Effect of carbohydrate type and concentration on polyhydroxy alcohol and trehalose content of conidia of three entomopathogenic fungi

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The entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces farinosus* were cultured on solid agar media containing different carbohydrate components (glycerol, glucose, trehalose or starch) at concentrations of ≤ 142.7 g added carbon l^{-1} for 30 d at 25 °C. The water activity (a_w) of the media ranged from 0.925 to 0.998. Growth of *M. anisopliae* and *P. farinosus* was stimulated between 0.975 and 0.995 a_w on glucose media and that of *P. farinosus* at 0.975 a_w on glycerol media. At < 0.970 a_w , growth of each fungal species was significantly reduced ($P < 0.05$). Polyhydroxy alcohols (polyols) and trehalose were extracted from conidia produced on different media and quantified using HPLC. Total polyol content of conidia produced on glucose media varied between 5.2 and 52.2 mg g^{-1} for *B. bassiana*, 77.3 and 90.3 mg g^{-1} for *M. anisopliae*, and 26.7 and 76.1 mg g^{-1} for *P. farinosus*. The amounts of specific polyols in conidia varied significantly from media of different glucose concentrations. Mannitol was the predominant polyol in conidia of all three species, with conidia of *M. anisopliae*, for example, containing as much as 75.2 mg mannitol g^{-1} when cultured on glucose media. The amount of the lower molecular mass polyols glycerol and erythritol was greater in conidia produced on glucose media with > 50.0 g added carbon l^{-1} than that in conidia produced at lower glucose concentrations. Conidia contained between 10.8 and 20.8 mg glycerol plus erythritol g^{-1} on glucose media with 142.7 g added carbon l^{-1} , depending on species. Conversely, conidia of *B. bassiana* and *P. farinosus* contained maximum amounts of trehalose (≤ 23.5 mg g^{-1}) when produced on glucose media with < 50.0 g added carbon l^{-1} , and trehalose content was considerably less at higher glucose concentrations. There were accumulations of glycerol and erythritol in conidia of all three species when grown on glycerol media with > 25.0 g added carbon l^{-1} ; conidia of *B. bassiana* contained up to 154.0 mg glycerol plus erythritol g^{-1} . When *B. bassiana* and *P. farinosus* were grown on trehalose media, conidia contained up to 222.1 mg trehalose g^{-1} . By contrast, conidia of *M. anisopliae* contained < 17.0 mg trehalose g^{-1} under all conditions tested. The water availability of solutions of different polyols is discussed in relation to their potential to act in osmotic adjustment during germination. The ability to manipulate polyol and trehalose content of fungal propagules may be critical in enhancing the storage life and efficacy of biological control agents.

Keywords: entomopathogenic fungi, polyhydroxy alcohol, trehalose, conidia, water activity

INTRODUCTION

Fungal biological control agents can be ineffective if

conidia are unable to germinate because water availability is suboptimal (Walstad *et al.*, 1970; Ferron, 1977; Doberski, 1981; Gillespie & Crawford, 1986; Hsiao *et al.*, 1992) or if they have inadequate carbohydrate reserves (Lane *et al.*, 1991a; Harman *et al.*, 1991). One way to overcome these problems is to modify the carbohydrate

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Abbreviation: a_w , water activity.

content of the fungal inocula (Harman *et al.*, 1991; Hallsworth & Magan, 1994). Polyhydroxyl alcohols (polyols) and trehalose can be important for successful germination and survival of desiccation during storage of propagules (Al-Hamdani & Cooke, 1987; Harman *et al.*, 1991; Gornova *et al.*, 1992). Polyols are known to accumulate to high concentrations in fungi at low water availability, and so reduce intracellular water activity (a_w), while at the same time preventing enzyme inhibition due to dehydration (Brown, 1978; Yancey *et al.*, 1982; Carpenter & Crowe, 1988a). The disaccharide trehalose can replace water in membranes at reduced a_w (Crowe *et al.*, 1984; Tsvetkov *et al.*, 1989; Crowe & Crowe, 1993) and is known to stabilize enzyme structure during desiccation (Carpenter & Crowe, 1988a, b; Colaco *et al.*, 1992). Polyols and trehalose are compatible with fungal metabolism; high intracellular concentrations do not destabilize or inhibit enzymes, so they are referred to as compatible solutes (Brown, 1976; Jennings & Burke, 1990; Rudolph *et al.*, 1993).

Ballio *et al.* (1964) reported on the polyol content of conidia (of *Penicillium chrysogenum*) but they did not consider the effects of carbohydrate source or water availability. Hallsworth & Magan (1994) detailed changes in polyol and trehalose content of aerial conidia of *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces farinosus*. These conidia were obtained from cultures grown on media modified with the ionic solute KCl to give a range of water availabilities. Two recent studies examined the effect of medium composition on carbohydrate storage in total biomass and blastospores of the entomopathogen *B. bassiana* when grown in liquid culture (Bidochka *et al.*, 1990; Lane *et al.*, 1991a). Although Bidochka *et al.* (1990) found that the content of specific polyols in mycelium was enhanced by carbohydrate supplementation of the medium, no recognition was given to the role of polyols in osmotic adjustment and as compatible solutes.

An excess of exogenously supplied carbohydrate during nitrogen-substrate limitation can result in increased carbohydrate storage. Pfyffer & Rast (1980) showed that nitrogen-substrate limitation enhanced polyol content in mycelia (or vegetative cells) of seven non-entomopathogenic fungi. Bidochka *et al.* (1990) and Lane *et al.* (1991a) found increased carbohydrate content of mycelia and blastospores of the entomopathogen *B. bassiana* under similar conditions. However, propagules such as blastospores and submerged conidia are less able to survive storage and resist environmental stress than aerial conidia, so the latter are of higher potential value for agricultural use (Lane *et al.*, 1991b; Hegedus *et al.*, 1992).

When the carbohydrate content of a medium is increased there is a reduction in water availability. Previous studies of the effect of carbohydrate type or concentration on the physiology of biological control agents have neglected consideration of this effect (Bidochka *et al.*, 1990; Dillon & Charnley, 1990; Jackson & Bothast, 1990). Conversely, when a_w has been adjusted by varying carbohydrate

concentration in media, the potential effects of excess carbohydrate (e.g. those due to nitrogen-substrate limitation) have been ignored when considering the accumulation of specific polyols (Gervais *et al.*, 1988; Van Eck *et al.*, 1993).

The objectives of this study were to determine the effects of changing the concentration of glycerol, glucose, trehalose or starch in solid media, and of the resultant changes in a_w , on growth and accumulation of polyols and trehalose in conidia of *B. bassiana*, *M. anisopliae* and *P. farinosus*.

METHODS

Organisms and media. Fungal isolates were obtained from Horticulture Research International, Wellesbourne, UK (*B. bassiana* 206) and Rothamsted Experimental Station, Harpenden, UK (*M. anisopliae* V90 and *P. farinosus* V51). Stock cultures were stored at 5 °C and had been subcultured up to eight times on Sabouraud dextrose agar (SDA) (Oxoid) after passage through an insect. Experimental media, based on SDA, contained (per l): 15.0 g agar no. 2 (Oxoid), 10.0 g mycological peptone (Lab M), a carbohydrate component (see below) and 21.3 g MES (Sigma). The pH of media was adjusted by addition of 2.5 M NaOH to give a final value of 5.8 ± 0.3 . Media were autoclaved for 15.0 min at 121 °C, poured into 9.0 cm Petri plates (15.0 ml) and cooled to 25 °C. The surface pH of media was measured using a pH stick (Gallenkamp). Either glycerol (Merck), glucose (Sigma), trehalose (Sigma) or soluble starch (Merck) was incorporated in media to give a range of 2.7 to 142.7 g added carbon l⁻¹, resulting in a range of 0.925 to 0.999 a_w at 25 °C (Table 1). The a_w of each medium was measured immediately before inoculation using a Novasina Humidistat IC II as theoretical predictions of a_w , such as those made by Ellis *et al.* (1991), do not account for water lost upon pouring and cooling of media.

Culture conditions, growth assessment and collection of conidia. Petri plates were inoculated with 2.0 mm diameter mycelial plugs taken from the margin of 2-week-old cultures grown on SDA at 25 °C, and plates of the same a_w were incubated in sealed polyethylene bags at 25 °C without light. Colony diameter was measured at intervals of 3 d and radial

Table 1. Water activity (a_w) of media with different carbohydrate components

Values were means of three replicate measurements and variation was within $\pm 0.001 a_w$ unit. NA, Not applicable.

Added carbon (g l ⁻¹)	Water activity			
	Carbon source:			
	Glycerol	Glucose	Trehalose	Starch
2.67	0.994	0.998	NA	NA
15.85	0.986	0.995	0.999	0.999
31.70	0.975	0.988	NA	NA
43.59	NA	NA	0.993	0.993
55.48	0.957	0.973	NA	NA
95.10	0.925	0.959	0.980	0.982
142.66	NA	0.939	0.970	0.975

growth (mm d^{-1}) calculated from the linear portions of the curves plotted from these values. Radial growth rate of filamentous fungi on solid media has been shown to relate directly to dry weight production over a range of a_w (Griffin, 1977; Inch & Trinci, 1987). This method of growth assessment was preferred to dry weight determination because the latter does not account for the substantial quantities of stored carbohydrates that may have been present in some treatments during this study. The dry weight of such storage material does not represent structural growth.

After 30 d conidia were collected. This was carried out initially by two methods: wet- and dry-harvesting. Conidia were wet-harvested by flooding cultures with 10.0 ml AnalaR water (Merck) containing 1% (v/v) Tween 80 (Merck), dislodged by agitation with a glass rod and filtered through glass wool (see later). Dry-harvesting was carried out by gently scraping conidia from the surface of colonies using a spatula to avoid any possible dissolution of carbohydrates from media in the AnalaR water. Upon removal from the Petri plate, these conidia were placed into AnalaR water containing 1% Tween 80, filtered, centrifuged and rinsed as described below. Suspensions of conidia were filtered through glass wool into 30.0 ml Universal bottles and centrifuged immediately for 12.0 min at 4000 r.p.m. in a MSE Cenatur 2 centrifuge fitted with a four-place swing-out rotor. The glass wool was rinsed with AnalaR water prior to filtration to remove any loose fragments. Conidia were then resuspended in AnalaR water, recentrifuged as above, frozen to -80°C upon removal from the centrifuge and then lyophilized in an Edwards EF4 Modulyo freeze dryer (Edwards High Vacuum International). There were three replicates of each treatment.

Polyol and trehalose content of conidia from cultures grown on 95.1 or 142.7 g added carbon l^{-1} was determined and there was no significant difference ($P < 0.05$) between that of wet- or dry-harvested conidia. Collection of conidia was subsequently carried out by wet-harvesting.

Extraction and detection of polyols and trehalose. Dry conidia (5 mg) were put into 2.0 ml microfuge tubes containing 1.0 ml AnalaR water and sonicated for 120 s at an amplitude of 28.0 μm using a Fisons Soniprep 150, fitted with a 3.5 mm diameter exponential probe. After immersion in a boiling water bath for 5.5 min, extracts were passed through a 0.2 μm filter (Anatop).

Polyols and trehalose are weakly acidic at pH 14 and were separated by anion exchange using a Dionex series 4500 HPLC instrument fitted with a CarboPac PA1 (4×250.0 mm) column, a CarboPac PA1 guard column and a Dionex pulsed electrochemical detector. The mobile phase (flow rate 1.0 ml min^{-1}) was prepared by degassing AnalaR water with He for 25.0 min after which NaOH was added to give a 100 mM solution (pH approx. 14). Contamination of the mobile phase with Na_2CO_3 would accelerate elution of polyols and trehalose from the column and reduce resolution. Therefore, since NaOH pellets have a surface layer of Na_2CO_3 , NaOH was obtained as a preprepared 50% (w/w) solution (Merck). The Na_2CO_3 content of a 50% NaOH solution precipitates, so a pipette was inserted into the centre of the solution to avoid contamination with any precipitate on the inner surface of the container and, as a further precaution, the final third of each bottle was discarded. A 700 mM NaOH solution was prepared in the same way and used to flush the column daily and the electrode surface was cleaned with a plastic eraser. Injection volume was 50.0 μl ; peak areas were determined with a Hewlett-Packard 3390A integrator,

with attenuation set at 10. Detector sensitivity was adjusted to allow for variations of polyol and trehalose concentration. The limits of detection were 1.6 and 4.2 $\mu\text{g ml}^{-1}$ for polyols, and 2.8 and 5.9 $\mu\text{g ml}^{-1}$ for trehalose.

Standard solutions of glycerol (Merck), erythritol, arabitol, mannitol and trehalose (Sigma) were prepared and diluted to give a range of concentrations so that each injection of conidial extract was preceded and succeeded by injections of standards that could be detected at the same sensitivity. The polyol and trehalose content of conidia were calculated using the mean values of peaks from traces of standards that came immediately before and after sample traces, and values were expressed as mg (g conidia) $^{-1}$. Each analysis was carried out with three replicates per treatment.

RESULTS

Growth rate on different media

Radial growth rate of each species of entomopathogen varied with carbohydrate type and concentration (Fig. 1). At < 31.7 g added carbon l^{-1} *B. bassiana* and *M. anisopliae* grew significantly more slowly on glycerol than on glucose, trehalose or starch media ($P < 0.05$). Intermediate concentrations of glucose or trehalose resulted in a stimulation of growth of *M. anisopliae* and *P. farinosus*, and the same phenomenon was seen on glycerol with *P. farinosus*. As the glycerol or glucose content of media was increased to > 40.0 g added carbon l^{-1} , there was a decrease in growth rate of each species. The decrease in growth rate on glucose media was less than on glycerol media at equivalent carbon concentration or a_w . On trehalose and starch media, as carbon content was increased, the growth rate of each fungus was not greatly reduced at > 40.0 g added carbon l^{-1} as it was on glycerol and glucose media.

Polyols and trehalose in conidia from glycerol media

On glycerol media with 95.1 g added carbon l^{-1} , insufficient conidia of *B. bassiana* and *M. anisopliae* were produced for polyol and trehalose determination. As glycerol concentration was increased there were accumulations of glycerol and erythritol in conidia of each species. These were greatest for *B. bassiana*, where conidia contained as much as 96.0 mg erythritol g^{-1} and a total of 154.0 mg glycerol plus erythritol g^{-1} (Fig. 2). There were only trace amounts of polyols and trehalose in conidia obtained from cultures grown at 2.7 g added carbon l^{-1} , except for those of *M. anisopliae*, which contained 115.0 mg mannitol g^{-1} . The decrease in the mannitol content in conidia of *M. anisopliae* from cultures grown at higher carbon concentrations was approximately equal in value to the combined increase in glycerol and erythritol in these conidia.

Polyols and trehalose in conidia from glucose media

Conidia of *B. bassiana* contained only trace amounts of polyols on media with 2.7 g added carbon l^{-1} (Fig. 3). As glucose concentration was increased to > 50.0 g l^{-1} , the

Fig. 1. Radial growth rates of (a) *B. bassiana*, (b) *M. anisopliae* and (c) *P. farinosus* grown on buffered SDA with a modified carbohydrate component at 25 °C. ○, Glycerol; ●, glucose; ■, trehalose; □, starch. Results are means of three replicates; the least significant differences ($P < 0.05$) of growth rates on glycerol, glucose, trehalose and starch media were: (a) 0.13, 0.19, 0.18 and 0.11; (b) 0.45, 0.17, 0.22 and 0.14; and (c) 0.18, 0.22, 0.35 and 0.23, respectively.

mannitol content of conidia of all three fungi decreased from between 42.0 and 75.2 mg g⁻¹ to between 22.0 and 37.2 mg g⁻¹, depending on species, and this decrease was accompanied by increases in erythritol and/or arabitol of approximately equal value. At low carbon concentrations, conidia of *B. bassiana* and *P. farinosus* contained as much as 23.5 mg trehalose g⁻¹.

Polyols and trehalose in conidia from trehalose media

As the trehalose content of media was increased, the erythritol or arabitol content of conidia increased in each

Fig. 2. Polyols and trehalose extracted from conidia of (a) *B. bassiana*, (b) *M. anisopliae* and (c) *P. farinosus* grown on buffered media with a variable glycerol content, after 30 d at 25 °C. ○, Glycerol; △, erythritol; □, arabitol; ▲, mannitol; ■, trehalose. Results are means of three replicates; the least significant differences ($P < 0.05$) for glycerol, erythritol, arabitol, mannitol and trehalose were: (a) 6.53, 9.67, 0.74, 5.64 and 2.02; (b) 10.73, 5.16, 2.07, 9.66 and 1.96; and (c) 7.59, 9.29, 1.45, 3.70 and 2.17, respectively.

species. Conidia of *M. anisopliae* contained 67.4 mg arabitol g⁻¹ at 142.7 g added carbon l⁻¹ (Fig. 4). In *M. anisopliae*, the mannitol content of conidia decreased as arabitol content increased. Conidia of *B. bassiana* and *P. farinosus* contained significantly increased amounts of trehalose on media with ≥ 95.1 g added carbon l⁻¹ when compared to those from other media: 161.7 to 222.1 mg g⁻¹, respectively.

Polyols and trehalose in conidia from starch media

Mannitol was the predominant polyol in conidia of each

Fig. 3. Polyols and trehalose extracted from conidia of (a) *B. bassiana*, (b) *M. anisopliae* and (c) *P. farinosus* grown on buffered media with a variable glucose content, after 30 d at 25 °C. ○, Glycerol; △, erythritol; □, arabitol; ▲, mannitol; ■, trehalose. Results are means of three replicates; the least significant differences ($P < 0.05$) for glycerol, erythritol, arabitol, mannitol and trehalose were: (a) 1.78, 1.25, 0.73, 4.33 and 2.78; (b) 1.54, 3.45, 2.84, 6.71 and 1.32; and (c) 1.69, 4.69, 1.88, 5.76 and 4.43, respectively.

Fig. 4. Polyols and trehalose extracted from conidia of (a) *B. bassiana*, (b) *M. anisopliae* and (c) *P. farinosus* grown on buffered media with a variable trehalose content, after 30 d at 25 °C. ○, Glycerol; △, erythritol; □, arabitol; ▲, mannitol; ■, trehalose. Results are means of three replicates; the least significant differences ($P < 0.05$) for glycerol, erythritol, arabitol, mannitol and trehalose were: (a) 1.03, 2.11, 0.50, 5.90 and 11.65; (b) 3.00, 2.28, 5.22, 7.24 and 4.34; and (c) 1.53, 2.97, 1.63, 5.87 and 17.36, respectively.

species on starch media (Fig. 5). Conidia of *B. bassiana* contained as much as 49.2 mg trehalose g^{-1} at 15.9 g, added carbon l^{-1} , but lesser amounts when cultures were grown at higher carbon concentrations. As trehalose content of *B. bassiana* conidia decreased at higher carbon concentrations, mannitol content increased by a similar quantity.

DISCUSSION

Growth rate on different media

Nutrient utilization during fungal growth is most efficient

when carbon and nitrogen are present in a ratio of about 8:1 (Roels, 1983). In this study, the total nitrogen content of all media was 1.3 g l^{-1} and the carbon component from the mycological peptone constituted 6.1 g (l medium) $^{-1}$. A balanced medium, therefore, would contain about 4.0 g added carbon l^{-1} , in the form of glycerol, glucose, trehalose or starch. In this study media containing ≥ 15.9 g added carbon l^{-1} contained an excess of carbon. As the added carbon content of trehalose and starch media was increased there was not a proportional increase in growth rate of the fungi, despite the high a_w of all media (≥ 0.970). This suggests that growth was nitrogen-substrate limited.

Fig. 5. Polyols and trehalose extracted from conidia of (a) *B. bassiana*, (b) *M. anisopliae* and (c) *P. farinosus* grown on buffered media with a variable starch content, after 30 d at 25 °C. ○, Glycerol; △, erythritol; □, arabitol; ▲, mannitol; ■, trehalose. Results are means of three replicates; the least significant differences ($P < 0.05$) for glycerol, erythritol, arabitol, mannitol and trehalose were: (a) 1.11, 1.11, 1.61, 8.37 and 5.76; (b) 2.74, 1.63, 1.41, 8.86 and 5.65; and (c) 3.13, 1.72, 2.12, 5.43 and 3.92, respectively.

Stimulation of growth of *P. farinosus* at intermediate a_w values (0.975–0.990) was similar to that reported on media modified with KCl (Hallsworth & Magan, 1994), but not as marked. The stimulation of growth on media modified with KCl was seen in each fungal species and may have been due to a slight salt requirement (Larsen, 1986). Growth of *B. bassiana*, *M. anisopliae* and *P. farinosus* decreased progressively as the water availability was reduced to < 0.970 a_w as reported by Gillespie & Crawford (1986) and Hallsworth & Magan (1994). The reduction in growth rate per 0.010 reduction of a_w was greater on glucose or KCl than on glycerol media for each

species; this was most apparent in *B. bassiana*, where growth was reduced by 0.32 mm d⁻¹ on glucose media, 0.25 mm d⁻¹ on KCl media (Hallsworth & Magan, 1994) but only 0.13 mm d⁻¹ on glycerol media.

The relatively high growth rates at reduced a_w on glycerol media may have been due to the intracellular accumulation of glycerol taken up from the medium which can act intracellularly in osmotic adjustment and as a compatible solute. Glycerol has frequently been used to reduce the water availability of media (Pitt & Hocking, 1977; Hocking & Pitt, 1979; Gillespie & Crawford, 1986; Gervais *et al.*, 1988; Mutasa *et al.*, 1990). In these reports, the effects of reduced a_w have been considered without recognition that fungi can utilize exogenously-supplied glycerol which may accumulate intracellularly and act as a compatible solute, thus mitigating the effects of reduced water availability.

Polyols and trehalose in conidia from different media

This study shows that the carbohydrate component and water status of the substrate significantly affect the quantities of specific polyols and trehalose in conidia of entomopathogens. Conditions under which conidia contained higher amounts of these compatible solutes were often not those under which optimal growth occurred; Bidochka *et al.* (1990) also made this observation in mycelia of liquid cultures of *B. bassiana*. HPLC was used to quantify polyols and trehalose in the present study and our previous study (Hallsworth & Magan, 1994). By using this method it is possible to detect polyols and trehalose simultaneously. Furthermore, HPLC analysis avoids the derivatization of polyols that is necessary for GC detection.

In general, the greatest accumulation of polyols and trehalose in conidia occurred in the presence of excess carbon. Intracellular accumulation of carbohydrate reserves in nitrogen-substrate-limited liquid cultures of entomopathogens has been reported by Inch *et al.* (1986), Bidochka *et al.* (1990) and Lane *et al.* (1991a). In this study, the maximum content of polyols in conidia was 158.9, 138.0 and 112.4 mg g⁻¹ for *B. bassiana*, *M. anisopliae* and *P. farinosus*, respectively. By contrast, conidia from cultures of these fungal species grown on media with modified water availability contained considerably smaller amounts of polyols (Hallsworth & Magan, 1994). Conidia of *P. chrysogenum* were found to contain 81.0 mg polyols g⁻¹, but no attempt was made to increase the carbon concentration or reduce the water availability of the medium (Ballio *et al.*, 1964). Al-Hamdani & Cooke (1987) found that sclerotia (mycelial propagules) of the plant pathogen *Sclerotinia sclerotiorum* contained as much as 269.7 mg polyols g⁻¹ when grown on media modified with NaCl. Bidochka *et al.* (1990) found that mycelia of the entomopathogen *B. bassiana* contained ≤ 43.2 mg polyols g⁻¹, but the water availability of the media was not determined. Comparisons between the polyol content of conidia and that of mycelia or sclerotia are, however, of limited value due to the fundamental difference in the nature of these structures.

Glucose has commonly been used as a carbohydrate source to culture *B. bassiana*, *M. anisopliae* and *P. farinosus* (Smith & Grula, 1981; Gillespie & Crawford, 1986; Humphreys *et al.*, 1989; Hywel-Jones & Gillespie, 1990; Lane *et al.*, 1991a; Hallsworth & Magan, 1994). In this study, as glucose concentration was increased, trehalose content of conidia of *B. bassiana* and *P. farinosus* decreased, whereas erythritol content increased to between 10.9 and 20.5 mg g⁻¹, depending on species. These trends were probably a response to a reduction of a_w rather than an effect of increased carbohydrate availability as they were identical to those seen when media were modified with KCl to reduce a_w (Hallsworth & Magan, 1994). They were also reported in sclerotia from cultures of *S. sclerotiorum* grown over a range of a_w on solid media modified with KCl or NaCl (Al-Hamdani & Cooke, 1987). By contrast, Harman *et al.* (1991) found that the trehalose content of conidia of *Trichoderma harzianum* increased as a_w was reduced when the medium was modified with polyethylene glycol (PEG) 6000.

The polyol component of conidia of *M. anisopliae* was composed of progressively lower M_r compounds as a_w was reduced on glucose media, (this study) with KCl (Hallsworth & Magan, 1994) or with PEG 600 (J. E. Hallsworth, unpublished data). Accumulation of progressively lower M_r polyols in fungi grown over a range of decreasing a_w has been found in mycelia of non-entomopathogenic fungi (Beever & Laracy, 1986; Kelly & Budd, 1991). Although accumulations of low M_r polyols have frequently been reported in xerophilic fungi at reduced a_w (Adler *et al.*, 1982; Luard, 1982; Gadd *et al.*, 1984; Hocking & Norton, 1983; Hocking, 1986), water relations of such extremophiles do not typify the situation in other fungi. Protein structure in halophiles is, for example, different from that of non-halophilic organisms such as the entomopathogens in the present study (Yancey *et al.*, 1982; Kushner, 1986). Adler *et al.* (1982) suggested that intracellular glycerol content of fungal cells may increase during salt stress whereas sugar-rich media may promote the accumulation of higher M_r polyols such as arabitol. This idea is not supported by the results of the present study or one in which the response to different media of the same a_w were compared (Hallsworth & Magan, 1994).

Van Eck *et al.* (1993) gave recognition to the ability of the lower M_r polyol glycerol to increase intracellular viscosity more effectively than higher M_r polyols, thereby acting as a more compatible solute (Chirife *et al.*, 1984). Kelly & Budd (1991) suggested that accumulations of lower M_r polyols may serve to economize on the carbon source for growth and pointed out that shorter chain-length polyols have been shown to be more compatible (Brown, 1978). None of these studies, however, differentiated between the relative ability of different polyols to depress intracellular a_w and this difference may, in part, account for the accumulation of lower M_r polyols in mycelia or propagules of cultures grown at reduced a_w .

Unsaturated solutions containing an equal amount of

mannitol, arabitol, erythritol, and glycerol (in g l⁻¹) have different a_w , and these polyols differ greatly in solubility, so their potential to depress intracellular a_w varies. Mannitol is the least soluble, with a saturated solution having an a_w of 0.978 at 25 °C (Chirife *et al.*, 1984). In conidia obtained from cultures grown on media at < 0.978 a_w , mannitol would not enable depression of intracellular a_w sufficiently to allow mycelial growth or germ tube elongation. Mannitol is, however, known to act as a compatible solute (Brown, 1978; Jennings & Burke, 1990).

Arabitol is freely soluble and a saturated solution has an a_w of 0.819 (Chirife *et al.*, 1984). At a given concentration a solution of glycerol or erythritol would have a lower a_w than that of arabitol or mannitol. The solubility of erythritol is limited; a saturated solution has an a_w of 0.914 (J. E. Hallsworth, unpublished). However, erythritol is sufficiently soluble to depress intracellular a_w to less than 0.950, a value below which conidia of *B. bassiana*, *M. anisopliae* and *P. farinosus* cannot normally germinate (Gillespie & Crawford, 1986). Glycerol is miscible with water and, at a given concentration, a glycerol solution has a slightly lower a_w than that of erythritol (J. E. Hallsworth, unpublished data). For this reason, in part, it seems likely that accumulations of lower M_r compounds such as erythritol and glycerol can confer greater osmotic tolerance than polyols such as mannitol.

It is noteworthy that the largest accumulations of low M_r polyols in this study were found in conidia obtained from glycerol media at growth-limiting a_w . Attempts to optimize growth of fungi in the agricultural production of inocula will not necessarily result in the production of good quality propagules, as low M_r polyols seem to accumulate in propagules obtained from growth-limited cultures grown at reduced a_w . Al-Hamdani & Cooke (1987) compared germination of sclerotia obtained from such cultures grown on media of reduced a_w , with enhanced polyol content, with those from cultures grown at higher a_w . Sclerotia with enhanced polyol content germinated more vigorously than other sclerotia when tested on water agar, but germination was not assessed on media with reduced a_w .

Conidia from cultures grown on starch media (≥ 0.970 a_w) contained predominantly mannitol and very little erythritol and glycerol. This supports the idea that the accumulation of low M_r polyols in conidia obtained from cultures grown on glucose or trehalose media with > 50.0 g added carbon l⁻¹ was a response to reduced a_w and not a result of increased carbon concentration.

In his study, conidia obtained from cultures grown on trehalose media were found to contain up to 222.1 mg trehalose g⁻¹, considerably more than those obtained from cultures grown on SDA modified with KCl (Hallsworth & Magan, 1994). Trehalose is known to enhance desiccation tolerance. For example, conidia of *T. harzianum* and *Aspergillus japonicus* with an increased trehalose content retained viability during storage better

than those containing less trehalose (Harman *et al.*, 1991; Gornova *et al.*, 1992). As conidia of *B. bassiana* and *P. farinosus* were found to accumulate trehalose significantly more than those of *M. anisopliae*, the potential to improve the storage life of agricultural inocula may vary between species. It is interesting to note that trehalose is the main carbohydrate in the insect haemolymph and so may be an important natural carbohydrate source (Mullins, 1985). Conidia produced on the insect cadaver may contain more trehalose than those produced either on glucose-based media such as SDA, or on starch-based substrates used in the production of agricultural inocula.

Conidia from all media were found to be viable and work is in progress to assess germination and virulence at low water availability. The ability to manipulate polyol and trehalose content of fungal propagules may be critical in the development of inocula of enhanced quality which have improved desiccation tolerance and storage life, and greater efficacy in the field.

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