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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 ( $29.2 \times 10^5$  chlamyospores  $\text{mL}^{-1}$ ) than chlamyospore yields  
12 harvested from 7-day-old liquid cultures ( $1.07 \times 10^5$   
13 chlamyospores  $\text{mL}^{-1}$ ) 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<sup>®</sup>, 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-mm<sup>2</sup> 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  $-80^{\circ}\text{C}$  was  
4 inoculated onto a PDA plate, incubated for 2-3 weeks at  $25^{\circ}\text{C}$   
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  $25^{\circ}\text{C}$  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:  $\text{K}_2\text{HPO}_4$ ,  
19 1.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0 g;  $\text{KCl}$ , 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,  $8.8 \text{ gL}^{-1}$ ) and sodium nitrate (Sigma Chemical,  
22 St. Louis, MO, USA,  $5.74 \text{ g L}^{-1}$ ) 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 ( $4 \times 10^3$  conidia mL<sup>-1</sup>). 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  $7 \pm 0.5$  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<sup>-1</sup>.

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<sup>-1</sup>. 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<sup>-1</sup>.

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<sup>-1</sup>). 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 ( $5 \times 10^6$  propagules  $\text{mL}^{-1}$ ) 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  $\text{W m}^{-2}$  at a distance of 30 cm of the lamp.

23 Duplicate 200- $\mu\text{L}$  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- $\mu$ L were taken from the 200- $\mu$ 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<sup>-2</sup>, 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<sup>-2</sup>;  
4 UV-A dose = 0.4 kJm<sup>-2</sup>) 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<sup>-2</sup>) 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 ( $29.2 \times 10^5$  chlamyospores  $\text{mL}^{-1}$  and  $7.8 \times 10^6$   
6 conidia  $\text{mL}^{-1}$  (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  $93.5 \pm 2.2$  and  $95.8 \pm 0.8$  %

1 germination), germination of conidia harvested from 7-day-old  
2 cultures decreased dramatically after UV exposure ( $3.6 \pm 0.6$ )  
3 compared to germination of conidia harvested from 4-day-old  
4 cultures ( $27.6 \pm 2$ ) (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

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16

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**REFERENCES**

BARDIN M., SULIMAN M.E., SAGE-PALLOIX A-M., MOHAMED Y. F., & NICOT P.C.(2007) Inoculum production and long-term conservation methods for cucurbits and tomato powdery mildews. *Mycological Research* **111**, 6, 740-747

CLIQUET S., ASH G., & COTHER, E. (2004) Conidial and chlamyospore production of *Rhynchosporium alismatis* in submerged culture. *Biocontrol Science and Technology* **14**, 8, 801-810.

CLIQUET S., & ZEESHAN K. (2008) Impact of nutritional conditions on yields, germination rate and shelf-life of *Plectosporium alismatis* conidia and chlamyospores as potential candidates for the development of a mycoherbicide of weeds in rice crops. *Biocontrol Science and Technology* **1**, 1-11

COTHER E.J., & GILBERT R.L. (1994) Pathogenicity of *Rhynchosporium alimatis* and its potential as a mycoherbicide on several weed species in the *Alismataceae*. *Australian Journal of Experimental Agriculture* **34**, 1039-1042.

COTHER E.J., & VAN DE VEN R. (1999) The influence of nutrition on conidial production by *Rhynchosporium alismatis* and on

1 their subsequent infectivity to *Alisma lanceolatum*. *Biocontrol*  
2 *Science and Technology* **9**, 395-407.

3

4 CRUMP N.S., COTHER E.J., & ASH G.H. (1999) Clarifying the  
5 nomenclature in microbial weed control. *Biocontrol Science and*  
6 *Technology* **9**, 89-97.

7

8 ELZEIN A., & KROSCHEL J. (2004) Influence of agricultural by-  
9 products in liquid culture on chlamyospore production by the  
10 potential mycoherbicide *Fusarium oxysporum* Foxy 2. *Biocontrol*  
11 *Science and Technology* **14**, 8, 824- 836.

12

13 FRIEDBERG E.C., WALKER G.C., & SIEDE W. (1995) DNA repair and  
14 mutagenesis, American Society for Microbiology Press,  
15 Washington D.C.

16

17 GARDNER, K., WIEBE, M.G., GILLESPIE A.T., & TRINCI, A.P.J.  
18 (2000) Production of chlamydospores of the nematode-trapping  
19 *Duddingtonia flagrans* in shake flask culture. *Mycological*  
20 *Research* **104**, 205-209.

21

22 GARRAWAY M.O., & EVANS, R.C. (eds) (1984) Fungal nutrition and  
23 physiology. Wiley and Sons Inc., N-Y.

24

1 GHAJAR F., HOLFORD P., COTHER E., & BEATTIE A. (2006a) Effects  
2 of ultraviolet radiation, simulated or as natural sunlight, on  
3 conidium germination and appressorium formation by fungi with  
4 potential as mycoherbivores. *Biocontrol Science and Technology*  
5 **16**, 451-469.

6

7 GHAJAR F., HOLFORD P., COTHER E., BEATTIE A. (2006b) Enhancing  
8 survival and subsequent infectivity of conidia of potential  
9 mycoherbivores using UV protectants. *Biocontrol Science and*  
10 *Technology* **16**, 8, 825-839.

11

12 GIBBS P.A., SEVIOUR R.J., & SCHMID F. (2000) Growth of  
13 filamentous fungi in submerged culture: problems and  
14 solutions. *Critical Reviews in Biotechnology*, **20**, 1, 17-48.

15 GRAHAM R.J., PRAT R.J., PRATLEY, J.E., SLATER, P.D. & BAINES  
16 P.R. (1996) Herbicide resistant aquatic weeds, a problem in  
17 New South Wales, in Proceedings of 11th Australian Weeds  
18 Conference (SHEPHERD, C.H.,Ed.), 156-158. Weed Science Society  
19 of Victoria, Melbourne, Australia.

20

21 HALL R.A., PETERKIN D.D., ALI B., & LOPEZ V. (1994) Influence  
22 of culture age on rate of conidiospore germination in four  
23 deuteromycetous entomogenous fungi. *Mycological Research* **98**,  
24 763-768

25

1 HEBBAR K.P., LEWIS J.A., POCH S.M. & LUMSDEN R.D. (1996)  
2 Agricultural by-products as substrates for growth conidiation  
3 and chlamydospore formation by a potential mycoherbicide,  
4 *Fusarium oxysporum* strain EN4. *Biocontrol Science and*  
5 *Technology* **6**, 263-275.

6

7 JACKSON M.A., & SCHISLER D.A. (1992) The composition and  
8 attributes of *Colletotrichum truncatum* spores are altered by  
9 the nutritional environment. *Applied and Environmental*  
10 *Microbiology*, **58**, 7, 2260-2265

11

12 JACKSON M.A., & SCHISLER D.A. (2002) Selecting fungal  
13 biocontrol agents amenable to production by liquid culture  
14 fermentation. In *Proceedings of the 7th meeting of the IOBC*  
15 *OILB*, Kusadasi, Turkey IOBC/WPRS Bulletin

16

17 JACKSON M.A., CLIQUET S. & ITEN L.B. (2003) Media and  
18 Fermentation Processes for the Rapid Production of High  
19 Concentrations of Stable Blastospores of the Bioinsecticidal  
20 Fungus *Paecilomyces fumosoroseus*. *Biocontrol Science and*  
21 *Technology* **13**, 23-33.

22

23 JAHROMI F.G., COTHER E.J., & ASH G.J. (2002) Early infection  
24 process of *Damasonium minus* by the potential mycoherbistat



1 *Rhynchosporium alismatis*. *Canadian Journal of Plant Pathology*  
2 **24**, 131-136

3

4 Jahromi F.G. (2007) Effect of environmental factors on disease  
5 development caused by the fungal pathogen *Plectosporium*  
6 *alismatis* on the floating-leaf stage of starfruit (*Damasonim*  
7 *minus*), a weed of rice. *Biocontrol Science and Technology*, **17**,  
8 8, 871-877

9

10 LANOISELET, V., COTHER, E.J., ASH G.J., & VAN DE VEN R. (2001)  
11 Production, germination and infectivity of chlamydospores of  
12 *Rhynchosporium alismatis*. *Mycological Research* **105**, 4, 441-  
13 446.

14

15 MADRONICH S., MCKENZIE R.L., CALDWELL M.M., & BJORN L.O. 1995.  
16 Changes in ultraviolet radiation reaching the earth's surface.  
17 *Ambio* 24, 143-152

18

19 MOODY S.A., NEWSHAM K.K., AYRES P.G., & PAUL N.D. (1999)  
20 Variation in the responses of litter and phylloplane fungi to  
21 UV-B radiation (290-315 nm). *Mycological Research* **103**, 11,  
22 1469-1477

23

1 MYANISK M., MANASHEROB M., BEN-DOV E., ZARITSKY A., MARGALITH  
2 Y., & BARAK Z. (2001) Comparative sensitivity to UV-B  
3 radiation of two *Bacillus thuringiensis* subspecies and other  
4 *Bacillus* sp. *Current Microbiology*, **43**, 2, 140-143  
5  
6 NAKASONE K.K., PETERSON S.W., & SHUNG-CHANG J. (2004)  
7 Preservation and distribution of fungal cultures. In:  
8 *Biodiversity of fungi, Inventory and Monitoring methods*, p 41.  
9 Mueller, Bills and Foster Eds. Elsevier, Oxford, UK.  
10  
11 RANGEL D.E.N., ANDERSON A.J. & ROBERTS D.W. (2006) Growth of  
12 *Metarhizium anisopliae* on non-preferred carbon sources yields  
13 conidia with increased UV-B tolerance. *Journal of Invertebrate*  
14 *Pathology* **93**, 2, 127-134

SHABANA Y.M., MÜLLER-STRÖVER D., & SAUERBORN J. (2003) Granular pest formulation of *Fusarium oxysporum* f.sp. *orthoceras* for biological control of sunflower broomrape: efficacy and shelf-life. *Biological Control* **26**, 189-201

YPSILOS K.I., & MAGAN N. (2004) Impact of water-stress and washing treatments on production, synthesis and retention of endogenous sugar alcohols and germinability of *Metarhizium anisopliae* blastospores. *Mycological Research* **108**, 11, 1337-1345

#### LEGENDS OF FIGURES AND TABLES

Figure 1. The effect of UV radiation, either unfiltered (irradiance =  $2.30 \text{ W m}^{-2}$  in the range [260-400 nm] with a 312 nm peak) or transmitted through plastic lid (irradiance =  $1.64 \text{ W m}^{-2}$  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<sup>-1</sup>; and sodium nitrate : 5.74 gL<sup>-1</sup>

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<sup>-1</sup>; and sodium nitrate : 5.74 gL<sup>-1</sup>

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

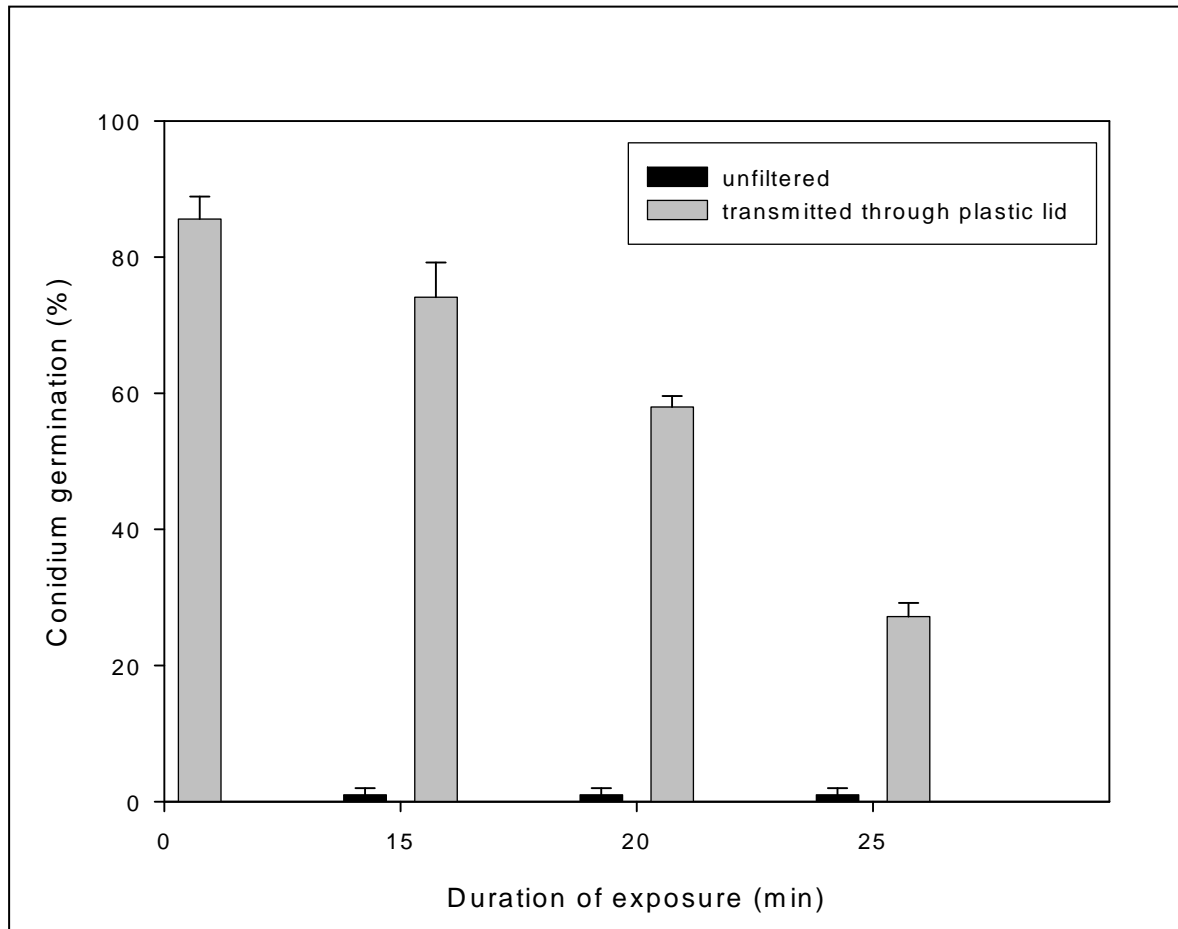


Figure 1.

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

Culture age <sup>a</sup>	Dry Weights (mg mL <sup>-1</sup> )	Conidia (mL <sup>-1</sup> )	Conidial germination <sup>b</sup> (%)	Total chlamydo-spores (mL <sup>-1</sup> ) <sup>c</sup>	Chlamyospore germination (%)
7 days	1.76 (b) <sup>d</sup>	3.85 x 10 <sup>6</sup> (b)	85.1 (ab)	1.07 x 10 <sup>5</sup> (b)	72.7 (a)
14 days	2.92 (a)	7.8 x 10 <sup>6</sup> (a)	79.1 (b)	29.2 x10 <sup>5</sup> (a)	55.3 (b)
21 days	2.92 (a)	5.8 x 10 <sup>6</sup> (ab)	91.7 (a)	6.7 x 10 <sup>5</sup> (ab)	71.7 (a)

<sup>a</sup> *P. alismatis* was grown in 8.8 g L<sup>-1</sup> malt extract and 5.74 g L<sup>-1</sup> sodium nitrate in submerged culture at 150 rpm and 25°C.

<sup>b</sup> Germination of fresh propagules was evaluated after 8 h incubation on cellophane squares placed on water agar at 25°C

<sup>c</sup> Production of chlamydo-spores during growth was expressed as total chlamydo-spore counts. A count represents either a chain of chlamydo-spores, a single-celled chlamydo-spore or a double-celled chlamydo-spore.

<sup>d</sup> Pairs of treatments with a letter into brackets in common do not differ significantly (P<0.05) based on a pair-wise LSD test



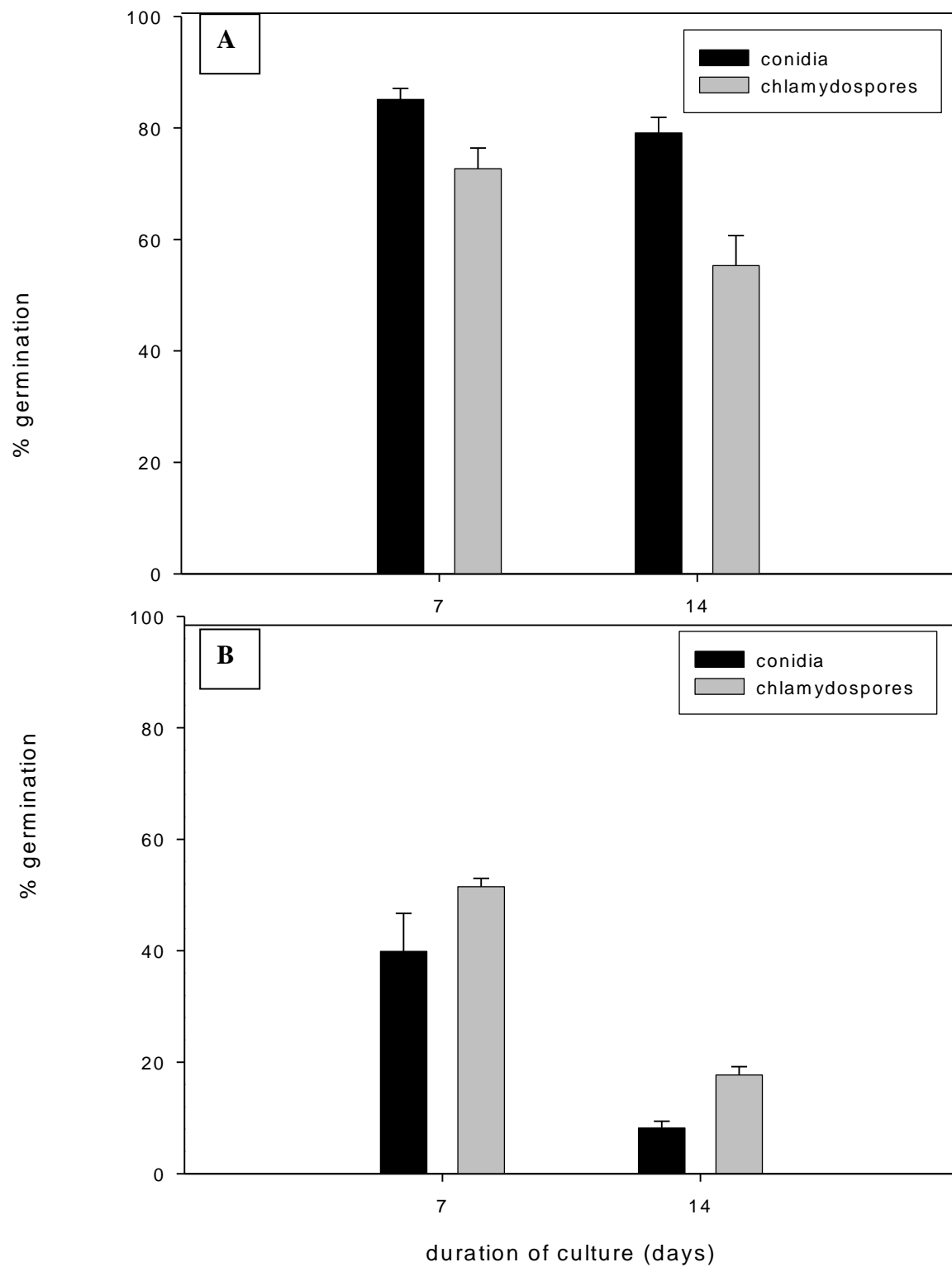


Figure 2.



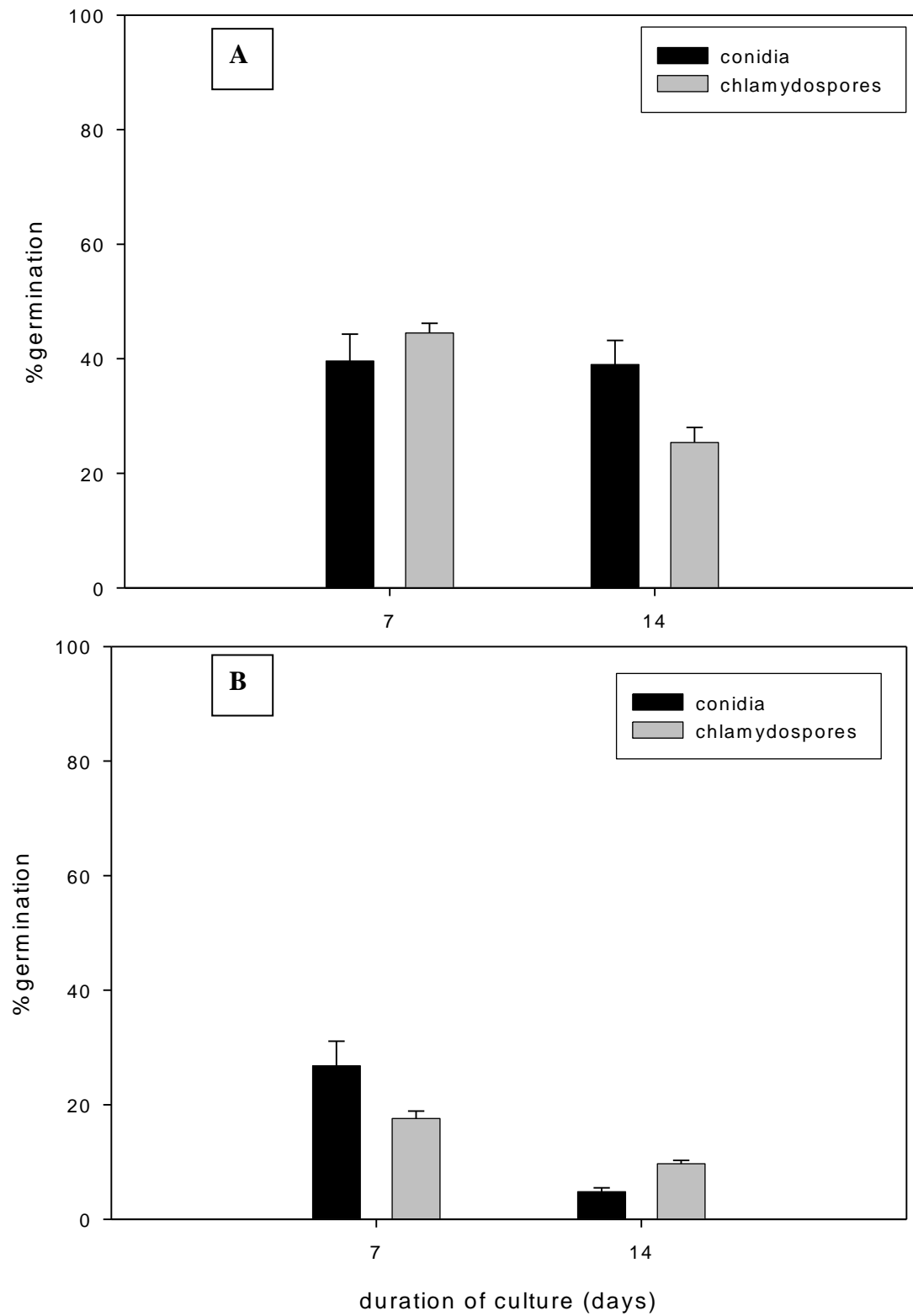


Figure 3.

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

