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

3

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8

9 Key words: *Plectosporium alismatis*, mycoherbicide, age, UV
10 tolerance, conidia, chlamydospores

11

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

14

1 **SUMMARY**

2

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

7 *P. alismatis* was grown in a liquid basal medium supplemented
8 with malt extract and sodium nitrate and harvested after 7, 14
9 or 21 days incubation. Although chlamyospore yields harvested
10 from 14-day-old liquid cultures were significantly higher
11 (29.2×10^5 chlamyospores mL^{-1}) than chlamyospore yields
12 harvested from 7-day-old liquid cultures (1.07×10^5
13 chlamyospores mL^{-1}) or from chlamyospore yields harvested
14 from 21-day-old liquid cultures, the germination of freshly-
15 harvested chlamyospores from 7-day-old cultures (72.7%) was
16 significantly reduced when propagules were grown for 14 days
17 (55.3%). When exposed to UV-radiation, conidia and
18 chlamyospores harvested from 14-day-old cultures germinated
19 at a lower rate (<20%) than conidia and chlamyospores
20 harvested from 7-day-old cultures (>40%). When conidia and
21 chlamyospores were dried and subsequently exposed to UV, less
22 than 30% of propagules harvested from 7-day-old germinated,
23 whereas less than 10% of propagules harvested from 14-day-old
24 cultures germinated. A 3 way analysis of variance including
25 cultural age, UV exposure and type of propagules confirmed
26 that the cultural age had more impact on the germination of

1 fresh or dry propagules ($P = 0.00001$ and $P = 0.0004$,
2 respectively) than the type of propagules considered ($P =$
3 0.5).

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

8

1 INTRODUCTION

2

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

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

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

24 We also showed that *P. alismatis* was able to produce
25 chlamydospores in a malt extract, sodium nitrate medium after
26 7-day incubation (Cliquet, Ash & Cother, 2004). Based on this

1 medium, nutritional studies led to the development of a liquid
2 medium containing appropriate malt extract and sodium nitrate
3 concentrations for optimal chlamyospore yields.
4 Furthermore, recent studies demonstrated that 10%
5 chlamyospores produced in this malt-extract, sodium nitrate
6 based medium were able to germinate after 4-month storage at
7 25°C, while conidia produced under the same culture conditions
8 showed poor survival (0% after 2-month storage (Cliquet &
9 Zeeshan, 2008). Propagules are required to survive drying in
10 order to maintain the viability of a dry preparation (Jackson
11 & Schisler, 2002). Chlamyospores are therefore promising
12 candidates for the development of a mycoherbistat. However,
13 under our cultural conditions, *P. alismatis* chlamyospore
14 yields obtained in submerged cultures were significantly lower
15 than conidia yields. Since we had already defined the optimal
16 nutritional conditions for high chlamyospore yields, (Cliquet
17 *et al.* 2004), our work focused on non-nutritional conditions
18 that may further increase chlamyospore yields. Because long
19 incubation periods (2-3 weeks) are generally required to reach
20 high levels of chlamyospores (Hebbar, Lewis, Poch, and
21 Lumsden, 1996), we investigated how cultural age may impact
22 chlamyospore yields in the present study.
23 In general, modifications of cultural conditions are known to
24 have significant consequences on fungal morphology and spore
25 qualities. Numerous studies have been conducted on the impact
26 of nutritional conditions on fungal desiccation tolerance

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

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

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

25

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

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

10

11

12 **MATERIALS and METHODS**

13

14 **Isolate**

15 *Plectosporium alismatis* was obtained from the culture
16 collection of the New South Wales Department of Primary
17 Industries with reference number DAR 73154. The fungal
18 pathogen was originally isolated from *Damasonium minus* (R.Br)
19 Buch. and maintained in a soil:sand mixture (Jahromi, Cother,
20 & Ash, 2002). In order to minimize any physiological or
21 morphological variation, one single conidium culture was
22 produced on potato dextrose agar (PDA, Difco, Detroit, MI,
23 USA) for 2 weeks at 25°C, cut into 2-mm² agar plugs and stored
24 in 10% glycerol at -80°C as recommended for successful
25 preservation of fungi (Nakasone, Peterson, & Shung-Chang,
26 2004).

1

2 **Inoculum production**

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

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

15

16 **Medium composition and growth conditions**

17 The basal mineral composition for *P. alismatis* growth was
18 derived from a Czapek-Dox composition which contained: K_2HPO_4 ,
19 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g; KCl , 0.5 g; $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$, 0.018 g;
20 deionized water: 1 L. For chlamydospore production, malt
21 extract (Difco, 8.8 gL^{-1}) and sodium nitrate (Sigma Chemical,
22 St. Louis, MO, USA, 5.74 g L^{-1}) were used (Cliquet et al.,
23 2004).

1 Flasks containing the malt extract sodium nitrate medium
2 (100 mL medium in 250 mL baffled flasks) were inoculated with
3 conidia (4×10^3 conidia mL⁻¹). Cultures were placed at 150
4 rpm, 25°C on a rotary shaker incubator (Infors HT Bottmingen,
5 Switzerland).

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

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

10

11 **Determination of propagule yields and dry weights**

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

20

21 **Preparation of propagule suspension for UV tolerance and** 22 **desiccation tolerance studies**

23

24 Conidial suspensions from solid media

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

7

8 Conidial and chlamyospore suspensions from liquid media

9 Liquid fungal cultures from duplicate flasks were poured onto
10 2 cheese-cloth layers in order to retain most of the mycelium.
11 Microscopic observation showed that no mycelium remained in
12 suspensions and that conidia were not aggregated.

13 Conidial suspensions were placed on a cellulose filter, rinsed
14 and concentrated with sterile distilled water to $5 \pm 2 \times 10^6$
15 conidia mL⁻¹. Mycelium remaining on cheese-cloth was rinsed to
16 remove most conidia, and homogenised to release
17 chlamyospores. The homogenised suspension was filtered on
18 cellulose filter to rinse and concentrate the aqueous
19 suspension to $5 \pm 2 \times 10^6$ chlamyospores mL⁻¹.

20

21 **Chlamyospore and Conidial Germination**

22 Drops of the propagule suspension were placed on four 2-cm
23 square pieces of cellophane on the surface of water agar
24 plates (granulated agar, Difco, 20 g L⁻¹). Plates were
25 incubated at 25°C as previously described (Cliquet *et al.*,
26 2004), although incubation time was reduced from 12h to 8 h to

1 prevent any possible microcycle conidiation that may occur as
2 previously mentioned (Cliquet *et al.*, 2004; Ghajar *et al.*,
3 2006a). Germination was evaluated microscopically after
4 staining cellophane pieces with lactophenol cotton blue.

5

6 **Drying experiments**

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

13

14

15 **Exposure of conidia and chlamydospores to UV radiation**

16 Irradiation experiments were conducted in a dark cabinet. A
17 312 nm-UV-lamp (Vilber Loumat, Marne la Vallée, France)
18 supplied 95% radiation ranged in wavelengths from 260 to 380
19 nm with a peak at 312 nm. The UV-A and UV-B irradiances were
20 measured using a UVX radiometer (UVP, LLC, Upland, CA, USA)
21 and a UV 31 and a UV 36 sensors. The total irradiance was 2.30
22 W m^{-2} at a distance of 30 cm of the lamp.

23 Duplicate 200- μL droplets of each propagule suspension were
24 placed in a Petri dish (89 mm diameter) (Greiner Bio-one 34/15
25 with vents, Courtaboeuf, France) at pre-specified position at
26 a distance of 30 cm under the UV-lamp, and droplets were

1 simultaneously placed in a dark cabinet with no lamp
2 (controls). In order to prevent dehydration, distilled water
3 was placed underneath the plates. Temperature and relative
4 humidity in the dark cabinets with or without the UV-lamp were
5 recorded (Testo probe 175, Forbach, France) and remained
6 constant (21°C, RH = 75%) during the experiment.

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

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

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

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

24 Germination increased from 30 to 70%, depending on exposure
25 time (Fig.1). Accordingly, wavelengths < 315 nm have been

1 reported to reduce conidium germination, with the most marked
2 effect with wavelengths < 290nm (Ghajar et al., 2006a).

3 A 25 min exposure time to UV radiation (UV-B dose = 2 kJm⁻²;
4 UV-A dose = 0.4 kJm⁻²) was selected for further experiments on
5 the impact of UV on solid- or liquid- cultures. This UV-B dose
6 is in the lower range of UV-B levels (0 to 26 kJm⁻²) recorded
7 after exposure to full-spectrum sunlight at different times of
8 the day (Richmond, NSW, Australia, 18/03/02, Ghajar et al.
9 2006a)

10

11 **Statistical analysis**

12

13 The propagule yield experiment was repeated once.

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

26

1 RESULTS AND DISCUSSION

2

3 Propagule yields harvested from liquid cultures

4 Maximal chlamyospore and conidia yields were reached at 14
5 days incubation (29.2×10^5 chlamyospores mL^{-1} and 7.8×10^6
6 conidia mL^{-1} (Table 1). Similarly, other studies on *Fusarium*
7 *oxysporum* growth kinetics showed that chlamyospores were
8 formed on day 5 and that chlamyospore yields reached a peak
9 10-14 days after inoculation (Hebbar *et al.*, 1996; Elzein &
10 Kroschel, 2004). In a previous time course experiment (0-10 d
11 incubation) in which propagule yields were recorded each day
12 (Cliquet *et al.*, 2004), maximum chlamyospore yields were
13 obtained after 72 h incubation and remained constant until day
14 10. Increasing culture duration to 2-3 weeks is therefore
15 required for time course chlamyospore yields determination,
16 as generally reported in studies on chlamyospore production
17 (Gardner, Wiebe, Gillepsie, & Trinci, 2000); Shabana, Muller-
18 Strover & Sauerborn, 2003).

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

22

23 Germination of propagules freshly-harvested from liquid 24 cultures

25

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

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

18 The germination of chlamydospores harvested from 14-day-old
19 cultures was significantly reduced (55.3%) compared to the
20 germination of chlamydospores harvested from 7-day-old
21 cultures (72.7%). Similarly, 71% of 8-day-old chlamydospores,
22 60% of 3-month-old chlamydospores, and 34% of 6-month-old
23 chlamydospores germinated (Lanoiselet, Cother, Ash, & van de
24 Ven, 2001), indicating how time of harvest may impact fungal
25 germination. In our experimental conditions, most
26 chlamydospores are produced in intercalary chains inside

1 hyphal aggregates in which substrate and oxygen diffusion is
2 likely limiting, as reported in a large number of fermentation
3 studies on filamentous fungi (Gibbs, Seviour & Schmid, 2000).
4 Stress due to lack of substrate and oxygen may increase with
5 incubation length and substrate depletion, thus affecting the
6 physiological state of chlamyospores.

7

8 **Germination of UV-exposed propagules freshly harvested from** 9 **liquid cultures**

10 The germination of conidia and chlamyospores harvested from
11 7-day-old cultures following UV exposure decreased, (40% and
12 50% germination, respectively) whereas only 10 to 20% of UV
13 exposed propagules harvested from 14-day-old cultures
14 germinated (Fig. 2B).

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

24 A 3-way analysis of variance (Table 2) clearly indicates that
25 the germination of freshly-harvested propagules after UV
26 exposure is related to the cultural age ($P < 10^{-5}$) rather than

1 to the type of propagules considered ($P = 0.51$). How cultural
2 age impacts UV tolerance of conidia and chlamyospores is
3 unclear. During the germination process, stored reserve
4 materials such as lipids, trehalose, or mannitol are broken
5 down and used for energy production and the synthesis of new
6 cellular material (Garraway & Evans, 1984). Since we evaluated
7 germination by spraying conidia and chlamyospores on water
8 agar plates overlaid with cellophane membranes, endogenous
9 reserves were initially the sole source of nutrients available
10 to *P. alismatis* conidia and chlamyospores during the
11 germination process. Accumulation of endogenous lipids and
12 proteins (Jackson & Schisler, 1992), as well as mannitol or
13 trehalose (Ypsilos & Magan, 2004), have been reported to be
14 related to the age of cultures. Therefore, differences in UV
15 tolerance related to the culture age may be due, at least
16 partially, to the variations in availability of endogenous
17 reserves, among which mannitol or trehalose are known as
18 protecting the cell against oxidative stress (Rangel et al.,
19 2006), while endogenous protein may provide an amino acid pool
20 necessary for protein synthesis and facilitate rapid
21 germination (Jackson & Schisler, 1992).

22

23 **Germination of UV-exposed conidia freshly harvested from solid** 24 **cultures (PDA)**

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

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

9 **Germination of propagules harvested from liquid cultures and** 10 **dried**

11 Conidia and chamydospores produced in our liquid culture,
12 malt-extract sodium nitrate medium, and harvested either at 7
13 day or at 14 days incubation were dried. As previously
14 recorded for freshly-harvested propagules, the germination of
15 dry propagules after U-V exposure decreased as cultural age
16 increased (Fig. 3). The age of culture had a significant
17 impact (Table 2) on dry propagule germination rate ($P = 4.10^{-}$
18 4), regardless of the type of dry propagules considered ($P =$
19 0.48).

20 Generally, studies on propagule survival examine the impact of
21 environmental factors on a one factor-at-a-time basis. As
22 commercial economical application of the mycoherbicide
23 requires to produce high yields of stable, dry propagules able
24 to produce weed disease (Jackson & Schisler, 1992), it appears
25 realistic to consider at the same time fungal tolerance to
26 desiccation and tolerance to UV exposure. In the present work,

1 the combination of these 2 stresses led to at least 50%
2 decrease in germination.

3

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

11

12

13

14

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16

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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 chlamyospore-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 chlamyospore-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 chlamyospore 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

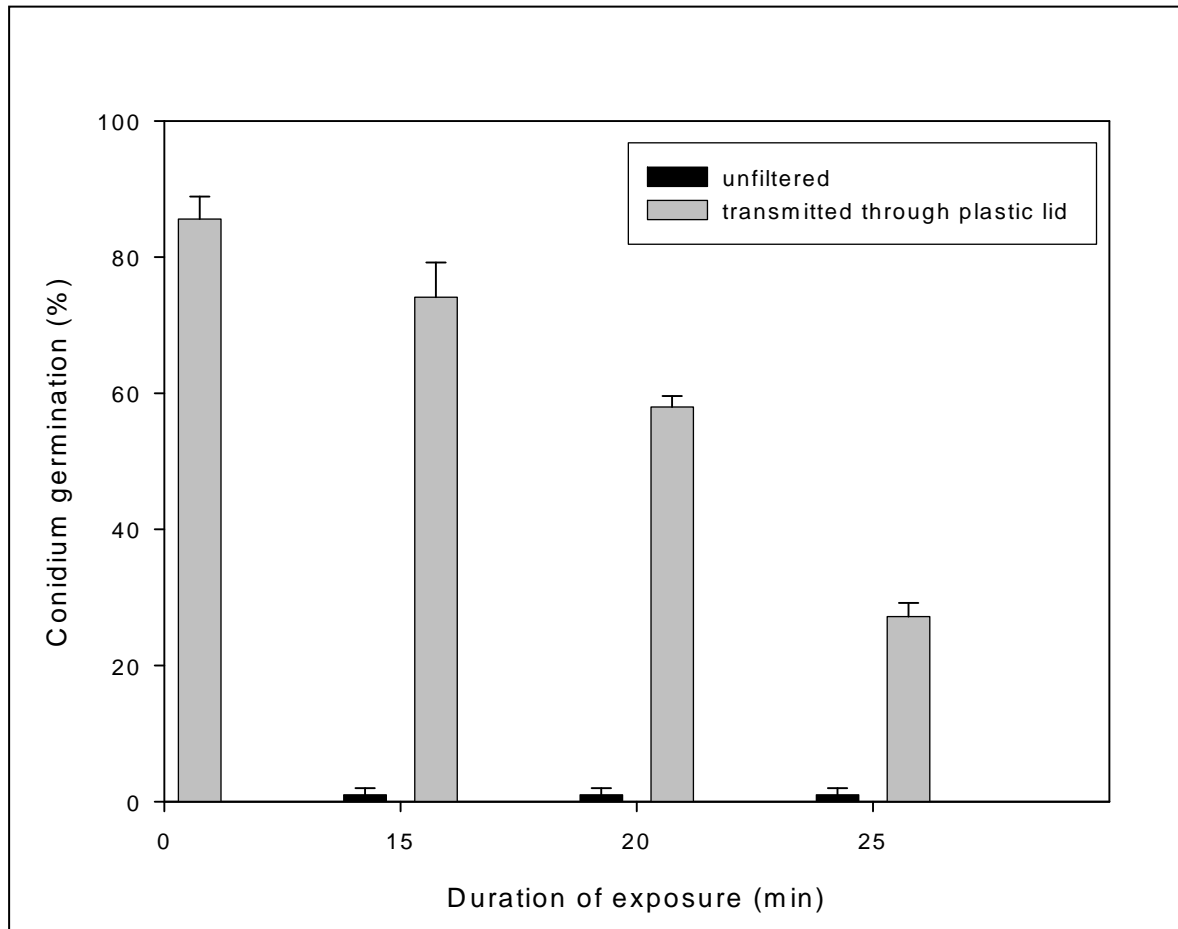


Figure 1.

Table 1. The impact of culture age on conidial and chlamyospore 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 x 10 ⁶ (b)	85.1 (ab)	1.07 x 10 ⁵ (b)	72.7 (a)
14 days	2.92 (a)	7.8 x 10 ⁶ (a)	79.1 (b)	29.2 x10 ⁵ (a)	55.3 (b)
21 days	2.92 (a)	5.8 x 10 ⁶ (ab)	91.7 (a)	6.7 x 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

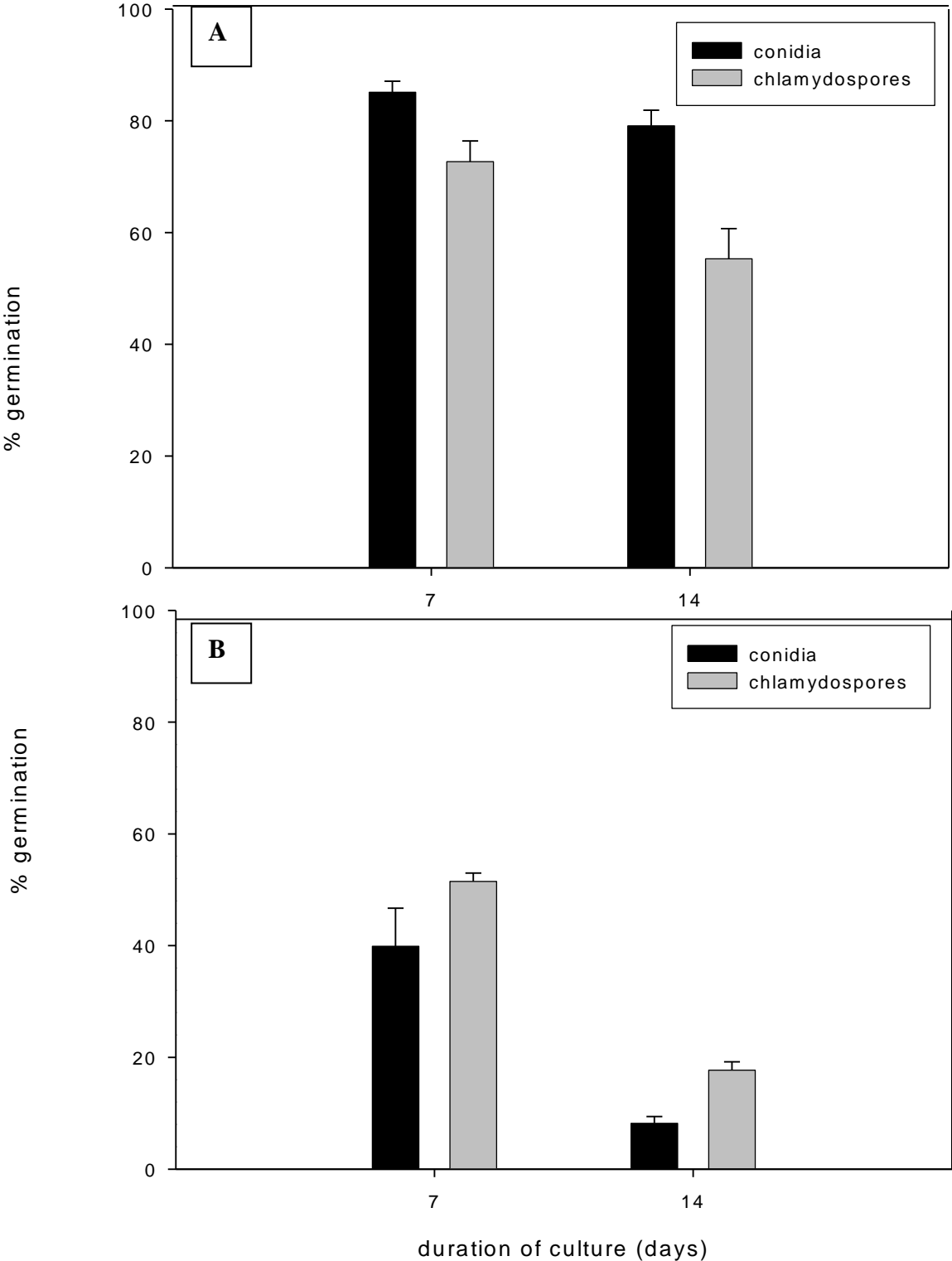


Figure 2.

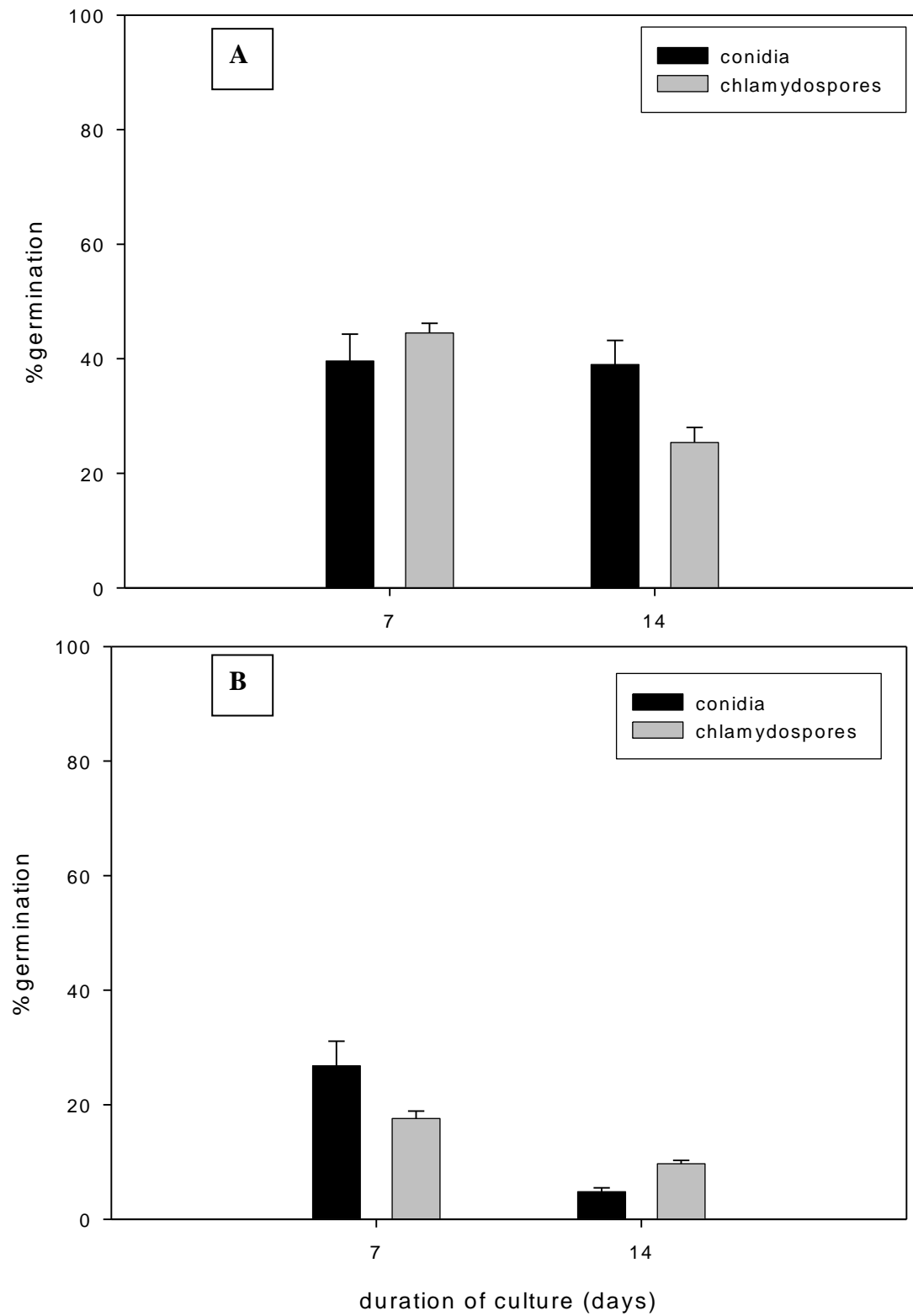


Figure 3.

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

