccodes* and *Helminthosporium solani*, respectively, are tuber blemish diseases affecting quality in the fresh and pre-pack potato industry. In the last 20 years, the importance of high-quality tuber appearance has increased considerably due to the growing demand for washed and pre-packed potatoes in the UK. Changing climate characterised by rising temperatures and wetter summers is a threat as this will favour the development of pathogens such as *C. coccodes* in the soil increasing the risk of food spoilage. Moreover, both diseases can develop not only in the field but also after harvest, with postharvest storage temperatures being a crucial factor in controlling fungal growth. Furthermore, anecdotal evidence showed differences on the aggressiveness of black dot depending on its origin (i.e. England and Scotland) on potato tubers. Silver scurf and black dot are difficult to differentiate as they present similar phenotypes characterised by silvery lesions making it challenging for managers to take the necessary corrective action during storage. Hence, the aim of this study was to give a general insight into the ecological conditions affecting the establishment of the causal agent of potato black dot in the field, and black dot and silver scurf during the supply chain. Therefore, *in vitro* experiments were designed to study the growth rate and lag times simulating both scenarios respectively: on soil extract agar (SEA) media at different temperatures (4, 11, 15 °C) and matric potentials (control [unmodified] and − 1.4 MPa [modified]); and on natural potato dextrose agar (NPDA) for different temperatures (4, 11, 15 and 20 °C) at 99 % relative humidity (RH) for 25 days. When simulating the field environment, drier conditions (matric potential = −1.4 MPa) reduced fungal growth for both isolates by 0.1 cm day^{-1} at the temperature of 15 °C, suggesting temperature as the main limiting factor for the growth of *C. coccodes* in the soil. The causal agent of black dot exhibited a faster growth rate under retailer-like conditions (i.e., 15 °C) compared to *H. solani*. Understanding the environmental influence on both the pathogen and the crop is vital for proper disease management to help reduce food loss and waste.

1. Introduction

Colletotrichum species are plant pathogens causing anthracnose diseases in different plant crops around the world. Among them, *C. coccodes* infects potato causing black dot disease. The main symptom of potato black dot disease is the presence of black microsclerotia on infected tissues which can be observed upon lifting (Johnson et al., 2018). These sclerotia survive over winter in both soil or in contaminated debris, and are capable of persisting for several years becoming the predominant inoculum source of this soil borne disease (Dillard and Cobb, 1998; Read and Hide, 1988). However, during postharvest cold storage, which for the fresh and pre-pack market can be up to ten months at 2.5 to 3.5 °C, the disease develops further resulting in significant lesions. Although the skin blemishes do not impact the taste and nutritional properties of potato tubers, they compromise the marketability and overall quality as this cosmetic defect can lead to severe economic losses to production (Lees and Hilton, 2003). Affected tubers might show irregular patches of the lesions, either revealing sclerotia or having them covered by the lesions (Jellis and Taylor, 1974) (Fig. 1A).

Silver scurf, a seed borne disease caused by *Helminthosporium solani*, is another important postharvest blemish disease that can often develop alongside black dot. Silver scurf lesions present conidiophores showing as short black thread-like structure causing a more regular lesion shape (Fig. 1B), whereas black dot lesions are characterised by discrete black

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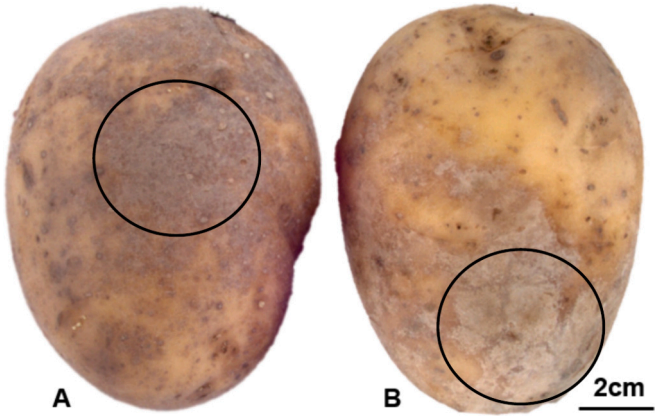

Fig. 1. Potato black dot and silver scurf disease symptoms on entire potato tubers cv. 'Maris Piper'. Black dot symptoms appear in potato tuber skin as irregular brown lesions (A). Silver scurf symptoms appear in potato skin as regular shape silvery lesions (B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dots (Errampalli et al., 2001; Jellis and Taylor, 1974; Read, 1993).

As a soil borne disease, field conditions, such as temperature, soil water availability, initial fungal load and fungicide application are important factors affecting the incidence of black dot (Johnson et al., 2018; Lees and Hilton, 2003). Projected climate changes, including rising temperatures and increased humidity in Northern Europe, pose future risks for potato production (Bebber et al., 2013) as these changes may favour the development of tuber pathogens. *In vitro* studies (Dillard, 1988) described that the optimal temperature for conidia germination of *C. coccodes* was 22 °C while optimum growth for sclerotia was 28 °C on water agar plates. Previous surveys (Read, 1993) conducted >20 years ago, observed differences in the incidence of black dot grown in England and Scotland. Originally this was linked to cooler temperatures and longer soil rotations in Scottish seed growing areas (Lees and Hilton, 2003). Yet, no research has been done on how *C. coccodes* from both origins would adapt to upcoming changing climate. In the case of *H. solani*, conidial germination on plant tissue and subsequent tuber infection occurs under high humidity conditions (> 95 % RH) and temperature between 15 and 32 °C (Errampalli et al., 2001). Silver scurf and black dot present similar phenotypes characterised by silvery lesions which are sometimes difficult to differentiate during storage. The biotic and abiotic variables involved in the interaction of the pathogen and the potato are not thoroughly understood (Johnson et al., 2018). Understanding how the causal agents of blemish diseases behave under different field and storage-like conditions is crucial for better disease management. Therefore, the aim of this study was to give a general insight into the conditions affecting the survival and establishment of the causal agent of potato black dot in the field, and black dot and silver scurf during the simulated supply chain conditions *in vitro*. For that, the objectives of this study were: i) to elucidate the effect of two-way interacting climatic factors i.e., temperature (4, 11, 15 °C) and water availability (−0.7 MPa and − 1.4 MPa), on fungal growth on simulated soil conditions using soil extract agar media for black dot isolates from two origins (England and Scotland); and ii) to evaluate the impact of temperature (4, 11, 15, and 20 °C) on the growth of *C. coccodes* and *H. solani* using natural potato dextrose agar media, when simulating different stages of the potato supply chain (i.e., storage, retailer and consumer stages).

2. Materials and methods

2.1. Fungal strains

C. coccodes strains were isolated from potato tubers cv. 'Maris Piper' harvested in Perthshire, Scotland, and Norfolk, England. All potato tubers were supplied by Albert Bartlett, UK, and harvested in September 2020. The pathogen responsible for silver scurf was purchased from CABI library for reference purposes (Table 1) and subcultured in potato dextrose agar (PDA) (full strength) (Thermo Fisher Scientific, USA) for seven days at 25 °C in the dark.

2.2. Isolation methodology

A total of 50 potato tubers were washed with tap water, and visually assessed for black dot and silver scurf diseases. Those tubers with black dot and silver scurf lesions were immersed for 15 min in the sterilisation solution (sodium hypochlorite, NaOCl, 0.5 %), following the protocol described elsewhere Gutiérrez-Pozo, 2021 - PhD thesis). Under a laminar flow bench, a small piece of the tuber skin containing the blemish was removed with a scalpel and centrally placed on PDA plates amended with chloramphenicol (0.1 g L^{-1}, Thermo Fisher Scientific, USA). The plates were then incubated at 25 °C for seven days, and four-millimetre diameter plugs were taken from all the isolates, placed in a solution of glycerol: water (70:30, *v/v*) and kept at −20 °C to ensure the use of the same inoculum for further experiments as well as for molecular identification.

2.3. Morphological and molecular identification of Colletotrichum coccodes strains isolated from potato tubers

Black dot isolates were observed under microscope (Olympus System Microscope, Model BX40, Tokyo, Japan).

Molecular identification of *Colletotrichum coccodes* strains isolated from potato tubers from Scotland and England was carried out following the protocol described in Cullen et al. (2002). Both Scottish and English strains of *C. coccodes* were grown in PDA plates for seven days at 25 °C. Four-millimetre diameter plugs containing mycelia and microsclerotia of black dot were taken and placed in malt extract broth in 50 mL centrifuge tubes. Tubes were left in the dark for seven days at 25 °C in a constant shake to stimulate their growth and avoid sedimentation. After this period, tubes were centrifuged at 7000 rpm at 20 °C where supernatant was discarded and the pellet containing the fungal material was kept at −20 °C until the DNA extraction. DNA was extracted using the peqGold Plant DNA Mini Kit (Avantor®, VWR International, UK). The eluted DNA was stored at −20 °C until the PCR amplification. Genomic DNA was amplified using primers designed previously by Cullen et al. (2002) (Table 2).

2.4. Media preparation

2.4.1. Preparation of soil extract agar media

Field conditions were simulated using soil extract agar (SEA) (HIMedia, India). Based on Steuter et al. (1981), adding polyethylene glycol (PEG) modifies the matric potential. Therefore, an appropriate amount of PEG 8000 (Melford, UK) was added using the equation of Kaufmann and Michel (1973) and described by Magan (1988), resulting in matric potential of −1.4 MPa. In this study, 8.5 cm sterile circular

Table 1
Information of black dot and silver scurf isolates used for the in vitro studies. *Helminthosporium solani* was bought from CABI (supplier collection) for reference purposes whilst *Colletotrichum coccodes* was isolated from potato tubers harvested from two different regions in England and Scotland.

Strain	Disease	IMI number	Supplier/origin
Helminthosporium solani	Silver scurf	189115	CABI Genetic Resources Collection, UK
Colletotrichum coccodes	Black dot	Isolated from tuber	Perthshire, Scotland, UK
Colletotrichum coccodes	Black dot	Isolated from tuber	Norfolk, England, UK

Table 2
PCR primers specific for *Colletotrichum* spp. and *C. coccodes*. PCR primer sets designed by Cullen et al., 2002. The first primer set consists of genus-specific outer primers (Cc1F1/Cc2R1) designed to target regions shared among various *Colletotrichum* spp. The second primer set includes species-specific nested primers (Cc1NF1/Cc2NR1) designed to bind to sequences that are unique to *C. coccodes*.

Primers		Sequence (5′-3′)	Size of product (bp)
Colletotrichum spp.	Cc1F1	ACCTAACTGTTGCTTCGGCG	447
	Cc2R1	AAATTTGGGGGTTTTACGGC	
C. coccodes	Cc1NF1	TGCCGCCTGCGGACCCCCCT	349
	Cc2NR1	GGCTCCGAGAGGGTCCGCCA	

Table 3
Treatments used for simulated field and storage scenarios. The growth of *C. coccodes* and *H. solani* isolates was studied under simulated field and postharvest storage conditions.

	Simulated scenarios			
	Field		Postharvest storage	
Isolates	*C. coccodes* English *C. coccodes* Scottish		*C. coccodes* English *C. coccodes* Scottish *H. solani* (reference)	
Environmental factors	Temperature (°C)	Water potential (-MPa)	Temperature (°C)	Relative humidity (%)
Levels	4, 11, 15	0.7, 1.4	4, 11, 15, 20	99

disks of capillary matting (Harrod Horticultural, Lowestoft, UK) were placed in sterile 9 cm Petri dishes where 15 mL of the cooled SEA media was added. The matting was overlaid with sterile disk of black polyester lining cloth (0.15 mm thick) and a cellophane disk (Innovia Films Ltd., Wigton, UK) (Fig. 2). The water activity of representative samples of media was checked with an AQUALAB 4TE water activity meter (Decagon Instruments, USA) and further converted to water potential.

2.4.2. Preparation of potato-based semi-synthetic media

A semi-synthetic growth media, natural potato dextrose agar (NPDA), was prepared to mimic the natural crop commodity frequently contaminated by the pathogens under study during postharvest conditions. The media was formulated following the optimised NPDA media described by Gutiérrez-Pozo (2021, PhD thesis) by mixing 2 % of glucose (Fisher Scientific, USA), 1.5 % of agar (Sigma-Aldrich, Dorset, UK) and 30 % of mashed potatoes in 1 L of potato infusion. The prepared media was autoclaved for 15 min at 121 °C and further poured into 9 cm Petri dishes (15 mL per plate).

2.5. Experimental design, inoculation and fungal growth measurements

2.5.1. Inoculation

Isolates were centrally inoculated on the NPDA and SEA media plates using a sterile 4 mm cork-borer. Five replicates were used per treatment. Inoculated plates were placed in polyethylene bags and stored in sterile boxes and were incubated at the different temperatures.

2.5.2. Experimental design

For the experiment mimicking field conditions, a three-way factorial design was followed (Table 3), where temperatures were selected according to the average soil temperature in Perthshire, Scotland (11 °C) and in Norfolk, England (15 °C) (Met Office) during the tuberization period which falls between April and May, depending on the planting date. Moreover, a third temperature, 4 °C, was also chosen to simulate conditions in the soil over winter periods. Two matric potentials, −0.7 MPa (media not modified) and − 1.4 MPa were used to represent two possible scenarios in the field depending on the moisture in the soil. Matric potential of −1.5 MPa is considered the permanent wilting point for plants (O'Geen, 2013). Thus, −0.7 MPa was considered the 'Control', while −1.4 MPa was regarded as the lowest threshold for potato plant growth. However, this value was still considered to represent dry conditions simulating potential future climate change scenarios.

The second experiment followed a two-way factorial design. Isolates were also incubated under different temperatures simulating varied supply chain conditions (4, 11, 15 and 20 °C) in NPDA. The lower temperature corresponded to industrial storage conditions, whilst 11 and 15 °C simulated the temperature range at the retailer stage, and 20 °C mimicked domestic conditions.

2.5.3. Fungal growth

Measurements of colony diameters were taken in two perpendicular directions to each other for each isolate and under the different interacting abiotic conditions for each nutritional media. Measurements started once the first sign of fungal growth was shown and they continued until the mycelium reached the edge of the plate (ca. 25 days). Two different growth parameters were calculated: maximum growth rate (μ_m, cm of diameter per day) and lag time (λ, days). Those parameters were obtained by plotting the diameters against time (days) and applying the primary linear model to those time points that represented the linear phase of the growth curve, using Microsoft Excel® (Microsoft Corporation, USA). The slope of the regression line was considered as the growth rate, while the lag time was estimated as the interception between the regression line and the *x*-axis taking into account the initial inoculum size (0.4 mm) (Garcia et al., 2009). Experiments were carried out with five replicates per treatment.

2.6. Statistical analysis

Statistical analyses were performed using JMP® 17 (SAS Institute Inc., 2022, Cary, NC, USA). Data sets (maximum growth rate [μ_{max}] and lag time [λ]) were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene test, respectively. Non-normality and variance homogeneity was confirmed even after performing multiple transformations. Thus, the non-parametric test, Kruskal–Wallis, was performed. When significant differences were found (p-value < 0.05), each pair were compared by a *post-hoc* Wilcoxon method. All plots were done

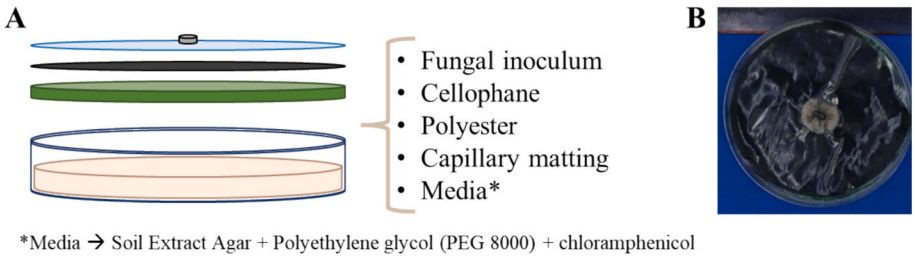

Fig. 2. Schematic of the overlay with matting (A) and example of the set up (B). Layout of the plates used for the matric potential experiments. Set up includes media (soil extract agar plus polyethylene glycol to modify matric potential and chloramphenicol to avoid bacterial growth), capillary matting, a polyester lining cloth, a cellophane disk, and the fungal inoculum.

using SigmaPlot (Systat Software, Inc. London, UK), unless otherwise stated.

3. Results

3.1. Molecular identification of C. coccodes strains isolated from potato tubers

Prior to molecular identification, black dot isolates were observed under microscope (Olympus System Microscope, Model BX40, Tokyo, Japan) (Fig. 3) where the typical conidia of *C. coccodes* were found.

PCR products were visualised under UV light (Syngene G:Box, India) on 1 % agarose Tris-Borate-EDTA (TBE) gels and presented in Fig. 4. Bands for both black dot isolates (English and Scottish) were observed in the first-round PCR (genus-specific primers) (bands A and C, from Fig. 4) and also in the second round (species-specific primers) (bands E and G, from Fig. 4). Therefore, the identification of the isolates *C. coccodes* isolated from potato tubers cv. 'Maris Piper' from England and Scotland was confirmed.

3.2. Effect of matric potential and temperature factors on C. coccodes growth on soil extract agar

Effects of temperature and matric potential on the growth rate of *C. coccodes* on soil extract agar plates are shown in Fig. 5. *C. coccodes* isolates from both origins, England and Scotland, grew faster at temperatures of 11 °C and 15 °C than at 4 °C, whilst water potential had no significant impact on fungal growth under cold conditions (4 °C). Under modified media simulating drier conditions (−1.4 MPa), the Scottish black dot isolate showed lower maximum growth rate than the isolates kept under unmodified conditions while drier conditions did not affect the growth of English isolates at 11 °C. Yet, both English and Scottish isolates growth was reduced by drier conditions at 15 °C. Scottish black dot showed a reduced growth compared to the English isolates across both matric potential conditions (Control and − 1.4 MPa) under the temperature of 15 °C.

English isolate took five days more to start growing in drier conditions than the Scottish one at 4 °C. However, when temperature was higher, no significant differences ($p > 0.05$) between isolates were found. That said, the agent of black dot isolated from Scotland had a lower lag time (one day and a half) when grew on the control media, and it took four days to start growing at the water potential of −1.4 MPa at higher temperature (15 °C) (Fig. 6).

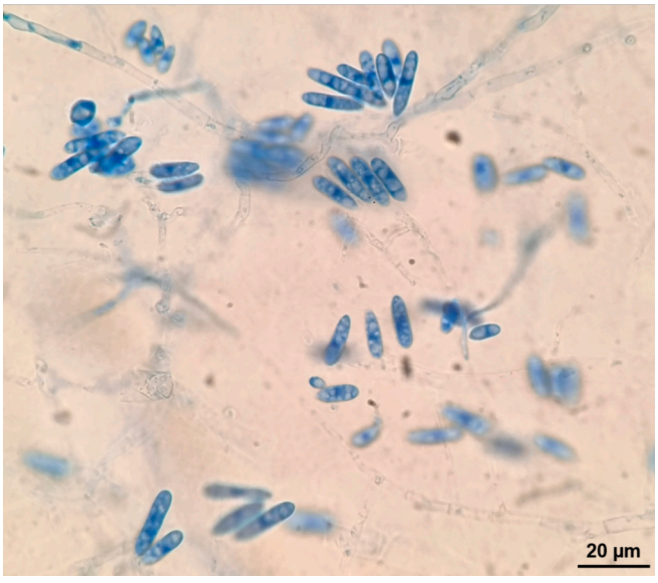

Fig. 3. Microscope view of *C. coccodes* strain isolated from potato tubers from Norfolk, England after 10 days of incubation at 25 °C. Conidia of *C. coccodes* can be observed at 1000× magnification in samples prepared with methylene blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

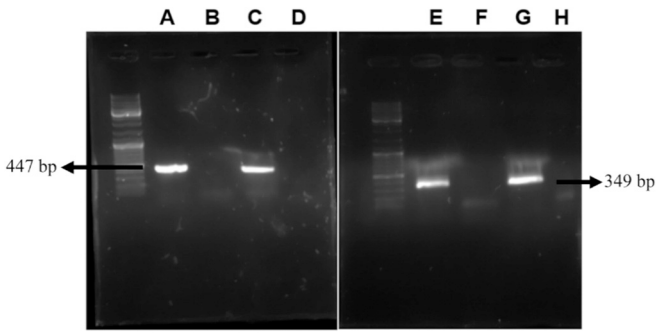

Fig. 4. UV visualisation of PCR products after their amplification for the regions Cc1F1 and Cc2R1 (*Colletotrichum*-specific, gel in the left) and Cc1NF1 and Cc2Nr1 (*C. coccodes*-specific, gel in the right). Different band letters correspond from left to right: A: amplification with Cc1F1/Cc2R1 *Colletotrichum coccodes* English isolate, B: negative control (dH_2O), C: amplification with Cc1F1/Cc2R1 *Colletotrichum coccodes* Scottish isolate, D: negative control (dH_2O), E: amplification with Cc1NF1/Cc2NR1 *Colletotrichum coccodes* English isolate, F: negative control (dH_2O), G: amplification with Cc1NF1/Cc2NR1 *Colletotrichum coccodes* Scottish isolate, H: negative control (dH_2O).

3.3. Effect of temperature on the growth of C. coccodes and H. solani strains on natural potato dextrose agar

Maximum growth rate and lag time for black dot and silver scurf isolates under different temperatures cultured in NPDA plates are showed in Fig. 7. Maximum growth rate for both isolates of *C. coccodes* was achieved at the highest temperature with a value of 0.66 cm diameter per day. No significant differences ($p > 0.05$) were found between isolates from different origins at any of the temperatures regarding growth rate. However, Scottish black dot started to grow after eight days post inoculation at 4 °C, while English black dot took nine days. The same pattern was repeated under the highest temperature studied (20 °C). There were no significant differences on the lag time between isolates at the temperatures of 11 and 15 °C, where the isolates started to grow after two or one day of the inoculation, respectively.

Maximum growth rate of black dot isolates was three-fold higher than the silver scurf isolate under all the temperatures. In general, the reference strain of silver scurf showed a higher lag time than black dot isolates under all temperatures studied, where it started to grow 1–2 days later than black dot isolates at temperatures of 11, 15 and 20 °C and it took one week more to start growing at 4 °C. Colonies growth in Petri dishes after 0, 4, 11, and 21 days at 20 °C are shown in Fig. 8.

4. Discussion

In this study, the effect of temperature on two *C. coccodes* strains and *H. solani*, and the effect of the interaction temperature x water potential on two *C. coccodes* strains was investigated in vitro using two culture media to simulate different conditions throughout the potato supply chain, including pre- and postharvest stages. The growth parameters (μ_{max} and λ) were directly affected by temperature in both experiments. Soil inoculum is known to be the major source of *C. coccodes* inoculum, which plays a significant role in determining the prevalence of black dot disease on daughter tubers; moreover, this inoculum spreads during the postharvest storage period (Denner et al., 1998; Lees et al., 2010; Lucas and Christ, 2006). Silver scurf is believed to be essentially a seedborne disease, even though infected seed tubers can contaminate the field introducing conidia hyphae of *H. solani* (Errampalli et al., 2001).

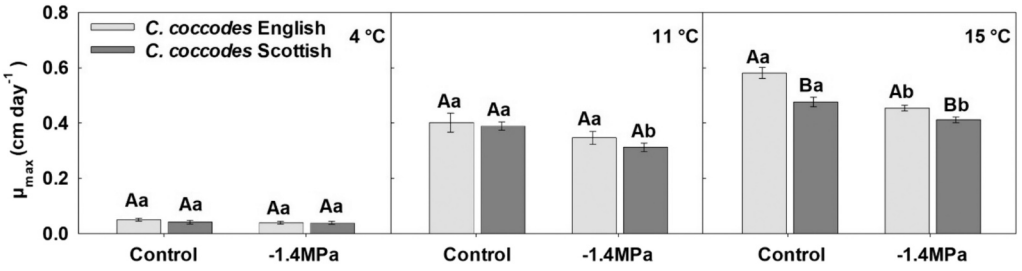

Fig. 5. Effect of temperature (4, 11, 15 °C) and water potential [Control (−0.7 MPa), −1.4 MPa] on maximum growth rate (μ_{max}) of *C. coccodes* strains from England and Scotland on soil extract agar media. Data shows means of five replicates with standard error bars of the means. Different capital letters indicate significant differences between isolates (English black dot and Scottish black dot) at each specific temperature and water potential. Different lower-case letters indicate significant differences between water potentials at each temperature and isolate (Wilcoxon, *p*-value<0.05).

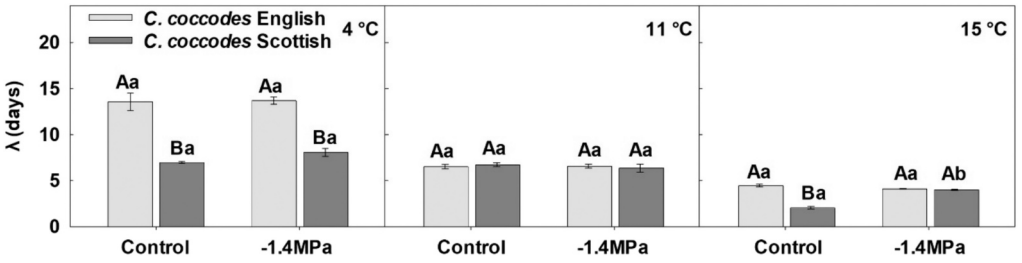

Fig. 6. Effect of temperature (4, 11, 15 °C) and water potential [Control (−0.7 MPa), −1.4 MPa] on lag time (λ) of *C. coccodes* strains from England and Scotland on soil extract agar media. Data shows means of five replicates with standard error bars of the means. Different capital letters indicate significant differences between isolates (English black dot and Scottish black dot) at each specific temperature and water potential. Different lower-case letters indicate significant differences between water potentials at each temperature and isolate (Wilcoxon, p-value<0.05).

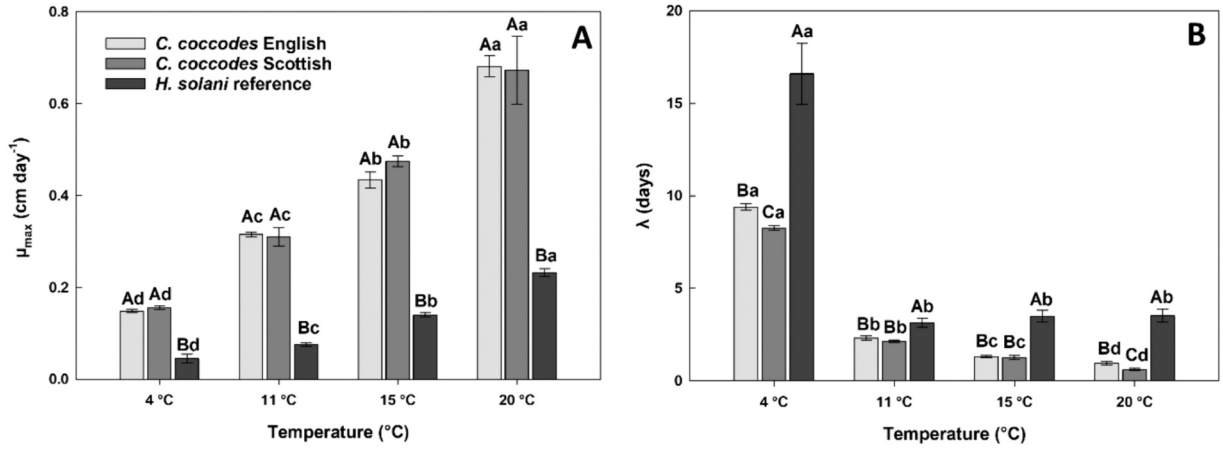

Fig. 7. Effect of temperature (4, 11, 15, 20 °C) on maximum growth rate (μ_{max}) (A) and lag time (λ) (B) of *C. coccodes* strains from England and Scotland and *H. solani* on potato-based media. Data shows means of 5 replicates with standard error bars of the means. Different capital letters indicate significant differences between isolates (English black dot and Scottish black dot) at each specific temperature. Different lower-case letters indicate significant differences between temperatures for each isolate (Wilcoxon, *p*-value<0.05).

However, it does not last long in the soil, diminishing the importance of soil inoculum in silver scurf incidence. This is the reason why *H. solani* was not considered in the soil-like conditions experiment. The temperatures used in the matric potential experiment were chosen to simulate representative soil temperatures during potato tuberization in both origins (Scotland and England) and over winter (11, 15 and 4 °C, respectively). Based on anecdotal evidence, it has been observed that sclerotia produced by *C. coccodes* on potato tubers of cv. 'Maris Piper' grown in England are more prominent and readily identifiable compared to those in tubers grown in Scotland. The extent to which the pathogen can proliferate through the soil to reach the host is not well-established (Lees et al., 2010). Harris et al. (2003) studied the effect of soil structure (modifying soil bulk density) on the spatial exploration of soil by the fungus *Rhizoctonia solani* (causal agent of black scurf on potatoes). They found that fungal mycelia expanded throughout the heterogenous networks of pores to find nutrients and the volume of soil explored by the fungus was higher with increasing bulk density, suggesting that soil structure may influence the final soil inoculum.

Between isolates, no significant differences were found in temperatures higher than 11 °C. Yet, at simulated cold conditions (4 °C) black dot isolated from Scotland seemed to take less time to start growing than the English one. This observation may be attributed to the generally lower temperatures experienced in Scotland throughout the year, possibly leading to an adaptation of the Scottish strain to these

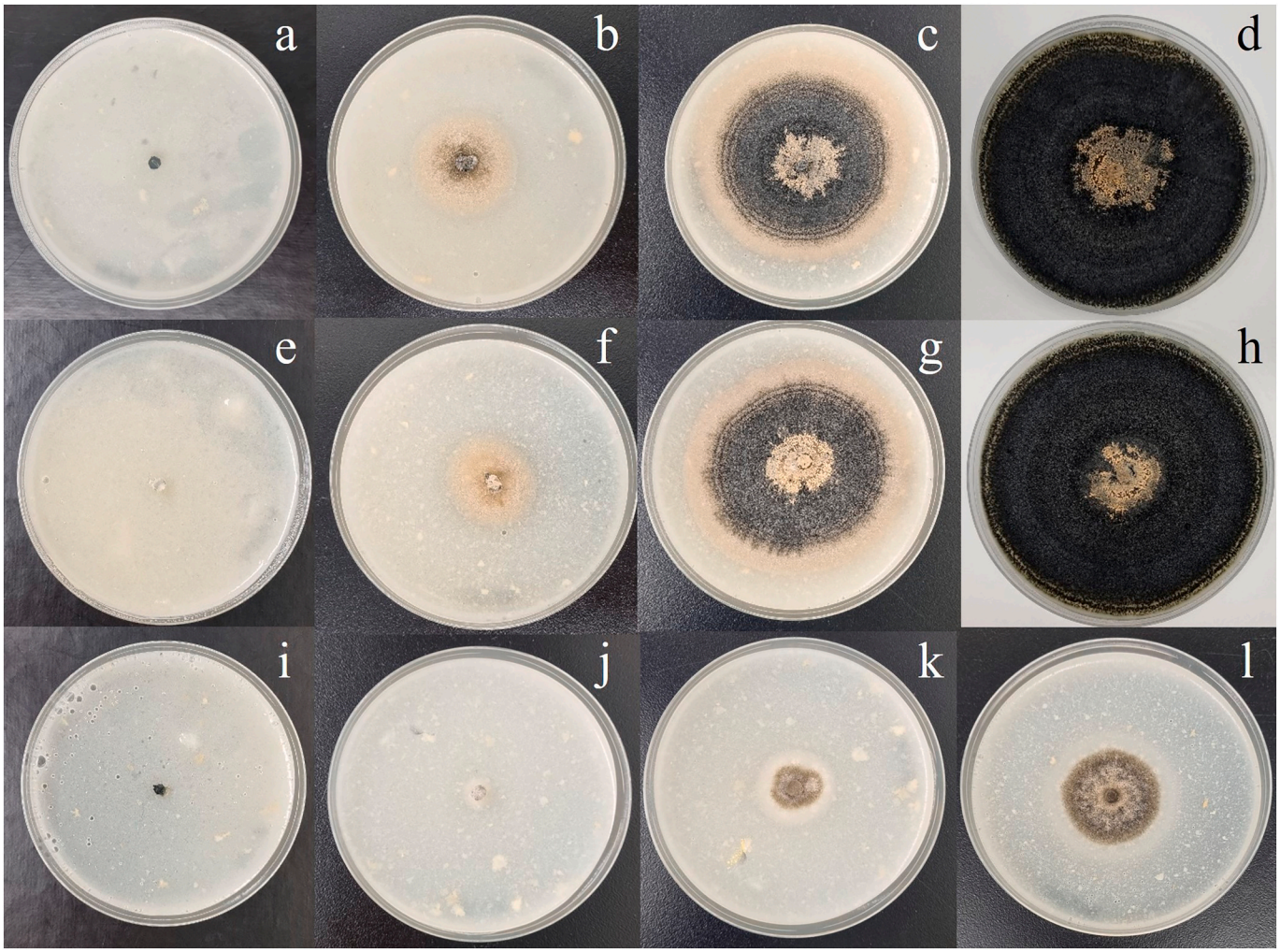

Fig. 8. Growth of *C. coccodes* isolated from Scottish tubers (a, b, c, d), from English tubers (e, f, g, h) and *H. solani* (i, j, k, l) strains on NPDA plates. Pictures taken on the inoculation day (a, e, i) and after 4 days (b, f, j), 11 days (c, g, k), and 21 days (d, h, l) of inoculation at 20 °C.

conditions. Surveys carried out in 1987–1990 in Eastern England revealed that by 1989–90, black dot had become a common disease across all regions of Britain, with the exception of Scotland where it was less frequent in ware potatoes (Read, 1993). Later, in a crop survey conducted in 1999 and 2000, regional differences in the incidence of black dot within the UK were already highlighted. Nevertheless, with the anticipated rise in temperatures due to climate change, both strains are expected to adapt in a similar way. This discrepancy could be also related to the great genetic variability existing in the species *C. coccodes*, with several Vegetative Compatibility Groups (VCGs) identified, mostly isolated from potato grown in different continents (Nitzan et al., 2002). Moreover, Nitzan and Tsror (2003) indicated that *C. coccodes* from different VCGs were characterised by different physiological behaviour (density of the sclerotia) in response to temperature (21, 25 and 30 °C). The variations observed among the VCGs imply a potential ecological adaptation within the population of the pathogen, possibly influenced by geographic locations. Hence, our study aimed to investigate the distinctions between two strains (*C. coccodes* isolated from English tubers vs. Scottish tubers).

Under low temperatures (4 °C) on soil extract agar media, no significant difference ($p > 0.05$) was observed in maximum fungal growth across both matric potential stress conditions, suggesting that field temperature has a more predominant impact than water availability on black dot development. Fungal germination and growth generally have been shown to be more sensitive to matric than osmotic potential stress (Brownell and Schneider, 1985; Magan, 1988). However, Dillard (1988) observed that maximum germination of conidia and growth from sclerotia of *C. coccodes* occurred at the highest osmotic potential. The selected matric potentials for this study, −0.7 MPa (unmodified) and −1.4 MPa, correspond to 0.995 and 0.99 of water activity (a_w), respectively. However, a matric potential of −1.5 MPa (0.99 a_w) is the wilting point for plants. Thus, this experiment facilitated the observation of the pathogen's behaviour under conditions to simulate the tuberization stage of the potato plant. The ability to grow under low water potentials would be advantageous in dry soil and would be of value to vascular pathogens after the death of the plant when they spread to other host tissues and form new survival structures before the colonisation of these tissues by soil saprophyte. It is known that black dot is more common in wet than dry seasons (Schmiedeknecht, 1956).

Results on fungal growth under storage-like conditions (NPDA media experiments) showed temperature is correlated with fungal growth of both silver scurf and black dot isolates. These findings are supported by Glais-Varlet et al. (2004) who studied the in vitro development of *C. coccodes* on malt agar at temperatures ranging from 5 to 27 °C. They recorded an increase of growth rates of >0.5 cm day^{-1} at higher temperatures (20 to 27 °C). At lower temperatures (5 °C), the growth rate of black dot isolates was below 0.2 cm day^{-1}, similar values to the ones obtained in the present study. The severity of symptoms is expected to increase with higher temperatures or prolonged storage durations, particularly in conditions where tubers are subjected to humidity

(Massana-Codina et al., 2021). Yet, in the UK washed and pre-pack potato market, the optimum temperature for long-term storage is ca. 2.5–3.5 °C where development of the fungal disease are slowed down (Cunnington and Pringle, 2012; Peters et al., 2016). The causal agent of black dot exhibited a faster growth rate under retailer-like conditions (i.e., 15 °C) compared to *H. solani*. Based on anecdotal data from industry, potato black dot and silver scurf seem to present similar infection patterns at their early developmental stages, and it becomes difficult to quantify the area of the tuber affected by black dot and/or silver scurf lesions later on during storage since sometimes they overlap. The development of fungal disease in plants is dependent on many complex interactions between host, pathogen and environment. Water activity, temperature and pH are significant factors in the growth of soilborne plant pathogens including *C. coccodes* and *H. solani*. Competition and antagonism between pathogens for host tissue may also affect disease development and this may occur between *C. coccodes* and *H. solani* on potato tubers (Read, 1993). Therefore, further research should aim to investigate the competition and interaction dynamics between both isolates.

5. Conclusions

The growth rate of various strains of *C. coccodes* has been subject to prior investigation across different growth medias and temperature conditions, thereby determining the optimal temperature range for fungal growth (Dillard, 1988). Yet, the current study is the first to report the use of natural potato dextrose agar, to closely mimic the properties of potato tubers and provide a more accurate description of the potential development of the disease. Moreover, this is the first time that the effect of matric potential has been studied on different strains of *C. coccodes* to simulate soil conditions under a potential climate change scenario, highlighting the importance to understand the abiotic factors affecting the development of potato pathogens. When simulating field environment, drier conditions (matric potential = −1.4 MPa) reduced fungal growth for both isolates by 0.1 cm day^{-1} at the temperature of 15 °C, suggesting temperature as the main limiting factor for the growth of *C. coccodes* in the soil. The causal agent of black dot exhibited a faster growth rate under retailer-like conditions (i.e., 15 °C) compared to *H. solani*. Investigating the behaviour of the causal agents of black dot and silver scurf in controlled in vitro conditions can be considered as the first step before studying the development of the pathogen (and the diseases) in vivo in larger-scale field or storage trials. Such information is essential for the development of preventive measures as part of an integrated approach of controlling and managing potato black dot including optimal storage conditions, and irrigation.

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CRediT authorship contribution statement

Marta Sanzo-Miró: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Angel Medina:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Leon A. Terry:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **M. Carmen Alamar:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

None.

Data availability

The datasets generated and analysed in this study can be found in CORD using the following link http://doi.org/10.17862/cranfield.rd.25145684.

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