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UV-C irradiation compromises conidial germination, formation of appressoria, and induces transcription of three putative photolyase genes in the barley powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*

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ABSTRACT

UV-C irradiation is known to compromise germination of *Blumeria graminis* conidia and to reduce powdery mildew infestation. However, only scarce information is available on the effects of UV-C irradiation on *B. graminis* appressorium formation. Applying a Formvar® resin-based *in vitro* system allowed for analyzing *B. graminis* germination and appressorium formation in absence of plant defense. UV-C irradiation more strongly affected the differentiation of appressoria than conidial germination. *In vivo* and *in vitro*, a single dose of 100 J m^{-2} UV-C was sufficient to reduce germination to less than 20 % and decrease appressorium formation to values below 5 %. UV-C irradiation negatively affected pustule size and conidiation. White light-mediated photoreactivation was most effective immediately after UV-C irradiation, indicating that a prolonged phase of darkness after UV-C treatment increases the efficacy of *B. graminis* control. UV-C irradiation increased transcript levels of three putative *B. graminis* photolyase genes, while mere white light or blue light irradiation did not contribute to the transcriptional up-regulation. Thus, UV-C irradiation effectively controls *B. graminis* infestation and proliferation by restricting prepenetration processes. Nevertheless, photoreactivation plays an important role in UV-C-based powdery mildew control in crops and hence has to be considered for planning specific irradiation schedules.

1. Introduction

Blumeria graminis is an obligate biotrophic ascomycete that infests relevant crop species such as wheat and barley. It causes a destructive foliar disease that inflicts severe economic losses. Hence, this organism is regarded as the world's sixth most important fungal phytopathogen (Dean et al., 2012). Currently, *B. graminis* is most effectively controlled applying a range of fungicides, which may pose environmental problems. Moreover, *B. graminis* is considered as a plant pathogen showing a high risk of development of resistance to fungicides (FRAC Pathogen Risk List, www.frac.info). A more sustainable, biological control of *B. graminis* is therefore in the focus of current research efforts (Kiss, 2003; Laur et al., 2018). However, in recent years a physical alternative, the application of ultraviolet (UV) radiation, has shown great potential in the management of powdery mildew disease in several crops and ornamental plants (Janisiewicz et al., 2016; Suthaparan et al., 2012a; Willocquet et al., 1996). However, our knowledge of the effects of UV-light on *B. graminis* remains scarce. Buxton et al. (1957) reported that a single UV-C dose of approx. 54 J m^{-2} resulted in a 95 % reduction of infectivity in *B. graminis*, while the germination of *B. graminis* conidia dusted onto ordinary glass slides prior to irradiation was reduced by more than 90 %. A similar study corroborated a more prominent impact of UV-C irradiation on infectivity rather than on conidial germination on water agar and suggested a higher efficacy of UV-treatment before the establishment of fungal infection (Moseman and Greeley, 1966). UV-irradiation not only affects early events of powdery mildew infection but also stimulates plant defense (Mintoff et al., 2015; Urban

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et al., 2018). In barley leaves, UV-irradiation leads to the accumulation of the potential defense compound tryptamine, which was shown to inhibit germination of *B. graminis* conidia *in vitro* (Miyagawa et al., 1994). UV-light also induced transcription of a chalcone synthase gene involved in flavonoid phytoalexin production (Christensen et al., 1998). Therefore the contribution of direct or indirect effects of UV-irradiation to the observed anti-mildew effect remains vague, particularly since a strongly UV-absorbing pigment, 3-hydroxykynurenine, has been identified in *B. graminis* (Wilson et al., 2003). While melanin has not been detected in mildew, 3-hydroxykynurenine, abundantly present in germinating conidia and hyphae, might act as an effective UV-protectant (Wilson et al., 2003). Since foliar plant pathogens are subject to sunlight, they must have evolved effective means for protection against UV.

B. graminis mainly propagates asexually via the production of wind-dispersed conidia. When a conidium lands on a suitable host surface, it germinates and produces a short primary germ tube, which consecutively penetrates the plant cuticle (Edwards, 2002; Yamaoka et al., 2006). Then, a secondary germ tube forms, stretches, swells and differentiates into an appressorial germ tube that develops into a lobed and septate infection structure, the appressorium. The fully differentiated appressorium subsequently attempts to forcefully penetrate both host cuticle and epidermal cell wall to form the feeding structure, the haustorium, which provides the fungal pathogen with water and nutrients required for the formation of secondary hyphae and for the growth of an extended mycelium on the host leaf surface. The initial phases of infection, germination and differentiation, summarized as prepenetration processes, are usually completed within 18 h post-inoculation (Zhang et al., 2005). For efficient germination and appressorium formation, *B. graminis* conidia require a hydrophobic surface in combination with specific cuticular wax components (Hansjakob et al., 2010; Zabka et al., 2008; Zhu et al., 2017). Therefore, analyzing the germination behavior of *B. graminis* on hydrophilic agar or glass surfaces in absence of the germination and differentiation inducing wax constituents results in abnormal prepenetration and consequently in misleading or inconsistent data (Yarwood, 1957). By applying a Formvar®-based *in vitro*-system, essentially consisting of a Formvar® membrane containing hydrophobic host leaf wax or host leaf wax constituents that induce the *B. graminis* prepenetration processes, a possible interference with plant defense during prepenetration can be fully excluded (Hansjakob et al., 2012; Hildebrandt et al., 2018). Currently, there is no information available concerning the effects of UV irradiation on the differentiation of appressorial germ tubes and formation of functional appressoria in *B. graminis*.

UV-C has been shown to have deleterious effects on proteins and DNA. Absorption of UV-C photons by DNA induces two major products indicative of DNA damage: cyclobutane-pyrimidine dimers and pyrimidine(6–4)pyrimidone photoproducts. Although both photoproducts lead to mutagenesis, the major UV-C photoproduct is the cyclobutane pyrimidine dimer (CPD), which typically accounts for about 75 % of UV-C photoproducts in DNA (Manova et al., 2016). The photoproducts inhibit transcription, prevent genome replication and thus cell division. As efficient countermeasures, organisms have developed a number of highly conserved repair mechanisms such as base excision repair, nucleotide excision repair, mismatch repair, and photoreactivation that uses blue or near UV light energy for repair (Rastogi et al., 2010). Photoreactivation reverses cyclobutane pyrimidine dimers and 6-4 photoproducts into the intact monomer bases, catalyzed by a light-dependent photolyase enzymatic process (Thiagarajan et al., 2011). Photoreactivation has also been documented for *B. graminis* and other powdery mildew fungi after UV-C irradiation (Buxton et al., 1957; Suthaparan et al., 2018). In the closely related gray mold fungus *Botrytis cinerea* white and blue light irradiation strongly induced two UV-damage repair-related genes in a *Bcwcl1* blue light receptor-dependent manner (Zhu et al., 2018). The annotated *B. graminis* f. sp. *hordei* DH14 genome (Spanu et al., 2010) contains three members of the cryptochrome/DNA photolyase gene family encoding proteins that might be involved in photoreactivation processes. The first gene, BGHDH14_bgh00666, codes for a protein (Genbank: CCU77936.1) orthologous to the CPD DNA photolyase CRY1 (amino acid identity of 57 %) from *B. cinerea*, where this protein constitutes the major photolyase activity in photoreactivation (Cohrs and Schumacher, 2017). The product of the second gene BGHDH14_bgh01427 (Genbank: CCU75653.1), however, shows a 58 % amino acid identity to the 6-4 DNA photolyase Cry1 of *Trichoderma reesei* (Guzmán-Moreno et al., 2014) and hence may also exhibit 6-4 photoproduct repair activity. Both genes constitute the EggNog ortholog group: OG5_127186 in *B. graminis* (Huerta-Cepas et al., 2016). A third gene, BGHDH14_bghG001129 000001001, encodes a putative Cry-DASH protein (Genbank: CCU75260.1), whose *B. cinerea* ortholog *Cry*2 (59 % amino acid identity), as in other fungi, appears to be largely dispensable for photorepair (Cohrs and Schumacher, 2017). However, Cry-DASH proteins were shown to repair CPD lesions in single-stranded DNA and loop structures of double-stranded DNA (Pokorny et al., 2008; Selby and Sancar, 2006). To date, however, there is no information available concerning photolyase gene expression and transcriptional regulation in *B. graminis* and other powdery mildew pathogens.

By applying the Formvar® *in vitro*-system, the central aim of the present study was to analyze the dose-dependent effects of UV-C irradiation on the prepenetration processes of *B. graminis*, in absence of living host tissue and thus without possible interference with plant defense. We further aimed at determining the capability of conidial photoreactivation *in vitro* and *in vivo*, and at analyzing the transcriptional responses of the putative *B. graminis* f.sp. *hordei* photolyase genes upon UV-C irradiation under different light conditions using a qRT-PCR based assay.

2. Materials and methods

2.1. Plant and fungal material

Barley (*Hordeum vulgare* L. cv. Stendal) caryopses were sown in plastic posts (⌀,9 cm) filled with standard potting soil (Typ ED73; SteuderComp, Schermbeck, Germany) and kept in growth chambers in a 16 h: 8 h (light/dark) photoperiod (white light intensity, 230 ± 10 μmol photons m^{-2} s^{-1}) at 20 °C: 18 °C and 70 % relative humidity. The white light inside the growth chamber was provided by fluorescent bulbs (F17T8/TL741, Philips, USA). *B. graminis* (DC.) E.O. Speer f. sp. *hordei* Em. Marchal [isolate A6] (*Bgh*) was propagated on its host (barley, *H. vulgare* L. cv. Stendal) under the same conditions as described above. One day before conidia were required for experimentation, heavily infected leaves were cautiously shaken to remove older conidia in order to obtain freshly produced spores available for subsequent experimentation.

2.2. Preparation of surfaces

Total leaf wax extracts of barley were isolated by dipping about sixty 12–14 d old entire leaves for 60 s into 50 ml chloroform (>99 %, Roth, Karlsruhe, Germany). In order to remove the solvent and residual water, the chloroform/wax solutions were dried under a continuous stream of nitrogen gas. The wax extracts were then redissolved in chloroform and finally added to the 0.5 % solution of Formvar® resin (polyvinyl formal, CAS 63450-15-7, Applichem). The final concentrations of wax were 0.5–1 mg ml^{-1} (Hansjakob et al., 2010).

Standard microscopy glass slides or Histobond® glass slides were thoroughly cleansed with Deconex® 11 UNIVERSAL detergent (Applichem, Darmstadt, Germany) solution (v/v, 1:200), subsequently rinsed off with distilled water, immersed in isopropanol for 30 s, then in distilled water for 10 min and finally dried at room temperature.

The dried standard glass slides were dipped into a solution of 1 % (w/v) polyvinylpyrrolidone 40 (PVP40) (Sigma–Aldrich, Darmstadt, Germany) and dried for 24 h at room temperature. The PVP40-coated slides were then dipped into a 0.5 % Formvar® (polyvinylformal, Applichem, Darmstadt, Germany) solution in chloroform supplemented with barley leaf wax extract (0.5 mg ml^{-1}) and subsequently dried for 24 h at room temperature to remove solvent traces. The edges of the slides were carefully scratched by fine sandpaper and then slowly immersed in distilled water at an angle of about 45°. This treatment led to the solubilization of the PVP coating and consequently to the release of Formvar®/barley leaf wax membranes floating on the water surface. One side of a glass slide was covered with 500 μl of 2 % Bacto water agar. After gelling of the agar, the slide was submersed below the floating membrane. The slide was carefully pulled out again at a 45° angle, resulting in the transfer a Formvar®/barley leaf wax membrane attached to the agar surface. Excess water was gently dabbed off by touching the edges of the slide with filter paper.

The Histobond® glass slides were directly dipped into the 0.5 % Formvar® solution in chloroform supplemented with barley leaf wax extract and subsequently dried for 24 h at room temperature to remove solvent traces. The Formvar®/barley wax coated Histobond® glass slides were subsequently inoculated with *B. graminis* conidia, which were then used for isolation of total RNA.

As surface hydrophobicity is known to play an important role for the initiation of *B. graminis* prepenetration processes, the contact angles of 2 μL droplets of distilled water deposited on the Formvar®/barley leaf wax membrane/agar coated glass surfaces were determined with an OCA 15 contact angle system and SCA20 software system (Dataphysics Instruments, Filderstadt, Germany). Five measurements were performed on each coated slide. Only slides exhibiting contact angles of 105 ± 10° were subjected to further experimentation.

2.3. *Inoculation with* B. graminis *conidia*

The Formvar®/barley leaf wax/agar coated glass slides were used to investigate *B. graminis* prepenetration processes *in vitro*. In addition, adaxial surfaces of detached 12-d-old barley primary leaves were applied to study prepenetration *in vivo*. For inoculation, the coated glass slides and detached barley leaves were fixed at the base of a settling tower. Conidia from infected barley leaves were blown into the tower using pressurized air to ensure an even distribution at a density of $1.5–2 \times 10^3$ conidia cm^{-2}. In order to achieve a relative humidity of ≥90 %, coated glass slides and primary barley leaves were placed on moist filter paper and kept in glass Petri-dishes sealed with Parafilm®M (Sigma–Aldrich, Darmstadt, Germany).

2.4. *UV-C irradiation*

A BLX-254 UV crosslinker (Vilber Lourmat, Marne la Vallée, France) equipped with five fluorescent lamps of 8 W each ($\lambda = 254$ nm), and a radiometer that permanently measured the emitted UV energy was used as the UV-C light source. Inoculated conidia were treated with different doses of UV-C light for *in vitro* and *in vivo* experiments. Conidia not subjected to UV-C irradiation were used as control. Conidial germination and differentiation were analyzed after incubation periods of 18 or 48 h in darkness at 20 ± 1 °C.

2.5. *Photoreactivation*

After UV-C irradiation inoculated surfaces were immediately transferred to the growth cabinet and incubated under white light (230 ± 10 μmol photons m^{-2} s^{-1}) for 2 h and subsequently in darkness for 16 h. In order to avoid a greenhouse effect within the sealed Petri-dishes under light, the growth chamber temperature was set at 18 ± 2 °C, which resulted in surface temperatures of 20 ± 1 °C.

To evaluate the efficacy of photoreactivation after a prolonged period of darkness, conidia were treated with 100 J m^{-2} or 200 J m^{-2} UV-C, incubated in darkness for 6 h and subsequently under white light for 12 h or 18 h. Conidial germination and differentiation were analyzed *in vitro* and *in vivo*. In order to determine infection rates on detached barley leaves upon UV-C irradiation, inoculated conidia were incubated under a photoperiod (dark 6 h/light 18 h) for 3–5 d.

2.6. *Microscopic analysis*

Individual conidia *in vitro* were directly analyzed by light microscopy (Leica DMR with Leica IM1000 software package, Wetzlar, Germany). With their adaxial surface up, inoculated barley leaf surfaces were transferred onto filter paper moistened with bleaching solution (ethanol: acetic acid, 1:1, v/v) until they appeared transparent and then placed onto filter paper moistened with lactoglycerol (lactic acid: glycerol: water, 1:1:1, v/v/v) for 4 h. The fungal structures on the leaf surfaces were stained with droplets of 0.05 % (w/v) Trypan Blue (Merck, Darmstadt, Germany) in acetic acid/glycerol/water (1:1:1, v/v/v) for 45 min.

By microscopic inspection, it was determined whether conidia had remained non-germinated (ng), had formed a primary germ tube only (pgt), a non-swollen secondary germ tube (sgt), a swollen appressorial germ tube (agt) or a septate appressorium (app) (Fig. 1). Also the number of conidia that were damaged, burst or desiccated was recorded (aberr). *In vivo* we additionally analyzed whether conidia had formed a haustorium in a host epidermal cell or whether it had formed a colony with secondary hyphae. Only single, well-separated conidia were monitored and counted in order to eliminate the possibility of inhibition caused by crowding.

2.7. *Photolyase gene expression upon UV-C irradiation*

In order to investigate the transcriptional regulation of three putative *B. graminis* photolyase genes *in vitro*, conidia inoculated onto Histobond® Formvar®/wax coated glass slides were treated with or without UV-C light (100 J m^{-2}) and then incubated in darkness, under white light or blue light conditions. The blue light source was a LED ($\lambda_{max.}$ 475 nm, Philips, USA) at a fluence rate of 80 μmol photons m^{-2} s^{-1}. After incubation periods of 30 min, 2 h and 6 h, conidia were harvested by painting surfaces with 5 % (w/v) cellulose acetate dissolved in acetone. After evaporation of acetone, cellulose-acetate strips were collected, immediately frozen in liquid nitrogen and finally stored at −80 °C for further experimentation. The frozen strips were ground in liquid nitrogen with extraction buffer (50 mM Tris/Cl pH 9.0, 150 mM NaCl, 5 mM EDTA, 5 % SDS) followed by phenol/chloroform/isoamylalcohol (Roti PCI, 25:24:1, Roth, Karlsruhe, Germany) and chloroform/isoamylalcohol (24:1 (v/v) extraction. The RNA was precipitated with 3 M sodium acetate (pH 5.2) and 100 % ethanol. After DNA digestion (DNAseI kit RNeasy Mini Elute Cleanup, Quiagen, Hilden, Germany) the RNA was finally

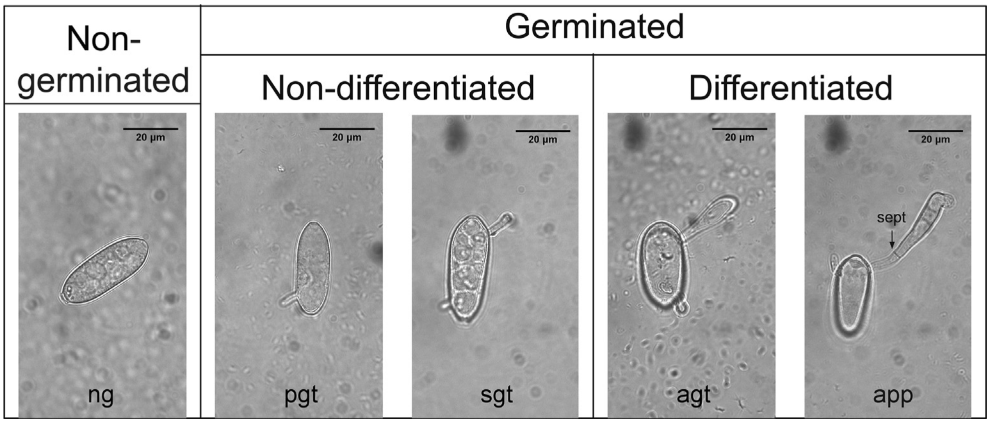

Fig. 1. Developmental stages of prepenetration processes of *B. graminis* conidia: ng, non-germinated conidium; pgt, germinated conidium with a primary germ tube only; sgt, conidium with an elongated secondary germ tube; agt, conidium with a swollen appressorial germ tube; app, conidium with a fully differentiated appressorium.

re-suspended in 15 µl diethyl pyrocarbonate-treated water. The concentration was determined by a NanoDrop™ One/OneC spectral photometer (Thermo Scientific, Darmstadt, Germany). Total RNA from three biological replicates was used for cDNA synthesis. The integrity of RNA used for complementary DNA (cDNA) synthesis was confirmed by denaturing agarose gel electrophoresis. Reverse transcription was carried out with SuperScript® III First-Strand Synthesis System for RT-PCR (Thermo Fisher Scientific, Darmstadt, Germany) according to the manufacturers protocol using oligo $(dT)_{20}$ primer. The synthesized cDNA was stored at −20 °C until further use.

For qPCR the Maxima SYBR Green qPCR Master Mix (2X) (Thermo Fisher Scientific, Waltham, MA, USA) was used according to the manufacturer's protocol with 300 nM gene specific primers in technical triplicates on a C1000™ Thermal Cycler with a CFX96™ Real-Time System (Bio Rad, Munich, Germany). A two-step cycling protocol was performed (initial denaturation 3 min at 95 °C followed by 40 cycles 10 s at 95 °C and 30 s at 60 °C) in combination with a melting curve analysis. The efficiency of the primers was determined and optimized for 90–110 % using the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH, BGHDH14_bgh00075, Genbank X99732) as internal control (Pennington et al., 2016). Gene expression was calculated according to the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Primers used for RT-qPCR were as follows:

BGHDH14_bgh00666 (5′ − 3′): PHLF (TGATAGGGCGTGTGAGTGTG) and PHLR (TCGTCAAGCAGCAAAAAGCC). BGHDH14_bgh01427 (5′ − 3′): CRYF (TGACGATGAGGATTGGGCTG) and CRYR (CCGCCACCTCTACATGGTTT); BGHDH14_bghG001129000001001: DASHF (GAGCATCCCAACACGAATGC) and DASHR (TCAGCGGAGCAAATTGAGGT); GAPDH (5′ − 3′): GAPDHF (GGAGCCGAGTACATAGTAGAGT) and GAPDHR (GGAGGGTGCCGAAATGATAAC).

2.8. Statistics

The basis for statistical analyses of conidial development was n = 3 independent biological experiments, where n = 1 represents six technical replicates (100 monitored conidia each) for *in vitro* experiments and three technical replicates (100 monitored conidia each) for *in vivo* experiments, respectively. Significant differences ($P < 0.05$) between multiple datasets were tested by Kruskal–Wallis test followed by a Bonferroni multiple comparison *post hoc* test (significant differences of normality was <0.05, Shapiro test) or one-way ANOVA test followed by a Tukey *post hoc* test (both significant differences of normality and homogeneity of variance were >0.05, Shapiro test and Levene's test). Pairwise comparisons were tested by a Student's T test (significant differences of normality was >0.05, Shapiro test). The statistical analyses were performed with IBM SPSS statistics (version 23).

3. Results

3.1. Effects of UV-C irradiation on B. graminis prepenetration processes

Conidia on Formvar®/wax/agar coated glass slides (*in vitro*) or conidia on detached barley leaves (*in vivo*) were exposed to different UV-C doses followed by an incubation in darkness for 18 h in order to assay the effects of UV-C irradiation on conidial germination and appressorium formation (differentiation). UV-C irradiation not only affected conidial germination but also the formation of appressoria, which was significantly compromised (Fig. 2). All UV-C treatments significantly increased the proportions of non-germinated conidia *in vitro* up to 76 ± 6 % at 100 J m^{-2} and concomitantly reduced the proportions of conidia that had formed appressoria when compared with non-UV-C controls in a dose-dependent manner. After an initial lag phase from 0 to 30 J m^{-2} the proportion of non-germinated conidia *in vitro* and *in vivo* almost linearly increased with ascending UV-C doses (Fig. 2C). With respect to differentiation a similar lag phase at doses up to 30 J m^{-2} was recorded (Fig. 2D). *In vitro*, the maximal rate of differentiation inhibition was achieved at 80 J m^{-2} and *in vivo* at 100 J m^{-2} (Fig. 2D). Irrespective of the underlying surface, conidia did no longer form mature functional appressoria when irradiated with a dose of 100 J m^{-2}. However, *in vivo*, on the barley leaf surface, the rates of germination and appressorium formation were generally distinctly higher than on the Formvar®/wax/agar coated glass slides.

3.2. Prolonged incubation in darkness after UV-C exposure hampered pre-penetration

In order to determine whether and to which degree *B. graminis* could repair the functional damage caused by UV-C irradiation in darkness, we treated conidia *in vitro* and *in vivo* with 100 J m^{-2} or 200 J m^{-2} UV-C light and a subsequent incubation for 48 h in the absence of light (Fig. 3). The proportions of non-germinated conidia treated with 100 J m^{-2} and 200 J m^{-2} were 54 ± 9 % and 79 ± 4 %, respectively, *in vitro*, and 52 ± 7 % and 78 ± 8 %, respectively, *in vivo*. The proportions of non-germinated conidia in the non-irradiated

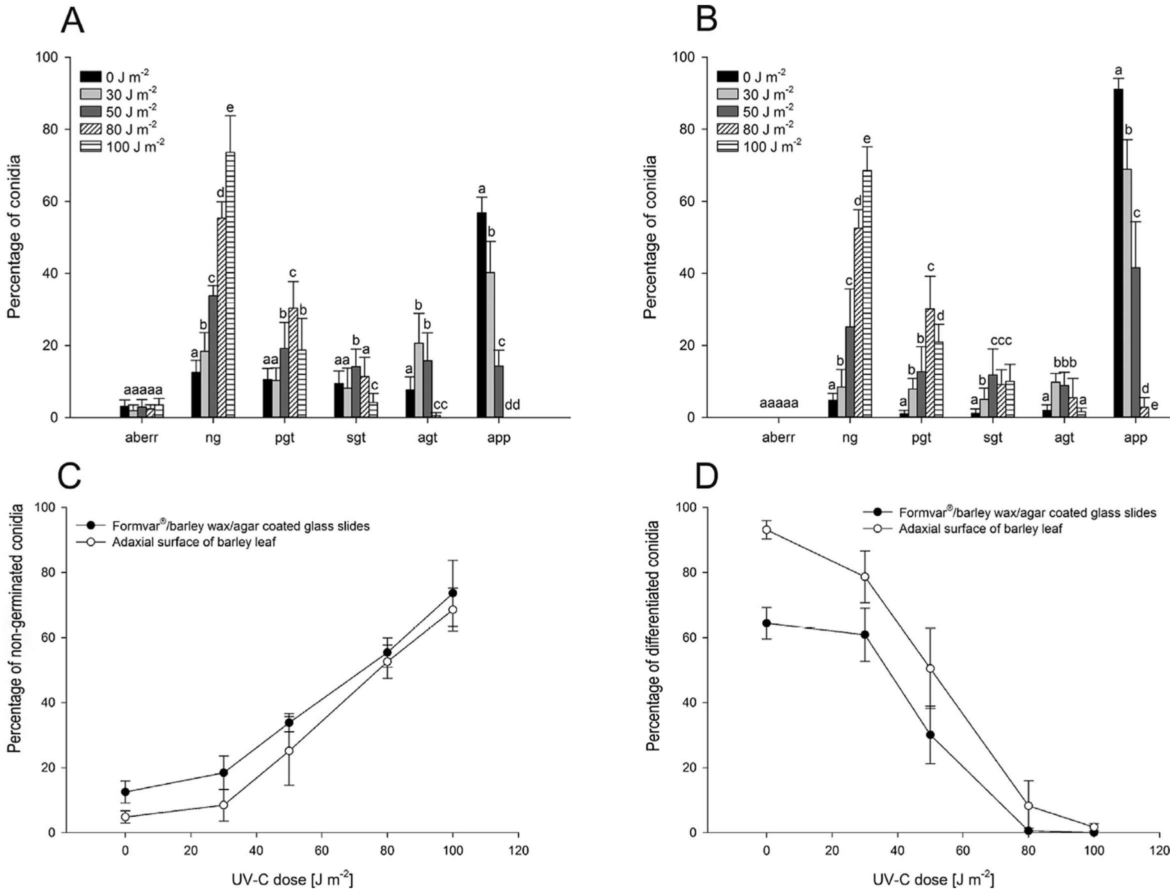

Fig. 2. Development of *Blumeria graminis* f. sp. *hordei* conidia after UV-C treatment and an incubation period of 18 h in darkness on (A) Formvar®/barley wax/agar coated glass slides and (B) adaxial surface of barley leaf. Dose-response curves showing the proportions of non-germinated conidia (C) and of differentiated conidia (agt and app) on Formvar®/barley wax/agar coated glass slides and on primary barley leaves. Prepenetration developmental stages of *B. graminis* conidia: aberr, burst, dead conidia; ng, non-germinated conidia; pgt, germinated conidia with a primary germ tube only; sgt, conidia with an elongated secondary germ tube; agt, conidia with a swollen appressorial germ tube; app, conidia with a fully differentiated appressorium. In (A) and (B), each value is given as mean ± SD of six replicates (100 conidia each) of three independent biological experiments. In (C), each value is given as mean ± SD of three replicates (100 conidia each) of three independent biological experiments. Significant differences were determined in a Kruskal–Wallis test, with *post hoc* comparison by Bonferroni multiple comparison test: different letters indicate significant differences ($P < 0.05$).

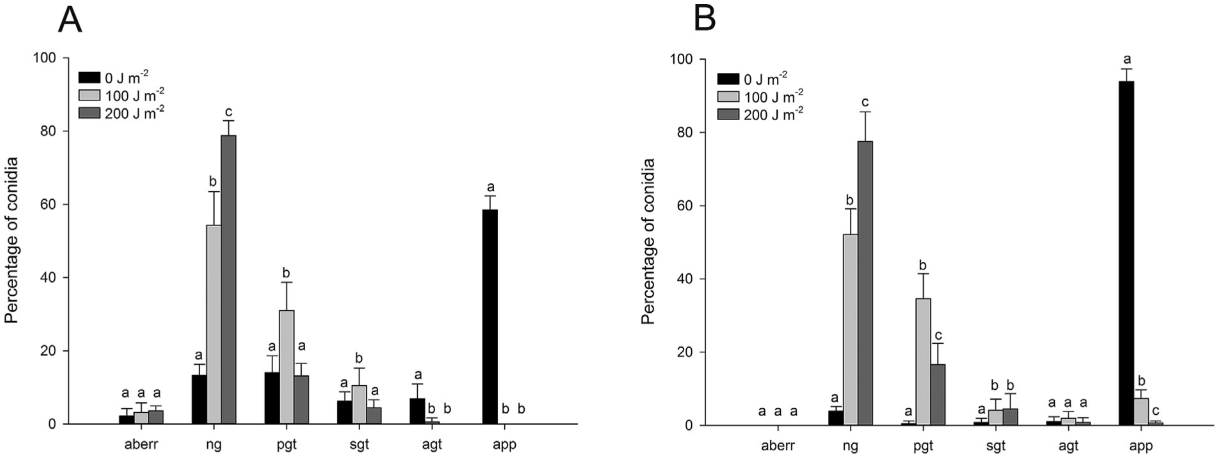

Fig. 3. Development of *Blumeria graminis* f. sp. *hordei* conidia after UV-C treatment and an incubation period of 48 h in darkness on (A) Formvar®/barley wax/agar coated glass slides and (B) on adaxial barley leaf surfaces. Developmental stages of *B. graminis* conidia: aberr, burst, dead conidia; ng, non-germinated conidia; pgt, germinated conidia with a primary germ tube only; sgt, conidia with an elongated secondary germ tube; agt, conidia with a swollen appressorial germ tube; app, conidia with a fully differentiated appressorium. In (A), each value is given as mean ± SD of six replicates (100 conidia each) of three independent biological experiments. In (B) each value is given as mean ± SD of three replicates (100 conidia each) of three independent biological experiments. Significant differences were determined in a Kruskal–Wallis test, with *post hoc* comparison by Bonferroni multiple comparison test: different letters indicate significant differences ($P < 0.05$).

control treatments were significantly lower than those with UV-C irradiation. *In vitro* none of the irradiated conidia differentiated appressoria. However, 7 ± 2 % of the conidia treated with 100 J m^{-2} UV-C light and 1 ± 1 % of conidia exposed to 200 J m^{-2} UV-C irradiation on the barley leaf surface still differentiated an appressorium. The penetration efficiency of conidia on barley leaves treated with 100 J m^{-2} or 200 J m^{-2} UV-C light, representing the percentage of conidia with a primary haustorium, was 2 ± 2 % and 0 %, respectively. Conidia that were not irradiated with UV-C light exhibited a penetration efficiency of 60 ± 7 %. The percentage of conidia remaining non-germinated after exposure to 100 J m^{-2} UV-C light and an 18 h dark incubation was 76 ± 6 % *in vitro* and 69 ± 7 % *in vivo*, respectively (Fig. 2). A dark incubation period of 48 h after irradiation reduced these proportions significantly to 54 ± 9 % *in vitro* and 52 ± 7 % *in vivo* (Fig. 3). Likewise the proportions of conidia forming appressoria increased by extending the period of dark incubation *in vivo*. Irrespective of the incubation time in darkness, only very few if any of the conidia that were treated with ≥100 J m^{-2} were able to successfully penetrate and form a haustorium.

3.3. Photoreactivation by white light irradiation

In order to determine to which extent *B. graminis* conidial germination and differentiation after UV-C irradiation is restored by a subsequent white light treatment, conidial development was analyzed after 2 h of white light irradiation followed by 16 h in darkness (Fig. 4). White light treatment resulted in a significant increase in the proportions of germinated and differentiated conidia. While a UV-C dose of 100 J m^{-2} and a subsequent incubation of 18 h in darkness completely prohibited the formation of appressoria *in vitro* (Fig. 2), a 2 h white light treatment increased the proportion of conidia with mature appressoria to 25 ± 7 %, which is 42 % of the value obtained without UV-C irradiation (Fig. 4). *In vivo* on the detached barley leaves 2 h of white light incubation after UV-C irradiation resulted in a recovery rate of appressorium formation to 46 % of the value without UV-C irradiation. The degree of recovery therefore negatively correlated with the UV-C dosage level. However, UV-C irradiation followed by 6 h darkness/12 h light resulted in distinctly reduced proportions of germinated and differentiated conidia (Fig. 5), when compared with the 2 h white light incubation immediately after UV-C irradiation (Fig. 4). At a dose of 100 J m^{-2} UV-C followed by 6 h darkness/12 h light the proportion of conidia with mature appressoria increased to only 7 ± 4 % *in vitro*, which is 12 % of the value obtained without UV-C irradiation. However, a prolonged light phase of 18 h after 6 h in darkness, resulted only in a marginal increase in the proportions of conidia with mature appressoria. *In vivo* similar results were obtained with conidia exposed to a single UV-C dose of 100 J m^{-2}. Under 6 h darkness/12 h light a dose of 200 J m^{-2} UV-C fully prevented appressorium formation *in vitro*, whereas *in vivo* at best 1 ± 1 % of the conidia still formed an appressorium. The penetration efficiency of UV-C irradiated conidia *in vivo* was analyzed after an incubation period of 24 h in darkness or 6 h darkness/18 h light regime. 14 ± 7 % of non-irradiated conidia had formed haustoria, while only 1 ± 1 % of conidia irradiated with 100 J m^{-2} UV-C and none of the conidia exposed to 200 J m^{-2} UV-C successfully penetrated the underlying host cuticle and epidermal cell wall.

3.4. UV-C irradiation reduces pustule size and affects conidiation

To analyze the suppressing effect(s) of UV-C light on *B. graminis* infection and pustule (colony) formation under more natural incubation conditions, conidia inoculated onto detached barley leaves were irradiated with 100 J m^{-2} or 200 J m^{-2} UV-C and subsequently incubated under a 6 h dark/18 h light regime for 3–5 d (Fig. 6).

After 5 d, inoculated leaves without UV-C irradiation (control) showed a pronounced infection with apparent, conidiating pustules, whereas irradiated *B. graminis* conidia rarely produced haustoria, secondary hyphae and consequently pustules (Fig. 6A). The colonies emerging from UV-C irradiated conidia 3 d after inoculation were distinctly smaller than those derived from untreated conidia (Fig. 6B, D). Doubling the UV-C dose from 100 to 200 J m^{-2} reduced pustule size by roughly 50 %. While after 4 d the first colonies derived from non-UV-C irradiated conidia started to form conidia, colonies from conidia exposed to UV-C irradiation did not exhibit conidiation even at 5 d post inoculation. In contrast to control leaves, UV-C irradiated leaves allowed for analyzing the different developmental stages that the conidia had attained and to determine the infection rates of *B. graminis* upon UV-C irradiation (Fig. 6C, D). Under the given light regime UV-C irradiated conidia partially recovered and completed the pre-penetration processes in a dose-dependent manner. Conidia exposed to 100 J m^{-2} UV-C light still infected their host leaves however at 23 ± 9 % and the formed pustules had not started to form conidia. At 200 J m^{-2} UV-C irradiation the percentage of successful penetration was decreased to 7 ± 4 %.

3.5. UV-C irradiation activates putative photolyase genes

RT-qPCR analyses were employed in order to explore the transcriptional regulation of the three *B. graminis* genes coding for proteins putatively exhibiting photolyase activity (BGHDH14_bgh00666, BGHDH14_bgh01427 and BGHDH14_bghG001129000001001) upon UV-C irradiation followed by different light conditions. Conidia exposed to 100 J m^{-2} UV-C light were incubated for different time periods in darkness, constant white light, or under blue light on Formvar®/wax coated glass slides *in vitro* (Fig. 7). Our initial analyses showed that the transcript levels of the putative photolyase gene BGHDH14_bgh00666 30 min and 2 h after UV-C irradiation were increased about threefold, irrespective of whether the conidia had been incubated in white light or in darkness (Fig. 7A, B). However, after 6 h in light the transcript levels in UV-C irradiated conidia decreased to values seen for the non-UV-C irradiated conidia, while the transcripts in the UV-C irradiated conidia incubated in darkness were roughly eightfold in number and thus remained at a distinctly increased level (Fig. 7C). For the putative Cry-DASH gene BGHDH14_bghG001129000001001 induction profiles were rather similar to BGHDH14_bgh00666 and BGHDH14_bgh01427 30 min after UV-C irradiation (Fig. 7D, G). The six-fold increase after 2 h post UV-C irradiation in darkness (Fig. 7E) was followed by a more than 20-fold up-regulation 6 h after UV-C irradiation (Fig. 7F). White light treatment, however, resulted in decreased transcript numbers at 2 and 6 h post UV-C irradiation. Transcripts of the putative 6-4 DNA photolyase gene BGHDH14_bgh01427 showed only a 2-fold increase 2 h after UV-C irradiation (Fig. 7H) and subsequent incubation in darkness while after 6 h the levels were increased by factor 6 (Fig. 7I). Incubation under white light irradiation resulted only at 6 h post UV-C irradiation in a substantial decrease of transcript numbers when compared with the non-UV-C irradiated controls.

Since photoreactivation depends on the absorption of blue light by photolyases we decided to substitute white light by a more defined blue light source (LED; $\lambda_{max.}$ 475 nm) and selected an intermediate incubation time of 2 h after UV-C irradiation (Fig. 8). For BGHDH14_bgh00666 we obtained almost the same pattern as for the previous 30 min and 2 h white light incubation. Transcripts were distinctly increased upon UV-C irradiation irrespective of subsequent light conditions (Fig. 8A). For BGHDH14_bgh01427 and BGHDH14_bghG001129000001001 incubation in darkness after

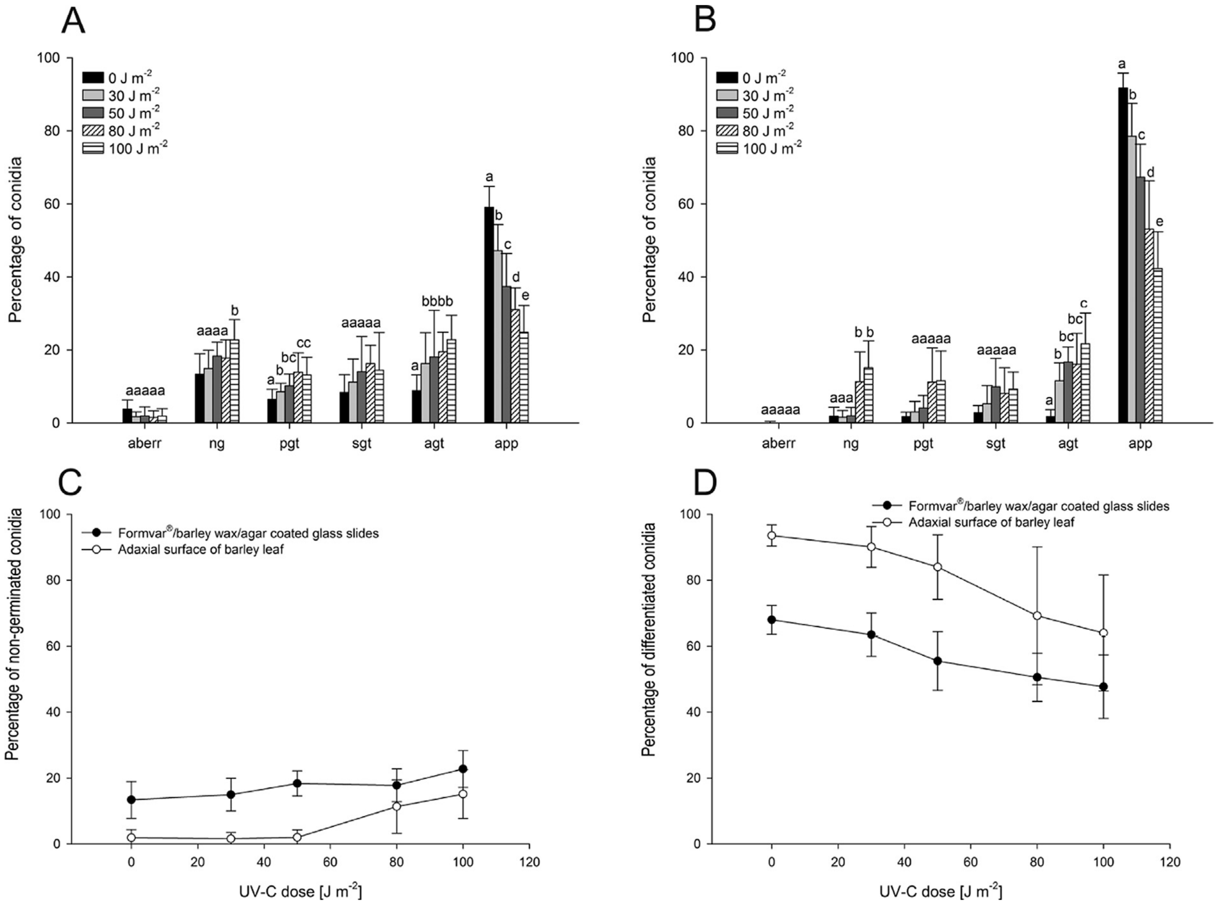

Fig. 4. Development of *Blumeria graminis* f. sp. *hordei* conidia after UV-C treatment and an incubation period of 2 h light and 16 h in darkness on (A) Formvar®/barley wax/agar coated glass slides and (B) adaxial surface of barley leaf. Dose-response curves showing the proportions of (C) non-germinated conidia and (D) of differentiated conidia (agt and app) on Formvar®/barley wax/agar coated glass slides or on primary barley leaves. Photon fluence rate was 230 ± 10 μmol $m^{-2}s^{-1}$. Prepenetration developmental stages of *B. graminis* conidia: aberr, burst, dead conidia; ng, non-germinated conidia; pgt, germinated conidia with a primary germ tube only; sgt, conidia with an elongated secondary germ tube; agt, conidia with a swollen appressorial germ tube; app, conidia with a fully differentiated appressorium. Each value is given as mean ± SD of six replicates (100 conidia each) of three independent biological experiments. Significant differences were determined in a Kruskal–Wallis test, with *post hoc* comparison by Bonferroni multiple comparison test: different letters indicate significant differences ($P < 0.05$).

UV-C irradiation resulted in increased values (twofold and eightfold, respectively). The blue light incubation, however, resulted in a distinct decrease of transcripts to levels not different from the non-UV/darkness controls (Fig. 8B, C). LED blue light irradiation (λ_{max}. 475 nm) did not result in a substantial transcriptional up-regulation of the putative *B. graminis* photolyase genes.

4. Discussion

4.1. *UV-C mediated suppression of* B. graminis *prepenetration*

Detrimental effects of UV-C light on *B. graminis* development had been reported previously (Buxton et al., 1957; Moseman and Greeley, 1966). Most studies, however, focused on disease reduction or on effects on spore germination and rarely dealt with the effects on developmental progress and/or the formation of functional infection structures. To date, there is only scarce information available concerning the hampering effects of UV-C irradiation on the pre-penetration processes of the economically important powdery mildew pathogen *B. graminis*. The present study primarily aimed at the characterization of UV-C irradiation effects on pre- but also on post-penetration stages of *B. graminis* infection. By applying the Formvar® *in vitro*-system, providing a hydrophobic surface and the germination and differentiation inducing very-long-chain aldehydes, direct host plant-derived effects such as plant defense were largely excluded.

Our results show a distinct effect of UV-C irradiation on germination and differentiation of infection structures *in vivo* as well as *in vitro*. Although the non-germination and differentiation values are generally lower *in vitro* (Hansjakob et al., 2012), the almost parallel run of the dose-response curves, albeit at a higher level *in vivo* (Fig. 2C, D), suggests that the direct physical impact of UV-C irradiation rather than UV-induced plant defense is responsible for the reduced proportions of germinated and differentiated conidia with increasing doses of UV-C irradiation. However, within the range of doses applied, germination and differentiation follow two different types of dose-response curves. After an initial lag phase the values of conidia remaining non-germinated increase almost linearly with higher doses of UV-C irradiation. For differentiation, however, the curve exhibits a sigmoidal shape. The initial lag phase is followed by a phase of more or less constant decrease ending in a plateau phase. *In vitro* appressoria were no longer formed at doses of 80 and 100 J m^{-2}, whereas these doses still allowed for the germination of 30 % of the conidia. This initial lag phase, however, is not reflected by earlier data on *B. graminis* germination and infectivity, indicating an instant linear decrease in germination with increasing UV-C doses followed by a plateau phase, and a linear decrease of infectivity (Buxton et al., 1957).

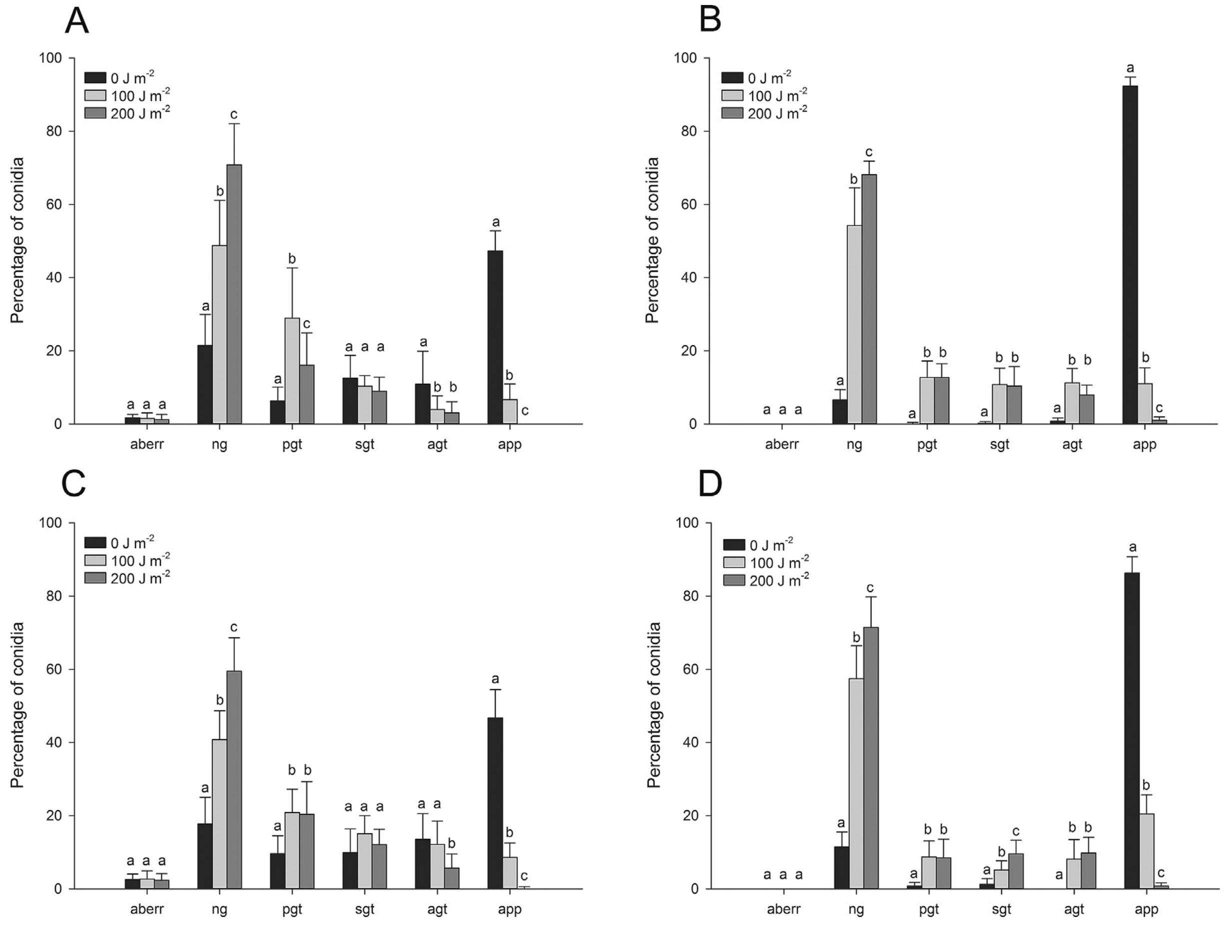

Fig. 5. Development of *Blumeria graminis* f. sp. *hordei* conidia after UV-C treatment and an incubation period of 18 h (dark/light: 6 h/12 h) (A and B) or 24 h (dark/light: 6 h/18 h) (C and D) on different substrata. (A) Conidia on Formvar®/barley wax/agar coated glass slides; (B) Conidia on adaxial barley leaf surface; (C) Conidia on Formvar®/barley wax/agar coated glass slides; (D) conidia on adaxial barley leaf surface. Photon fluence rate was 230 ± 10 μmol $m^{-2}s^{-1}$. Developmental stages of *B. graminis* conidia: aberr, burst, dead conidia; ng, non-germinated conidia; pgt, germinated conidia with a primary germ tube only; sgt, conidia with an elongated secondary germ tube; agt, conidia with a swollen appressorial germ tube; app, conidia with a fully differentiated appressorium. In (A) and (C), each value is given as mean ± SD of six replicates (100 conidia each) of three independent biological experiments. In (B) and (D) each value is given as mean ± SD of three replicates (100 conidia each) of three independent biological experiments. Significant differences were determined in a Kruskal–Wallis test, with *post hoc* comparison by Bonferroni multiple comparison test: different letters indicate significant differences ($P < 0.05$).

Nevertheless, our results suggest that the fungal differentiation is more susceptible to UV-C irradiation than germination. The more pronounced decrease in differentiation is probably a consequence of conidial cell cycle arrest in response to UV-damaged DNA (Suthaparan et al., 2016). Since the formation of the primary germ tube is a fairly speedy process, with the majority of conidia germinating within the first 45 min after surface contact, the formation of the secondary germ tube and the differentiation of an appressorium take more time and depend on the success of a nuclear division preceding cytokinesis (Hansjakob et al., 2012). As DNA replication is an integral part of appressorium formation, this process might be more prone to UV-C irradiation than the formation of the primary germ tube, which is independent of a preceding replication. Recently, it was shown that UV-C irradiation significantly suppressed nuclear division in conidia of the tomato powdery mildew *Oidium neolycopersici* (Suthaparan et al., 2018). Since cycloheximide, a potent inhibitor of protein biosynthesis, only slightly affected the formation of primary germ tubes but strongly prevented the formation of appressoria (Hansjakob et al., 2012) it is tempting to speculate that at least some of the components required for germination are indeed preformed and thus *de novo* protein biosynthesis becomes less important, which in turn might contribute to the observed lower UV-C susceptibility of primary germ tube formation. However, the initial lag-phase in the dose-response curves might also reflect the potential UV-shielding function of the tryptophan derivative 3-hydroxykynurenine present in *B. graminis* conidia and hyphae (Wilson et al., 2003).

Very-long-chain aldehydes, promoting *B. graminis* germination and differentiation, were shown to be easily oxidized to form acids when exposed to air during storage (Hwang et al., 2002). Since aliphatic aldehydes all possess an absorption spectrum in the region of 230–370 nm (Martinez et al., 1992), UV-C irradiation may induce the photooxidation of very-long-chain aldehydes, potentially affecting physical and functional wax properties. In order to assay whether photooxidation of the wax aldehydes affected *B. graminis* prepenetration, Formvar®/wax/agar coated glass slides were irradiated with 200 J m^{-2} UV-C prior to inoculation. Neither germination nor differentiation was affected by this treatment, demonstrating that photooxidation did not play a distinct role within the range of doses applied (Supplemental Fig. 1).

While a UV-C dose of 12.36 J m^{-2} was shown to sufficiently control the strawberry powdery mildew fungus *Posdosphaera aphanis* (Janisiewicz et al., 2016), distinctly higher doses ≥100 J m^{-2} completely inhibited the formation of *B. graminis* appressoria *in vitro* and *in vivo*, which is also close to the reported 54 J m^{-2} dose that resulted in a 90 % decrease of infectivity in *B. graminis* (Buxton et al., 1957). For the powdery mildew fungi, *Podosphaera leucotricha* and *Sphaerotheca macularis* daily doses of

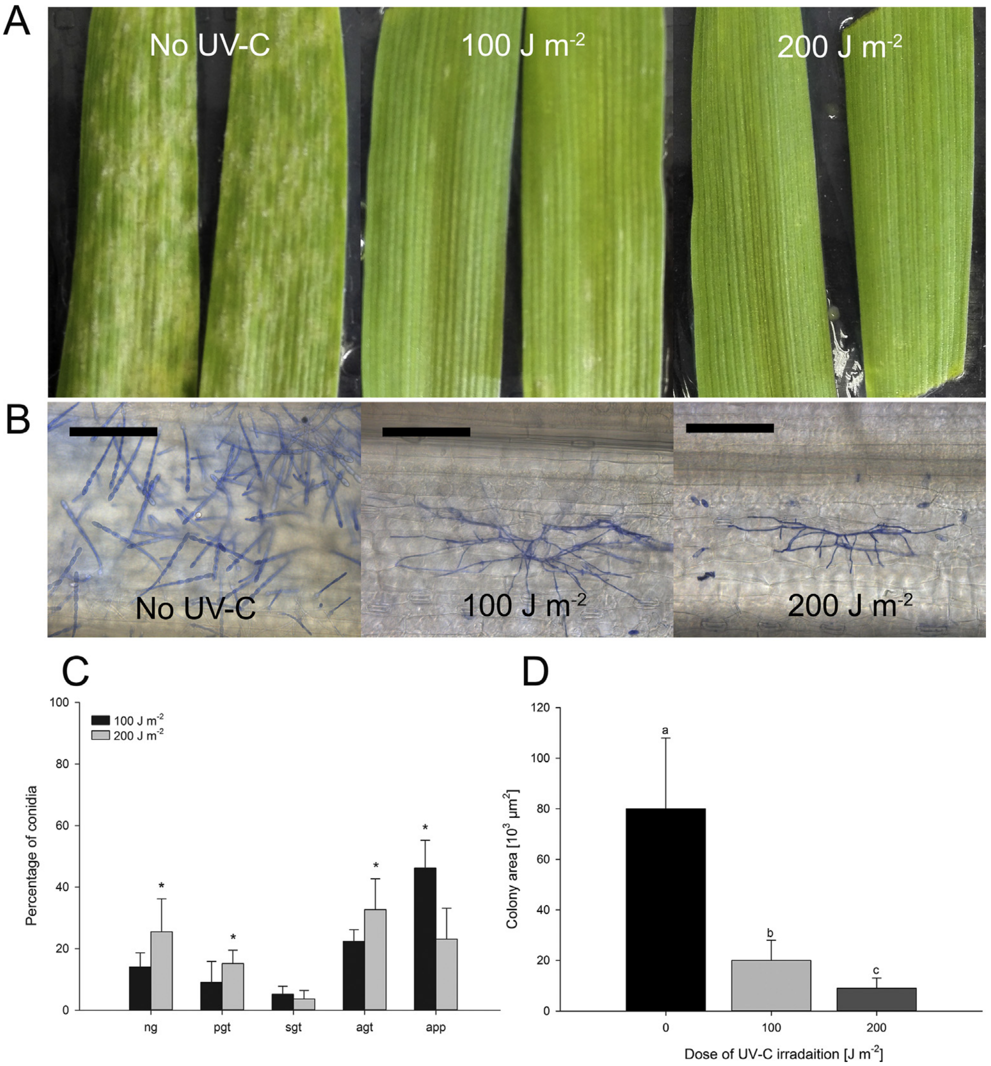

Fig. 6. Development of *Blumeria graminis* f. sp. *hordei* conidia after UV-C treatment and an incubation period of 120 h (dark/light: 6 h/18 h) on adaxial barley leaf surfaces. The photon fluence rate was 230 ± 10 μmol $m^{-2}s^{-1}$. (A) Pustules on barley leaves after UV-C treatments; from left to right: two non-treated inoculated leaf segments, two segments irradiated with 100 J m^{-2} and two segments irradiated with 200 J m^{-2} UV-C. (B) Trypan-blue stained pustules on adaxial barley leaf surfaces after UV-C treatments; from left to right: non-treated control, 100 J m^{-2}, 200 J m^{-2}. (C) Conidial prepenetration development on adaxial barley leaf surfaces. (D) Pustule size after UV-C treatment. Developmental stages of *B. graminis* conidia: ng, non-germinated conidia; pgt, germinated conidia with a primary germ tube only; sgt, conidia with an elongated secondary germ tube; agt, conidia with a swollen appressorial germ tube; app, conidia with a fully differentiated appressorium; In (C), each value is given as mean ± SD of three replicates (100 conidia each) of three independent biological experiments. Significant differences were determined in a Student's T test: * indicates significant differences ($P < 0.05$). In (D), each value is given as mean ± SD of 50 colonies. Significant differences were determined in a One-way ANOVA, with *post hoc* Tukey test: different letters indicate significant differences ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

300–600 J m^{-2} proved to be most effective in a greenhouse study with apple seedlings and strawberry plants (Van Hemelrijck et al., 2010). These data indicate a broad range of sensitivity towards UV-C irradiation among powdery mildew species. This may be explained by the multifactorial nature of UV tolerance, which does not depend only on the amount of DNA lesions (Nascimento et al., 2010). Most powdery mildew species possess only white mycelium that remains colorless throughout the season. The mycelium of *B. graminis*, however, is usually greyish to occasionally ochre. This specific coloration might be due to the presence of the UV-absorbing pigment hydroxykynurenine in hyphae and conidia (Wilson et al., 2003). So far, *B. graminis*, is the only member of the Erysiphaceae known to abundantly contain this pigment. However, the mycelium of *P. aphanis* and of other species is also known to turn brownish with age, which might indicate the presence of a possibly UV-absorbing pigment at later stages of development (Braun et al., 2002). More research is needed to analyze the specific role(s) of 3-hydroxykyurenine or of other potentially present UV-absorbing compounds and their specific role(s) in the powdery mildews.

Even a prolonged period of darkness (48 h) after irradiation with a dose of 100 J m^{-2} UV-C resulted only *in vivo* in the formation of very few appressoria, while *in vitro* the formation of appressoria was still completely abolished. Hence, one may conclude that *B. graminis* conidia cannot reverse the detrimental effects of UV-C irradiation by a prolonged incubation in the dark. Under conditions of prolonged darkness 200 J m^{-2} UV-C widely prevented not only germination and differentiation but also the formation of haustoria and hence the formation of *B. graminis* pustules on the detached leaves. In line with Buxton et al. (1957) UV-C irradiation dramatically reduced the infectivity of *B. graminis* conidia. Our results also show that still a substantial portion of appressoria penetrated between 24 and 48 h post inoculation.

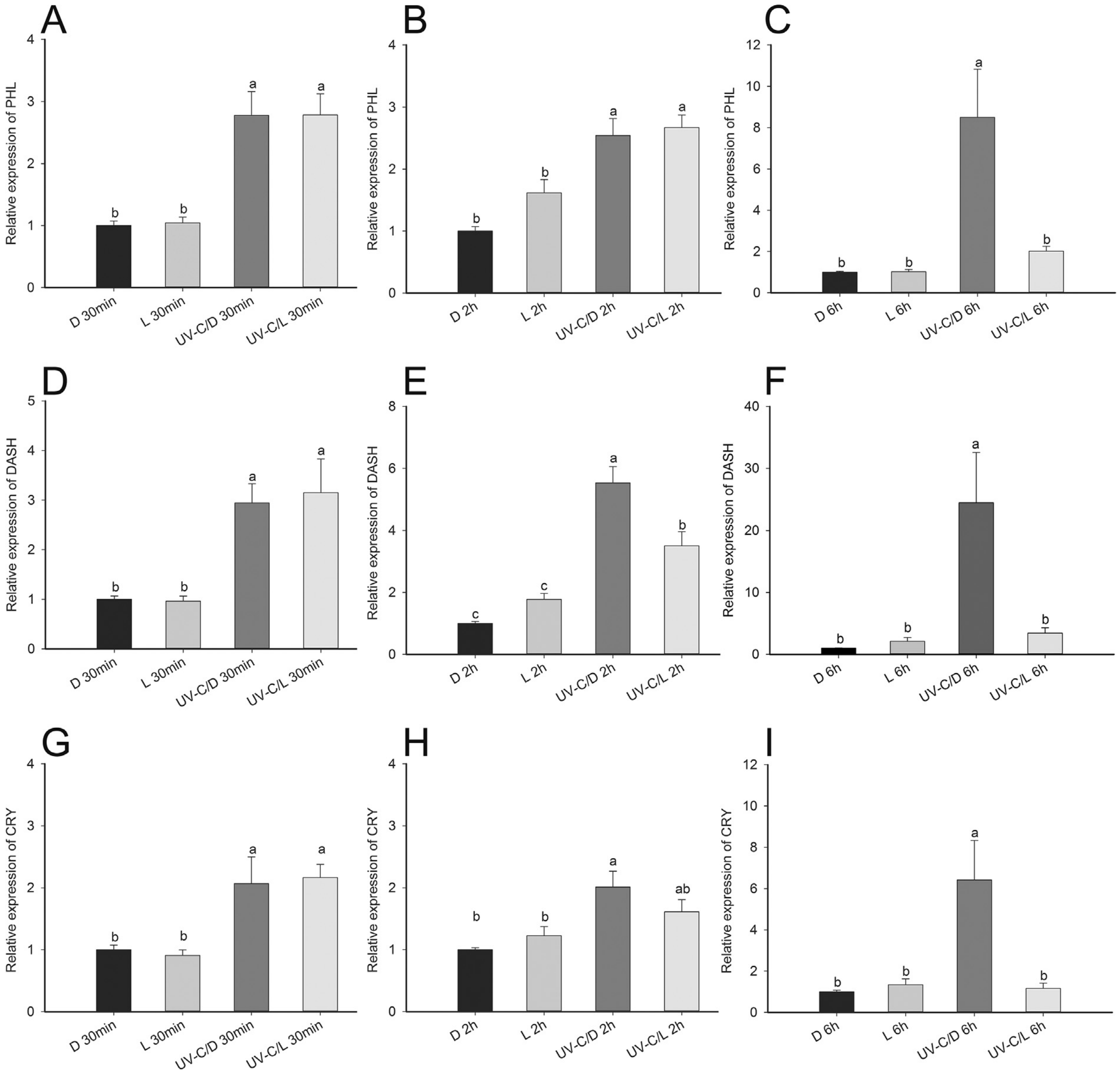

Fig. 7. RT-qPCR analysis of putative photolyase gene expression in *Blumeria graminis* f. sp. *hordei* conidia after UV-C exposure *in vitro*. (A, B, C) Relative transcript levels of the putative CPD-photolyase gene BGHDH14_bgh00666, (D, E, F) of the putative Cry-DASH encoding gene BGHDH14_bghG001129000001001, and (G, H, I) of the putative 6-4 DNA photolyase gene BGHDH14_bgh01427. Conidia on Formvar®/barley wax coated glass slides were irradiated or not with UV-C light (100 J m^{-2}) and then incubated with/without white light exposure (230 ± 10 μmol $m^{-2}s^{-1}$) for (A, D, G) 30 min, (B, E, H) 2 h or (C, F, I) 6 h. D: dark; L: white light. GAPDH gene (BGHDH14_bgh00075, Genbank X99732) was employed as reference gene. Each value is given as mean ± SE of three independent biological experiments. Significant differences were determined in a One-way ANOVA test, with *post hoc* Tukey test: different letters indicate significant differences ($P < 0.05$).

4.2. The role of photoreactivation

In order to assay the effects of photoreactivation in *B. graminis* conidia, two hours of incubation with white light after UV-C irradiation were followed by 16 h in darkness. The 2 h white light treatment resulted in distinctly higher proportions of germinated and differentiated conidia, which unambiguously demonstrates the effectiveness of photoreactivation in *B. graminis* conidia. Dark incubation after UV-C irradiation resulted in a sigmoidal dose-response curve for differentiation and in a two-phased curve for germination. The 2 h white light treatment changed these curves to more linear functions without defined lag and plateau phases. *In vitro* and *in vivo* the 2 h white light incubation after UV-C irradiation resulted in roughly 45 % restoration of appressorium formation. Our findings corroborate the key role of photoreactivation in the recovery of UV-mediated damage in the powdery mildew fungus *B. graminis* (Buxton et al., 1957). Likewise, photo-incubation was shown to have a significant germination restoring effect on UV-irradiated conidia of *O. neolycopersici* (Suthaparan et al., 2017).

Plants usually grow in a light:dark cycle, where light phases are much longer than 2 h. Therefore we analyzed the photoreactivation under conditions mimicking a more natural light:dark cycle. The conidia were incubated for six hours in darkness after UV-C irradiation and subsequently for 12 or 18 h exposed to white light. Despite 12 h of white light irradiation, the restoration effect of this treatment regarding appressorium formation was only 12 %. This finding suggests that the first hours after UV-C irradiation are critical for the efficacy of UV-C treatment. Our results are therefore in full accordance with data from *O. neolycopersici* demonstrating the importance of a sufficiently long dark period between UV treatment and possible photoreactivation (Suthaparan et al., 2018). It was also reported that the colonization of UV-treated *P. aphanis*

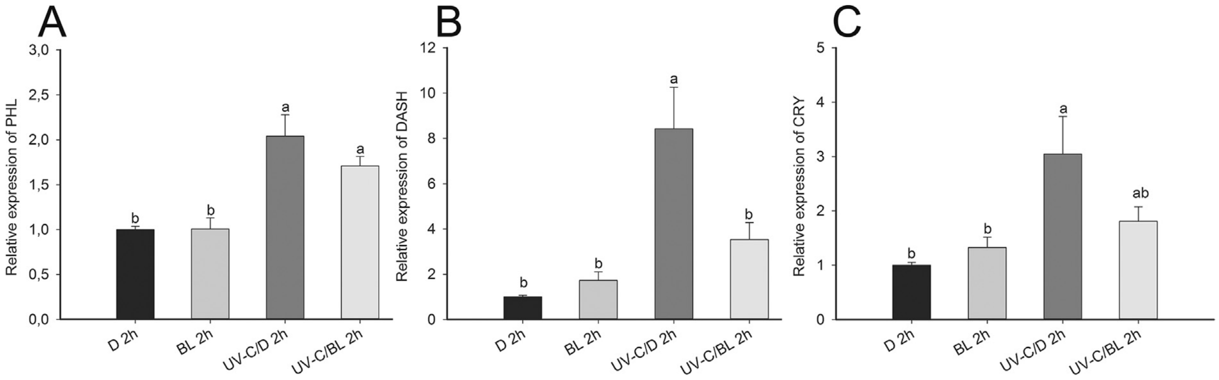

Fig. 8. RT-qPCR analysis of putative photolyase gene expression in *Blumeria graminis* f. sp. *hordei* conidia *in vitro* on Formvar®/barley wax coated glass slides after UV-C exposure (100 J m^{-2}) followed by 2 h irradiation with/without blue light (80 μmol $m^{-2}s^{-1}$). Relative transcript levels of (A) a putative CPD-photolyase gene BGHDH14_bgh00666, (B) of the putative Cry-DASH encoding gene BGHDH14_bghG001129000001001, and (C) of the putative 6-4 DNA photolyase gene BGHDH14_bgh01427. D: darkness; BL: blue light. Each value is given as mean ± SE of three independent biological experiments. Significant differences were determined in a One-way ANOVA test, with post hoc Tukey test: different letters indicate significant differences ($P < 0.05$).

was negatively affected by dark incubation ≥4 h (Janisiewicz et al., 2016). Nevertheless, extending the light phase to 18 h resulted in a slight increase in *B. graminis* appressorium formation which suggests a (minor) role for expanded day-length in photoreactivation. For the most efficient control of powdery mildew under field/greenhouse conditions we suggest, in line with others, to irradiate plants with UV-C during the first half of the night in order to fully exploit night-time darkness (Suthaparan et al. 2012b, 2014).

After germination on the host leaf it takes 3–5 d for newly formed *B. graminis* pustules/colonies to release fresh conidia. In order to observe colony/pustule formation of UV-C irradiated conidia, detached, inoculated barley leaves were subjected to single doses of 100 or 200 J m^{-2} UV-C and subsequently incubated in a 6 h darkness/18 h light photocycle for up to 5 d. Despite UV-C irradiation and most probably due to photoreactivation a significant proportion of conidia still germinated and formed appressoria although in a dose-dependent manner. The prolonged light phases allowed for a more efficient photoreactivation. A greater proportion of conidia remained viable for longer than 24 h and were able to proceed with prepenetration and infection until the formation of pustules. Our results show a strong negative correlation between pustule size and UV-C dose applied. Within the given time frame UV-C treatment completely abolished conidiation, thus preventing asexual propagation. In line with this, UV-C application dramatically reduced visible symptoms and production of conidia on strawberry plants inoculated with *P. aphanis* (Janisiewicz et al., 2016). So far, the exact reason for this phenomenon remains to be unveiled. One possible explanation is that the host epidermal cells might be damaged to such an extent that the host/pathogen interaction might be disturbed or that the plant can no longer provide sufficient nutrients to its fungal pathogen. The latter would be in agreement with data from barley coleoptiles where the formation of secondary hyphae after haustorium formation was severely affected upon UV-irradiation of the coleoptile tissue (Yamaoka et al., 1993). Since this effect could be reversed by supplementing sugars to the coleoptile floating solution the authors speculated that the energy supplies from the plant might be blocked for expression of resistance. Taken together our results suggest a retardation of conidial and pustule development upon UV-C exposure. This view is further supported by a previous report demonstrating a delayed development of secondary hyphae of *B. graminis* f.sp. *tritici* on wheat leaves upon UV-C irradiation later than 6 h after inoculation (Mount and Ellingboe, 1968). Consequently, several irradiation treatments in the time frame from germination to conidiation are suggested for the efficient control of *B. graminis* by UV-C light. However, the overall dose should remain at a distinctly sub-lethal level for the host plants. In barley, doses ≥5 k J m^{-2} UV-C resulted in the increased membrane and DNA damage (Georgieva et al., 2015). However, the barley photorepair system was shown to nearly completely remove CPDs within 2 d post-irradiation (Manova et al., 2016).

4.3. Photolyase gene expression is induced upon UV-C exposure

Most fungi show a light-induced expression of genes which protect against or repair UV-induced lesions, and formation of pigments which filter visible light and/or UV (Braga et al., 2015). Our RT-qPCR results indicate a transcriptional activation of the three putative *B. graminis* f. sp. *hordei* photolyase genes upon UV-C irradiation. For none of the three genes, a subsequent incubation in only white light resulted in distinctly elevated transcript levels. 30 min and 2 h white light photoreactivation did not result in reduced transcript levels of bgh0666 and bgh01427, while at 6 h after irradiation in white light a distinct downregulation became discernible. Incubation in blue light ($\lambda_{max.}$ 475 nm) resulted in downregulation of BGHDH14_bgh01427 and BGHDH14_bghG001129000001001 but not of BGHDH14_bgh0666 already two hours after UV-C irradiation. This might indicate that the blue light could be more efficiently used by the gene products of BGHDH14_bgh01427 and BGHDH14_bghG001129000001001 and/or that these are faster or more efficient in the repair of specific DNA damage patterns than the damage repaired by BGHDH14_bgh0666. Both treatments, irradiation with blue light ($\lambda_{max.}$ 475 nm) or with white light were apparently sufficient for photoreactivation but were ineffective in inducing the transcriptional upregulation of the putative photolyase genes. These results are in contrast to the situation in other fungi, such as *B. cinerea*, where *Bcuve1*, which encodes a UV-damage endonuclease and *Bcphr1* (=*Bccry1*) that codes for a CPD-photolyase are strongly induced by white and blue light irradiation, dependent on the expression of a white collar 1 homolog, the blue light photoreceptor gene *Bcwcl1* (Zhu et al., 2018). In *Trichoderma atroviride*, the CPD-photolyase gene *phr1* is strongly and rapidly upregulated by blue light, dependent on the white collar blue light regulator homologs *blr1* and *blr2* (Berrocal-Tito et al. 2000, 2007). Likewise, in the basidiomycete *Ustilago maydis*, the causative agent of corn smut disease, the blue light induction of photolyase (λ_{max} 471 nm) is mediated by white collar 1 (Brych et al., 2016). Therefore it is tempting to speculate that an induction of the three *B. graminis* photolyase genes may not be mediated by the only white collar ortholog present in *B. graminis* f. sp. *hordei*, annotated as putative blue-light receptor PCMADA (BGHDH14_bgh01221; CCU76947). Nevertheless, it remains to be determined whether a blue light-inducible UV-damage endonuclease plays a role in protecting *B. graminis* from UV-damage. Our results are, however, in accordance with observations with *Podosphaera xanthii* on cucumber, where a certain level of blue photons in growth light before UV treatments minimized the risk of UV mediated damage for the host plant, while it maintained the high efficacy of UV-C irradiation against powdery mildew (Suthaparan et al., 2017). Nevertheless, even without UV-C treatment transcripts of the three putative *B. graminis* photolyase genes were clearly detectable, at least for conidia produced and inoculated under white light conditions. This might suggest a more or less constitutive expression at basal levels that can be further increased upon UV-irradiation, which could explain the unresponsiveness regarding up-regulation to irradiation with blue or white light. However, it remains to be determined whether the three putative photolyase genes are expressed in conidia that were produced in complete darkness.

5. Conclusions

In the reproductive vectors of land plants, pollen and spores, UV absorption occurs primarily by phenolic constituents of the sporopollenin polymer located in their exine or outer wall (Rozema et al., 2009). Fungal spores use pigments, such as melanins, located in or on the cell wall, and endogenous or extracellular UV-absorbing metabolites that act as UV-sunscreens (Braga et al., 2015). Despite of their aerial mode of dispersal, conidia and ascospores of the barley powdery mildew fungus *B. graminis* show at best a slightly greyish to ochre coloration. The more or less hyaline outer appearance of *B. graminis*, with the exception of its brownish-black pigmented chasmothecia, generally suggests a higher susceptibility to UV-irradiation in comparison to other fungal species. Taken together, our results in combination with previous data (Buxton et al., 1957) indeed confirm a distinctly higher susceptibility to UV-C irradiation, particularly, when compared to the high doses (0.8×10^3–10^4 J m^{-2} UV-C) required to fully inactivate conidia of the plant pathogenic fungi *B. cinerea* and *Monilinia fructigena* (Marquenie et al., 2002; Zhu et al., 2018). Nevertheless, the specific role(s) of the UV-C absorbing compound 3-hydroxykynurenine in and/or on conidia of *B. graminis* (Wilson et al., 2003) remain(s) to be elucidated. Application of the Formvar® *in vitro*-system, which excludes potential effects of host plant defense, demonstrated that UV-C irradiation after surface contact affected the prepenetration processes of *B. graminis* conidia in almost the same way as on leaves. However, substantial inhibitory contributions of plant defense at later stages of pustule growth cannot be ruled out.

The single-celled conidia of *B. graminis* are particularly sensitive to UV-C irradiation immediately after germination and before the onset of nuclear division. In this crucial phase of fungal development, UV-C irradiation can largely prevent the formation of penetration competent appressoria and hence infection. Pustules originating from the few surviving conidia that successfully infected the host plant after UV-C irradiation exhibited a distinctly retarded growth and a dramatically reduced competence for vegetative propagation. In order to increase the efficacy of UV-C irradiation, the repair capabilities of photoreactivation should be minimized by implicating prolonged phases of darkness after UV-C irradiation. This approach is further supported by the expression pattern of the putative photolyase genes in *B. graminis* being insensitive to transcriptional up-regulation by white light. Our data thus shed new light on the relevance of photoreactivation for UV-C irradiation of *B. graminis* spores and hyphae. The specific optimization of dose, frequency and timing could turn UV-C irradiation into one of the key strategies to control *B. graminis* on the agriculturally highly relevant crops wheat and barley, particularly with respect to modern controlled-environment indoor farming concepts.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2018.12.002.

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