ORIGINAL ARTICLE

Effect of germicidal UVC light on fungi isolated from grapes and raisins

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Abstract

Aims: To examine how UVC affects the different genera of fungi commonly isolated from grapes, with the aim of understanding changes in mycobiota during grape ripening and possible applications for preventing grape decay during storage.

Methods and Results: Spores of *Aspergillus carbonarius*, *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium janthinellum* and *Alternaria alternata* (between 100–250 spores/plate agar) were UVC irradiated for 0 (control), 10, 20, 30, 60, 300 and 600 s. Plates were incubated at 25°C and colonies were counted daily up to 7 days. *Alternaria alternata* and *Aspergillus carbonarius* were the most resistant fungi. Conidial germination in these species was reduced by approx. 25% after 10 s of exposure, compared with greater than 70% reduction for the remaining species tested. *Penicillium janthinellum* spores were the most susceptible at this wavelength. UVC exposures of 300 s prevented growth of all isolates studied, except for *Alternaria alternata*.

Conclusions: UVC irradiation plays a major role in selecting for particular fungi that dominate the mycobiota of drying grapes.

Significance and Impact of the Study: The UVC irradiation of harvested grapes could prevent germination of contaminant fungi during storage or further dehydration.

Introduction

Grapes are susceptible to insect attack and to fungal diseases, especially grey rot, downy mildew and black rot. Damaged grapes are vulnerable to further diseases such as summer bunch rot, which may be caused by *Aspergillus niger*, *Alternaria tenuis*, *Cladosporium herbarum*, *Rhizopus arrhizus*, *Penicillium* spp. and other fungi. Fungal invasion depends on grape maturity. *Alternaria*, *Cladosporium*, *Botrytis* and *Rhizopus* are common at early veraison, whereas *Aspergillus* and *Penicillium* are more frequently found at harvest and during sun drying (Magnoli *et al.* 2003; Bellí *et al.* 2004; Valero *et al.* 2005). *Aspergillus carbonarius* and *Aspergillus niger* are know to produce ochratoxin A (OTA) in grapes and raisins (Abarca *et al.* 2003; Bellí *et al.* 2004).

Changes in the mycobiota of grapes are affected by weather, solar irradiation, rising temperatures and decreases in the water activity of berries due to increasing berry sugar content. Grapes before harvest and especially during drying, are exposed for long periods to sunlight. Ultraviolet radiation from the sun is divided into three wavebands: UVA, UVB and UVC.

Ultraviolet radiation has been known for many years to affect micro-organisms. UVC (100–280 nm) is highly germicidal and is commonly used for sterilisation of surfaces, water and air; however, quantification of these effects has been difficult. Levetin *et al.* (2001) reported that UVC radiation applied to air-conditioning systems significantly reduced the incidence of *Cladosporium* spp. and *Aspergillus versicolor*. Green *et al.* (2004) reported 35 and 54 mJ cm^{-2} [UV dose (mJ cm^{-2}) = Irradiance (μW cm^{-2}) · Irradiation time (s)·10^{-3}] as the doses of ultraviolet germicidal irradiation (225–302 nm) necessary to inactivate 90% of the spores of *Aspergillus flavus* and *Aspergillus fumigatus*,

respectively. Jun *et al.* (2003) modelled the effect of pulsed UV-light on the inactivation of *Aspergillus niger* spores in corn meal and reported that for a 100 s treatment time, 3 cm of distance from the UV strobe and with 3800 V input gave a 4·9 $\log_{10}$ reduction of *Aspergillus niger*. In contrast, Nigro *et al.* (1998) found that irradiating grape berries with UVC light had no effect on filamentous fungi and even increased the incidence of yeasts and bacteria.

The effect of solar radiation on some genera of airborne fungi, such as *Cladosporium*, *Penicillium*, *Alternaria*, *Epicoccum* or *Aspergillus* among others, was studied by Ulevičius *et al.* (2004), who found that *Aspergillus niger* was the species most commonly isolated after treatment. Given these findings, we examined how the most energetic wavelength from sunlight, UVC, could affect the different genera of fungi commonly isolated from grapes, with the aim of understanding changes in mycobiota during grape ripening and possible applications for preventing grape decay during storage.

Materials and methods

Fungi used in this study were isolated from grapes and dried vine fruits collected from Australia and Spain: *Aspergillus carbonarius* (UdLTA 3·122) capable of OTA production, *C. herbarum* (UdLTA 3·129), *Penicillium janthinellum* (UdLTA 3·126), *Aspergillus niger* (FRR 5694) OTA positive and *Alternaria alternata* (FRR 4780). UdLTA strains were from the culture collection of the Food Technology Department, Lleida University, Spain and FRR strains were from the culture collection at CSIRO Food Science Australia, North Ryde, NSW Australia.

For spore production, each species was inoculated onto Malt Extract Agar (MEA; Raper and Thom 1949) and onto Dichloran Chloramphenicol Malt Agar for *Alternaria alternata* (DCMA; Andrews 1992) and incubated for 7 days at 25°C. Spores were harvested into aqueous Tween 80 (0·5%), quantified using a haemocytometer and diluted to give a final suspension of 10^3 spores ml^{-1}.

The growth medium was Synthetic Nutrient Medium (SNM), chosen because its composition is similar to that of grapes between veraison and ripeness (Delfini 1982), adjusted to 0·97 a_w (Valero *et al.* 2005). Aliquots (0·1 ml) of each 10^3 spore suspension were inoculated onto SNM by spreading uniformly on the agar surface and afterwards irradiated with UVC (254 nm), for 0 (control), 10, 20, 30, 60, 300 and 600 s. Plates without the lids were set upside at 10 cm from the lamp (G20T10 Sankyo Denki, Co., Ltd, Tokyo, Japan) in a closed chamber previously sterilised. Nominal lamp power was 19 W and 75·8 μWcm^{-2} of irradiance (at 1 m of distance). Each combination of species and irradiation time had four

replicates. Plates were incubated at 25°C for 7 days and colonies counted daily up to 7 days.

Survival fractions (S) of the five microorganisms were plotted against UVC irradiation time and an equation describing the one-stage exponential decay curve

$$S = exp(-kt)$$

where k = decay rate (s^{-1}); t = irradiation time, was generated using GraphPad Prism version 4·00 for Windows (GraphPad Software, San Diego, California, USA). Covariance analysis of germination percentages *vs* time and related Duncan's multiple range tests were performed using SAS (SAS Institute Inc., Cary, NC, USA).

Results

Alternaria alternata and *Aspergillus carbonarius* were the most resistant fungi when irradiated with UVC for 10 or 20 s (Fig. 1). Conidial germination in these species was reduced by approx. 25% after 10 s of exposure, compared with greater than 70% reduction for the remaining species tested. For UVC exposures longer than 20 s, *Alternaria alternata* was the only species capable of growing after 300 and 600 s exposure to UVC light. In contrast, *P. janthinellum* spores were the most susceptible at this wavelength, with total conidial inactivation after 30 s of exposure. UVC exposure of 60 s reduced survival of all fungi tested by more than 90%.

The exponential decay of the survival fractions was modelled so as to generate a single equation describing the UVC resistance for each isolate against exposure time (Fig. 2). More resistant fungi showed lower decay rates (k).

UVC irradiation at a distance of 10 cm for 30 s prevented *Aspergillus* growth for 3 days and UVC exposures of 300 s prevented growth of all isolates studied, apart from *Alternaria alternata*, for longer than 7 days (Table 1). The capacity to repair damaged spores was affected by UVC irradiation in all species tested, as longer incubation times were required for all viable spores to germinate when irradiated with higher cumulative UVC dosages. In fact all viable spores that were not irradiated germinated between 2–3 days, while the irradiated spores germinated differently, up to the sixth day, depending on the strains (Fig. 3). UVC irradiation for 10 s produced a significant reduction ($P < 0.05$) in germination of all fungi assayed, compared with germination of non-irradiated spores. Generally, subsequent exposures of 10 s led to successive significant reductions.

Discussion

Integration of these findings on resistance to UVC with data on the effects of temperature and water activity on
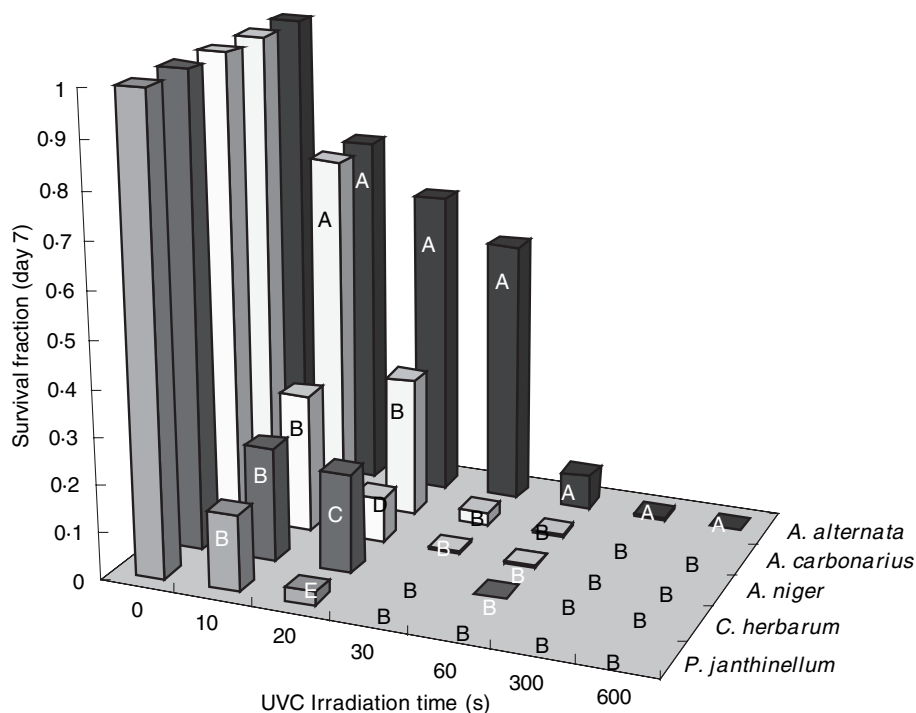
Figure 1 Survival fraction of the five isolates (*Alternaria alternata*, *Aspergillus carbonarius*, *Aspergillus niger*, *Cladosporium herbarum* and *Penicillium janthinellum*) after incubation for 7 days (average of four replicates). For comparison of species within each time point, bars with different letters are significantly different ($P < 0.05$).
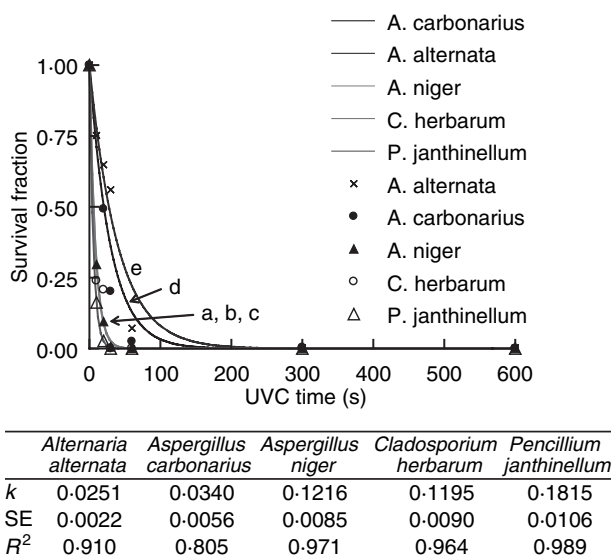

	Alternaria alternata	*Aspergillus carbonarius*	*Aspergillus niger*	*Cladosporium herbarum*	*Pencillium janthinellum*
k	0·0251	0·0340	0·1216	0·1195	0·1815
SE	0·0022	0·0056	0·0085	0·0090	0·0106
R^2	0·910	0·805	0·971	0·964	0·989

Figure 2 Models for single stage exponential decay curves of five isolates as influenced by UVC irradiation. Exponential decay rates for each curve are detailed in table: *Survival fraction* = exp $(-kt)$. k, decay constant (s^{-1}); t, UVC irradiation time (s). SE: standard error, R^2: regression coefficient. Isolates: a ($\triangle$), *Penicillium janthinellum*; b (▲), *Aspergillus niger*; c ($\bigcirc$), *Cladosporium herbarum*; d (◆), *Aspergillus carbonarius*; e (×), *Alternaria alternata*.
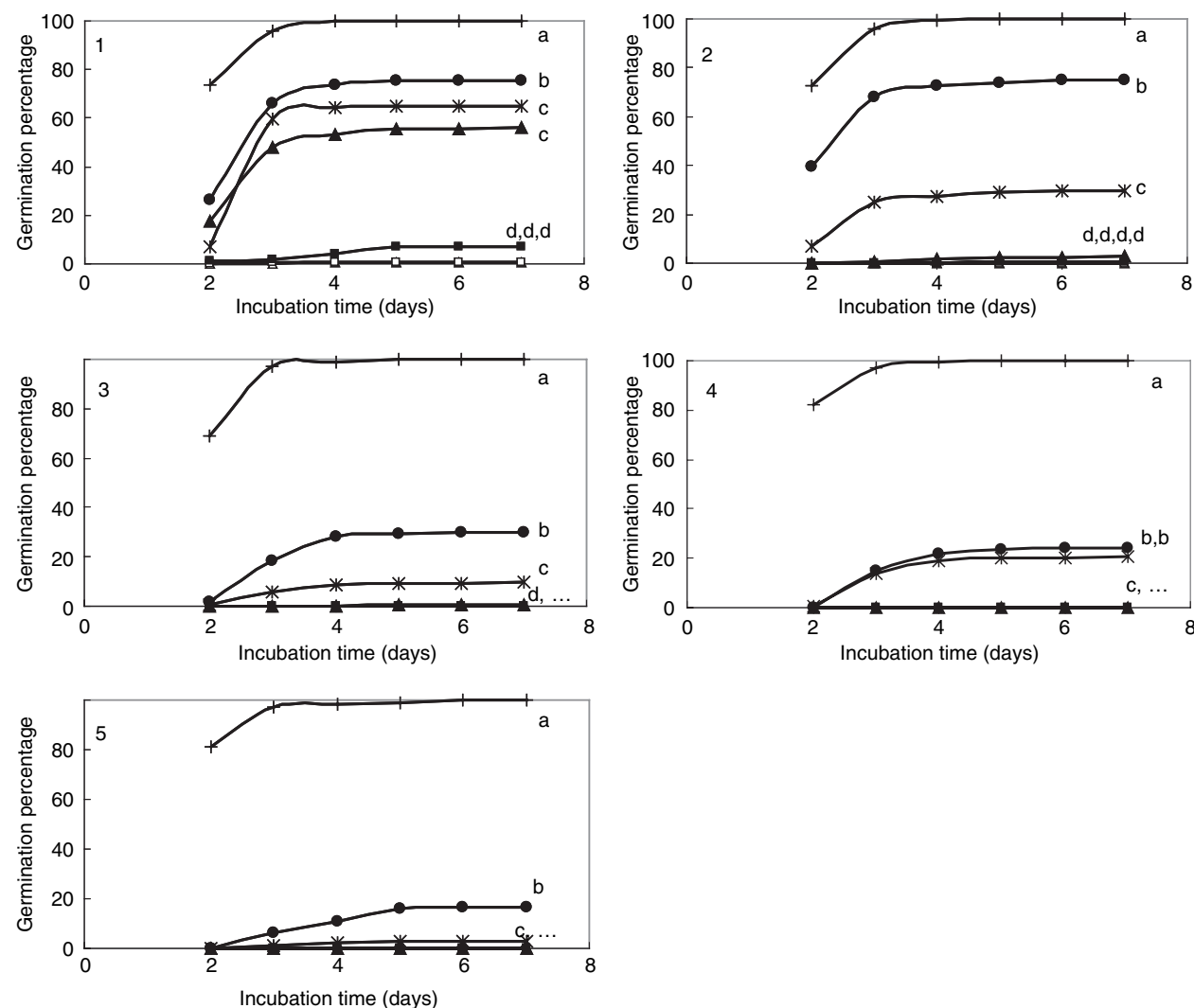
fungal growth and survival provides a good explanation of the incidence of fungi found on both fresh and sun-dried grapes. *Alternaria* is the most common fungal genus found on grapes, followed by *Penicillium*, *Aspergillus*, *Epicoccum* and *Cladosporium* (Sage *et al.* 2002; Bellí *et al.* 2004; Valero *et al.* 2005). Leong *et al.* (2006) reported a decrease (10^5) in *Aspergillus carbonarius* spore viability on grapes after an exposure to UV irradiation equivalent to 1 week of high UV intensity with cloudless skies in Sydney, NSW Australia, a cumulative energy estimated in 3.6×10^3 joules (10 mWh, milliwatt hour). When grapes are dried in intense sunlight, the most commonly found fungi are *Aspergillus*, *Penicillium* and *Alternaria* (Romero *et al.* 2005; Valero *et al.* 2005). Likewise, Ulevičius *et al.* (2004) isolated fungal propagules from air on the Lithuanian coast at different hours during the day and found that *Aspergillus niger*, *Aletrnaria alternata*, *Cladosporium* spp., *Arthrinium phaerosporum* and dematiaceous sterile mycelium were prevalent after exposure to solar radiation.

Our data demonstrating the resistance of *Alternaria alternata* to UVC agree with the findings of Rotem and Aust (1991), who noted that propagules of *Alternaria* sp. were the most resistant to ultraviolet radiation or sunlight, followed by *Mycosphaella* sp., *Aspergillus niger* and *Botrytis cinerea*. *Alternaria alternata* has multi-celled spores with

Table 1 Lag phase (days) of five isolates of fungi as influenced by duration of UVC irradiation

	Duration of UVC irradiation (s)						
	0	10	20	30	60	300	600
Alternaria alternata	≤2	≤2	≤2	≤2	≤2	3	4
Aspergillus carbonarius	≤2	≤2	≤2	3	4	n.g.	n.g.
Aspergillus niger	≤2	≤2	≤2	4	3	n.g.	n.g.
Cladosporium herbarum	≤2	≤2	≤2	n.g.	5	n.g.	n.g.
Penicillium janthinellum	≤2	3	3	n.g.	n.g.	n.g.	n.g.

n.g., no growth.

for the survival and longevity of fungal spores (Bell and Wheeler 1986), whereas non-melanin compounds are poor protectants against ultraviolet radiation (Durrell and Shields 1960). Grishkan *et al.* (2003) found a significant correlation between areas receiving high solar irradiation and the incidence of melanin-containing fungal species among soil microfungi isolated in Israel.

Aspergillus carbonarius and *Aspergillus niger* produce single-celled conidia with melanin and aspergilline in their cell walls (Duguay and Klironomos 2000; Babitskaya and Shcherba 2002), but differ in their UVC resistance

Figure 3 Germination curves following radiation of different fungi genera. 1, *Alternaria alternata*; 2, *Aspergillus carbonarius*; 3, *Aspergillus niger*; 4, *Cladosporium herbarum*; 5, *Penicillilum janthinellum*. UVC irradiation times: +, 0 s; ●, 10 s; ✳, 20 s; ▲, 30 s; ■, 60 s; □, 300 s; △, 600 s. Lower case letters denote final germination percentages that are significantly different (*P* < 0·05).

thick, melanised walls that are believed to confer tolerance to sunlight (Carzaniga *et al.* 2002). The photoprotective properties of melanin are believed to be important

and their incidence on grapes. *Aspergillus carbonarius* spores are thought to possess thicker walls than the 90–160 nm thick wall of *Aspergillus niger* (Tiedt 1993). The

greater UVC resistance displayed by *Aspergillus carbonarius* spores than *Aspergillus niger* spores provides a logical explanation for the high numbers of *Aspergillus carbonarius* on grapes subjected to prolonged sun exposure. In a study of fungal contamination of grapes after sun drying, *Aspergillus* section *Nigri* spp. were found in more than 80% of dried grapes (Valero *et al.* 2005). *Aspergillus carbonarius* occurred in increased frequency compared with *Aspergillus niger* on raisins and dried vine fruits (Leong *et al.* 2004; Romero *et al.* 2005; Valero *et al.* 2005; Gómez *et al.* 2006). This is somewhat surprising, as *Aspergillus niger* is more tolerant than *Aspergillus carbonarius* to low water activities (growth limits of 0·77 a_w and 0·87 a_w, respectively; Valero *et al.* 2007) and high temperatures (optimum growth at 35–40°C and 30°C, respectively; Panasenko 1967; Palacios-Cabrera *et al.* 2005; Valero *et al.* 2007). Resistance to UVC provides a competitive advantage for *Aspergillus carbonarius* during grape drying that overrides the superior growth of *Aspergillus niger* in hot and dry conditions.

Cladosporium herbarum produces conidia with a melanin-like pigment (Margalith 1992). The thickness of its conidial wall is unknown, but the pigmented conidia allow *Cladosporium* to survive under high solar irradiation, which explains their commonly occurrence on grapes and raisins (Romero *et al.* 2005; Valero *et al.* 2005), even though its maximum growth temperature is approximately 32°C (Domsch *et al.* 1980; Valero *et al.* 2007).

Some *Penicillium* species are known to produce melanin-like compounds; however, there is a little information regarding *P. janthinellum* (Youngchim *et al.* 2004). This species produces single-celled slightly pigmented conidia and hyaline mycelium hence, has few protective mechanisms against UV radiation.

Both the spatial distribution of pigments and highly efficient DNA-repair mechanisms have a cumulative effect of ecological significance, conferring on those microorganisms an advantage in surviving on grape surfaces (Wynn-Williams *et al.* 2002; Ulevičius *et al.* 2004).

Our results show that UVC irradiation plays a major role in selecting for particular fungi that dominate the mycobiota of drying grapes. Temperature and water activity are also important. In addition, the UVC irradiation of harvested grapes could prevent germination of contaminant fungi during storage or further dehydration that may lead to production of undesired substances such as mycotoxins.

Acknowledgements

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