Culture age impacts Plectosporium alismatis propagule yields and subsequent desiccation and UV-radiation tolerance

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Cultural age impacts *Plectosporium alismatis* propagule yields and subsequent desiccation and UV-radiation tolerance

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Running title: effects of culture age on *P. alismatis* propagules

SUMMARY

The effect of cultural age was studied on yields, desiccation tolerance and resistance to ultraviolet radiation of *Plectosporium alismatis, a potential* mycoherbistat of aquatic weeds in Australian rice fields.

P. alismatis was grown in a liquid basal medium supplemented with malt extract and sodium nitrate and harvested after 7, 14 or 21 days incubation. Although chlamydospore yields harvested from 14-day-old liquid cultures were significantly higher (29.2×10^5 chlamydospores mL^{-1}) than chlamydospore yields harvested from 7-day-old liquid cultures (1.07×10^5 chlamydospores mL^{-1}) or from chlamydospore yields harvested from 21-day-old liquid cultures, the germination of freshly-harvested chlamydospores from 7-day-old cultures (72.7%) was significantly reduced when propagules were grown for 14 days (55.3%). When exposed to UV-radiation, conidia and chlamydospores harvested from 14-day-old cultures germinated at a lower rate (<20%) than conidia and chlamydospores harvested from 7-day-old cultures (>40%). When conidia and chlamydospores were dried and subsequently exposed to UV, less than 30% of propagules harvested from 7-day-old germinated, whereas less than 10% of propagules harvested from 14-day-old cultures germinated. A 3 way analysis of variance including cultural age, UV exposure and type of propagules confirmed that the cultural age had more impact on the germination of

fresh or dry propagules ($P = 0.00001$ and $P = 0.0004$, respectively) than the type of propagules considered ($P = 0.5$).

These results demonstrate that the cultural age impacts significantly propagule yields and germination of *P. alismatis* conidia and chlamydospores, particularly after a stress caused by dehydration and/or exposure to UV-B radiation.

INTRODUCTION

The deuteromycete *Plectosporium alismatis* (Oudem) W.M. Pitt, W. Gams & U. Braun (synonym *Rhynchosporium alismatis*, *Spermosporina alismatis*), is a pathogen of starfruit (*Damasonium minus* (R. Br.)Buch. and of several other Alismataceae aquatic weeds in Australian rice crops. The potential of *P. alismatis* for control of aquatic weeds has been shown by Cother & Gilbert (1994) as an alternative to the utilization of Londax®, a bensulfuron herbicide which is likely to have contributed to the development of herbicide resistant weeds (Graham, Prat, Pratley, Slater, & Baines, 1996).

For weed control, *P. alismatis* will be applied inundatively and its effect is likely to be static rather than cidal (Crump, Cother, & Ash, 1999). Consequently, the appropriate term for *P. alismatis* is mycoherbistat rather than mycoherbicide. Our current studies aim at growing *P. alismatis* in submerged cultures with the goal of producing propagules with the most fitted potential for the development of a mycoherbistat.

In previous work, we showed that *P. alismatis* was able to produce high yields of conidia in a casamino-acids, glucose based medium (Cliquet & Zeeshan, 2008).

We also showed that *P. alismatis* was able to produce chlamydospores in a malt extract, sodium nitrate medium after 7-day incubation (Cliquet, Ash & Cother, 2004). Based on this medium, nutritional studies led to the development of a liquid medium containing appropriate malt extract and sodium nitrate concentrations for optimal chlamydospore yields.

Furthermore, recent studies demonstrated that 10% chlamydospores produced in this malt-extract, sodium nitrate based medium were able to germinate after 4-month storage at 25°C, while conidia produced under the same culture conditions showed poor survival (0% after 2-month storage(Cliquet & Zeeshan, 2008). Propagules are required to survive drying in order to maintain the viability of a dry preparation (Jackson & Schisler, 2002). Chlamydospores are therefore promising candidates for the development of a mycoherbistat. However, under our cultural conditions, *P. alismatis* chlamydospore yields obtained in submerged cultures were significantly lower than conidia yields. Since we had already defined the optimal nutritional conditions for high chlamydospore yields, (Cliquet *et al*. 2004), our work focused on non-nutritional conditions that may further increase chlamydospore yields. Because long incubation periods (2-3 weeks) are generally required to reach high levels of chlamydospores (Hebbar, Lewis, Poch, and Lumsden, 1996), we investigated how cultural age may impact chlamydospore yields in the present study.

In general, modifications of cultural conditions are known to have significant consequences on fungal morphology and spore qualities. Numerous studies have been conducted on the impact of nutritional conditions on fungal desiccation tolerance

(Jackson, Cliquet & Iten, 2003; Jackson & Schisler, 1992). However, literature is scarce on the impact of *P. alismatis* cultural age on fungal attributes, except for a few articles (Hall, Peterkin, Ali & Lopez, 1994 ; Bardin, Suliman & Sage-Palloix, 2007) and, to our knowledge, no study has been reported on the impact of cultural age on fungal desiccation tolerance.

Ultra Violet (UV) radiation is an additional environmental stressfull factor for fungal species (Moody, Newsham, Ayres & Paul, 1999). While UV-C radiation (200-280 nm) does not reach the ground level due to oxygen and ozone (Madronich, McKenzie, Caldwell & Bjorn, 1995), recent studies on *P. alismatis* have shown that UV-A (315-400 nm) stimulate appressoria formation while UV-B reduce significantly conidial germination (Ghajar, Holford, Cother, & Beattie, 2006a). The detrimental impact of UV-B has been reported on fungal insect pathogens and is related to the medium composition, and in particular, to the type of carbon sources present in growth medium (Rangel, Anderson, & Roberts, 2006).

According to Myanisk, Manasherob, Ben-Dov, Zaritsky, Margalith, & Barak (2001), the age of *Bacillus thuringiensis* may impact their resistance to UV-B; however, to our knowledge, the impact of cultural age of fungal cultures on tolerance to UV radiation has not been investigated.

The current study investigates whether the cultural age impacts conidia and chlamydospore yields of *Plectosporium alismatis* produced in liquid culture and whether there are differences in tolerance to UV exposure and/or to desiccation of propagules (i.e. conidia and chlamydospores) harvested from cultures at different periods of time.

In addition, since most studies on UV tolerance examine conidia produced on solid media, the impact of cultural age on UV-B tolerance of conidia produced on PDA was examined.

MATERIALS and METHODS

Isolate

Plectosporium alismatis was obtained from the culture collection of the New South Wales Department of Primary Industries with reference number DAR 73154. The fungal pathogen was originally isolated from *Damasonium minus* (R.Br) Buch. and maintained in a soil:sand mixture (Jahromi, Cother, & Ash, 2002). In order to minimize any physiological or morphological variation, one single conidium culture was produced on potato dextrose agar (PDA, Difco, Detroit, MI, USA) for 2 weeks at 25°C, cut into 2-mm^2 agar plugs and stored in 10% glycerol at -80°C as recommended for successful preservation of fungi (Nakasone, Peterson, & Shung-Chang, 2004).

Inoculum production

A frozen suspension from stock culture at -80°C was inoculated onto a PDA plate, incubated for 2-3 weeks at 25°C until profuse sporulation occurred and renewed every month. Sub-cultures on PDA were produced from the sporulated plate (one serial transfer).

Conidia for use as aqueous conidial suspension were produced by inoculating PDA sub-culture onto PDA and growing these inoculated PDA plates at 25°C for 4 days or 7 days. Four-day-old plates were gently washed with 3 mL distilled water and the conidial suspension inoculated into shake flasks. Conidia harvested from 4-day old and 7-day-old plates were tested for UV tolerance.

Medium composition and growth conditions

The basal mineral composition for *P. alismatis* growth was derived from a Czapex-Dox composition which contained: K_2HPO_4, 1.0 g; $MgSO_4.7H_20$, 1.0 g; KCl, 0.5 g; $Fe_2SO_4.7H_2O$, 0.018 g; deionized water: 1 L. For chlamydospore production, malt extract (Difco, 8.8 gL^{-1}) and sodium nitrate (Sigma Chemical, St. Louis, MO, USA, 5.74 g L^{-1}) were used (Cliquet *et al.*, 2004).

Flasks containing the malt extract sodium nitrate medium (100 mL medium in 250 mL baffled flasks) were inoculated with conidia (4 x 10^3 conidia mL^{-1}). Cultures were placed at 150 rpm, 25°C on a rotary shaker incubator (Infors HT Bottmingen, Switzerland).

The pH of cultures was maintained at 7 ± 0.5 during growth by addition of 1 N NaOH or 1 N HCl.

Four replicate flasks were harvested after 7, 14 or 21 days incubation, respectively.

Determination of propagule yields and dry weights

Cultures were vacuum-filtered on cellulose filter papers (110-mm diameter, Whatman plc, Brentford, UK) to remove the spent medium. Filtered cultures were rinsed with 50 mL deionized water and allowed to dry on the bench top for 12 h until constant weight. Dry mats were weighed and suspended in 21.5 ml distilled water. The suspension was fragmented in a Potter homogeniser (Fisher Scientific Bioblock, Illkirch, France). Propagule counts were performed using a haemocytometer.

Preparation of propagule suspension for UV tolerance and desiccation tolerance studies

<u>Conidial suspensions from solid media</u>

Conidia harvested from 4-day-old or 7-day-old PDA plates were gently washed with sterile distilled water. Aqueous suspensions were filtered through 2 cheese-cloth layers to remove any mycelium fragment, placed on a cellulose filter and washed with sterile distilled water in order to rinse and concentrate conidial suspensions to 5 ± 2 x 10^6 conidia mL^{-1}.

Conidial and chlamydospore suspensions from liquid media

Liquid fungal cultures from duplicate flasks were poured onto 2 cheese-cloth layers in order to retain most of the mycelium. Microscopic observation showed that no mycelium remained in suspensions and that conidia were not aggregated.

Conidial suspensions were placed on a cellulose filter, rinsed and concentrated with sterile distilled water to 5 ± 2 x 10^6 conidia mL^{-1}. Mycelium remaining on cheese-cloth was rinsed to remove most conidia, and homogenised to release chlamydospores. The homogenised suspension was filtered on cellulose filter to rinse and concentrate the aqueous suspension to 5 ± 2 x 10^6 chlamydospores mL^{-1}.

Chlamydospore and Conidial Germination

Drops of the propagule suspension were placed on four 2-cm square pieces of cellophane on the surface of water agar plates (granulated agar, Difco, 20 g L^{-1}). Plates were incubated at 25°C as previously described (Cliquet *et al.*, 2004), although incubation time was reduced from 12h to 8 h to prevent any possible microcycle conidiation that may occur as previously mentioned (Cliquet *et al*., 2004; Ghajar *et al*, 2006a). Germination was evaluated microscopically after staining cellophane pieces with lactophenol cotton blue.

Drying experiments

Conidia and chlamydospore suspensions harvested from liquid media (5 x 10^6 propagules mL^{-1}) were filtered on autoclaved cellulose filters and placed at room temperature until constant weights (12h). Suspensions were prepared by gently washing dry filters with sterile distilled water and spore germination evaluated using the method described above.

Exposure of conidia and chlamydospores to UV radiation

Irradiation experiments were conducted in a dark cabinet. A 312 nm-UV-lamp (Vilber Loumat, Marne la Vallée, France) supplied 95% radiation ranged in wavelengths from 260 to 380 nm with a peak at 312 nm. The UV-A and UV-B irradiances were measured using a UVX radiometer (UVP, LLC, Upland, CA, USA) and a UV 31 and a UV 36 sensors. The total irradiance was 2.30 W $m-^{2}$ at a distance of 30 cm of the lamp.

Duplicate 200-µL droplets of each propagule suspension were placed in a Petri dish (89 mm diameter) (Greiner Bio-one 34/15 with vents, Courtaboeuf, France) at pre-specified position at a distance of 30 cm under the UV-lamp, and droplets were simultaneously placed in a dark cabinet with no lamp (controls). In order to prevent dehydration, distilled water was placed underneath the plates. Temperature and relative humidity in the dark cabinets with or without the UV-lamp were recorded (Testo probe 175, Forbach, France) and remained constant (21°C, RH = 75%) during the experiment.

In order to evaluate the impact of the UV-lamp on conidial germination, suspensions obtained from 4-day-old PDA cultures were exposed for 0, 15, 20 or 25 min.

At the end of UV exposure, 100-µL were taken from the 200-µL droplet, placed on a cellophane piece and propagule germination assessed after 8h incubation.

No germination was observed after direct exposure to UV radiation (Fig. 1).

Plates (Greiner Bio-one 34/15) were covered with their plastic lid and exposed to UV radiation. To determine the transmission of UV radiation, pieces of plastic lids were examined in a spectrophotometer (Hitachi 2000, France). The plastic lid formed a useful cut-off filter, transmitting less than 1% of wavelengths <280 nm, corresponding to UV-C radiation. Transmission of UV-B was maintained (280-315 nm) as well as UV-A transmission (315-400 nm). The total irradiance was 1.64 W m^{-2}, 80% of which produced by UV-B in the range 280-340 nm.

Germination increased from 30 to 70%, depending on exposure time (Fig.1). Accordingly, wavelengths < 315 nm have been reported to reduce conidium germination, with the most marked effect with wavelengths < 290nm (Ghajar et al., 2006a).

A 25 min exposure time to UV radiation (UV-B dose = 2 kJm^{-2}; UV-A dose = 0.4 kJm^{-2}) was selected for further experiments on the impact of UV on solid- or liquid- cultures. This UV-B dose is in the lower range of UV-B levels (0 to 26 kJm^{-2}) recorded after exposure to full-spectrum sunlight at different times of the day (Richmond, NSW, Australia, 18/03/02, Ghajar et al. 2006a)

Statistical analysis

The propagule yield experiment was repeated once.

Evaluation of tolerance of propagules to UV was performed using conidia and chlamydospore suspensions prepared from duplicate flasks. Duplicate droplets of suspensions were used in UV exposure tests, and the whole experiment repeated once. Data from each experiment (first and repeated) were analysed separately through a one-way analysis of variance (Statgraphics 4.0, Toulouse, France). Least significant difference (LSD) was used to separate means ($P < 0,05$). Since results from one way anova were similar in both experiments (block effect >0.05), data were pooled. A 3 factor analysis of variance including UV effect, age of culture and propagule type was run with pooled data.

RESULTS AND DISCUSSION

Propagule yields harvested from liquid cultures

Maximal chlamydospore and conidia yields were reached at 14 days incubation (29.2 x 10^5 chlamydospores mL^{-1} and 7.8 x 10^6 conidia mL^{-1} (Table 1). Similarly, other studies on *Fusarium oxysporum* growth kinetics showed that chlamydospores were formed on day 5 and that chlamydospore yields reached a peak 10-14 days after inoculation (Hebbar *et al.*, 1996; Elzein & Kroschel, 2004). In a previous time course experiment (0-10 d incubation) in which propagule yields were recorded each day (Cliquet *et al.*, 2004), maximum chlamydospore yields were obtained after 72 h incubation and remained constant until day 10. Increasing culture duration to 2-3 weeks is therefore required for time course chlamydospore yields determination, as generally reported in studies on chlamydospore production (Gardner, Wiebe, Gillepsie, & Trinci, 2000); Shabana, Muller-Strover & Sauerborn, 2003).

The germination of freshly-harvested, or dry propagules harvested at 7 days or 14 days incubation and exposed to UV radiation was examined.

Germination of propagules freshly-harvested from liquid cultures

In our experimental conditions, 85 % freshly-harvested conidia germinated after 8 h incubation regardless of cultural age

(Figure 2A). In a previous work, we reported that 4 days of growth were required for conidia to reach 80% germination, corresponding to the time needed to produce new conidia in large numbers, this germination remaining constant until 10 days of growth (Cliquet *et al.*, 2004). Since 80% of *P. alismatis* conidia germinate after 14 days of incubation, we may conclude that *P. alismatis* germination, under our growth conditions, is not affected by cultural age in a range 5-14 days. According to Hall *et al.* (1994), the impact of cultural age on conidial germination appears to be strain-dependant, some fungal spores from 2-3 old cultures germinating more rapidly (40-60% germination) than those taken from 14-old cultures, (10% germination) probably as a consequence of a first-formed conidial effect. Additionnal experiments with various *P. alismatis* isolates are needed to specify whether differences in germination of conidia harvested from various incubation periods are strain-dependant.

The germination of chlamydospores harvested from 14-day-old cultures was significantly reduced (55.3%) compared to the germination of chlamydospores harvested from 7-day-old cultures (72.7%). Similarly, 71% of 8-day-old chlamydospores, 60% of 3-month-old chlamydospores, and 34% of 6-month-old chlamydospores germinated (Lanoiselet, Cother, Ash, & van de Ven, 2001), indicating how time of harvest may impact fungal germination. In our experimental conditions, most chlamydospores are produced in intercalary chains inside hyphal aggregates in which substrate and oxygen diffusion is likely limiting, as reported in a large number of fermentation studies on filamentous fungi (Gibbs, Seviour & Schmid, 2000). Stress due to lack of substrate and oxygen may increase with incubation length and substrate depletion, thus affecting the physiological state of chlamydospores.

Germination of UV-exposed propagules freshly harvested from liquid cultures

The germination of conidia and chlamydospores harvested from 7-day-old cultures following UV exposure decreased, (40% and 50% germination, respectively) whereas only 10 to 20% of UV exposed propagules harvested from 14-day-old cultures germinated (Fig. 2B).

The adverse impact of UV radiation on *Plectosporium alismatis* germination has been reported (Ghajar *et al.*, 2006a). As germination proceeds, some of the most basic pathways involved are those concerned with the synthesis of DNA, RNA, and protein (Garraway & Evans, 1984). A major effect of UV-B radiation on fungi is direct damage to DNA, with possible production of reactive oxygen, especially hydrogen peroxide (Friedberg *et al.* 1995), generating probably a delay in protein synthesis and oxidative stress (Rangel *et al.*, 2006).

A 3-way analysis of variance (Table 2) clearly indicates that the germination of freshly-harvested propagules after UV exposure is related to the cultural age ($P < 10^{-5}$) rather than to the type of propagules considered (P = 0.51). How cultural age impacts UV tolerance of conidia and chlamydospores is unclear. During the germination process, stored reserve materials such as lipids, trehalose, or mannitol are broken down and used for energy production and the synthesis of new cellular material (Garraway & Evans, 1984). Since we evaluated germination by spraying conidia and chlamydospores on water agar plates overlaid with cellophane membranes, endogenous reserves were initially the sole source of nutrients available to *P. alismatis* conidia and chlamydospores during the germination process. Accumulation of endogenous lipids and proteins (Jackson & Schisler, 1992), as well as mannitol or trehalose (Ypsilos & Magan, 2004), have been reported to be related to the age of cultures. Therefore, differences in UV tolerance related to the culture age may be due, at least partially, to the variations in availability of endogenous reserves, among which mannitol or trehalose are known as protecting the cell against oxidative stress (Rangel *et al.*, 2006), while endogenous protein may provide an amino acid pool necessary for protein synthesis and facilitate rapid germination (Jackson & Schisler, 1992).

Germination of UV-exposed conidia freshly harvested from solid cultures (PDA)

While conidia harvested at 4 days or 7 days had similar germination rates (respectively 93.5 ± 2.2 and 95.8 ± 0.8 %

germination), germination of conidia harvested from 7-day-old cultures decreased dramatically after UV exposure (3.6 ± 0.6) compared to germination of conidia harvested from 4-day-old cultures (27.6 ± 2)(data not shown). These results confirm that the cultural age has a significant impact on UV tolerance and should be further considered for enhancement of UV tolerance together with adjuvant addition as proposed (Ghajar *et al.*, 2006b).

Germination of propagules harvested from liquid cultures and dried

Conidia and chamydospores produced in our liquid culture, malt-extract sodium nitrate medium, and harvested either at 7 day or at 14 days incubation were dried. As previously recorded for freshly-harvested propagules, the germination of dry propagules after U-V exposure decreased as cultural age increased (Fig. 3). The age of culture had a significant impact (Table 2) on dry propagule germination rate ($P = 4.10^{-4}$), regardless of the type of dry propagules considered ($P = 0.48$).

Generally, studies on propagule survival examine the impact of environmental factors on a one factor-at-a-time basis. As commercial economical application of the mycoherbicide requires to produce high yields of stable, dry propagules able to produce weed disease (Jackson & Schisler, 1992), it appears realistic to consider at the same time fungal tolerance to desiccation and tolerance to UV exposure. In the present work, the combination of these 2 stresses led to at least 50% decrease in germination.

As a conclusion, the finding that conidia and chlamydospore tolerance to UV and desiccation may vary in relation to cultural age illustrates the importance of growth parameters in the development of our bioherbicide. Further work is needed to find optimal growth conditions that take into account yields, spore attributes and time of incubation compatible with economical requirements.

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LEGENDS OF FIGURES AND TABLES

Figure 1. The effect of UV radiation, either unfiltered (irradiance = 2.30 W m^{-2} in the range [260-400 nm] with a 312 nm peak) or transmitted through plastic lid (irradiance = 1.64 W m^{-2} in the range [275-400 nm]) on *Plectosporium alismatis* conidial germination.
Conidia were produced on PDA for 4 days. Bars representing conidium germination are standard error bars

Figure 2. Effect of cultural age on the germination rate of *Plectosporium alismatis* freshly-harvested propagules, not exposed to UV radiation (**A**) or following exposure to UV (**B**)

P. alismatis was grown for 7 or 14 days in a chlamydospore-supporting liquid culture medium based on malt extract : 8.8 gL^{-1}; and sodium nitrate : 5.74 gL^{-1}

Bars represent means ± standard error

Figure 3. Effect of culture age on the germination rate of *Plectosporium alismatis* dried propagules, not exposed to UV radiation (**A**) or following exposure to UV (**B**).

P. alismatis was grown for 7 or 14 days in a chlamydospore-supporting liquid culture medium based on malt extract : 8.8 gL^{-1}; and sodium nitrate : 5.74 gL^{-1}

Bars represent means ± standard error

Table 1. The impact of cultural age on conidial and chlamydospore yields and germination by *Plectosporium alismatis*

Table 2. The effect of type of propagules, cultural age, UV exposure and factor interactions on the germination rate of *Plectosporium alismatis* propagules expressed as a 3 way analysis of variance

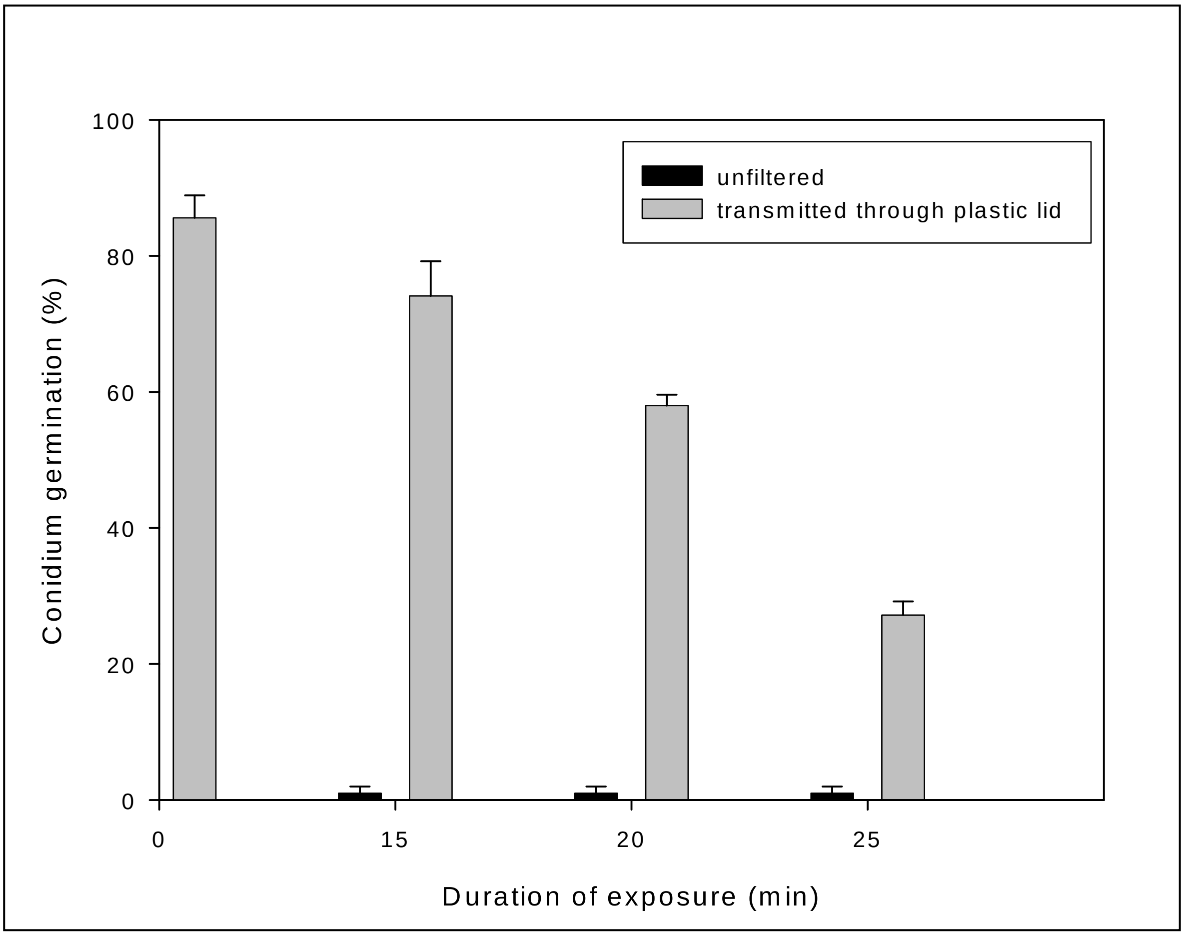
Conidium germination (%)
100
80
60
40
20
0
unfiltered
transmitted through plastic lid
0
15
20
25
Duration of exposure (min)

Figure 1.

Table 1. The impact of culture age on conidial and chlamydospore yields and germination by *Plectosporium alismatis*

Culture age [a]	Dry Weights (mg mL-1)	Conidia (mL^{-1})	Conidial germination [b] (%)	Total chlamydospores (mL^{-1}) [c]	Chlamydospore germination (%)
7 days	1.76 (b)[d]	3.85×10^{6} (b)	85.1 (ab)	1.07×10^{5} (b)	72.7 (a)
14 days	2.92 (a)	7.8×10^{6} (a)	79.1 (b)	29.2×10^{5} (a)	55.3 (b)
21 days	2.92 (a)	5.8×10^{6} (ab)	91.7 (a)	6.7×10^{5} (ab)	71.7 (a)

[a] *P. alismatis* was grown in 8.8 g L^{-1} malt extract and 5.74 g L^{-1} sodium nitrate in submerged culture at 150 rpm and 25°C.
[b] Germination of fresh propagules was evaluated after 8 h incubation on cellophane squares placed on water agar at 25°C
[c] Production of chlamydospores during growth was expressed as total chlamydospore counts. A count represents either a chain of chlamydospores, a single-celled chlamydospore or a double-celled chlamydospore.
[d] Pairs of treatments with a letter into brackets in common do not differ significantly (P<0.05) based on a pair-wise LSD test

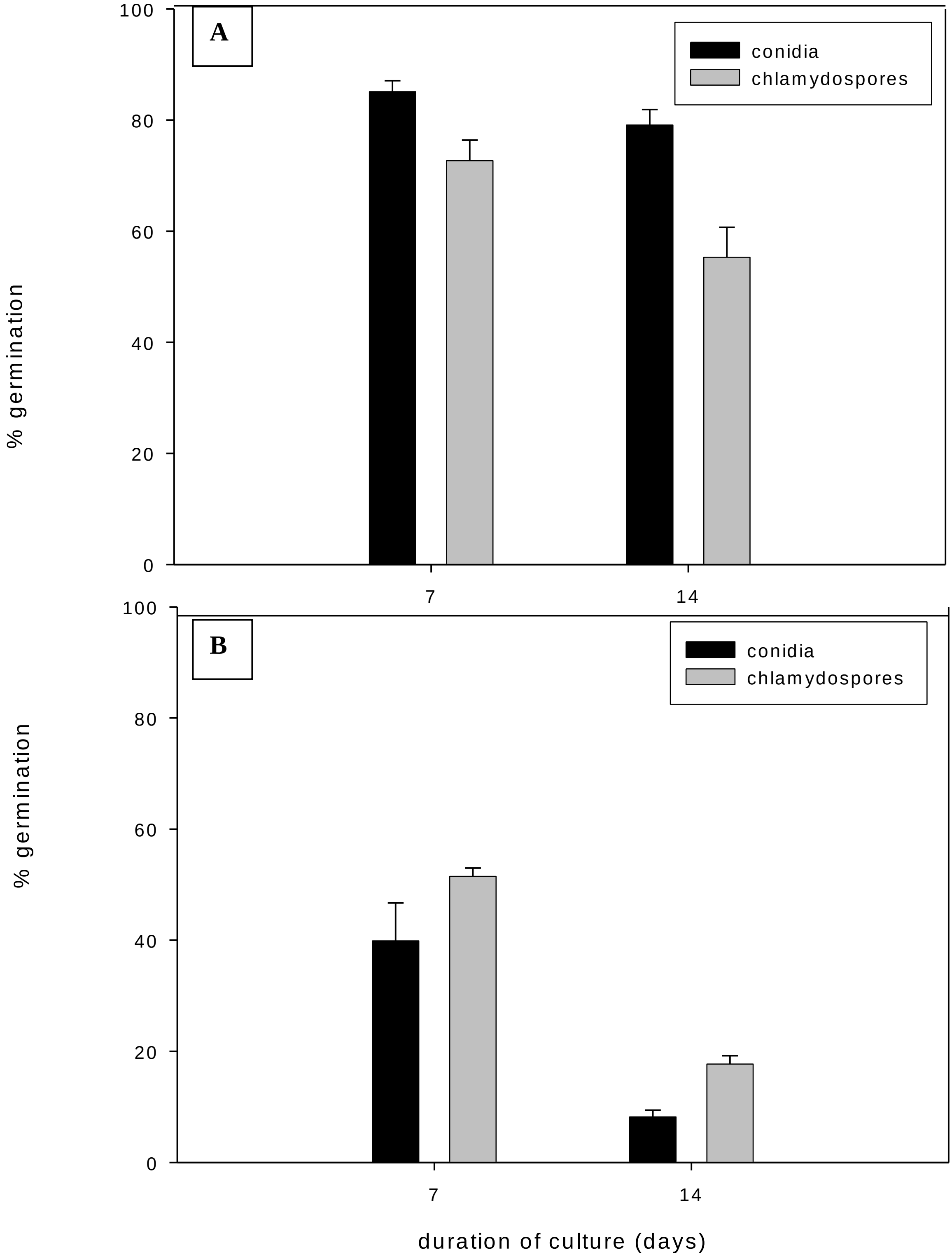
A
conidia
chlamydospores
% germination
100
80
60
40
20
0
7
14
B
conidia
chlamydospores
% germination
100
80
60
40
20
0
7
14
duration of culture (days)

Figure 2.

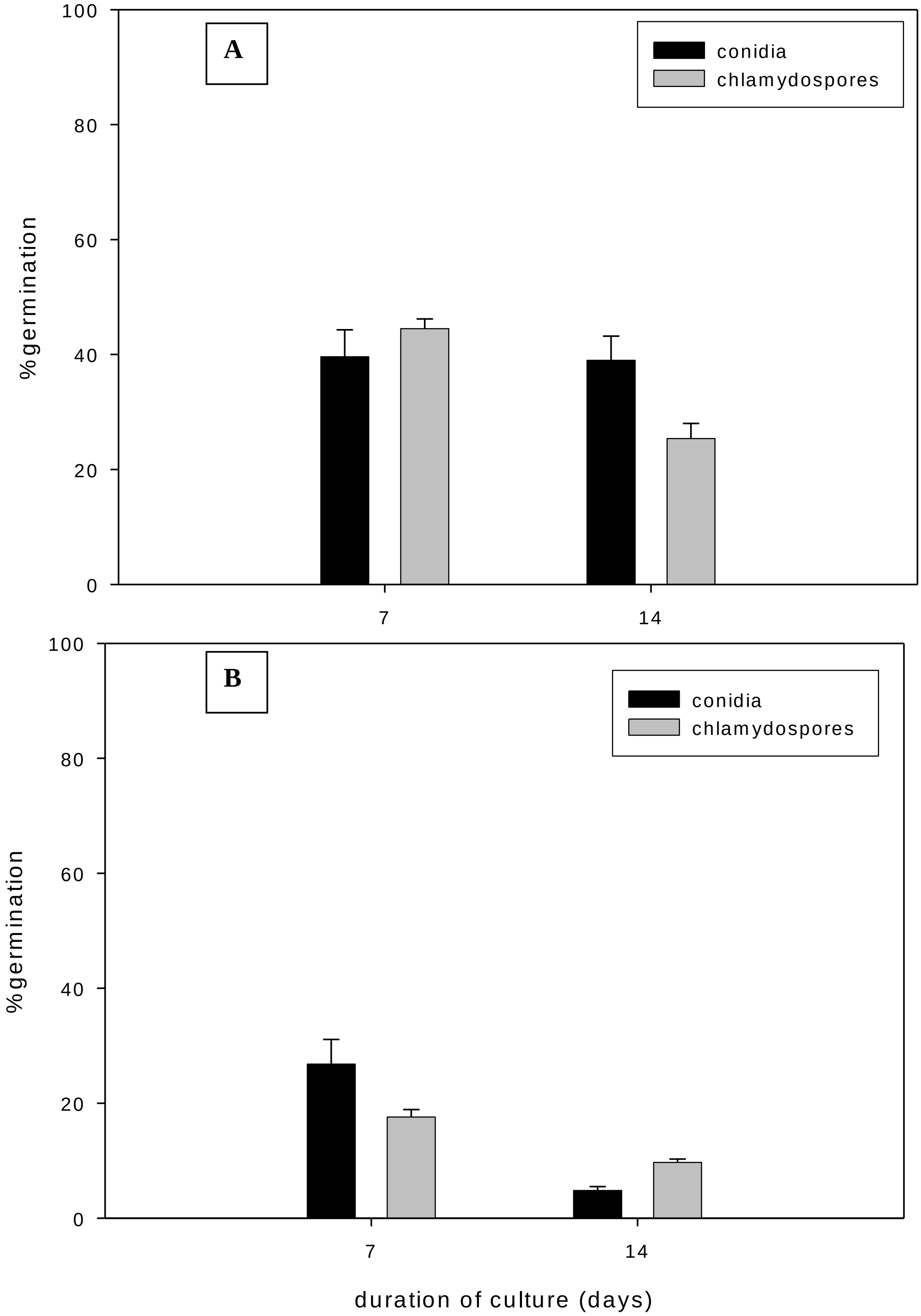
A
conidia
chlamydospores
% germination
100
80
60
40
20
0
7
14
B
conidia
chlamydospores
% germination
100
80
60
40
20
0
7
14
duration of culture (days)

Figure 3.

Table 2. The effect of type of propagules, cultural age, UV exposure and factor interactions on the germination rate of *Plectosporium alismatis* expressed as a 3-way analysis of variance

Main effect and interaction	Freshly-harvested propagules		Dried propagules	
	F-ratio	Probability	F-ratio	Probability
A: type of propagules (conidia or chlamydospores)	0.44	0.51	0.49	0.48
B: UV exposure (transmission of UV-B and UV-A)	172.8	0.00001	58.8	0.00001
C: Age of culture (7 or 14 days)	38.6	0.00001	14.4	0.0004
AB: Propagules x UV	18.3	0.0001	0.89	0.34
AC: Propagules x age	2.2	0.14	0.04	0.84
BC: UV x Age	10.1	0.002	0.17	0.68
ABC:Propagule x UV x Age	0.51	0.47	9.2	0.0036