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Survival and germinability of *Rhynchosporium secalis* conidia exposed to solar radiation

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Summary *Rhynchosporium secalis*, the causal agent of barley scald disease, is a fungus commonly found in the environment. Disease spread within a field and between fields occurs through the aerial dispersal of the fungal spores. However, not much is known about the survival potential of fungal conidia exposed to solar radiation. In the present study, detached conidia of *R. secalis* were exposed simultaneously in the field to direct sunlight or placed in an adjacent ventilated enclosure in the dark for periods ranging from 0.5 to 8h. In addition, conidia were either exposed or not exposed to UV-C light (254 nm) for periods ranging between 0.5 and 60 min in the laboratory. After exposure, conidia were placed on water agar Petri dishes and allowed to germinate for 24h. Germinability of conidia was reduced by up to 94% after 8h of exposure to solar irradiance (670-860 Wm^{-2}) in the field in comparison to the non-exposed control. Germinability of conidia in the laboratory was reduced up to ~100% by doses of UV-C light of 3.2±0.7 Wm^{-2}. The results of this study will contribute to a better understanding of the relationship between climatic conditions and barley scald epidemics.

Additional keywords: barley scald, climate, spore survival, UV-C light, virulence

Introduction

Aerial dispersal of inoculum is considered an important factor in the epidemiology of many fungal plant diseases (Aylor, 2003; Stanosz *et al.,* 2016). *Rhynchosporium secalis* (Oudem) J. J. Davis, the causal agent of scald disease, is an important pathogen of barley (*Hordeum vulgare* L.) worldwide (Zhan *et al.,* 2008). *R. secalis* is considered economically important because it can cause marked reduction in crop yield and quality (Yahyaoui, 2003). The appearance of scald disease in a field with no previous history of barley cropping can be attributed either to the use of infected seed or to the transportation of airborne putative ascospores from sources outside the field. Once infection is established in a crop, conidia and/or putative ascospores can then be easily dispersed throughout the crop by rain splashing or wind (Fountaine *et al.,* 2010). Therefore, the recent resurgence of the scald in new barley cultivation areas due to the arrival of more aggressive isolates of the pathogen underscores the need for renewed and improved management of the disease. Clearly, forecasts could be improved if they included knowledge of the presence or absence of the pathogen in an area.

Not much is known whether *R. secalis* conidia could be transported to a field from an outside source, and, if transported, whether they would initiate disease. Several researchers have determined the potential of *R. secalis* for *in vitro* spore production (Stedman, 1980; Avrova and Knogge, 2012). However, to our knowledge, there have been no published studies related to the escape of *R. secalis* conidia in the air and their capacity to survive exposure to direct sunlightand incite disease.

The purpose of the present work was to investigate the survival potential of *R. secalis* conidia exposed to solar radiation in the field, as measured by their ability to germinate after exposure to direct sunlight for various lengths of time. The effect of UV-C light on the germinability of *R. secalis* conidia under controlled conditions was also evaluated.

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Materials and Methods

Experimental material

Rhynchosporium secalis pathotype Rs 22, which is one of the most virulent Syrian pathotypes to all barley genotypes available so far (Arabi *et al.,* 2010), was used in this study. At the end of the growing season, symptomatic barley leaves, naturally infected by pathotype Rs22, were collected from the field. In the laboratory, leaf sections measuring 1.5 cm x 1 cm bearing well-developed scald lesions were cut, placed at 18 °C in the dark and wetted twice a day using a high-pressure sprayer in order to stimulate the production of conidia. Sporulation on leaf lesions was checked under a light microscope. After 7 days, produced conidia were transferred from diseased leaf sections to coverslips (18 x 18 x 0.14 mm) by lightly pressing the coverslip on the surface of a sporulating lesion. The coverslips were then placed (conidia facing up) on a plastic window screen stretched on a lightweight wooden frame (20 cm^2). The coverslips were fixed on the screen with a small piece of transparent, double-sided adhesive tape (Aylor and Sanogo, 1997).

Exposure tests in the field

The experiments were performed under field conditions during the summer of two consecutive years (2013-2014). Samples (coverslips carrying the conidia) were exposed either to direct sunlight or to darkness for time periods ranging from 0.5 to 8 h. For each exposure period, two sets of samples were used: one set was exposed to direct sunlight and another one to darkness (control). For exposing the samples to direct sunlight, the plastic window screen carrying the coverslips with the conidia was placed in the field 1.1 m above the ground under full sunlight conditions. For exposing the second set of samples to darkness in the field, the window screen with the coverslips was placed in a darkened enclosure with ventilation. Six coverslips per treatment were used as replicates. At the beginning of each exposure time, conidia were collected from each replicate coverslip, placed immediately on 1.5% water agar Petri dishes, incubated at 20-22°C in the dark for 24h, and served as non-exposed controls (G_0). The temperature of the coverslips carrying conidia was determined with a Multi-thermometer apparatus with copper thermocouple wires fixed to the bottom of two additional coverslips without conidia (one coverslip for each treatment) placed in the same conditions as those that carried the conidia.

Exposure tests in the laboratory

Conidia produced on barley leaves, using the method described above, were transferred to coverslips and were handled as described for the field tests. The coverslips with conidia were exposed in the laboratory at room temperature (22 to 25°C) to short-wavelength (254 nm) UV light (UV-C light) emitted from ultraviolet tubes (TUV-30 W/G T8-UV-C; Philips, The Netherlands) for 0.5, 5, 15, 30, and 60 min. The distance between the conidia and the UV-C light tubes was ~15 cm. The UV irradiance at the level of the conidia was 3.2±0.7 Wm^{-2} as measured with a UVX-CR radiometer with a UVX-25 sensor (Ultraviolet Products, Cambridge, UK). Three replicate coverslips were used for each exposure period and treatment (with and without exposure to UV-light). The non-exposed to UV-C light coverslips (control) were kept under room temperature. The experiment was repeated three times.

Assessment of conidial viability

At the end of each exposure time in both the field and the laboratory tests, exposed and non-exposed coverslips with conidia were collected and placed (conidia facing down) on 1.5% water agar medium contained in 9-cm plastic Petri dishes (6 Petri dishes per treatment) and incubated at 20-22°C in the dark for 24h to allow germination. Germinated conidia were counted in random fields at x 100 magnification with a light microscope. A total of 100 to 200 conidia was examined on each coverslip. A conidium was considered germinated if the length of the germ tube was greater than or equal

to the length of the conidium (12.5-14.3 µm) (Manners, 1966). For calculating the percentage of conidial germination, the following formula was used:

Germination (%) = (Number of germinated conidia x 100)/total number of conidia examined.

Meteorological data

Solar irradiance, air temperature, relative humidity (RH) and wind speed were measured in the field during the whole experimental period. Solar irradiance (Wm^{-2}) was measured with a pyranometer (LI 200 SB, Li-COR. Int., Lincoln, NE 68504) located at a height 1.1m above the ground. Air temperature and RH were measured with a platinum resistance-thermometer sensor (Pt100) of DIN EN 60751, Class B (ES Electronic Sensor GmBH, Germany) and wind speed with a cup anemometer (model 014A, COMBILOG, Theodor Friendrichs & Co. Hamburg, Germany). All instruments were located at the same height above the ground as the coverslips carrying the conidia (i.e. 1.1 m). The meteorological data were recorded at 1h intervals using a data logger (COMBILOG 1020, Theodor Friendrichs & Co. Hamburg, Germany).

Virulence tests

The virulence of conidia exposed for 8h to solar radiation and those that were not exposed was tested in the field using three barley cultivars; WI 2291 from Australia, and Arabi Abiad and Tadmor from Syria. Those cultivars were selected for their different levels of resistance to scald disease (Arabi *et al.*, 2010). The experiment was located at a site with favorable environmental conditions for the development of the disease. Seeds were sown under rainfed conditions (500 mm annual rainfall) in a completely randomized block design, with three replicate plots for each treatment (50 plants per replicate and cultivar). Each replicate plot was 1 × 1 m, with 1m wide buffer zone around it. Each plot consisted of five rows, 25 cm apart, with 10 seeds sown per row. Inoculum of pathotype Rs22 was prepared from conidia produced on 2- to 3-week-old cultures grown on Lima Bean Agar (LBA) medium. Conidia were suspended in water and their concentration was adjusted with a hematocytometer to 0.5×10^6 conidia/mL. A surfactant (polyoxyethylene-20-sorbitan monolaurate) was added (100 µL/L) to the conidial suspension to facilitate dispersion of the inoculum over the leaf surfaces.

Inoculations were timed to correspond with periods of free moisture when possible. Three inoculations were performed using a fine mist hand-held sprayer. The first inoculation was applied when plants began to tiller (GS 31) and the last when plants were at least 30 cm height (Zadoks *et al.,* 1974). Non-inoculated plants (control) were sprayed with distilled water. Disease severity was assessed as a percentage of the second leaf lamina showing symptoms, 17 days post-inoculation, according to the method used by Ceolini (1980).

Statistical analysis

Data were analyzed using the STAT-ITCF programme (MICROSTA, realized by ECOSOFT, 2nd Version, Institut Technique des Céréales et des Fourrages Paris). Analysis of variance (Newman-Keuls test) was conducted to test for differences among exposure periods in test sets. The germination for conidia exposed to sunlight was calculated as G_S/G_0, and for conidia that were not exposed to sunlight (controls) as G_{NS}/G_0 where, G_S: the average of absolute germination percentage for exposed conidia, G_{NS}: the average of absolute germination percentage for non-exposed conidia, and $G_{0:}$ the average of absolute germination percentage for non-exposed conidia at the beginning of the exposure period (time 0). The germinability of conidia exposed to sunlight was compared to that of conidia not exposed to sunlight using the formula: G_{NS}-G_S/G_{NS}.

Results and Discussion

The environmental conditions that prevailed in the field during the exposure of *R. secalis* conidia to direct sunlight are presented

in Table 1. Germinability of *R. secalis* conidia was significantly (P<0.001) reduced by up to 94% after 8h of exposure to direct sunlight in comparison to the non-exposed controls (Table 2). Results showed a highly significant decrease in the germination of conidia with increasing exposure time to direct sunlight (Table 2). In addition, significant differences (P<0.05) in the virulence between exposed and non-exposed to sunlight conidia were observed (Table 3).

Survival of conidia is of paramount importance in the build-up of *R. secalis* inoculum, as conidia transported from infected plant residues *via* wind or rain can germinate and infect healthy barley plants. Very little is known about the effect of sunlight in the survival of these conidia. In the present work, conidia collected on coverslips and either exposed or not exposed to solar radiation were used to represent conidia naturally deposited on the upper surface of a barley leaf. Although there may have been differences in the ability of conidia of different ages to germinate the germination of non-exposed conidia were used as a baseline (control) to compensate these differences. Under the conditions of our study, germinability of *R. secalis* conidia was reduced significantly by exposure to solar radiation. These results agree with those of earlier studies that have demonstrated a significant effect of light on the germinability of plant pathogens in other pathosystems (Rotem and Aust, 1991; Ben-Yephet and Shtienberg, 1994; Braga *et al.,* 2015; Cordo *et al.,* 2017).

Our findings clearly indicate that solar radiation significantly affects *R. secalis* conidial viability, which mainly depends on the duration of exposure. Considering the impacts of these findings on the epidemiology and the spread of *R. secalis* conidia with a potential of causing infection at a given location, the later could be reasonably predicted through detailed analyses of the available solar radiation data along with the travel path of the conidia (Isard *et al.,* 2005). On the other hand, conidial pigmentation has been reported to play an essential role in solar radiation protective mechanisms (Swan, 1974; Ignoffo and Garcia, 1992; Butler and Day, 1998; Fuller *et al.,* 2015), which might be a possible explanation of *R. secalis* tolerance to solar exposure up to 8 h.

UV-C exposure (3.2±0.7 Wm^{-2}) significantly (P<0.001) reduced the germinability of *R. secalis* conidia (Fig. 1). The reduction in germinability increased with increasing time of exposure to UV irradiation. It is well known that the UV-C wavelength is highly efficient

Table 1. Environmental conditions that prevailed in the field during the exposure of *Rhynchosporium secalis* conidia to direct sunlight.

Variable	Range (Unit)
Air temperature	26-38 (°C)
Relative humidity	30-47 (%)
Average wind speed at sample height	0.9-4.1 (m/s)
Irradiance of incident solar radiation	670-860 (Wm^{-2})

Table 2. Germination (%) of *Rhynchosporium secalis* conidia exposed (Gs) and not exposed (G_{NS}) to direct sunlight in the field for periods ranging from 0.5 to 8 h.

Treatment	Time (h)								
	0.5	1	2	3	4	5	6	7	8
G_S	A83.3b[x]	B74.4b	C65.7b	C60.3b	D50.1b	E37.9b	E32.2b	E25.6b	F11.1b
G_{NS}	A94.3a	A94.6a	A97.5a	A98.5a	A98.8a	A98.9a	A98.9a	A98.1a	A98.3a
$(G_{NS}-G_S/G_{NS})$	0.12	0.21	0.32	0.39	0.49	0.62	0.67	0.74	0.89

[x] Means preceded by different capital letters (column) and followed by different lowercase letters (row) differ significantly at P<0.001 according to Newman-Keuls test.

Table 3. Mean scald disease severity (% of the second leaf lamina showing symptoms) 17 days after the inoculation of three barley (*Hordeum vulgare*) cultivars with *Rhynchosporium secalis* conidia, previously exposed (Gs) or not exposed (G_{NS}) to direct sunlight for 8 h under field conditions.

Cultivar	Disease severity (%)[x]	
	G_{NS}	G_S
WI2291	A89.3a[y]	A77.57b
Arabi Abiad	B76.7a	B57.43b
Tadmor	C17.1a	C11.10b

[x] Mean of three replicates.
[y] Means preceded by different capital letters (column) and followed by different lowercase letters (raw) are significantly different at (P<0.05) by Newman-Keuls test.

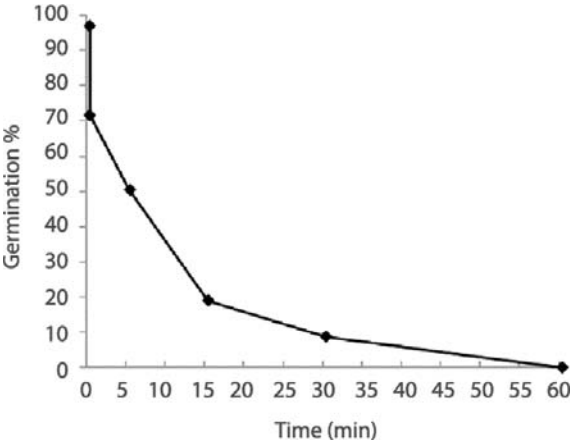

Figure 1. Germination (%) of *Rhynchosporium secalis* conidia exposed to UV-C light (254 nm) for different time periods at room temperature (22-25°C). The UV irradiance at the level of conidia was 3.2±0.7 Wm^{-2}.

in killing most micro-organisms. This method has been used extensively in sterilization procedures with low energy germicidal lamps, which emit radiation principally at 254 nm. These results are consistent with reports on other fungi, such as *Aspergillus carbonarius*, *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium janthinellum, Alternaria alternata* and *Venturia inaequalis* (Aylor and Sanogo, 1997; Valero *et al.,* 2007).

Overall, the present study, which is part of an on-going project on the epidemiology of scald disease on barley in Syria, provides new information on the effect of solar radiation on the survival of *R. secalis* conidia during their aerial dispersal. Solar radiation affects the viability of *R. secalis* conidia, and thus, it is an important factor in their survival while being airborne. This knowledge is important because dispersal limitation of the *R. secalis* population may drive very interesting ecological dynamics. Models of airborne dispersal of plant pathogens show that the rate of long-distance dispersal depends strongly on the longevity of propagules in the atmosphere (Aylor, 2003; Wilkinson *et al.,* 2012). The influence of environmental factors, such as cloud cover, wind, etc. on solar radiation and their effect on conidia viability need to be also considered.

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Επιβίωση και βλαστικότητα κονιδίων του μύκητα *Rhynchosporium secalis* μετά από έκθεση στην ηλιακή ακτινοβολία

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Περίληψη Ο φυτοπαθογόνος μύκητας *Rhynchosporium secalis*, που προκαλεί στο κριθάρι την ασθένεια ρυγχοσπορίωση, απαντάται συχνά στο περιβάλλον. Η εξάπλωση της ασθένειας εντός του αγρού ή μεταξύ των αγρών επιτυγχάνεται μέσω των αερομεταφερομένων σπορίων του μύκητα. Εντούτοις, δεν υπάρχουν επαρκείς πληροφορίες σχετικά με την επιβίωση των κονιδίων του μύκητα που εκτίθενται στην ηλιακή ακτινοβολία. Στην παρούσα μελέτη, κονίδια του *R. secalis* εκτέθηκαν ταυτοχρόνως και σε γειτνιάζουσα θέση στον αγρό είτε απευθείας στο ηλιακό φως είτε στο σκοτάδι (εντός ενός επαρκώς αεριζόμενου κλειστού θαλάμου), για περιόδους που κυμαίνονταν από 0,5 έως 8 ώρες. Επιπροσθέτως, κονίδια του μύκητα εκτέθηκαν ή δεν εκτέθηκαν (μάρτυρας) σε UV-C ακτινοβολία (254 nm) για περιόδους που κυμαίνονταν από 0,5 έως 60 λεπτά, στο εργαστήριο. Μετά την έκθεση, τα κονίδια τοποθετήθηκαν σε τρυβλία Petri που περιείχαν άγαρ και αφέθηκαν να βλαστήσουν για 24 ώρες. Η βλαστικότητα

των κονιδίων που εκτέθηκαν για 8 ώρες στην ηλιακή ακτινοβολία (670-860 Wm^{-2}) στον αγρό μειώθηκε έως και 94% σε σχέση με εκείνη του μη εκτεθειμένου μάρτυρα. Σε συνθήκες εργαστηρίου, δόση UV-C ακτινοβολίας 3.2 ± 0.7 Wm^{-2} μείωσε τη βλαστικότητα των κονιδίων του μύκητα έως και ~100%. Τα αποτελέσματα της παρούσας μελέτης θα συμβάλουν στην καλύτερη κατανόηση της σχέσης μεταξύ των κλιματικών συνθηκών και της εμφάνισης επιδημιών της ασθένειας.

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