Effects of solar radiation and relative humidity on germination of Botryosphaeriaceae species conidia

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Abstract Viability and germination of conidia of three Botryosphaeriaceae species, which are important grapevine pathogens, were significantly affected by exposure to different levels of sunlight and relative humidity (RH). After 7 h exposure to non-filtered sunlight (+UV), filtered sunlight (-UV) and shade, germination differed between light levels, being 35, 57 and 81%, respectively, and after 70 h exposure it was 0, 21 and 65%, respectively. Non-germinated conidia were unable to germinate when placed in a moist environment, indicating that they had been killed. High RH favoured germination since 91 and 70%, respectively, germinated after 3 h in 100 and 97% RH. However, in 93% RH, only 44% germinated by 24 h and in 84% RH no conidia germinated. The inhibition of germination was temporary since replacement in a moist environment caused conidia to germinate. Light and RH effects were similar for the three species.

Keywords *Neofusicoccum luteum*, *Neofusicoccum australe*, *Diplodia mutila*, solar radiation, humidity, conidia.

INTRODUCTION

Botryosphaeriaceae species are important fungal pathogens of woody plants worldwide (Slippers & Wingfield 2007; Lazzizzera et al. 2008), but the effects of environmental conditions on their disease cycles are not well understood. In New Zealand, the conidia of *Neofusicoccum* and *Diplodia* species, which have been found throughout the year in rain water runoff collected on grapevines (Amponsah et al. 2009), are able to infect freshly pruned grapevine shoots or canes leading to dieback (Amponsah et al. 2008). The ability of these conidia to survive in the environment is an important factor in determining their infection success, especially if repeated rain events cause further dispersal after their initial release from pycnidia. However, there

has been little reported research on the survival of Botryosphaeriaceae species conidia exposed to solar radiation or dry conditions.

The purpose of this study was to determine the effects of (i) different levels and periods of solar radiation on survival of conidia, as measured by their ability to germinate, and (ii) different relative humidities on conidial germination.

MATERIALS AND METHODS

Effect of solar radiation and temperature on conidium viability

The conidial tendrils of *N. luteum*, *N. australe* and *D. mutila* that oozed from inoculated, green grapevine shoots (Amponsah et al. 2008) were each removed with the tip of a sterilised scalpel,

mixed into a 20 µl drop of sterile water on a glass slide, spread to cover about a 1 cm² area and air-dried immediately within a laminar flow cabinet. The air-dried conidium-laden glass slides were laid into square Petri dishes (100 mm²) lined with five layers of moist facial tissue and supported by plastic drinking straws.

The conidia were then exposed to three levels of solar radiation by covering the Petri dishes with different materials, supported on wooden blocks to create a 50 mm high ventilation gap. The radiation levels were: (1) filtered sunlight (–UV) with 1.5 mm thick Makrolon® UV Polycarbonate that allows penetration of only visible white light, (2) non-filtered sunlight (+UV) with a 1.5 mm Acrylic sheet, A-cast™, which allows penetration of both UV and visible white light, and (3) shade with 1.5 mm thick brown cardboard. The temperature within each type of Petri dish was recorded every 30 min with TinyTag Talk 2 temperature loggers (Gemini Data Logger UK Ltd). There were three slides per species for each level of sunlight exposure. The light exposure times were achieved over continuous periods of 1 to 5 days and repeated twice on separate days during 10 to 28 February 2010 when daylight hours for the period lasted 13.5–14.5 h each day (from sunrise to sunset). For every solar radiation treatment of each species, an extra slide of conidia was made and immediately observed (within 30 min of harvest) under a light microscope for presence of germination. They were then incubated under 100% RH at 25°C and in continuous darkness for 12–24 h and observed again for viability. These samples served as non exposed (pretest) controls for comparison of percent germination with treatments.

Effect of relative humidity (RH) on conidial germination

To determine the effects of different relative humidities (RH) on conidial germination, saturated salts were prepared and poured into square Petri dishes that had been lined with five layers of facial tissue paper to soak up the saturated salt solution and to prevent spillage. Potassium chloride (KCl), potassium nitrate (KNO₃), potassium sulphate (KSO₄) and water

were used to achieve an approximated RH of 84, 93, 97 and 100% respectively, at 25°C (Greenspan 1977). The lids were replaced and sealed with Parafilm™. The containers were left to equilibrate for 1 h at 25°C, after which the conidium-laden slides of *N. luteum*, *N. australe* and *D. mutila*, prepared as described before, were each put into the equilibrated Petri dishes and incubated at 25°C for 3, 6, 24 or 48 h. The three replicates per treatment were set out in a completely randomised design.

Assessment of conidium viability and statistical analysis

At the end of every incubation period, the conidium-laden slides from both experiments were observed under the compound microscope at × 40 magnification. Between 150 and 200 conidia were examined over the slide centre to allow counting of germinated and non-germinated conidia. A conidium was considered germinated when its germ tube was at least half the conidium length. On slides with zero germination the conidia were suspended in a drop of sterile water and this was spread onto PDA plates for incubation in the dark at 25°C for 48 h, followed by examination under a stereo microscope at × 20 magnification to confirm that the conidia had been killed, rather than germination being temporarily inhibited by the light exposure or RH. The data of percent germination, with species and treatments as separate factors, were analysed separately for each experiment by ANOVA using GenStat version 11. Treatment means were compared by Fisher's protected LSD tests at $P \leq 0.05$.

RESULTS

Effect of solar radiation and temperature on conidia viability

The repeated experiments did not differ ($P > 0.05$) in overall effects and the effects of solar radiation exposure on percent germination from the two repeated trials did not differ ($P > 0.05$) between the species, *N. australe*, *N. luteum* and *D. mutila*, so these data were also combined for the second analysis. Germination of the conidia was

significantly affected by light levels ($P < 0.001$; $LSD = 1.94$), with shade, filtered solar radiation (-UV) and non-filtered solar radiation (+UV) having germination of 75.2, 28.1% and 12.2% respectively, compared to the levels in the preset controls, which were 96.3%. Increasing exposure times caused decreasing germination ($P < 0.001$; $LSD = 2.75$), with an overall mean germination of 58.0% after 7 h exposure being significantly higher than that of 28.6% recorded after 70 h exposure (Figure 1a). There was a highly significant ($P < 0.001$; $LSD = 4.75$) interaction between the exposure times and the radiation levels. The range of temperatures recorded under the three different radiation levels were 18–26°C for shade, 28–33°C for filtered solar radiation (-UV) and 21–28°C for non-filtered solar radiation (+UV). The highest temperature recorded under the radiation treatments was 41.3°C, although under shade it reached 35°C for 30 min and 39°C for 1 h, respectively, during the 7 and 14 h exposure times. Germination of conidia observed for 7–14 h exposure under shade did not differ significantly from the 28 h exposure. Non-germinated conidia from the slides with zero germination (+UV) failed to germinate after incubating on PDA for 3 days at 25°C in continuous darkness.

Effect of relative humidity (RH) on conidial germination

The effects of RH on percent germination did not differ ($P > 0.05$) between *N. australe*,

N. luteum and *D. mutila* and so these data were combined in the second analysis. The effects of the different RH levels and times on percent conidial germination were highly significant ($P < 0.001$; $LSD = 0.96$) (Figure 2), as were the effects of exposure times ($P < 0.001$; $LSD = 1.10$) and the interaction between the RH level and times ($P < 0.001$; $LSD = 1.91$). The RH of 100% resulted in the quickest and highest conidial germination of 91.8% within 3 h, whereas in 97% RH it took 6 h for germination to reach 67.2%, which was close to the maximum of 69.9% after 48 h. In 93% RH no conidia had germinated after 3 and 6 h but germination reached 43.9% after 24 h, and 48.9% after 48 h (Figure 2). Conidia placed in 100 and 97% RH formed mycelial networks by 6 h, but in 93% RH, the germinating conidia had not developed mycelium networks, even by 48 h.

DISCUSSION

The exposure of Botryosphaeriaceae conidia to light reduced their viability in relation to the level of light and exposure time. Non-filtered solar radiation (+UV) had the greatest effect compared with filtered solar radiation (-UV) and shade. The detrimental effect of UV was further shown by 0% germination after 56 h exposure with +UV compared to the 69% germination after the same time in the shade. Since the non-germinated conidia were still unable to germinate after plating onto PDA, this confirmed that they

Figure 1 (a) Mean percent germination of Botryosphaeriaceae species conidia given different exposure times and levels of solar radiation (shade, filtered sunlight (-UV) or non-filtered sunlight (+UV)) and (b) hours at which conidia were exposed to temperatures greater than 40°C under the three levels of solar radiation. The lines were log transformed to provide the best fit.

Figure 2 Mean percent germination of Botryosphaeriaceae species conidia during exposure to four levels of relative humidity (RH; 84, 93, 97 or 100%) at 25°C for 3, 6, 24 or 48 h. The lines were log transformed to provide the best fit.

had been killed by the light exposure. The greater sunlight levels were also associated with longer periods of temperatures above 41°C (Figure 1b), which may have reduced conidium viability, although the data seem to indicate that the effect was unlikely to be due to temperature alone. Further studies should use an experimental set-up that provides greater ventilation in an attempt to eliminate this potential effect. The damaging effects of UV light found in this study were also reported by Arabi et al. (2009) who observed that when conidia of *Pyrenophora graminea* were exposed to sunlight, decreased germinability and pathogenicity resulted. Similar observations were made on the lethal effects of UV in sunlight on germination of *Puccinia striiformis* uredospores (Maddison & Manners 1972), and conidia of *Alternaria solani* (Rotem et al. 1985), *Venturia inaequalis* (Aylor & Sanogo 1997), *Peronospora tabaci*, *Uromyces phaseoli* and *Mycosphaerella fijiensis* (Parnell et al. 1998).

The relatively high germinability of conidia kept in the shade treatment in this study was also observed by Maddison & Manners (1972). Their investigation, conducted in England on relatively clear days in midsummer, found that germination of *P. graminis* uredospores was reduced to 10% by 20 h exposure to sunlight, whilst 6–10 h caused a similar reduction in germination of *P. striiformis* uredospores. However, in the

present study germination of all three species of the Botryosphaeriaceae was not significantly different at the light levels tested.

The ability of conidia to germinate was also dependent on the supply of moisture through RH, since it was optimum at 100% RH, much reduced at 93% RH, with no germination at 84% RH even after 72 h at the optimum growth temperature of 25°C. This finding was in agreement with Arauz & Sutton (1989) who also observed that maximum germination of *D. seriata* conidia occurred in free water and declined as RH was reduced from 100 to 92%, with no germination at 88.5% RH. The conidia of another woody trunk pathogen, *Potentialmyces pyri*, were reported to have even more stringent requirements since no germination occurred at 95% RH, but 99% germinated at 98% RH (Liu & Xiao 2005).

The RH levels also affected the extent of mycelial growth after germination. At 93% RH the conidia that germinated after 24 h had not developed a mycelial network after another 24 h, indicating that more moisture was necessary for mycelium growth and development. Arauz & Sutton (1989) reported that conidia of *D. seriata* were very sensitive to drying and in no instance did the rewetted germ tubes resume growth following dryness periods of any duration – unfortunately they did not specify the minimum dryness period.

These studies indicated that disease cycles of Botryosphaeriaceae species involving conidia are most effective in rainy weather since they require moisture for germination. The conidia are released during high RH, but if they are not supplied with continued high RH or free water they will not germinate. However, they will not even survive if exposed to UV light for 3 days or longer. Conidia released in early summer mornings may be exposed to sunlight for long periods, whilst those released during rainy, cloudy conditions can germinate quite quickly. A previous conidial trapping experiment (Amponsah et al. 2009) showed that conidia of Botryosphaeriaceae species were abundant in rain-splash during winter pruning of grapevines when the free moisture allows germination and many wounds are available for infection. However, the overall effects of the resulting cane dieback on vine death have not been reported. Further studies should be done to investigate the effects of the cooler winter temperatures on these processes. During summer, some parts of the grapevine canopy may provide sufficient shade and high RH to ensure rapid germination of conidia that can infect through the wounds created by summer trimming, which is usually conducted several times during the season.

In conclusion, this study has clearly shown that RH and UV light play a major role on the Botryosphaeriaceae species conidium viability. While low RH alone caused delays in conidium germination, the effect of UV light, possibly in combination with the associated high temperatures, killed the conidia outright. Botryosphaeriaceae species are therefore more likely to infect grapevine wounds in rainy, cloudy weather than dry, sunny weather.

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