Vitis **44** (3), 149–150 (2005)

Research Note

Catalase activity and susceptibility of grapevine callus culture (cv. Cabernet Sauvignon) to *Botrytis cinerea* infection: Effects of UV-B exposure

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K e y w o r d s : *Vitis vinifera*, bunch rot, grey mould, host-pathogen interactions, climate change, oxidative metabolism, active oxygen species.

Introduction: Bunch rot of grapes (grey mould), caused by *Botrytis cinerea* is a serious fungal pathogen of grapevines worldwide. As with all diseases of plants, the occurrence of this pathogen is largely driven by climatic and other environmental factors. Consequently any future climate changes are likely to result in an altered distribution and severity of bunch rots (Barlow 2000). The amount of ultraviolet radiation in the 280-320 nm range (UV-B) reaching the surface of the earth is increasing as a result of ozone depletion from the stratosphere (Polle 1997; Schultz 2000). The net result of this increase in UV-B on plant function is largely unknown although a range of effects has been reported including alterations in growth and morphology, plant pigmentation and nitrogen metabolism (Polle 1997; Schultz 2000). Furthermore there are many reports in the literature on UV-B effects on the levels of enzymes and antioxidants involved in protecting plant tissues against the harmful effects of Active Oxygen Species (AOS) (Polle 1997), but little has been published specifically dealing with grapevines. Previous work using field-grown vines or vines grown in pots has looked at reducing incident UV-B radiation using polyester films and the potential effect of future increase in UV-B radiation deduced by extrapolation (Schultz 2000, Steel 2001, Keller *et al*. 2003). Based on similar studies Kolb *et al.* 2003 found that vines screened from UV-B have an altered phenolic profile. Phenolic synthesis has previously been implicated in vine-resistance to fungal attack (Pezet and Pont 1992).

The aim of this study was to investigate the susceptibility of vine tissues to *B. cinerea* that are grown in the presence of enhanced rather than diminished UV-B radiation. A second aim was to determine if infection of vine tissues leads to an alteration in activities of catalase, a ubiquitous enzyme that acts to protect tissues against oxidative damage. A tissue culture system was used to avoid the variability of field-grown vines and because of the adaptability of such a system to culture under a UV-B source in the laboratory.

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Material and Methods: A single spore isolate of *B. cinerea* used in this study was collected from a vineyard in Tumbarumba, NSW, Australia and maintained on slopes of Potato Dextrose Agar (PDA) at 25 ºC. Callus cultures were initiated from cv. Cabernet Sauvignon explants and maintained under dark conditions (25 ºC) as described previously (Keller *et al.* 2000). Callus cultures (5 per plate) were used 3 weeks after sub-culturing when they were approximately 12 mm^2 in size. For conidial production, mycelial fragments were aseptically transferred to PDA plates and cultures grown at 25 ºC. Conidia were harvested by washing the surface of the plate with sterile distilled water (5 ml). Spore concentration was determined using a haemocytometer and adjusted to 10^6 conidia per ml by diluting with sterile distilled water. Callus cultures were inoculated by placing a 10 µl drop (*i.e.* 10^4 conidia) on the surface of the callus culture. The degree of necrosis was assessed by measuring the area of necrosis (mm^2) microscopically 24 h post-inoculation. Results are the means of 5 separate cultures.

Experiments on the effects of UV-B radiation were conducted by exposing callus cultures to a UV-B source (Philips Fluorescent Lamp TL 20/12, 313 nm); the degree of exposure varied by altering the distance of the culture from the light source. UV-B exposure was quantified using a light radiometer (YSI Model 65 Radiometer, Ohio). Using a scanning spectrophotometer (Shimadzu UV-2101PC) the transmittance of the plastic of the petri dish lid was found to be 89 % at 420 nm, 64 % at 320 nm, 47 % at 300 nm and 0 % at 280 nm, and thus effectively acted as a screen for short wavelength UV radiation (*i.e.* UV-C). Catalase activity was assayed from callus cultures either grown in the dark or exposed to UV-B and either inoculated or un-inoculated with *B. cinerea*. Callus tissue was ground in liquid nitrogen and the frozen powder homogenised in Phosphate Buffer, (pH 7.0, 0.1M, 5 $ml \cdot g^{-1}$ fresh weight). The homogenate was centrifuged (12,000 g, 20 min, 4 ºC) and the supernatant collected. The catalase activity of extracts (3 ml) at 27 ºC was assayed using a Clark Oxygen electrode (YSI, Ohio) as previously described using H_2O_2 (50 µl; 0.24 % v/v) as a substrate (Steel 1996). Catalase units were defined as mmol oxygen released $min^{-1} \cdot g$ fresh $weight^{-1}$. Three determinations were made per culture and 8 separate cultures were assayed.

Results and Discussion: UV-B exposure increased the rate of necrosis development in grapevine callus cultures inoculated with a *B. cinerea* spore suspension in a dose dependent manner over the 0 - 1.2 x 10^{-5} J $cm^{-2} \cdot s^{-1}$ range (Fig. 1). This range is typical of anticipated increases in UV-B radiation likely to reach the surface of the earth in the future (Blumthaler and Ambach 1990). The amount of UV-B currently reaching the surface of the earth varies from location to location but is typically in the 45 kJ m^{-2} day^{-1} range (Grammatikopoulos *et al*. 2001; Kolb *et al.* 2003).

At exposure levels > 1.2 x 10^{-5} J cm^{-2} s^{-1} for periods > 24 h there was a general browning of the callus, making differentiation of the necrotic area difficult. A positive correlation between UV-B exposure and *Botrytis* susceptibility is consistent with observations on field-grown vines where incident UV-B radiation has been screened using a polyester film, *i.e.* screened vines have a lower incidence and se-

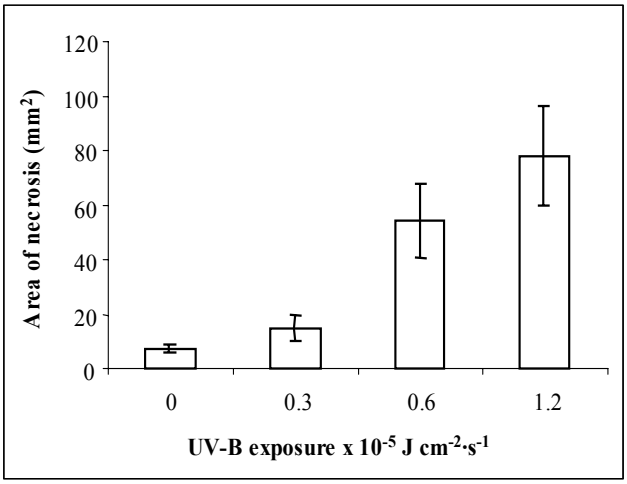

Fig. 1: Effect of UV-B exposure on the development of necrosis in grapevine callus (cv. Cabernet Sauvignon) inoculated with *Botrytis cinerea* (10^4 spores). Cultures incubated for 24 h at 25 ºC. Bars represent standard errors of the mean, n = 5. No necrosis was observed in callus tissue that was un-inoculated and cultured in the dark.

verity of *Botrytis* (Steel 2001). This contrasts with the work of Keller *et al*. (2003) who found that potted vines grown under ambient light conditions had a lower incidence of powdery mildew (a biotroph with a different biology and growth habit to a necrotrophic pathogen such as *B. cinerea)* than vines that were grown under a UV absorbing screen.

Catalase activity was elevated in callus cultures that had been either exposed to UV-B or inoculated with a *B. cinerea* spore suspension. A combination of both, UV-B exposure plus fungal inoculation led to a similar increase in catalase, although this was not significantly greater than when either of the two treatments were applied independently (Fig. 2). Catalase is responsible for the removal of H_2O_2 from cells, and the amount of H_2O_2 detected in bean leaf

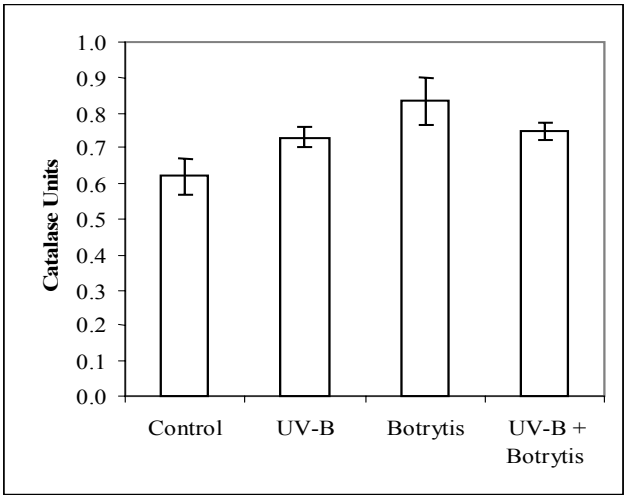

Fig. 2: Effect of UV-B exposure (0.6 x 10^{-5} J cm^{-2} s^{-1}) and/or inoculation with *Botrytis cinerea* (10^4 spores) on catalase activity of grapevine callus cultures incubated for 24 h at 25 ºC. Bars represent standard errors of the mean, n = 8. Control cultures were water inoculated with sterile distilled water.

tissue infected with *B. cinerea* correlates with the aggressiveness of the isolates (Tiedemann 1997). Tomato leaves infected with *B. cinerea* have increased activities of catalase (Patykowski and Urbanek 2003) although such an effect has not been recorded previously for vine tissues inoculated with *B. cinerea*.

Our observations indicate that grapevines of the future are likely to be more prone to attack by *B. cinerea* and that both fungal challenge and UV-B exposure lead to enhanced activities of catalase. This heightened disease susceptibility will be further magnified in areas of the world that are predicted to experience increases in the amount of rain falling during the harvest period (Barlow 2000).

Adj. Prof. P. Kriedemann is thanked for his assistance in the measurement of UV-B energy levels while the assistance of Mrs. J. Hatfield for initiation of callus cultures is gratefully acknowledged. This work was funded by a Charles Sturt University competitive grant; OPA 776.

Barlow, S.; 2000: Bunch rots: Is climate change the culprit? In: C. Davies, C. Dundon, R. Johnstone (Eds): Proc. ASVO Viticulture Seminar Managing Bunch Rots, 4-6. Mildura, Australia (ASVO: Adelaide).

Blumthaler, M.; Ambach, W.; 1990: Indication of increasing solar ultraviolet-B radiation flux in alpine regions. Science **248,** 206-208.

Grammatikopoulos, G.; Drilias, P.; Kyparissis, A.; Petropoulou, Y.; Manetas, Y.; 2001: Reduction of ambient UV-B radiation does not affect growth but may change the flowering pattern of *Rosmarinus officinalis* L. Plant Ecol. **154**, 119-122.

Keller, M.; Rogiers, S. Y.; Schultz, H. R.; 2003: Nitrogen and ultraviolet radiation modify grapevines' susceptibility to powdery mildew. Vitis **42,** 87-94.

Keller, M.; Steel, C. C.; Creasy, G. L.; 2000: Stilbene accumulation in grapevine tissues: Developmental and environmental effects. Acta Hortic. **514**, 275-286.

Kolb, C. A.; Kopecký, J.; Riederer, M.; Pfündel, E. E.; 2003: UV screening by phenolics in berries of grapevine (*Vitis vinifera*). Funct. Plant Biol. **30**, 1177-1186.

Patykowski, J.; Urbanek, H.; 2003: Activity of enzymes related to H_2O_2 generation and metabolism in leaf apoplastic fraction of tomato leaves infected with *Botrytis cinerea*. J. Phytopathol. **151**, 153-161.

Pezet, R.; Pont, V.; 1992: Differing Biochemical and Histological Studies of Two Grape Cultivars in the View of their Respective Susceptibility and Resistance to *Botrytis cinerea*. In: K. Verhoeff, N. E. Maltrakis, B. Williamson (Eds): Recent Advances in Botrytis Research, 169-178. Proc. 10th Int. Botrytis Symp., Heraklion, Crete, 1992. Pudoc Scientific Publ., Wageningen, The Netherlands.

Polle, A.; 1997: Defence against Photooxidative Damage in Plants. In: J. G. Scandalios (Ed.): Oxidative Stress and the Molecular Biology of Antioxidant Defences. Cold Spring Harbor Monograph Series **34**, 623-666. Cold Spring Harbour Laboratory Press, Woodbury, USA.

Schultz, H. R.; 2000: Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. Aust. J. Grape Wine Res. **6**, 2-12.

Steel, C. C.; 1996: Catalase activity and sensitivity to the fungicides, iprodione and fludioxonil in *Botrytis cinerea*. Lett. Appl. Microbiol. **22**, 335-338.

Steel, C. C.; 2001: Effects of altered UV light and climate change on the susceptibility of grapevines to fungal diseases. Aust. New Zealand Grapegrower Winemaker, **449,** 13-15.

Tiedemann, A. V.; 1997: Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea*. Physiol. Mol. Plant Pathol. **50**, 151-166.

Received February 25, 2005