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UV protectants for *Candida oleophila* (strain O), a biocontrol agent of postharvest fruit diseases

R. Lahlali*†, B. Raffaele and M. H. Jijakli

Plant Pathology Unit, Gembloux Agro-Bio Tech, Liege University, 5030 Passage de Déportés Gembloux, Belgium

This study investigated the influence of UV-B radiation (280–320 nm) on survival of *Candida oleophila* strain O, an antagonist yeast that prevents postharvest diseases caused by *Botrytis cinerea* and *Penicillium expansum* on apple and pear fruits. Lethal doses (LD_{50} and LD_{90}) were, respectively, 0·89 and 1·45 Kj m^{-2} for *in vitro* exposure and 3·06 and 5·5 Kj m^{-2} for *in vivo* exposure. A screening test of UV-B protectants for strain O was also evaluated under *in vitro* and *in vivo* conditions. The *in vitro* results showed that sodium ascorbate (0·1% and 0·01%), riboflavin (0·1%) and uric acid (0·1% and 0·01%) were the most effective and most suitable protectants. However, only riboflavin (0·1%) and uric acid (0·1%) were effective under *in vivo* conditions. The efficacy obtained with strain O against *P. expansum*, when subjected to UV-B radiation, was 75·0% and 49·2% for pathogen concentrations of 10^5 and 10^6 spores mL^{-1}, respectively. Adding riboflavin to strain O gave a similar efficacy (64·2%). Applying strain O together with uric acid (0·1%) was less active (47·7%). Nonetheless, its efficacy when applied with the antioxidants sodium ascorbate (71·1%) or ascorbic acid (82·5%) was the greatest. Riboflavin and uric acid were the most cost-effective protectants, and could be included in the final formulation of strain O when applied preharvest.

Keywords: *Candida oleophila* (strain O), *Penicillium expansum*, preharvest, UV-B protectants

Introduction

Postharvest diseases of fruits and vegetables caused by microbial pathogens account for enormous losses of agricultural products worldwide (Janisiewicz & Korsten, 2002). These losses have been estimated to exceed several billion dollars every year (Wilson & Wisniewski, 1989). Moreover, with increasing consumer demand for fresh and minimally processed fruits and vegetables free of microbial pathogens and pesticide residues, there is an urgent need for alternative protective control methods (Droby *et al.*, 1991).

Generally, postharvest diseases of fruits and vegetables are managed using synthetic fungicides either before or after harvesting (Lahlali *et al.*, 2009; Sharma *et al.*, 2009). Thiabendazole (TBZ) and imazalil are the most commonly used chemicals for preventing and controlling postharvest infections caused by *Penicillium* spp. on apples and citrus fruits (Jijakli *et al.*, 1993). Unlike preharvest treatments, these products are applied after harvesting by dipping or drenching (Lahlali *et al.*, 2009). However, this practice attracts criticism because of the possibility of fungicide resistance development and other environmental effects. Biological control of fruit diseases appears to be an alternative to chemical treatments because the environments of storage rooms (temperature and relative humidity) are stable and defined over time. Furthermore, fruits could command a higher market price, which could outweigh the higher cost of biological treatment (Fokkema, 1991; Jijakli *et al.*, 1993; Wilson & Wisniewski, 1994; Droby *et al.*, 1996).

The antagonistic potential of biocontrol agents (BCAs) in preharvest conditions is undoubtedly dependent on environmental conditions. Survival and biological activity are highly influenced by environmental conditions such as temperature, relative humidity, rainfall, UV radiation, pH and nutritional status (availability of sugars and amino acids) (Ippolito & Nigro, 2000; Magan, 2001). Therefore, the study of the response of BCAs to various environmental stresses under laboratory and natural conditions is a crucial step in identifying antagonistic activity and the factors limiting BCA survival when applied as preharvest treatments (Teixido *et al.*, 1999). This ecological study will directly improve the formulation or lead to improvement of the performance of the BCA and ensure a more reliable efficacy (Wisniewski & Wilson, 1992).

The antagonistic yeast *Candida oleophila* is considered to be a BCA model and has been studied worldwide for the control of postharvest fruit diseases (Jijakli *et al.*, 1993; Droby *et al.*, 1998; Lahlali *et al.*, 2004). *Candida oleophila* strain O was isolated from the surface of apple fruits and all steps necessary for biofungicide development (mode of action, monitoring, formulation and large-scale application) were studied (Jijakli *et al.*,

*E-mail: lahlali.r@gmail.com
†Present address: Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, S7N 0X2, Saskatoon, Saskatchewan, Canada.

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1993). Recently, Lahlali & Jijakli (2009) studied its response to water stress as well as low relative humidity and proposed a formulation based on skimmed milk to control the low availability of water contents. However, the use of UV protectants to protect antagonistic yeast, applied preharvest, has not been previously investigated. Few studies have been performed on the influence of UV sunlight on the survival of BCAs. Nevertheless, it has been reported that UV radiation could be a factor limiting the BCA effectiveness when applied preharvest. Consequently, different sunscreens have been selected and added to BCA formulations in order to protect them against the harmful effects of sunlight. The most used among them are zinc oxide, titanium oxide, silicate, talc, inositol, folic acid, *p*-amino benzoic acid (PABA), riboflavin, tyrosine, tryptophan, ascorbic acid, tinpol LPW and congo red (Dunkle & Shasha, 1989; Burges, 1998). Current research work on the influence of sunlight on antagonistic yeasts is rare. The aims of the present work were to determine the *in vitro* and *in vivo* effects of UV-B radiation on *C. oleophila* survival, and to screen and compare the ability of the most successful compounds used in previous work to promote the survival and effectiveness of strain O.

Materials and methods

Microorganisms

Candida oleophila (strain O) was isolated from apple (cv. Golden Delicious) fruits and stored at −80°C in glycerol 25% (Jijakli *et al.*, 1993). Before use, the yeast was successively subcultured onto potato dextrose agar (PDA, Merck) medium and incubated with a 16-h photoperiod for 24 h at 25°C. It was harvested with 0·85% NaCl solution by flooding a Petri dish and the cell concentration adjusted according to optical density (OD) measurements using a UltrospecII spectrophotometer (LKB Biochron Ltd) at 595 nm using the following equation: 10^6 CFU mL^{-1} = (OD − 0·015)/0·014 (Jijakli & Lepoivre, 1998).

Penicillium expansum (strain vs2) was isolated from decayed apple fruits. For long-term storage, the spore suspension was maintained at −80°C in tubes containing 25% glycerol. During experiments, the initial conidial inoculum was taken from Petri-dish cultures on PDA medium, preserved at 4°C for no more than 6 months. Conidial suspension was prepared from a 10 ± 1-day-old colony culture in sterile distilled water (SDW) containing 0·05% Tween 20, filtered through cheesecloth and adjusted to the desired concentration using a haemocytometer.

UV-B experimental design

To assess the effect of UV-B (280–320 nm) radiation on growth of *C. oleophila*, four UV-B Philips lamps (TL UV-B 40 W/12 RS) were used. These lamps were positioned 25 cm from the target (Petri dish or apple). In *in vitro* tests, the lids of Petri dishes were substituted by diacetate cellulose Clarifoil Standard filters (75 μm thick) (Clarifoil-France). Before use, these filters were presolarized for at least 100 h under the UV lamps in order to remove wavelengths below approximately 292 nm. Once presolarized, filters retain their properties for approximately 15 days. When tested *in vivo*, these filters were placed under UV-B lamps. Table 1 summarizes UV-B exposure time (h) and corresponding UV-B doses (Kj m^{-2}), and natural sunlight around the summer solstice (h).

In vitro effect of UV-B radiation on survival of *C. oleophila*

Petri dishes containing 20 mL suspension of *C. oleophila* at a concentration of 10^7 CFU mL^{-1} in triplicate, were subjected to different exposure times of UV-B [(0, 0·5 h (0·46 Kj m^{-2}), 1 h (0·93 Kj m^{-2}), 2 h (1·87 Kj m^{-2}), 2·5 h (2·33 Kj m^{-2}), 3 h (2·79 Kj m^{-2}) and 4 h (3·74 Kj m^{-2})]. For each exposure time, a control dish was kept at 25°C in the dark. The whole 20 mL of suspension were harvested after each exposure time and serially diluted to four 10-fold and 100-μL aliquots for each dilution, including stock, in four replicates for incubation on PDA in Petri plates at 25°C for 72 h (16-h photoperiod). There were four replicates for each plating serial dilution (20 plates per exposure time). This experiment was repeated twice and the results were expressed as percentage mortality rate (MR) using the formula MR(%) = [(UT−ET)/(UT)] × 100; where UT = average number of colonies not exposed to UV-B, and ET = number of colonies exposed to UV-B.

In vivo effect of UV-B radiation on survival of *C. oleophila*

Apple fruits were disinfected by soaking for 4 min in sodium hypochlorite solution (v/v 10%) and then rinsed twice in SDW. After drying for 1 h at ambient

Table 1 UV-B radiation dose (Kj m^{-2}) emitted by ultraviolet-B Philips lamps for each UV-B exposure time and their corresponding values of natural sunlight around the summer solstice (h) (Melendez, 2002). The UV-B dose was measured using Delta UV sensors (Delta-T Devices)

Exposure time (h)	UV-B dose (Kj m^{-2})	Natural sunlight (h)[a]
0·5	0·46	0·2
1	0·93	0·4
2	1·87	0·8
4	3·74	1·59
6	5·61	2·39
8	7·48	3·19

[a]Natural sunlight was estimated by assuming the average dose of UV-B radiation per day was 9·4 Kj m^{-2} and this dose was maintained for at least 4 h around noon. To determine the corresponding value for each UV-B dose, a linear relationship was established between natural sunlight (A) and UV-B dose (B) (A = 0·425*B, r^2 = 1).

temperature, apples were dipped in 400 mL of strain-O suspension (10^8 CFU mL^{-1}) for 2 min. Upon drying for 1 h, apples were subjected to different exposure times of UV-B radiation [(0, 1 h (0·93 Kj m^{-2}), 2 h (1·87 Kj m^{-2}), 3 h (2·79 Kj m^{-2}), 4 h (3·74 Kj m^{-2}) and 5 h (4·68 Kj m^{-2})], with a control treatment in darkness for each exposure time. At half exposure time, the apples were inverted in order to expose their entire surface to UV-B radiation. At the end of each exposure time, the apples were placed in freezer bags containing 1 L KPBT washing buffer [KH_2PO_4 (0·05 M), K_2HPO_4 (0·05 M) and 0·05% (w/v) Tween 80, pH 6·5] on a rotary shaker for 20 min at 120 r.p.m. for yeast recovery. Four serial 10-fold dilutions were prepared from 1 mL apple washing buffer and 100 μL plated in triplicate onto selective media (HST-PDA) consisting of PDA supplemented with two fungicides [Sumico (S) at 2·5 mg mL^{-1} and tetramethyl thiuram disulfide (T) 0·25 mg mL^{-1}] and one antibiotic [hygromycin (H) 416 mg mL^{-1}]. Four dishes were used for each exposure time and were incubated at 25°C for 72–96 h. This experiment was repeated twice and the results were recorded as mortality rate (%) versus UV-B dose (Kj m^{-2}) as described above.

In vitro screening of UV-B protectants

Based on an initial screening to determine any impact on the loss of viability together with cost (Table 2), four UV-B protectants: sodium ascorbate, ascorbic acid, uric acid and riboflavin, were selected for further studies. As described earlier for *in vitro* screening without protectants, Petri dishes containing 20 mL suspension of strain O adjusted to 10^7 CFU mL^{-1} at three concentrations (0·1%, 0·01% or 0·001% w/v) of each UV protectant were subjected to different UV-B exposure times (0, 0·5, 1, 2, 3 or 4 h). Strain O and each protectant were prepared separately at twice the desired concentration in SDW and then mixed together at the ratio of 10:10 (v/v). The concentrations of selected compounds were chosen based on their cost for practical feasibility and their impact on viability of strain O (Table 2). For each exposure time, 100 μL were incubated on PDA for 72 h at 25°C. Four dishes were used for each exposure-time–concentration combination. The number of colony forming units (CFU mL^{-1}) was determined for each protectant concentration and the log (CFU mL^{-1}) was plotted against exposure time. This assay was carried out twice and viability of strain O was recorded after 2 h of exposure using the formula: percentage viability = [(colony number in the presence of UV-B protectant)/(colony number in unexposed medium without protectant)] × 100.

Table 2 Effect of potential UV-B protectants on the viability of *Candida oleophila* (strain O) in relation to commercial cost

UV protectant	Concentration (%)	Price/concentration[a] (U) (w/w)	Viability[b]
Ascorbic acid	0·1	0·01	+++
Casein	0·5	0·06	++
Casein	0·1	0·01	++
Congo red	0·1	0·09	++
FB28	0·1	2·36	+++
Folic acid	1	1·53	+++
Folic acid	10	15·30	+++
Gelatine	0·5	0·04	++
Gelatine	0·1	0·09	++
Inositol	10	4·20	++
Lignin	0·5	???	+++
L-Tryptophan	0·1	0·11	++
L-Tyrosine	1	0·22	++
L-Tyrosine	10	2·20	+++
PABA	0·1	0·05	+++
Riboflavin	1	0·48	+++
Riboflavin	10	4·80	+++
Sodium ascorbate	0·1	0·02	+++
Titan dioxide	0·1	0·07	+
Tryptophan	1	1·14	++
Uric acid	10	5·80	+++

[a]Price calculated based on the concentration rate of the UV-B protectant in 100 g of final formulated product of *C. oleophila* (strain O). U = 1 Euro; ? = unknown.
[b]+++: >100% viability, ++: 30–60% viability, +: <30% viability.

In vivo screening of UV-B protectants

The concentration of *C. oleophila* strain O was adjusted to 10^8 CFU mL^{-1} for each UV-B protectant concentration (0·1%, 0·01% and 0·001% w/v). Disinfected apples were treated with each protectant concentration and then exposed to UV-B radiation for 2 h in the same way as described for the *in vivo* assay without protectant. At the end of the experiment, yeasts were recovered from the apple surface as described earlier. Four dishes were used per treatment and were incubated at 25°C for 72–96 h for colony counts. This experiment was repeated twice and viability of strain O was calculated using the same formula as above.

Efficacy test of formulated strain O against *P. expansum*

Disinfected apple fruits were wounded at two equidistant points on the equatorial zone (4 mm in diameter and 2 mm deep) using a cork borer and placed in plastic bags. Each wound was treated with 50 μL strain O suspension with or without protectant substances and was exposed to UV-B radiation for 2 h. One batch of apples was used as an exposed UV-B control and another batch as an unexposed control. For both these treatments, wounds were treated with 50 μL SDW. Four apples were used per treatment. Following UV-B exposure, wounds were inoculated with 50 μL pathogen suspension at 10^5 or 10^6 spores mL^{-1}. Each plastic bag was moistened with 3 mL SDW. Apple fruits were incubated at 24°C for 5 days and the experiment conducted twice. Lesion diameters were then recorded for each treatment.

The efficacy of treatments compared to control was calculated as percentage reduction in lesion diameter using

the equation $100 \times (D_t - D_y)/D_t$, where D_t and D_y are mean diameters of lesions on apples in the control group, and in the presence of the UV-B protectant, respectively.

Statistical analysis

Mortality rates (%) of *in vitro* UV-B doses (0·46, 0·93, 1·87 and 2·33 Kj m^{-2}) and *in vivo* UV-B doses (0·93, 1·87, 2·79, 3·74 and 4·68 Kj m^{-2}) were transformed to probit values as described by Bliss (1934) and then plotted against UV-B dose in order to estimate lethal doses (LD_{50} and LD_{90}). The equation of a linear line ($y = ax + b$, in which y = probit mortality, x = dosage of UV-B radiation, a = the increase of y per unit increase of x, and b = intercept) was used for determining the lethal doses LD_{50} and LD_{90}, which corresponded respectively to the probit values of 5 and 6·28. The ANOVA PROC of SAS software (SAS Institute), was performed on strain O viability (%) and lesion diameter (mm). When significant effects were observed, the Newman–Keuls test was used for the mean separation ($P \leq 0{\cdot}05$).

Results

In vitro effect of UV-B radiation on survival of strain O

The impact of UV-B radiation exposure time on *C. oleophila* strain O was evaluated under *in vitro* conditions (Fig. 1a). The mortality rate was 54·2% after 1 h and reached 100% after 2 h. There was a positive correlation between probit mortality and UV-B dose ($r = 0{\cdot}99$). The lethal doses, LD_{50} and LD_{90}, were 0·89 and 1·45 Kj m^{-2}, respectively, corresponding, respectively, to 0·39 and 0·62 h of natural sunlight around the summer solstice.

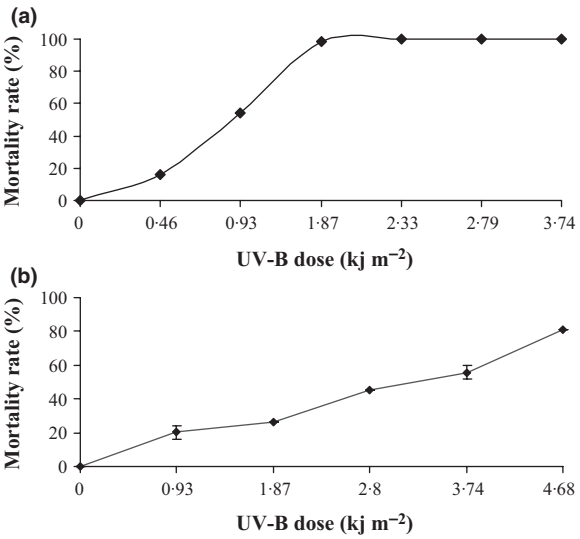

Figure 1 *In vitro* (a) and *in vivo* (b) mortality rates (%) of *Candida oleophila* (strain O) versus UV-B exposure dose. Values are the mean of two experiments with four replicates. Vertical bars represent standard errors corresponding to their respective means.

In vivo effect of UV-B radiation on survival of strain O

This trial was conducted under the same conditions as the *in vitro* experiment, but with apples. Mortality of strain O was plotted against exposure time (Fig. 1b). Mortality reached 20·4%, 26·2% and 45·3% after 1, 2 and 3 h of UV-B exposure, respectively. A mortality rate of 80·9% was observed after 5 h of UV-B exposure. Again, a positive correlation was observed between probit mortality and UV-B dose ($r = 0{\cdot}97$). LD_{50} and LD_{90} were 3·06 and 5·5 Kj m^{-2}, respectively, corresponding to 1·31 and 2·52 h of natural sunlight around the summer solstice (data not shown).

In vitro screening of UV-B protectants

Table 2 shows the results of the preliminary screening test used to assess the effectiveness of UV-B protectants for *C. oleophila* strain O under *in vitro* conditions. The compounds tested gave varying responses to UV-B radiation and the best results were obtained with higher percentages of UV-B protectant (1% and 10% v/v), except for inositol and tryptophan. Consequently, of the 16 UV-B protectants, sodium ascorbate, riboflavin, ascorbic acid and uric acid were selected and retained for further tests based on their higher degree of protection and lower cost. Their effectiveness was evaluated at lower concentrations (0·001%, 0·01% and 0·1% v/v) in suspensions of strain O.

Numbers of strain O cells (log CFU mL^{-1}) were plotted against UV-B radiation exposure time for each protectant concentration (Fig. 2). A fast decline in the strain O population was evident when exposed to UV-B in the absence of a UV-B protectant, with final cell viability varying from 0% to 50% of the initial population. However, the addition of UV-B protectants reduced population decline and resulted in significantly higher numbers of strain O cells compared with the unformulated strain O. This increase in strain O density remained statistically dependent on UV-B protectant level and was slightly lower than that of the unexposed strain O without protectant. Variance analysis demonstrated a significant effect ($P \leq 0{\cdot}0001$) of UV-B protectants on survival of strain O compared with the unformulated strain O (data not shown). The viabilities recorded at both protectant concentrations (0·1% and 0·01%) were not significantly different for ascorbic acid and riboflavin. However, for sodium ascorbate and uric acid, 0·01% did not significantly improve survival. Regardless of the UV-B protectant used, its lowest concentration (0·001%) was not effective in protecting strain O from the adverse effect of UV-B radiation (data not shown).

In vivo screening of UV-B protectants

There was a statistically significant effect of protectant concentration on the viability of strain O following UV-B exposure (Table 3). Strain O showed the highest viability

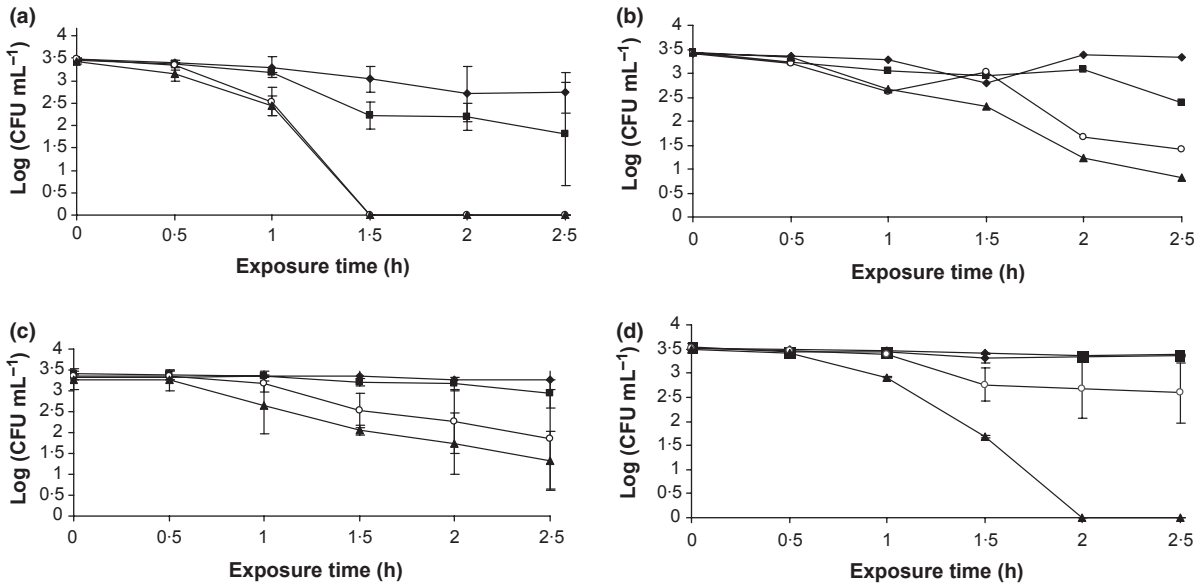

Figure 2 Recovery of *Candida oleophila* strain O (CFU mL^{-1}) after *in vitro* UV-B exposure as affected by UV-B protectants: ascorbic acid (a), riboflavin (b), sodium ascorbate (c) and uric acid (d) at three concentrations [0·1 (■), 0·01(◆) or 0·001% (○)]. Strain O exposed to UV-B radiation in the absence of protectant served as control (▲). The experiment was repeated twice with four replicates for each UV-B protectant concentration. Vertical bars represent the standard errors corresponding to their respective means.

with 0·1% UV-B protectants. For sodium ascorbate, the viabilities recorded at concentrations of 0·1% and 0·01% were not significantly different (30·9% and 33·6%). They did, however, differ significantly between the 0·001% treatment and the unformulated strain O (21·5% and 9·1%, respectively). Again, in the case of ascorbic acid, cell viability was statistically similar for concentrations of 0·1% and 0·01% (35·8% and 30·9%, respectively) and significantly different from that obtained at a concentration of 0·001% (20·0%). However, in the case of riboflavin and uric acid, the highest viability (87·1% and 98·3%, respectively) was reached at the 0·1% concentration. This viability was significantly higher than that obtained at a concentration of 0·01% (56·0% and 39·8%, respectively) or 0·001% (49·2% and 36·9%, respectively).

Table 3 Effect of potential UV-B protectants on the viability of *Candida oleophila* (strain O) cells (applied at 10^8 CFU mL^{-1}) 2 h after *in vivo* UV-B exposure at three protectant concentrations (0·001%, 0·01% or 0·1% w/v). The results are the mean of two experiments with four replicates. ANOVA was performed separately for each UV protectant. Values followed by the same letters are not significantly different according to the Newman and Keuls test ($P \leq 0.05$)

Protectant	Concentration (w/v)	Viability (%)
Ascorbic acid	0	14·4c
	0·001	20·0b
	0·01	30·8a
	0·1	35·8a
Riboflavin	0	11·1c
	0·001	49·27b
	0·01	**56·0b**
	0·1	**87·1a**
Sodium ascorbate	0	9·1c
	0·001	21·4b
	0·01	30·9a
	0·1	33·6a
Uric acid	0	29·8c
	0·001	36·8b
	0·01	39·8b
	0·1	**98·2a**

Viability values >50% are shown in bold.

Efficacy test of formulated strain O against *P. expansum*

The biocontrol efficacy of strain O applied in mixture with different UV-B protectants at 0·1% was evaluated against *P. expansum*. There was a significant effect of UV protectants and unformulated strain O on *P. expansum* lesion diameter compared with untreated controls (Fig. 3). Regardless of pathogen concentration, strain O combinations with UV-B protectants followed the same trends and no significant difference was observed between both control treatments. At a pathogen concentration of 10^5 spores mL^{-1}, ascorbic acid, sodium ascorbate and unformulated strain O were more effective than uric acid and riboflavin. The highest efficacy was observed for ascorbic acid (82·5%), followed by sodium ascorbate (71·5%), unformulated strain O (75%), riboflavin (64·2%) and uric acid (47·7%). However, at 10^6 spores mL^{-1}, no significant difference was observed between ascorbic acid, sodium ascorbate and unformulated strain O. Again, the best control was obtained when

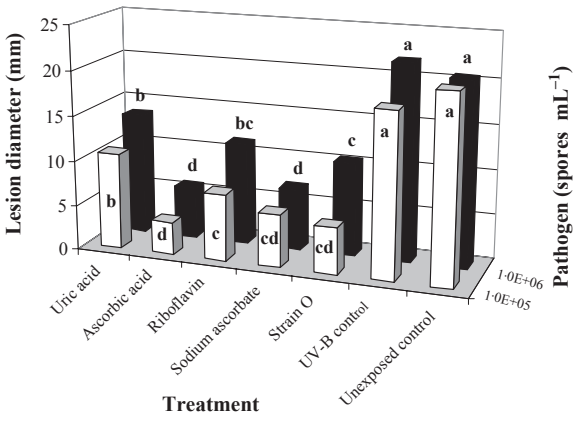

Figure 3 Lesion diameter (mm) of *Penicillium expansum* as affected by different formulations of *Candida oleophila* strain O with UV-B protectants and by pathogen concentration (spores mL^{-1}). This experiment was conducted twice with four replicates per treatment. For the same concentration of pathogen, treatments having the same letter are not significantly different according to the Newman and Keuls test ($P \leq 0{\cdot}05$).

strain O was applied together with ascorbic acid or sodium ascorbate.

Discussion

It is known that the BCAs used for controlling postharvest fruit disease are highly effective in postharvest conditions and become sensitive to environmental conditions when applied prior to harvesting. Therefore, the abiotic factors that most significantly limit BCA survival under field conditions are temperature, components of sunlight [mainly UV-A (320–400 nm) and UV-B (280–320 nm)], pH, relative humidity and water availability (Teixido *et al.*, 1999; Ippolito & Nigro, 2000; Magan, 2001). Various wavelengths of sunlight have been reported to be responsible for photodegradation and inactivation of various microbial fungicides (Hadapad *et al.*, 2009). UV-B is responsible for direct and indirect DNA damage. The direct damage induces the formation of cyclobutane pyrimide dimers in DNA that cause mutation or the failure of transcription (Griffiths *et al.*, 1998). Indirect DNA damage results from photo-oxidative processes and the generation of reactive oxygen species (ROS; Griffiths *et al.*, 1998). It is known that the detrimental effect of UV-B radiation on microorganisms is mediated by ROS (Wang *et al.*, 2008). To quench ROS, organisms develop efficient antioxidant systems to scavenge them, including antioxidant molecules and antioxidant enzymes. This study is the first investigation conducted to evaluate the effect of sunlight on the antagonistic yeast *C. oleophila* (strain O).

The results show that the antagonistic yeast here was more sensitive to UV-B radiation under *in vitro* conditions than *in vivo* conditions. *In vitro* exposure to UV-B radiation for 3 h resulted in complete loss of cell viability. Braga *et al.* (2002) studied the impact of four UV-B exposure times on the viability and germination of two species of entomopathogenic fungus, *Verticillium lecanii* and *Aphanocladium album*, and reported that viability was reduced to zero after 4 h. Lethal doses which halved each strain population were, respectively, 86 and 120 min, corresponding to 4·1 and 5·8 Kj m^{-2}. For strain O, doses which halved populations were 0·89 (24 min) and 3·06 Kj m^{-2} (1·3 h) under *in vitro* and *in vivo* conditions, respectively. Riesenman & Nicholson (2000) reported that the LD_{90} for a *Bacillus subtilis* mutant ranged from 31·3 to 28·0 Kj m^{-2} and explained that the greatest resistance was afforded by a particular bacterial cell wall structure. Hadapad *et al.* (2008) underlined that the exposure of *B. sphaericus* ISPC-8 and 1593 spores to UV-B radiation for 6 h resulted in complete loss of spore viability and 50% reduction in larvicidal activity. Therefore, the BCA in the present study appeared to be more sensitive to sunlight than *V. lecanii, A. album* and bacteria.

Several reports describe the ways in which various agents protect against UV-B damage (Dunkle & Shasha, 1989; Cohen *et al.*, 1991; Hadapad *et al.*, 2009). Different synthetic compounds such as congo red, uric acid, *p*-aminbenzoic acid (PABA), benzaldehyde, melanin and malachite, casein, gelatine sodium alginate starch, carboxymethycellulose have been evaluated as suitable UV protectants for various entomopathogens and bacteriophages (Dunkle & Shasha, 1989; Cohen *et al.*, 1991). The present study selected and tested different synthetic compounds as UV-B protectants. A slight difference was observed between them when compared for survival of strain O (Table 2). Consequently, four compounds were screened and evaluated at low concentrations based on their effectiveness and their lower cost (Table 3). These compounds improved survival of strain O under *in vivo* and *in vitro* conditions following UV-B exposure. This result is in agreement with that of Hadapad *et al.* (2009). Also, it was noticed that some protectants lost their efficacy when applied to the apple's surface. This may have been the result of their oxidative protective action against UV-B radiation (i.e. ascorbic acid and sodium ascorbate act as antioxidants). Thus, when they were tested *in vitro*, their exposure to oxygen was lower, resulting in higher efficacy. Sodium ascorbate was less sensitive to oxidation than ascorbic acid. Evidently, sodium ascorbate and ascorbic acid would not be useful for strain O formulations at the tested concentrations. They have not been proven effective in protecting strain O against sunlight. Ghajar *et al.* (2006) also concluded that the use of ascorbic acid or sodium ascorbate would not be economically feasible.

Uric acid and riboflavin gave the best results and appeared to be promising products for strain O formulation because of their high performance and low cost. Ignoffo & Garcia (1995) reported that UV-B protectants should also be cost effective to use as a spray-tank additive. Riboflavin did not act as an antioxidant but as a UV absorber. This mode of action would then be less susceptible to possible oxidation. Hadapad *et al.* (2009)

also reported that riboflavin gave better protection (73·2%) than ascorbic acid (47·8%), as did Ghajar *et al.* (2006), who reported that riboflavin at 1% gave better protection to conidia of *Plectosporium alismatis* than ascorbic acid and sodium ascorbate at 5%.

In the biocontrol experiment, it appeared that all UV-B protectants tested, with the exception of uric acid, did not significantly influence the effectiveness of strain O. The obtained results showed that sodium ascorbate (71·5% and 66·7%) and ascorbic acid (82·5% and 71·8%) provided the best efficacy against this pathogenic fungus, followed by riboflavin and uric acid. The results also demonstrated that the efficacy of strain O was influenced by the time interval between its application and pathogen inoculation. It also significantly decreased with increasing pathogen pressure. These results are in agreement with those reported by Jijakli *et al.* (1993) and Lahlali *et al.* (2004).

Undoubtedly, the four UV-B protectants evaluated in the present work greatly improved the survival and effectiveness of strain O. Nonetheless, some of them cannot be used in the final formulation of the strain because of their restricted market as additives (uric acid), their mode of action against UV-B radiation as antioxidants or their high cost (ascorbic acid and sodium ascorbate). Therefore, future studies will assess the effectiveness of strain O in combination with riboflavin, uric acid (at low concentrations) and skimmed milk (1%) under field conditions prior to harvesting.

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