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ORIGINAL ARTICLE

Effect of temperature and water activity on spore germination and mycelial growth of three fungal biocontrol agents against water hyacinth (*Eichhornia crassipes*)

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Abstract

Aims: To determine the effect of water activity (a_w = 0·880–0·960) and temperature (15–35°C) on the percentage of viable conidia and mycelial growth of three biocontrol agents effective against water hyacinth in Mali: *Alternaria* sp. isolate Mlb684, *Fusarium sacchari* isolate Mln799 and *Cadophora malorum* isolate Mln715.

Methods and Results: The fungi were grown *in vitro* on plates containing potato dextrose agar medium at different a_w values (glycerol being added to adjust the a_w). The percentage of viable conidia and radial growth rate decreased with decreasing water activity. Statistical analysis showed a significant effect of a_w, temperature and the $a_w \times$ temperature interaction on mycelial growth ($P < 0{\cdot}0001$). Water activity emerged as the factor exerting the greatest influence. Differences were observed between the fungi tested, the *C. malorum* appearing more tolerant to low a_w and the *F. sacchari* more tolerant to high temperature (35°C). Growth models predicting the combined effect of a_w and temperature were developed and response surfaces generated, showing fairly good agreement with the experimental values.

Conclusions: Our results confirm the previous finding that a_w has a greater influence than temperature on fungal growth. Under most conditions, variation of environmental factors has a detrimental influence on the percentage of viable conidia and mycelial growth rate of fungal isolates.

Significance and Impact of the Study: The developed models may contribute to predicting the best environmental conditions for use of these fungi as effective biocontrol agents against water hyacinth.

Introduction

Water hyacinth (*Eichhornia crassipes*), originally from South America, is the most prolific aquatic weed worldwide. Since its introduction in the 1990's as an ornamental plant into aquatic areas in Mali, it has become a main focus of intense control efforts (Dagno 2006).

Water hyacinth has become widespread and is viewed as the worst aquatic weed throughout the tropical and subtropical regions (El-Morsy 2004). The 'explosive' growth of the plant and its ability to infest a wide range of freshwater habitats has created enormous environmental and economical problems. Water hyacinth is considered a major aquatic weed in Africa (Niger, Benin, Congo, Egypt, Tanzania, Uganda, Mali and South Africa), Europe (Spain) and several Asian and American countries (Dagno *et al.* 2007). It causes widespread problems for millions of users of water bodies and water resources, and this is especially the case in Mali. The Malian authority spends many billion dollars each year to control water

hyacinth infestation (Dagno 2006). Several investigators have therefore focused on controlling water hyacinth by physical, chemical, or biological methods (Charudattan 2005). Among the control methods available, biological control is increasingly viewed as an attractive, eco-friendly method for use in agriculture (Lahlali and Hijri 2010).

Biocontrol by means of micro-organisms is an emerging strategy in many countries infested with water hyacinth. Several good arguments suggest that plant pathogens are worth consideration as biocontrol agents: pathogens can cause significant reductions in water hyacinth biomass, notably following natural disease after severe attacks by insects, or when used as inundative bioherbicides (Charudattan 2001). Several previous findings highlight the potential of *Acremonium zonatum*, *Alternaria eichhorniae* and *Cercospora piaropi* for controlling water hyacinth (i.e. reducing the weed's biomass) under controlled conditions (Shabana and Mohamed 2005). In Mali, research on the biological control of water hyacinth was initiated in 2006. It has led to the isolation and identification of *Alternaria* sp. (isolate Mlb684), *F. sacchari* (isolate Mln799) and *Cadophora malorum* (isolate Mln715) (K. Dagno, R. Lahlali, M. Diourté and M.H. Jijakli, unpublished data). Currently, there are no data available on large-scale trials carried out with these fungal isolates, used as aqueous conidial preparations. The variability of the performance of these isolates as biocontrol agents may be influenced by environmental factors that vary over time and from one area to another. Nanguy *et al.* (2010), Begoude *et al.* (2007), Plaza *et al.* (2003) and Patriarca *et al.* (2001) reported that water activity (a_w) and temperature are the principal abiotic parameters determining the germination and fungal growth. Accordingly, knowledge of the biology of biocontrol agents against water hyacinth, and notably regarding their germination and growth in relation to temperature and a_w, should be useful in developing a more effective mycoherbicide. Boyette *et al.* (2007) reported that these two environmental factors are the most important parameters influencing the efficacy of a mycoherbicide.

To optimize the practical use of a biological control agent, it is essential to understand how the physical environment affects the agent's survival, germination and growth (Sanogo *et al.* 2002). Response surface methodology (RSM) is the approach most often used to model relationships between a combination of factors and an organism's growth curve parameters (Devlieghere *et al.* 1998). To our knowledge, no report is available on the effects of temperature and water activity on the development of *F. sacchari*, or *C. malorum*. Regarding *Alternaria*, Pose *et al.* (2009) reported the increase in the germination and the growth rate of *Alternaria alternata* with increasing a_w values of substrate. They observed no growth or germination at the lowest a_w level evaluated (0·904) after 100 days of incubation at 6 °C and 15 °C. With the exception of *F. sacchari*, none of these fungi pose any risk to human, animal health or economically important crops in Mali and are viewed as attractive candidates for managing water hyacinth infestations in Mali. Hence, the aim of this work was to assess the effects of temperature and a_w on the percentage of viable conidia and mycelial growth of *F. sacchari*, *C. Malorum* and *Alternaria* sp. and to elaborate predictive models based on the collected mycelial growth data.

Materials and methods

Fungi

Fusarium sacchari isolate Mln799, *C. malorum* isolate Mln715 and *Alternaria* sp. isolate Mlb684 were identified by the Industrial Fungal & Yeast Collection (BCCM/MUCL- Louvain-la-Neuve, Belgium) and by Dr E.G. Simmons (USA). For long-term storage, the strains were placed at −70°C in tubes containing 25% glycerol at the Plant Pathology Unit (Gembloux Agro-Bio Tech, University of Liege). The initial conidial inocula used in the experiments were taken from Petri-dish cultures on potato dextrose agar (PDA; Merck, Darmstadt, Germany), preserved at 4°C for no more than 6 months and then subcultured at 25°C on different culture media before use.

Media

One specific medium was used for each fungal isolate, PDA for *F. sacchari*, V8 agar for *Alternaria* sp. and MA2 (Malt agar 20%) for *C. malorum*. The water activity was adjusted by addition of increasing amounts of glycerol to obtain levels of 0·960, 0·920 and 0·880 at 15, 25 and 35°C (Lahlali *et al.* 2008). The range of temperature and a_w was chosen according to their minimum and maximum averages recorded in Mali. The a_w of all media was measured with an AquaLab 3TE (Decagon Device, Inc., 2365 NE Hopkins Court Pullman, WA, USA).

Effect of temperature and a_w on the percentage of viable conidia and mycelial growth of fungal isolates

The effects of a_w and temperature on the percentage of viable conidia were studied for *F. sacchari*, *C. malorum* and *Alternaria* sp. Percentage of viable conidia was evaluated at three a_w values and three temperatures 4, 8 and 24 h after inoculation of Petri dishes containing the test media. For each a_w–temperature combination, there were three Petri dishes, and each Petri dish was seeded with

three individual 10-μl droplets (containing 1×10^5 spores ml^{-1}) of conidial suspension in separate wells. After inoculation, the Petri plates were sealed in polyethylene bags to prevent water loss and placed immediately in incubators set at the appropriate temperature. The preservation of water content in the media was checked by measuring the a_w of inoculated Petri dishes at the end of experiment, and no change in the a_w of any tested medium was detected. At each assessment time, the percentage of viable conidia was estimated by observation under the microscope (at 40× or 100× magnification) of 100 conidia from each droplet of inoculum, thus yielding a total of nine counts per treatment at each time (Xu *et al.* 2001). The spores were considered alive when the length of the germinate tube was equal to half of the diameter of the spore (Paul *et al.* 1992). To evaluate the effects on radial growth, a 10-μl aliquot of 10 μl 10^5 spores ml^{-1} was inoculated at the centre of Petri dishes containing a test medium. Petri plates were sealed and then incubated at each temperature. The average radial growth of each growing mycelial colony was measured daily (in mm) in two perpendicular directions without opening the Petri dishes, until the plates were completely colonized (Marin *et al.* 1996). Growth rates (mm day^{-1}) were calculated for each a_w–temperature combination by linear regression from the linear phase of the growth curve. This experiment was conducted three times with three replicates.

Statistical analyses

A fully factorial design run in triplicate was used to generate the percentage of viable conidia and growth rate of *F. sacchari* (isolate Mln799), *C. malorum* (isolate Mln715) and *Alternaria* sp. (isolate Mlb684) in modified media at three temperatures and three a_w levels. Variance analysis was used to assess the effects of temperature and a_w on the percentage of viable conidia and mycelial growth *in vitro*. Growth rates were subjected to the general linear model procedure of the Statistical Analysis System (SAS software ver 9.1. Cary, NC, USA). All statistical significances were estimated at $P = 0{\cdot}05$. Where ANOVA revealed significant differences, Duncan's multiple range tests were applied to the means. Percentages of viable conidia were modelled using a nonlinear equation $y = ax^2 + bx + c$, where y, x, (a and b) and c represent, respectively, the percentage of viable conidia, incubation temperature, model parameters and the response value of y for all factors equal to zero.

MINITAB – 15 ENGLISH was used to apply RSM to a 3^2 factorial design. Temperature (15, 25 and 35°C) and a_w (0·880, 0·920 and 0·960) were the studied factors, and the design included nine experiments with three replicates. The following quadratic polynomial model was fitted to the response:

$$Y = B_0 + \sum_{i=1}^{2} B_i X_i + \sum_{i=1}^{2} B_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1} B_{ij} X_i X_j$$

where Y is the response (growth rate in mm day^{-1}), B_0 is a constant coefficient, X_i are coded variables that can have three values (−1, 0, or 1), B_i are linear coefficients, B_{ij} are the second-order interaction coefficients, and B_{ii} are the quadratic coefficients. All model coefficient values were calculated by multiple regression analysis. Interpretation of the data was based on the sign (positive or negative effect on the response) and statistical significance ($P < 0{\cdot}05$) of each coefficient. The notion of major effects $\beta i.Xi$ assumes that the main effects are both positive and the interaction effect $\beta ijXiXj$ is antagonistic (negative) or synergistic (positive). R^2 (the coefficient of determination) and the regression coefficients (βi and Bij) were employed to evaluate regression model performance.

Results

Effects of temperature and a_w on the percentage of viable conidia

Figure 1 shows, for each isolate and at different incubation times, how the percentage of viable conidia (i.e. the percentage of conidia having germinated) varied at different water activities as a function of the incubation temperature. As expected, the percentage of viable conidia increased over time. At $a_w = 0{\cdot}88$, all three isolates germinated poorly, *Alternaria* sp. showing the lowest rates (17% after 24 h at 25°C) and *F. sacchari* the highest (52% after 24 h at 35°C). Overall, the percentage of viable conidia was found to improve with increasing water activity, reaching or approaching 100%, at the considered strain's optimal germination temperature, within 24 h at $a_w = 0{\cdot}96$ and $a_w = 0{\cdot}92$. The *C. malorum* and *Alternaria* sp. both germinated better at 25°C than at 15°C or 35°C, but *F. sacchari* seemed, under most conditions, to germinate best at 35°C. ANOVA showed the main effects of temperature and a_w on the percentage of viable conidia to be significant ($P < 0{\cdot}01$) (data not shown).

Effects of temperature and water activity on mycelial growth

Figure 2 presents the average radial growth rates (in mm day^{-1}) of the three strains under the conditions tested, along with the results of Duncan's multiple range analysis showing which growth-rate differences were statistically significant. *Alternaria* sp. and *F. sacchari* showed

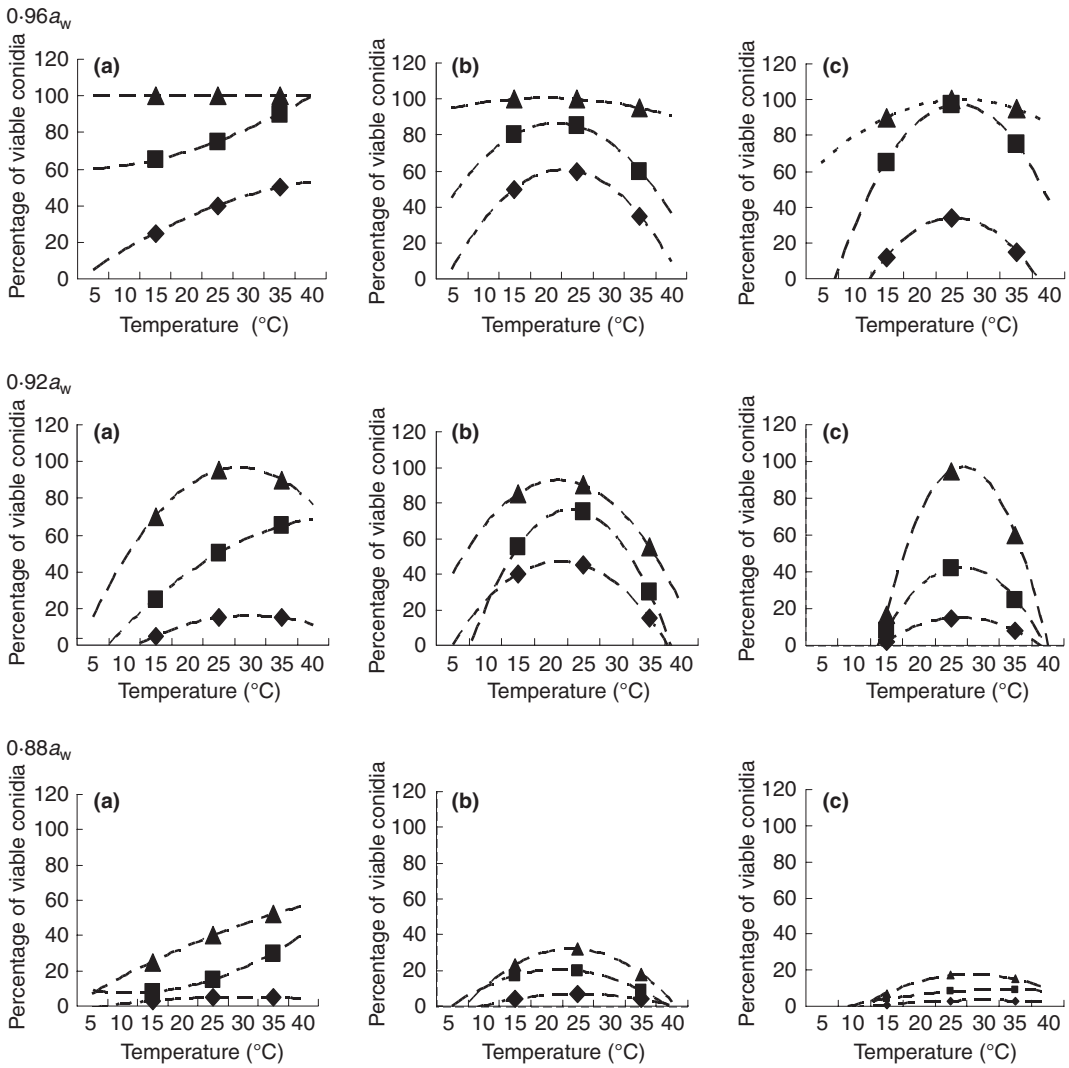

Figure 1 Mean germination rate as a function of temperature according to the strain, the water activity and the incubation time. (a) *Fusarium sacchari* (isolate Mln799), (b) *Cadophora malorum* (isolate Mln715), (c) *Alternaria* sp. (isolate Mlb684). The symbols ◆, ■, and ▲ indicate the germination time (4, 8 and 24 h, respectively). The dotted lines are fitted curves (equation in the text).

no growth at $a_w = 0{\cdot}88$ at any temperature (Fig. 2a,c), but the *C. malorum* did show some growth at this a_w (Fig. 2b). All three isolates were found to grow faster with increasing water activity. For *C. malorum*, an increase was observed only at $a_w = 0{\cdot}96$ (Fig. 2b). When growth was observed, it was always better at 25°C than at 15°C or 35°C, although in one case (*C. malorum* at $a_w = 0{\cdot}96$, Fig. 2b), the difference between 15°C and 25°C was not significant. The *Alternaria* sp. (isolate Mlb684) failed to grow at 35°C at any a_w (Fig. 2a). The highest growth rate (3·5 mm day^{-1}) was observed for *F. sacchari* at $a_w = 0{\cdot}96$ and 25°C (Fig. 2c). Variance analysis (Table 1) showed that temperature, a_w and their interaction significantly influenced the fungal growth rate.

Modelling the combined effect of temperature and a_w on the growth rates of fungal isolates

RSM was then used to model the effects of temperature and water activity on the growth rate of our three potential biocontrol agents against water hyacinth. For each strain, a quadratic polynomial model based on a 3^2 factorial design was fitted to the data by multiple regression analysis (see Materials and methods). For the temperature and water activities tested, there is fairly good agreement between the observed and predicted values (Table 2). Table 3 shows the respective R^2 values, 85·89, 77·31 and 93·40% for *C. malorum*, *Alternaria* sp. and *F. sacchari*, respectively, the determined model coefficients, and their

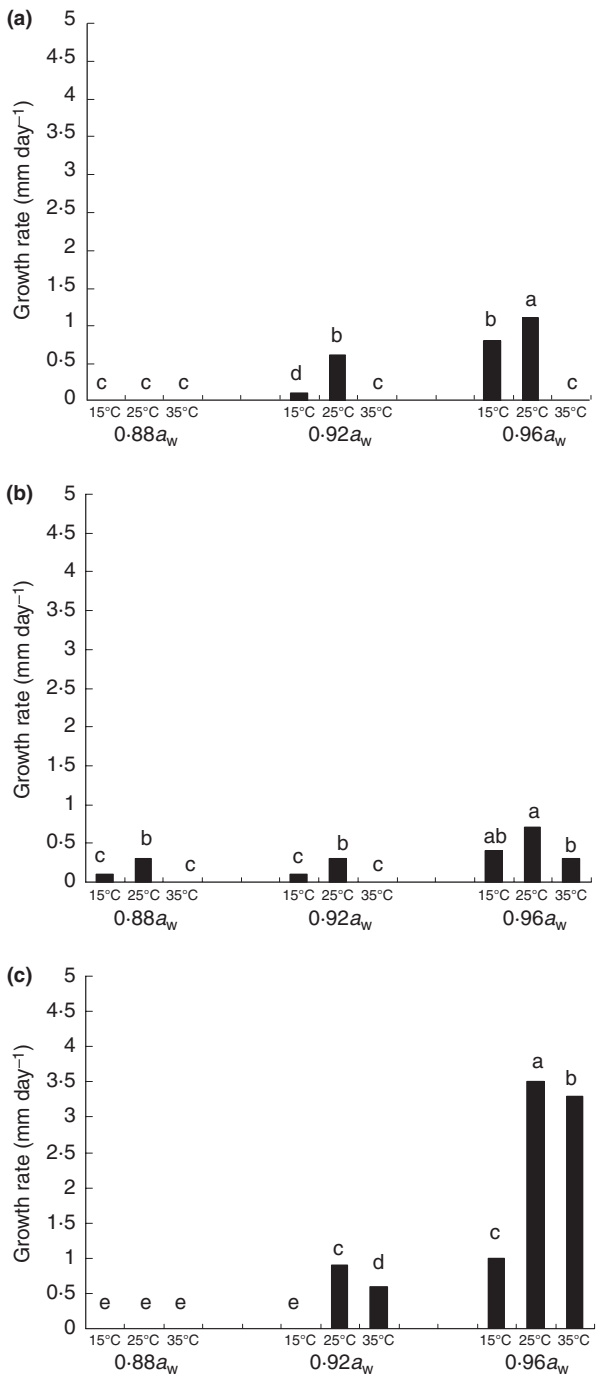

Figure 2 Average growth rates of (a) *Alternaria* sp. (isolate Mlb684), (b) *Cadophora malorum* (isolate Mln715) and (c) *Fusarium sacchari* (isolate Mln799) at different temperatures and water activities. Significance was determined by Duncan's multiple range tests. Results with the same letter are not significantly different ($P < 0{\cdot}05$).

levels of significance. Model parameters underlined that a_w had a greater effect than temperature on mycelial growth (Table 3). Water activity had positive linear and

Table 1 Variance analysis of the effects of water activity (a_w), temperature (T) and their interactions on the growth rate of *Alternaria* sp. (isolate Mlb684), *Cadophora malorum* (isolate Mln715) and *Fusarium sacchari* (isolate Mln799)

Isolate	Source of variation	df	MS	*F* statistic	Pr > *F*
Mlb684	a_w	2	0·008255	18·71	0·0001**
	T°C	2	0·0042	20·15	0·0001**
	$a_w \times T$°C	4	0·003997	9·06	0·0001**
Mln715	a_w	2	0·004130	36·75	0·0001**
	T°C	2	0·004250	35·60	0·0001**
	$a_w \times T$°C	4	0·000234	2·08	0·0001**
Mln799	a_w	2	0·176814	190·62	0·0001**
	T°C	2	0·164250	213·60	0·0001**
	$a_w \times T$°C	4	0·040472	43·63	0·0001**

MS, mean square; df, degrees of freedom; Pr, probability; **significant ($P < 0{\cdot}0001$).

quadratic effect on the growth rate of fungal isolates whereas the temperature had a negative linear effect for both fungal isolates *C. malorum* and *Alternaria* sp. and a positive linear effect for *F. sacchari*. Regardless of the fungal isolate, temperature had a negative quadratic effect on the growth rate. For all fungal isolates, the effect of the interaction temperature and a_w revealed to be antagonistic, suggesting that the effect of one factor is reduced as the value of the other increases.

Discussion

We have focused here on the percentage of viable conidia and mycelial growth rates of three fungal pathogens, *F. sacchari*, *C. malorum* and *Alternaria* sp., as possible indicators of the capacity of these pathogens to colonize the water hyacinth ecosystem. In greenhouse trials, these organisms have previously been found to induce 70% (isolates Mln799, Mln715) or 71% (isolate Mlb684) foliar lesions in this plant. Yet the efficacy of such pathogens tends to be greater and less variable in the greenhouse than in the field (Boyette *et al.* 2007), and environmental factors are believed to influence importantly the efficacy of weed biocontrol agents under field conditions. Temperature and water activity are suspected of being the major determining factors (Babu *et al.* 2003; Charudattan 2005; Nanguy *et al.* 2010), but with the exception of *Alternaria* (Pose *et al.* 2009), we have found no report in the literature describing the influence of these factors on the growth or germination of the above-mentioned strains. We have therefore studied the behaviour of these fungi *in vitro* on glycerol-supplemented media and modelled their growth.

In the present study, glycerol was the only solute used to adjust the a_w of the medium. This solute can support growth because it is a potential carbon source for

Table 2 Experimental and predicted values of the growth rate for *Fusarium sacchari* (isolate Mln799), *Cadophora malorum* (isolate Mln715) and *Alternaria* sp. (isolate Mlb684). To obtain the predicted values, a factorial design (3^2) was applied, the factors studied being temperature (T) and water activity (a_w)

					Radial growth rate (mm day^{-1})					
	Experimental value		Coded value		Observed value			Predicted value		
Experimental factors	a_w	T	a_w	T	Mln799	Mln715	Mlb684	Mln799	Mln715	Mlb684
E1	0·96	15	1	−1	1·00 ± 0·00	0·40 ± 0·00	0·80 ± 0·01	1·30	0·50	0·80
E2	0·96	25	1	0	3·50 ± 0·00	0·70 ± 0·00	1·10 ± 0·00	3·10	0·70	0·90
E3	0·96	35	1	1	3·30 ± 0·00	0·30 ± 0·00	0·00 ± 0·00	3·50	0·20	0·10
E4	0·92	15	0	−1	0·00 ± 0·00	0·10 ± 0·00	0·10 ± 0·00	0·00	0·10	0·20
E5	0·92	25	0	0	0·90 ± 0·00	0·30 ± 0·00	0·60 ± 0·01	1·00	0·40	0·50
E6	0·92	35	0	1	0·60 ± 0·01	0·00 ± 0·00	0·00 ± 0·00	0·80	0·00	0·00
E7	0·88	15	−1	−1	0·00 ± 0·00	0·10 ± 0·00	0·00 ± 0·00	0·00	0·10	0·00
E8	0·88	25	−1	0	0·00 ± 0·00	0·30 ± 0·00	0·00 ± 0·00	0·50	0·40	0·30
E9	0·88	35	−1	1	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00	0·00	0·00	0·00

Table 3 Coefficients of the models describing the growth rates of *Cadophora malorum* (isolate Mln715), *Alternaria* sp. (isolate Mlb684) and *Fusarium sacchari* (isolate Mln799) at different temperatures (T) and water activity (a_w), and significance thereof

Factor or interaction	Coefficient	Mln715	Mlb684	Mln799
	R^2	85·89	77·31	93·40
	$\beta 0$	0·044**	0·054**	0·103**
a_w	$\beta 1$	0·017**	0·033**	0·130**
T	$\beta 2$	−0·012**	−0·014*	0·050**
a_w^2	$\beta 11$	0·014*	$0·010^{ns}$	0·077**
T^2	$\beta 22$	−0·037**	−0·046**	−0·070**
$a_w \times T$	$\beta 12$	$-0·004^{ns}$	−0·018**	−0·070**

ns, not significant ($P > 0·05$); *significant ($P < 0·05$); **highly significant ($0·05 < P < 0·0001$).

micro-organisms (Parra *et al.* 2004). In addition, glycerol exhibits no inhibitory effect (Baxter *et al.* 1998) and can be used to reach lower a_w values than other solutes, such as NaCl (Lahlali *et al.* 2005).

From our experiments, water activity emerged as a crucial determinant of germination for all three strains, their germination being fastest at $a_w = 0·96$ (reaching 100% at 25°C within 24 h) and slowest at $a_w = 0·88$. At high water activity, their conidia could germinate fast over a wide range of temperatures, from 15 to 35°C. At $a_w = 0·96$ and 25°C, for example, it took only 4 h for 35–60% of the viable conidia to germinate, depending on the organism studied. This may explain why long wetness periods do not hinder infection of water hyacinth plant by these three fungal pathogens. Our germination data are in agreement with results previously reported for two apple brown rot fungi, *Monilinia fructigena* and *Monilinia fructicola* (Tamm and Fluckiger 1993; Xu *et al.* 2001). Pose *et al.* (2009) reported that the germination time of *A. alternata* increased with a reduction on a_w.

Our growth data show that temperature and a_w are key determinants of growth for the studied strains. Water activity emerges as the factor having the greatest influence on mycelial growth of fungal isolates. No growth was observed at a_w of 0·88 for both isolates *Alternaria* sp. and *F. sacchari* regardless of temperature while there was low growth for *C. malorum* at 15 and 25°C. Our results demonstrated that a_w has a greater influence than temperature on the growth rates of fungal isolates Mlb684, Mln715 and Mln799. These results are in agreement with the previous finding that the amount of available water in the substrate and the surrounding environment is very important for fungal growth (Lahlali *et al.* 2008). Furthermore, in accordance with Sparringa *et al.* (2002) on *Rhizopus oligosporus*, a significant interaction between the two studied factors was shown.

Both germination and growth are important for the efficacy of a fungus used as a biocontrol agent. Of the three organisms studied, both the *F. sacchari* and the *C. malorum* isolates appear to germinate better at $a_w = 0·88$ than the *Alternaria* sp. *F. sacchari*, moreover, shows better germination at temperatures above 35°C than either of the other strains, whose temperature optimum for germination is near 25°C. Pose *et al.* (2009) reported similar patterns of the influence of a_w and temperature on the germination and the growth rate of *A. alternata* with shortest germination at 35°C and no growth at $a_w = 0·90$ and temperature of 15°C. Regarding growth, the *F. sacchari* again shows better tolerance of higher temperature, whereas the *C. malorum* shows better tolerance of water stress. Our findings suggest that countering water stress could be a means of improving the

performance of fungal biocontrol agents under field conditions. Vegetable oil might be used for this purpose (Shabana 2004).

Data are available concerning the effects of temperature and water activity on a number of other fungi. Lasram *et al.* (2010) report optimal growth of *Aspergillus carbonarius* at temperatures ranging from 25 to 30°C and $a_w = 0{\cdot}99$. They mention that this fungus grows poorly at 15°C and at $a_w \leq 0{\cdot}90$. Romero *et al.* (2010) likewise report slow growth of *A. carbonarius* at low a_w and temperature (0·83 and 15°C). Similar effects of a_w and temperature were observed by Bekada *et al.* (2008) for the fungus *Mucor racemosus*, and Begoude *et al.* (2007) found that *Trichoderma asperellum* failed to grow at $a_w = 0{\cdot}88$, whatever the temperature.

On the basis of our radial growth data, we have developed models as tools for interpreting such data. Within the temperature and a_w ranges specified, the selected models predict fairly accurately the growth rates of these three strains. At $a_w = 0{\cdot}88$, for instance, the *C. malorum* model correctly predicts slight growth at 15 and 25°C but none at 35°C, and the *F. sacchari* and *Alternaria* sp. models correctly predict no growth of either strain at 15 or 35°C. Yet, there are some slight discrepancies between the observed and predicted values, such as the slight growth predicted for *F. sacchari* and *Alternaria* sp. at $a_w = 0{\cdot}88$ for 25°C.

Modelling the growth of these fungal isolates on a solid substrate is a first step towards simulating what happens when these biocontrol agents are applied to water hyacinth, extrapolating their behaviours to field conditions, and finding a formulation that takes into account their ecophysiological traits. Yet, it is crucial to emphasize that our glycerol models for *F. sacchari*, *C. malorum* and *Alternaria* sp. are based on data obtained under *in vitro* conditions. Our models might overestimate growth under natural conditions, because our strains were grown on a nutrient-rich artificial medium under good light conditions. Furthermore, environmental factors other than those studied here may be involved, such as relative humidity, UV, pH and interactions with organisms of the microflora present on the leaf surface of water hyacinth.

It may thus be mandatory to develop models based on *in vivo* conditions and taking into account the factors just mentioned. In this framework, it might be possible to integrate models such as ours into a broader study of the impact of environmental factors on the biocontrol agent – weed system studied here. Good models of fungal behaviour under field conditions could provide a basis for a more rational control strategy, possibly involving the use of a formulation protecting *F. sacchari*, *C. malorum* and *Alternaria* sp. against unfavourable environmental factors.

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References

Babu, R.M., Sajeena, A. and Seetharaman, K. (2003) Bioassay of the potentiality of *Alternaria alternata* (Fr.) keissler as a bioherbicide to control water hyacinth and other aquatic weeds. *Crop Prot* **22**, 1005–1013.

Baxter, C.J., Magan, N., Lane, B. and Wildman, H.G. (1998) Influence of water activity and temperature on *in vitro* growth of surface cultures of a *Phoma* sp. and production of the pharmaceutical metabolites, squalestatins S1 and S2. *Appl Microbiol Biotechnol* **49**, 328–332.

Begoude, B.A.D., Lahlali, R., Friel, D., Tondje, P.R. and Jijakli, M.H. (2007) Response surface methodology study of the combined effects of temperature, pH, and a_w on the growth rate of *Trichoderma asperellum*. *J Appl Microbiol* **103**, 845–854.

Bekada, A.M.A., Benakriche, B., Hamadi, K. and Bensoltane, A. (2008) Modelling of effects of water activity, pH and temperature on the growth rate of *Mucor racemosus* isolated from Soft Camembert Cheese. *World J Agric Sci* **4**, 790–794.

Boyette, C.D., Hoagland, R.E. and Weaver, M.A. (2007) Biocontrol efficacy of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) is enhanced with unrefined corn oil and surfactant. *Weed Biol Manag* **7**, 70–76.

Charudattan, R. (2001) Biological control of weeds by means of plant pathogens: significance for integrated weed management in modern agro-ecology. *Biocontrol* **46**, 229–260.

Charudattan, R. (2005) Ecological, practical and political inputs into selection of weed targets: what makes a good biological control target? *Biol Control* **35**, 183–196.

Dagno, K. (2006) *Évaluation des microorganismes fongiques en tant qu'agents de lutte Biologique contre Eichhornia crassipes (Martius) Solms-Laubach dans le bassin du fleuve Niger au Mali*. DEA doc. Sci. agron. Gembloux, Belgique: Gembloux Agricultural University – FUSAGx p. 102.

Dagno, K., Lahlali, R., Friel, D., Bajji, M. and Jijakli, M.H. (2007) Synthèse bibliographique: problématique de la Jacinthe d'eau, *Eichhornia crassipes* dans les régions tropicales et subtropicales du monde, notamment son éradication par la lutte biologique au moyen des phytopathogènes. *Biotechnol Agron Soc Environ* **11**, 299–311.

Devlieghere, F., Debevere, J. and Van Impe, J. (1998) Effect of dissolved carbon dioxide and temperature on the growth

of *Lactobacillus sake* in modified atmospheres. *Int J Food Microbiol* **41**, 231–238.

El-Morsy, E.M. (2004) Evaluation of microfungi for the biological control of water hyacinth in Egypt. *Fungal Divers* **16**, 35–51.

Lahlali, R. and Hijri, M. (2010) Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. *FEMS Microbiol Lett* **311**, 152–159.

Lahlali, R., Serrhini, M.N. and Jijakli, M.H. (2005) Studying and modelling the combined effect of water activity and temperature on growth rate of *P. expansum*. *Int J Food Microbiol* **103**, 315–322.

Lahlali, R., Bajji, M., Serrhini, M.N. and Jijakli, M.H. (2008) Modelling the effect of temperature, water activity and solute on the *in vitro* growth on the biocontrol yeast *Pichia anomala* strain K. *Biotechnol Agron Soc Environ* **12**, 353–359.

Lasram, S., Oueslati, S., Valero, A., Marin, S., Ghorbel, A. and Sanchis, V. (2010) Water activity and temperature effects on fungal growth and Ochratoxin A production by Ochratoxigenic *Aspergillus carbonarius* isolated from Tunisian Grapes. *J Food Sci* **75**, 89–97.

Marin, S., Sanchis, V., Teixido, A., Saenz, R., Ramos, A.J., Vinas, I. and Magan, N. (1996) Water and temperature relations and microconidial germination of *Fusarium moniliforme* and *F. proliferation* from maize. *Can J Microbiol* **42**, 1045–1050.

Nanguy, S.P.M., Perrier-Cornet, J.M., Bensoussan, M. and Dantigny, P. (2010) Impact of water activity of diverse media on spore germination of *Aspergillus* and *Penicillium* species. *Int J Food Microbiol* **142**, 273–276.

Parra, R., Aldred, D., Archer, D.B. and Magan, N. (2004) Water activity, solute and temperature modify growth and spore production of wild type and genetically engineered *Aspergillus niger* strains. *Enzyme Microb Technol* **35**, 232–237.

Patriarca, A., Vaamonde, G., Pinto, V.F. and Comerio, R. (2001) Influence of water activity and temperature on the growth of *Wallemia sebi*: application of a predictive model. *Int J Food Microbiol* **68**, 61–67.

Paul, G.C., Kent, C. and Thomas, C.R. (1992) Viability testing and characterisation of germination of fungal spores by automatic image analysis. *Biotechnol Bioeng* **42**, 11–23.

Plaza, P., Usall, J., Teixido, N. and Vinas, I. (2003) Effect of water activity and temperature on germination and growth of *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum*. *J Appl Microbiol* **94**, 549–554.

Pose, G., Patriarca, A., Kyanko, V., Pardo, A. and Fernández Pinto, V. (2009) Effect of water activity and temperature on growth of *Alternaria alternata* on a synthetic tomato medium. *Int J Food Microbiol* **135**, 60–63.

Romero, S.M., Pinto, V.F., Patriarca, A. and Vaamonde, G. (2010) Ochratoxin A production by a mixed inoculum of *Aspergillus carbonarius* at different conditions of water activity and temperature. *Int J Food Microbiol* **140**, 277–281.

Sanogo, S., Pomella, A., Hebbar, P.K., Bailey, B., Costa, J.C.B., Samuels, G.J. and Lumsden, R.D. (2002) Production and germination of conidia of *Trichoderma stromaticum*, a mycoparasite of *Crinipellis perniciosa* on cacao. *Phytopathology* **92**, 1032–1037.

Shabana, Y.M. (2004) The use of oil emulsions for improving the efficacy of *Alternaria eichhorniae* as a mycoherbicide for water hyacinth (*Eichhornia crassipes*). *Biol Control* **32**, 78–89.

Shabana, Y.M. and Mohamed, Z.A. (2005) Integrated control of water hyacinth with a mycoherbicide and phenylpropanoid pathway inhbitor. *Biocontrol Sci Technol* **15**, 659–669.

Sparringa, R.A., Kendall, M., Westby, A. and Owens, J.D. (2002) Effects of temperature, pH, water activity and CO2 concentration on growth of *Rhizopus oligosporus* NRRL 2710. *J Appl Microbiol* **92**, 329–337.

Tamm, L. and Fluckiger, W. (1993) Influence of temperature and moisture on growth, spore production and conidial germination of *Monilia laxa*. *Phytopathology* **83**, 1321–1326.

Xu, X.M., Guerin, L. and Robinson, J.D. (2001) Effects of temperature and relative humidity on conidial germination and viability, colonization and sporulation of *Monilinia fructigena*. *Plant Pathol* **50**, 561–568.