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Effect of temperature on *Botrytis cinerea*, *Colletotrichum acutatum* and *Greeneria uvicola* mixed fungal infection of *Vitis vinifera* grape berries

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Summary

Detached *Vitis vinifera* 'Cabernet Sauvignon' berries (12.5° Bé) were inoculated either singularly or in combination with spore suspensions of *Botrytis cinerea*, *Colletotrichum acutatum* and *Greeneria uvicola* and the degree of disease expression examined at either 20 or 27 °C. Berries were more susceptible to *B. cinerea* at 20 °C and to *G. uvicola* at 27 °C but were highly susceptible to *C. acutatum* at either temperature. In experiments involving inoculation of berries with mixtures of fungal organisms, *B. cinerea* infection was diminished at 27 °C by either *C. acutatum* or *G. uvicola* but only by *C. acutatum* at 20 °C. *G. uvicola* infection was diminished by *C. acutatum* at both temperatures investigated. *B. cinerea* reduced the level of infection of both *C. acutatum* and *G. uvicola* at 20 °C. The findings have implications for seasonal bunch rot management of grapes in relation to predicted changes in global temperature.

K e y w o r d s : *Vitis vinifera*, bunch rot, climate change, berry microflora, grey mould, ripe rot, bitter rot, *Botrytis cinerea, Colletotrichum acutatum, Greeneria uvicola.*

Introduction

Bunch rot of grapes caused by *Botrytis cinerea* (grey mould) is common in viticultural regions that encounter wet conditions close to harvest. In Australia the Hunter Valley is a region that has been historically associated with Botrytis bunch rot (Nair 1985). In recent years disease control measures used to control *B. cinerea*, such as the use of strategically applied fungicides and foliar management for fruit ventilation, have failed in some vineyards. Closer inspection revealed that *B. cinerea* was present at relatively low frequency in bunch rot affected grapes in a number of vineyards in some seasons (Steel and Greer 2008). The predominant organisms associated with bunch rots were *Colletotrichum acutatum* and *Greeneria uvicola*, responsible for ripe rot and bitter rot respectively (Steel *et al*. 2007). These bunch rots are commonly associated with grapes in sub-tropical regions (Yamamoto *et al*. 1999, Longland and Sutton 2008) and negatively impact on grape and wine quality by imparting "off" flavours that are difficult to correct (Meunier and Steel 2009). All *Vitis vinifera* commercial varieties so far examined are susceptible to *C. acutatum* and *G. uvicola* infection although there are some differences in the degree of susceptibility (Longland and Sutton 2008, Shiraishi *et al*. 2007).

An examination of temperature records suggests that seasonally high temperatures might account for the apparent shift in the incidence of all three fungal species responsible for bunch rot infections in the Hunter Valley. This view was supported by our earlier observation that fruit ventilation by foliar management, a well documented method for Botrytis bunch rot control (Gubler *et al.* 1987), appears to favour non-Botrytis bunch rots, particularly in situations where the practice leads to sunburn and an increase in fruit temperatures (Steel and Greer 2008). Grape temperatures of around 20 °C are known to favour infection by *B. cinerea* (Nair and Allen 1993) but less is known of the temperatures that predispose berries to other bunch rot pathogens.

In this study we investigate the impact of berry temperature on the susceptibility of grape berries to mixed infections with *B. cinerea, C. acutatum* and *G. uvicola* using detached fruit.

Material and Methods

I n f e c t i o n s t u d i e s u s i n g d e t a c h e d b e r r i e s : Berries were inoculated with *B. cinerea* (TN 030), *C. acutatum* (DAR 75574) and *G. uvicola* (DAR 77258) isolated from *V. vinifera* ('Cabernet Sauvignon') as previously described (Steel *et al.* 2007). Disease-free 'Cabernet Sauvignon' berries (12.5° Baumé) were collected from the Charles Sturt University vineyard at Wagga Wagga, Australia (35.06° S, 147.36° E). Berries were surface sterilised in sodium hypochlorite (1% v/v) plus Tween 80 (0.05% v/v) for 2 min, then successively rinsed three times with sterile deionised water. Single berries were placed into the wells of microtitre plates plus lids (24 well, flat-bottom, Iwaki Microplates) with 20 mL of water surrounding the wells to create high humidity. Fungal spore suspensions were prepared by dislodging spores from the surface of cultures growing on potato dextrose agar (PDA) into 3 mL of sterile deionised water using a sterile glass rod. Spore concentration was quantified using a haemocytometer and adjusted to 2 x 10^6 spores·mL^{-1} by diluting with sterile deionised water. Berries were inoculated with either single or combined isolates of the three fungi in a 10 µL droplet of the spore suspension (i.e. 10^4 spores) placed near the distal apex of the berry. Control berries were treated with 10 µL of sterile distilled water. Each treatment was

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replicated three times with 24 berries per replicate. Berries were incubated for 5 d at either 20 °C or 27 °C under a 12 h light / 12 h dark photoperiod and examined for fungal infection with a dissecting microscope. The three fungal genera could be differentiated from each other on the berry surface according to spore colour and morphology (Sutton and Gibson 1977, Dyko and Mordue 1979) and identification confirmed by aseptically removing spores or small areas of infected berry skin and sub-culturing onto Dichloran Rose Bengal Chloramphenicol (DRBC) agar with subsequent sub-culture to PDA. Results were expressed as the mean percentage of berries infected and analysed by ANOVA using GenStat (VSN International Ltd, Hemel Hempstead, UK).

Results and Discussion

Temperature significantly influenced the infection of grapes by each fungal species. More berries were infected with *B. cinerea* at 20 °C (92 %) than at 27 °C (65 %) (Figure, a). While *G. uvicola* infected berries at both temperatures, infection was significantly greater at 27 °C (99 % infection) than at 20 °C (57 % infection) (Figure, b). In contrast, *C. acutatum* was equally and highly infective at both temperatures (Figure, c).

Colonisation of grape berries by *B. cinerea* was reduced when co-inoculated with *C. acutatum* at either temperature but was only reduced with *G. uvicola* at 27 °C (Figure, a). Conversely, the percentage of berries infected with *C. acutatum* (Figure, c) and *G. uvicola* (Figure, b) was reduced by co-inoculation with *B. cinerea* at 20 °C but not at 27 °C. *G. uvicola* did not colonise berries at 20 °C when co-inoculated with *B. cinerea* despite its ability to infect berries at this temperature when it was the sole inoculum. Co-inoculation with *C. acutatum* and *G. uvicola* led to a significant reduction ($P < 0.01$) in the percentage of berries infected with *G. uvicola* at both temperatures but had no effect on infection by *C. acutatum*. These observations were confirmed by inoculating berries with all three bunch rot pathogens together. *B. cinerea* was the predominant pathogen at 20 °C, whereas at 27 °C, *C. acutatum* predominated.

B. cinerea is frequently associated with bunch rot of grapes in cool, wet climates and our results support previous findings regarding the optimum temperature of 20.8 °C for berry infection (Nair and Allen 1993). Our observations on mixed berry inoculations at 27 °C and our previous observations on the studies of hyphal growth of *C. acutatum* and *G. uvicola* on PDA at a range of temperatures (Steel *et al*. 2007), support our hypothesis that temperature influences the prevailing mix of bunch rotting pathogens in humid conditions.

C. acutatum and *G. uvicola* were first reported as bunch rot pathogens in Australia only recently (Steel *et al*. 2007). Long term historical records for bunch rot incidence in the Hunter Valley are lacking although earlier grape ripening patterns coupled with a recent increase in the frequency of high temperature seasons have been documented (Hall and Jones 2009). Rainfall events coupled with higher temperatures may explain observed changes in the bunch rot pathogen profile in the Hunter Valley, particularly in situations where rotting of the grape berries is due to a complex of different fungal organisms. Aside from the three bunch rot organisms described in this work, there are a number of other fungi that are largely opportunistic pathogens of

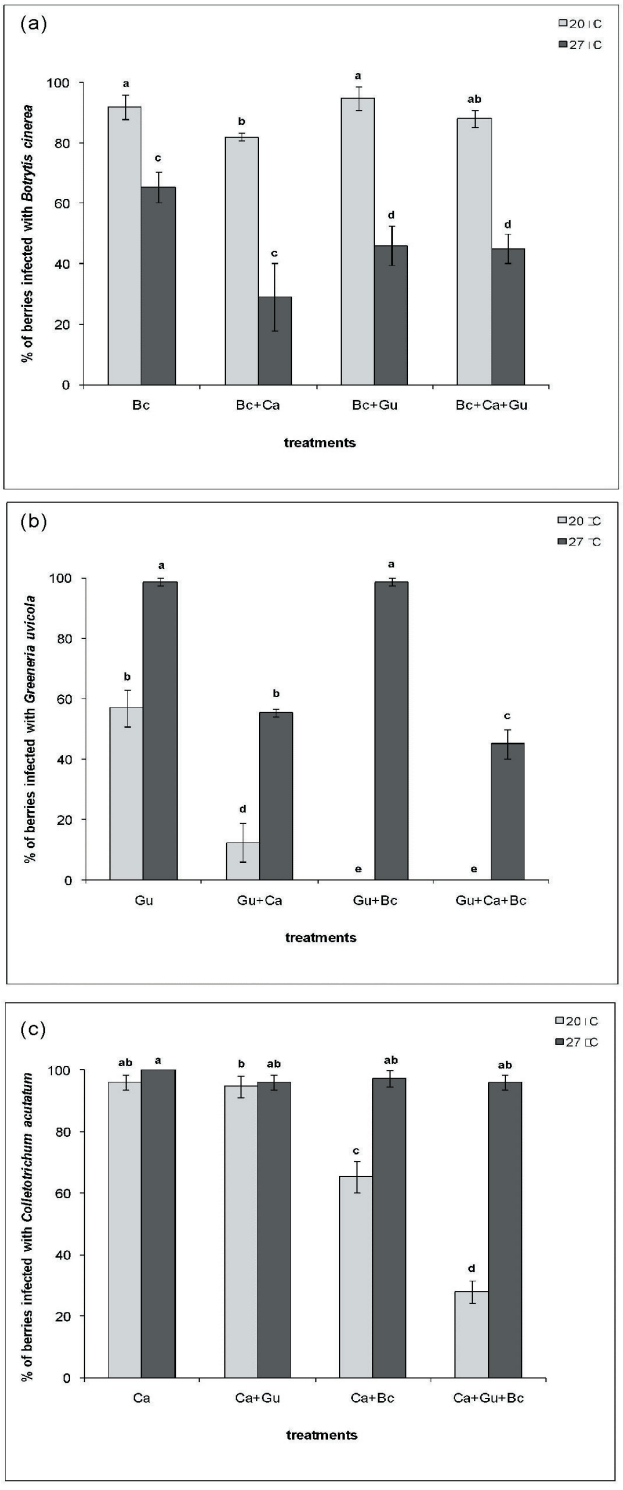

Figure: Effect of co-inoculating *Vitis vinifera* 'Cabernet Sauvignon' berries (12.5° Baumé) on (**a**) *Botrytis cinerea* (**b**) *Greeneria uvicola* and (**c**) *Colletotrichum acutatum* infection. Treatments relate to inoculation with each of the three organisms; Bc = *B. cinerea*; Ca = *C. acutatum*; Gu = *G. uvicola* either singularly or in combination at 20 or 27 °C. Letters indicate significant differences ($P < 0.01$). Bars represent standard errors of the mean.

grape berries (e.g. *Alternaria, Aspergillus, Penicillium*) (Pearson and Goheen 1988) and the interactions between each of these organisms, *B. cinerea* and the environment is unknown.

Our findings on the infection of grape berries by these three major fungal pathogens found in sub-tropical vineyards have broader ramifications for bunch rot management. *B. cinerea* is likely to become less of a problem in warmer, wetter regions, whereas other bunch rotting organisms such as *G. uvicola* and *C. acutatum* may predominate. However, proving this hypothesis conclusively would require extensive monitoring of field sites in conjunction with collection of micro climate data from the fruiting zone over several growing seasons. If predicted increases in temperature occur, adaptations to current control strategies are likely to be required. New strategies in fungicide selection and foliar canopy management will need to be considered, particularly as the well ventilated canopies used for *B. cinerea* control can expose fruit to temperatures favouring both bitter rot and ripe rot infection (Steel and Greer 2008).

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