Ethylene production by *Eucalyptus* clones in response to infection by hypovirulent and virulent isolates of *Cryphonectria cubensis*

Ethylene production was measured in the bark of two Eucalyptus *clones that were artificially inoculated with virulent and hypovirulent isolates of* Cryphonectria cubensis. *Trees inoculated with the hypovirulent isolate produced extremely low amounts of ethylene. No significant differences were found in ethylene production by a susceptible clone (ZG 14) and a disease-tolerant clone (TAG 5) inoculated with a hypovirulent isolate. However, clone ZG 14 produced significantly more ethylene than TAG 5 when inoculated with the virulent isolate of* C. cubensis. *This supports the view that trees more susceptible to fungal attack tend to produce greater amounts of ethylene than disease-tolerant clones. The amount of ethylene produced in response to* C. cubensis *infection may thus be a convenient and rapid means of distinguishing a susceptible from a resistant response. If heritable, the ability to produce small quantities of ethylene in response to infection instead of large quantities as in susceptible clones, may be a valuable factor in breeding for resistance to this pathogen.*

Canker and decay diseases are considered to have a negative impact on forest management and timber production throughout the world.[1,2] *Cryphonectria cubensis* (Bruner) Hodges is an important stem canker pathogen of *Eucalyptus* species.[3–6] The recent discovery of the pathogen in South Africa[6] has prompted concern regarding the effect that the disease could have on *Eucalyptus* plantations. This is especially because the forestry industry in South Africa depends largely on extensive plantations of *Eucalyptus* clones. Some of these clones are known to be highly susceptible to *C. cubensis* infection.[7]

Currently, the only means of controlling *Cryphonectria* canker in South Africa is through the planting of disease-tolerant clones. Trials to select clones on a wide variety of field sites are slow and cannot provide data fast enough to advise growers on appropriate clones for deployment. Screening using artificial inoculations in plantations is used, but this is time consuming and labour intensive. The South African forestry industry, therefore, requires a rapid yet reliable screening method, that reflects susceptibility and disease tolerance. One approach could be through the application of the findings of Shain and Wheeler[8] that oats resistant to victoria blight produced much less ethylene than susceptible oats when exposed to the toxin, victorin. The speed and ease with which ethylene can be measured could make it a useful measure of plant damage, indicating resistance or susceptibility to infection.[9]

Ethylene production is associated with stress and is involved in modulating a broad spectrum of physiological processes such as pathogenesis, senescence, flowering, fruit ripening, and the morphogenic response in seedlings called the 'triple response'.[10–16] Ethylene biosynthesis increases rapidly during plant–pathogen interaction or application of chemical elicitors.[17,18] The objective of this study was to gain information on ethylene production by susceptible and resistant *Eucalyptus* clones infected with a virulent isolate of *C. cubensis*, with a view to using this as an indication of susceptibility or resistance. Ethylene production by trees inoculated with a hypovirulent isolate of *C. cubensis*, infected with dsRNA, was also measured in order to determine whether or not ethylene was induced in the host.[19]

Materials and methods

Inoculations. Two *Eucalyptus* clones, known to be either moderately tolerant (TAG 5) or susceptible (ZG 14) to *C. cubensis* infection (Wingfield, unpub. data) were grown under controlled glasshouse conditions at 25°C. Twenty, two-year-old trees of each clone were inoculated with a hypovirulent (BSN 27.2) and 20 trees were treated with a virulent isolate (BSN 85.4) of *C. cubensis*. The hypovirulent isolate was previously shown to be infected with dsRNA and to display reduced virulence.[19] Twenty trees of each clone were also used as controls. Inoculum of *C. cubensis* was prepared by culturing the fungus on Potato Dextrose Agar (PDA) (20 g potato dextrose, Biolab; 20 g agar, Biolab; 1 litre distilled water) in Petri dishes, for seven days. All plants were wounded by removing a bark disc from the stems, with a 5-mm cork borer. A 5-mm disc from a 7-day-old PDA culture of *C. cubensis* was placed in each wound. A sterile PDA disk was used for the control treatments. All wounds were covered with Parafilm to reduce contamination and prevent the inoculum from drying out. After six weeks, the Parafilm was removed and isolations were made from discoloured cambium tissue, to verify the presence of *C. cubensis*.

Ethylene measurement. Bark plugs were removed, 2 cm above the canker lesion, using a 4-mm-diameter cork borer after six weeks. The plugs consisted of living bark tissue and thus contained both functioning and non-functioning secondary phloem, cortex and periderm. Dead outer bark (rhytidome) and xylem, if present, were removed from plugs before incubation. Each plug was immediately sealed in a 6-ml Vacutainer tube and the tubes cooled on ice until all plugs had been collected. Sealed Vacutainer tubes were then incubated in the dark for 20 h at 25°C. Ethylene production was measured by withdrawing 1 ml of gas and injecting it into a gas chromatograph equipped with a column of activated alumina and a flame ionisation detector. The oven temperature was 55°C. Ethylene production was expressed as nl h^{-1} g^{-1} dry weight of plug. All ethylene measurements were repeated three times and the means are presented in Table 1.

Statistical analysis. The amounts of ethylene produced in samples were statistically analysed for variances and differences among isolates and hosts. Means were tested for significance according to Tukey's procedure for comparison of means.[20]

Table 1. Rates of ethylene production by two *Eucalyptus* clones inoculated with a hypovirulent and a virulent isolate of *Cryphonectria cubensis*.

	Lesion length (mm)		Ethylene production[2]	
Isolate[1]	ZG 14	TAG 5	ZG 14	TAG 5
BSN 27.2 (H)	0.4[a3]	0.2[a]	3.6[a]	1.4[a]
BSN 84.5 (V)	138.0[c]	30.0[b]	46.8[c]	17.1[b]
Control	0	0	0[a]	0[a]

[1]BSN 27.2 (H) represents a hypovirulent isolate of *C. cubensis*. This isolate contains dsRNA and possesses a morphology typical of hypovirulent isolates. BSN 84.5 (V) represents a virulent isolate of *C. cubensis*, and is devoid of dsRNA and possesses a normal morphology.

[2]Amount of ethylene (C_2H_4) produced per g dry bark plug (nl h^{-1} g^{-1}) after a 20-h incubation period at 25°C in the dark.

[3]Each value represents the average for 20 trees. Values in each column followed by different letters differ significantly at $P = 0.01$ according to Tukey's procedure for comparison of means. Clone ZG 14 is known to be susceptible and clone TAG 5 moderately tolerant to *C. cubensis* infection.

Results

No lesions were produced on trees inoculated with sterile PDA, and the wounds became covered by callus tissue (Table 1). Samples from these trees showed no stimulation of ethylene production six weeks after inoculation. This was true for both the susceptible (ZG 14) and the moderately tolerant (TAG 5) clones (Table 1).

Ethylene production was significantly ($P = 0.01$) greater when clones were inoculated with a virulent (BSN 85.4) isolate of *C. cubensis*, compared to production of the gas after both the hypovirulent (BSN 27.2) and control inoculations. This was also true for both disease-tolerant (TAG 5) and disease-susceptible (ZG 14) clones (Table 1).

Inoculation of clones ZG 14 and TAG 5 with the hypovirulent isolate (BSN 27.2) showed that initial lesion expansion ceased after six weeks (Table 1). There was greater lesion development in ZG 14 than in TAG 5 (Table 1). There were no statistically significant ($P = 0.01$) differences in ethylene production between these clones and the controls (Table 1). Trees of clone ZG 14 produced extremely small quantities of ethylene compared to clone TAG 5, for which almost no measurable ethylene was detected.

Discussion

Results of this study have shown that a *Eucalyptus* clone (ZG 14), known to be highly susceptible to *C. cubensis* infection, and on which much larger cankers developed, produced significantly more ethylene than the disease-tolerant clone (TAG 5), when inoculated with the pathogen. Ethylene production in the bark of clones ZG 14 and TAG 5 was stimulated by *C. cubensis* when the cankers were expanding. These results are consistent with reports that the degree of ethylene production can reflect the extent of damage inflicted on the host by the pathogen.[9,21]

The relatively small amounts of ethylene produced in this study could be due to the repression of ethylene 2-cm away from the canker lesion. Experiments done by Hebard and Shain[9] showed that the stimulation of ethylene production was repressed near virulent chestnut blight cankers and up to 2 cm away from the cankers. This could reflect the impending death of host cells in that region, which occurs at least 350 µm in front of the advancing mycelial growth.[9] This situation is explained by the fact that ethylene synthesis is an active process requiring the metabolism of live cells.[9]

Clone ZG 14, which is highly susceptible to *Cryphonectria* canker, produced significantly greater amounts of ethylene in response to attack than the moderately resistant clone TAG 5. This is in agreement with previous reports suggesting a positive relationship between ethylene production and disease susceptibility.[8,21] A negative relationship has, however, also been reported by some authors,[11,14,22] who have shown that increased resistance can be reflected by enhanced ethylene production. This is in contrast to results of this study.

Our study indicates that there is a correlation between ethylene production after infection with *C. cubensis* and relative susceptibility or resistance of *Eucalyptus* clones to infection by the pathogen. It must also be noted that no ethylene was produced in clones ZG 14 and TAG 5 inoculated with the hypovirulent isolate. This is consistent with the knowledge that dsRNA has reduced the virulence of this isolate.

Although the work presented here is of a preliminary nature, there is sufficient evidence to suggest that ethylene production might provide a valuable tool in screening *Eucalyptus* clones for susceptibility to *Cryphonectria* canker. Additional experiments, including those associated with more clones and those inoculated on established trees in plantations, should follow.

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