Kino vein formation in *Eucalyptus globulus* and *E. nitens*

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Summary

Kino veins or pockets are characteristic defects of some *Eucalyptus* species and have long been recognised as sources of degrade in eucalypt timber. In order to investigate the causes of kino vein formation, the short-term responses of *Eucalyptus globulus* and *E. nitens* to mechanical, chemical (2-chloroethyl-phosphonic acid) and biological wounding treatments were examined. With the exception of the chemical treatment, the various wounding treatments did not consistently induce kino vein formation in either species. Instead, the new wound-associated wood and bark formed after wounding was characterised by the presence of dark extractives for both species. Although the dark extractives appeared similar to kino, microscopic examination showed they were formed in the less organised wound tissue and not in specialised kino veins. We suggest that the induction of tree exudates is part of a non-specific wound response and not necessarily a direct host response to invasion by microbial pathogens and insects.

*Keywords*: kinos; wood anatomy; injuries; bruising; ethylene; *Eucalyptus globulus*; *Eucalyptus nitens*

Introduction

The Tasmanian forest industry is increasingly applying intensive management practices to produce logs suitable for sawing from plantation eucalypts. Such practices include green pruning necessary for producing knot-free wood as well as thinning to encourage rapid diameter growth in retained trees (Dudzinski et al. 1992; Gerrand et al. 1997). However, one of the potential consequences of thinning and pruning operations is wounding to the retained trees, resulting in defects such as kino vein formation. Concern has been raised about the potential problem of sawlog downgrade due to a high incidence of kino veins (Gerrand et al. 1997; Somervile and Davies-Colley 1998; Vasiliauskas 2001).

Kino veins or pockets are commercially regarded as characteristic defects of some *Eucalyptus* spp. (Nelson and Hillis 1978; Bamber 1985). Kino veins, more commonly known as 'gum veins', can be the most severe forms of defect in the wood of some eucalypt species (Tippett 1986). Somervile and Davies-Colley (1998) found that kino pockets had the potential to seriously reduce recovery of veneer-grade product in a stand of *E. regnans*. Susceptibility to kino vein formation varies with species and environmental conditions (Doran 1975; Nelson and Hillis 1978; Tippett 1986). For example, trees growing on dry sites have apparently been observed to be severely affected by kino formation (Day 1959). The exact cause of the formation of kino veins is still not clearly understood, but it has been generally associated with stresses that apparently damage but do not kill the vascular cambium. The stresses include branch shedding, damage by biological agents (e.g. insects and fungi), physiological stress, fire and mechanical damage (Jacobs 1986). Most authors agree that kino veins generally play a role in wound defence as a tree resistance mechanism, acting as a 'barrier zone' (Wilkes 1986; Hillis 1987). Barrier zones have been defined as specialised tissue produced by the cambium in a non-specific response to infection as well as to mechanical wounding, and they serve to compartmentalise necrotic sapwood from living cambium (Tippett and Shigo 1981).

The present study investigated the causes of kino vein formation in eucalypt tree defence by comparing the responses of two commercially important *Eucalyptus* species (*E. globulus* and *E. nitens*) to various mechanical, chemical and biological wounding treatments. Both *E. globulus* and *E. nitens* are widely planted in Tasmania for pulp and sawlog purposes (Beadle and Mohammed 1999). Previous surveys of *E. nitens* sawlogs suggest that this species does not readily form kino veins (Nicholls and Pederick 1979; Yang and Waugh 1996a; Eyles and Mohammed 2002) while a small amount of kino has been observed in sawn timber of *E. globulus* (Yang and Waugh 1996b).

Materials and methods

Experimental site and wounding treatments

Studies were conducted at two experimental plantation sites growing both *E. globulus* and *E. nitens*. The first site was located at Esperance (about 2 km south of Dover, Tasmania — latitude 43°15'S, longitude 146°50'E). The altitude is 240 m a.s.l., and mean annual rainfall is 869 mm. The second site was at Lewisham (about 50 km east of Hobart, Tasmania — latitude 42°49'S, longitude 147°37'E). The altitude is 20 m a.s.l., and mean annual rainfall is 512 mm. Details of site establishment and tending for the Esperance and Lewisham sites are provided by Beadle et al. (1996) and White et al. (1998), respectively. At the Lewisham site, an additional study was carried out to explore the effect of drought stress on tree wound responses. The site had been divided into six blocks...
(replicates), three designated as ‘irrigated’ and three designated as ‘rain-fed’. The experimental design and irrigation treatment have been outlined elsewhere (Honeysett et al. 1996; Worledge et al. 1998). Briefly, the irrigated blocks received 1300–1500 mm y⁻¹ while the rain-fed blocks received 900–1100 mm y⁻¹ including supplementary irrigation. Trees at Esperance were 16 y of age with an average stand diameter of 24 cm and 26 cm for *E. nitens* and *E. globulus*, respectively. The trees at Lewisham were 9 y of age. The average stand diameters of the irrigated trees were 19 cm and 19.5 cm for *E. nitens* and *E. globulus*, respectively. The average stand diameters of the rain-fed trees were 16 cm and 16.3 cm for *E. nitens* and *E. globulus*, respectively.

In late summer of 2000, twelve trees (six *E. nitens* and six *E. globulus* at Esperance and ten trees each of *E. nitens* and *E. globulus* (five from the irrigated block and five from the rainfed blocks at Lewisham) were randomly selected for wounding treatments. The circumference of each tree was divided into four equal quadrants at about 1.3 m above ground-level. Four treatments were randomly assigned to the quadrants, one per quadrant as follows:

1. the impact force of a 3 kg steel rod (5.7 cm diameter × 15 cm long) attached to a rope 1 m long swinging through an arc of 90° (Fig. 1). This stimulated the ‘bruising’ type wound commonly inflicted on retained trees during thinning operations.

2. an injection of 0.5 mL of 0.2% CEPA aqueous solution (2-choroethyl-phosphonic acid, SIGMA) into a small wound (1 cm²) created by a chisel. CEPA releases ethylene under physiological conditions. The application of exogenous ethylene to induce production of tree secretions has been well documented in many tree species including *Eucalyptus* (Dowden and Foster 1973; Nelson and Hillis 1978; Hillis 1987). Anatomical and histochemical responses to CEPA treatment were recently reported for juvenile *E. globulus* and *E. nitens* (Eyles and Mohammed 2002).

3. a hole 10 mm in diameter drilled 3 cm into the outer sapwood to simulate the stem wounds commonly associated with stem-boring insects. A second hole with identical dimensions to the first was drilled 30 cm above the first hole and inoculated with a decay fungus previously isolated from a white-rot decay column in *E. nitens*. Inoculation involved inserting rectangular pieces, 2 cm × 1 cm, of a 2-week-old culture growing on malt extract agar into the drill hole and sealing it with a layer of lanolin wax. The decay fungus used for the inoculation is an unidentified basidiomycete known as ‘Isolate D’. The fungus is described in Barry (2001).

4. a block of dry ice (approx. 7 cm³) pressed firmly onto the bark for 5 min. This treatment attempted to damage the vascular cambium without physically disrupting the outer bark so as to minimise the exposure of injured tissue to the external environment (Robinson 1997).

About three months after treatment, three trees each of *E. nitens* and *E. globulus* at each of the Esperance and Lewisham sites were harvested. Billets ~50 cm in length that included the wounding treatments were taken to the laboratory. Each wounding treatment was excised from the billet by cutting longitudinally through the centre of the wounds using a bandsaw. The production of dark extractives was consistently observed as part of the wound response, to varying degrees, amongst all treatments. Therefore, responses were classified according to whether dark extractives (defined as non-structural components of wood and bark) were produced within specialised secretory cells, that is kino veins, or within new wound-associated wood formed directly adjacent to the wounding site (Figs 3 and 4). For clarity, we use the term ‘kino’ to refer to the exudate produced in kino veins and we refer to other exudates, not formed in kino veins, as ‘dark extractives’. In cases where kino veins had been formed, the length and width of the kino veins were recorded.

Wood samples of each wound response (a cube with edge of about 15 mm) were fixed in FAA (formaldehyde:acetic acid:70% ethanol, 5:5:90 v/v/v) for a minimum of 24 h at 4°C, dehydrated with an ethanol series and infiltrated in a LR White acrylic resin (ProSciTech, Brisbane) series. Samples were then polymerised in fresh 100% LR White resin for 8–10 h at 60°C (Eyles and Mohammed 2002). Transverse and longitudinal sections, 10 mm thick, were cut with a sledge microtome, dried onto glass slides, stained with a 1% aqueous solution of toluidine blue and permanently mounted in Cytoseal (ProSciTech, Brisbane). Tissue structure and cell changes were noted on a Zeiss Axioskop photomicroscope.

This procedure was repeated with the remaining trees, about 13 mo and 17 mo after setting up the experiments at Esperance and Lewisham, respectively.
Figures 2 and 3. Typical responses of various wound treatments (all are sections with a radial longitudinal orientation)

Figure 2a. Production of kino veins (arrow) in the xylem, 3 months after CEPA injection applied via chisel wound (*) in *E. globulus*.

Figure 2b. Microtome section of kino veins (KV) at a higher magnification showing the regular anatomical arrangement of a typical kino vein.

Figure 3a. Production of a continuous layer of dark extractives (arrow) in the bark of *E. nitens* 3 months after treatment with dry ice.

Figure 3b. Microtome section of dark extractives observed in Figure 3a at a higher magnification, showing continuous wound periderm (dashed line) and disorganised formation of traumatised parenchyma (*) at the interface between healthy and necrotic tissue.

Bar (2a and 3a) is about 1.5–2 cm. Bar (2b and 3b) is 500 µm. HT = healthy tissue, NT = necrotic tissue, PB = parenchyma bridge, P = phloem, VC = vascular cambium, WP = wound periderm, X = xylem.

The kino veins were 15–177 cm in length and 4–7 cm in width. Microscopic examinations showed that the general structure of kino veins produced in *E. globulus* was similar to that described for other eucalypt species including *E. obliqua* (Skene 1965), *E. radiata* (Dowden and Foster 1973), and *E. wandoo* and *E. marginata* (Tippett 1986). Typical kino veins consisted of a tangential series of radial and transverse parenchyma bridges that linked cavities filled with kino (Fig. 2b).

Data analysis

Since this qualitative study aimed to investigate the type of wounding treatments that might induce kino vein formation, the results were interpreted simply as positive or negative. Therefore, the formation of kino veins, regardless of size, was regarded as a positive response to the wounding treatment. Only data from the CEPA treatment was considered for statistical analysis, because kino veins were generally formed only in response to this chemical treatment. A χ² contingency analysis was used to test whether kino vein incidence was species-dependent. Due to the small number of replicates, the data obtained from trees harvested at 3 mo and those at 17 mo at both Esperance and Lewisham (including trees from the irrigated treatments only) were pooled.

Results and discussion

Response to CEPA treatment

The incidence of kino veins in response to CEPA treatment was significantly higher for *E. globulus* than for *E. nitens* (*P* < 0.001). Kino veins were induced in the xylem of all 11 *E. globulus* treated with CEPA at both Lewisham and Esperance (Fig. 2a and Table 1).
Nelson and Hillis (1978), studying the involvement of ethylene in kino vein formation in *E. regnans*, found the area of kino veins produced was a positive logarithmic function of the CEPA concentration. They recommended that 1 mL of 0.1% ethrel (a commercial preparation of CEPA) solution would be adequate for studies in the production of kino veins. It could be argued that application of higher concentrations than those used in the present study (0.5 mL of 0.2% CEPA) might have led to a higher incidence of kino veins, particularly in *E. nitens*. However, in another study, injection of even 0.3 mL of 1% CEPA solution failed to induce kino vein formation in juvenile *E. nitens* trees (Eyles and Mohammed 2002).

As mentioned earlier, in addition to observed genetic differences in kino vein occurrence between species, environmental factors may also contribute to the incidence of kino vein formation (Hillis 1987). Dry sites have been observed to be severely affected by kino vein formation, and Day (1959) suggested that water deficiency during periods of active growth might explain this observation. The effect of water stress on the incidence of kino vein formation has not been studied. In this study, kino veins were not induced in the xylem of any of the five *E. nitens* trees grown under simulated drought conditions. More significantly, kino veins were induced in only three out of the five *E. globulus* trees. While it is difficult to draw any firm conclusions from these

**Figures 4–7. Typical responses to different wound treatments**

**Figure 4.** (Section with radial longitudinal orientation). Formation of wound wood and dark extractives (arrow) 17 months after drill wounding (*), with decay restricted to the sapwood present at time of wounding.

**Figures 5 and 6.** (Tangential view). The area of impact (dotted oval, Fig. 5) created by the bruising treatment resulted in the splitting of the bark (arrow) in *E. nitens*. Removal of the bark (Fig. 6) revealed the discolouration of underlying xylem.

**Figure 7.** (Section with radial longitudinal orientation). Kino veins (arrow) induced by pruning wound in which the branch stub (*) was pushed out by subsequent growth in *E. globulus*. There is no sign of decay.

All bars are about 1.5–2 cm. P = phloem, VC = vascular cambium, X = xylem.
results, they appear contrary to Day’s explanation, especially considering that water deficits inhibit division of fusiform initials and xylem and phloem mother cells (Kozlowski and Pallardy 1997). The relationship between water stress and kino vein formation warrants further investigations, as future plantations may have to be established on water-limited sites.

**Response to dry ice treatment**

In a few trees, the dry ice treatment killed the underlying vascular cambium, but in the majority of trees damage by the dry ice treatment was restricted to the phloem. The severity of damage did not appear to be directly related to bark thickness. Instead, differences in surface area contact and pressure applied in the dry ice treatments might explain the varying severity of damage — even though every effort was made to ensure even contact between dry ice and bark. The responses were generally characterised by the production of callus tissue with the deposition of a layer of dark extractives at the interface of healthy bark and bark that had been killed by the dry ice application. The amount of deposition was variable, appearing either as a continuous layer of variable width (Fig. 3a) or as irregular deposits along the necrotic margin. Microscopic examinations showed that the lesion margin was composed of a completely differentiated wound periderm comprising multiple layers of thick-walled phellem in addition to traumatised parenchyma cells often filled with polyphenols, which comprising multiple layers of thick-walled phellem in addition to was composed of a completely differentiated wound periderm comprising multiple layers of thick-walled phellem in addition to traumatised parenchyma cells often filled with polyphenols, which had been derived from the de-differentiation of pre-existing phloem parenchyma cells (Fig. 3b). Both *E. globulus* and *E. nitens* trees responded in this manner. Some of the *E. nitens* trees at Lewisham, however, produced different coloured extractives in the xylem and phloem. Instead of the usual deposition of dark extractives, some *E. nitens* replicates produced a distinctly pink band of extractives located in the ray and axial parenchyma.

**Response to drill wounding with or without fungi inoculation**

Three months after treatment, both species had responded to drill wounding, with or without fungal inoculation, by producing callus tissue along the margins of healthy sapwood. The exposed callus was usually covered with a thin film of dark extractives. At Lewisham, the drill holes of irrigated trees were fully closed over by callus, unlike those trees growing under rainfed conditions. The slower rates of wound closure in trees subjected to drought could increase the time during which trees are exposed to microbial infection. Previous investigations of seasonal responses of *E. maculata* after artificial wounding found evidence that the rate of wound closure, whether by production of new wood or deposition of exudates, could be important in restricting the colonisation of wounds by decay fungi (Mireku and Wilkes 1989). Furthermore, a clonal trial selecting for tolerance of *E. grandis* to *Cryphonectria* canker found that clones with the fastest rate of wound closure were less susceptible to infection (Van Zyl and Wingfield 1999).

Seventeen months after injury, all drill hole wounds (even those trees exposed to drought at Lewisham) had fully occluded and the vascular cambium had been re-established. In both species, varying amounts of dark extractives were observed in the wound wood, whether fungal colonisation had occurred or not (Fig. 4). Kino veins had formed in response to drill wounding in four of the twenty-two *E. globulus* trees. Kino veins were not associated with drill wounding in any *E. nitens* trees. Drill holes inoculated with the unidentified decay fungus had formed decay columns generally >10 cm in length in both species. Fungal hyphae were visibly abundant in the drill hole and some were observed to be in intimate contact with the inner side of the dark extractives. Microscopic examination revealed that the hyphae had not spread into the wood formed after wounding, suggesting that the wound-associated wood may be an effective barrier against further fungal colonisation of wood formed subsequent to damage.

**Response to bruising treatment**

In both species, the impact from the 3 kg steel rod generally killed the bark as well as the underlying vascular cambium that was located directly under the area of impact and surrounding margins. Subsequent to the impact, the overlying bark separated from the vascular cambium, causing it to become detached from the bole while remaining on the stem (Fig. 5). Assessment of the level of damage was usually possible only after removal of the dead bark. In these cases, the bruising treatment was observed to cause extensive discolouration of the underlying xylem (Fig. 6). Furthermore, kino veins (<5 cm long) and/or dark extractives were common along the outer edges of the callus margins, particularly in *E. globulus* trees. Another feature consistently associated with this treatment was the occurrence of bark splitting, which provided potential entry points for insect and fungi (Fig. 5). Larvae and hyphae were observed in a number of trees in both species.

Despite the preliminary nature of this study, the results suggest that assessment of damage levels in retained trees after thinning operations may be underestimated since bark-covered wounds are not as visible as bark-torn wounds. This finding may have important implications to industry, particularly as decay and discolouration have been reported to occur at a greater rate in bark-covered than open wounds over a two-year study period (Dudzinski *et al*. 1992; White and Kile 1993).

**Role of kino and dark extractives in wound repair in eucalypts**

In previous studies of eucalypt host–pathogen interactions, any exudate produced by the tree was simply referred to as kino (Mireku and Wilkes 1989; Wardlaw 1999). There, kino was defined as a red-brown aqueous polyphenolic exudate with the main constituent being polymerised forms of proanthocyanidins otherwise known as condensed tannins’ (Hillis and Carle 1962; Hillis and Yazaki 1975). Recent studies using modern analytical techniques including High Performance Liquid Chromatography coupled with Electrospray Ionisation Mass Spectrometry (HPLC with ESI-MS) have shown the chemical composition of kino to be markedly different from that of dark extractives detected in wound wood (Eyles *et al*. 2002, 2003; Eyles unpublished data). Given that there are clear differences in the chemistry and origin of these extractives, we suggest that future studies investigating the role of exudates in a eucalypt’s defence should fully detail the nature of the exudates, particularly as these differences may reflect different roles in wound healing and defence. For example, in the present study, the production of dark extractives was shown to occur readily in the xylem and/or the phloem in both species — even in *E. nitens*, a species that does not usually form kino veins.
The production of tree exudates within specialised intercellular secretory spaces in the xylem and/or phloem is found in a range of woody tree species (e.g. latex from Hevea species, gum from Prunus species and resin from Pinus species; Hillis 1987). While they are generally implicated in protection against pests, diseases and damage, the precise nature of their involvement remains unclear (Pearce 1996). There is conflicting evidence of the role of kino vein formation as a defence mechanism in host–pathogen interactions. In one study, the kino veins were significantly larger in trees inoculated with various canker fungi than in control trees (Old et al. 1986). In another report, kino vein formation was not correlated with size of lesions formed in response to the root rot, Phytophthora cinnamomi in E. marginata (Tippett et al. 1983, 1985). Given that fungal hyphae were observed to grow intimately with dark extractives — as also seen by Wilkes (1986) — it would seem that the induction of exudates in eucalypts is primarily in response to wounding and not specifically against biotic agents such as fungi. Even other forms of wounding not considered in this experiment, such as pruning, were shown to induce kino veins in the absence of fungal decay (Fig. 7). Hanks et al. (1999) found no evidence to suggest that kino served to defend E. rudis trees against Phoracantha semipunctata (colonising phloem-boring larvae), particularly as production of kino veins usually requires days or even weeks whereas beetle larvae can penetrate the bark and reach the cambium within 24 h. Investigations of the wound reactions of Scots pine (Pinus sylvestris L.) to attacks by two bark beetles (Lieutier et al. 1995) similarly concluded that the induced production of resin was a wound reaction arising from mechanical stress due to insect boring, and not solely a defence reaction against biological agents such as fungal pathogens. This does not mean, however, that these dark extractives may not have a role in impeding the spread of fungi or insects. For example, kino veins have been observed to effectively prevent the outward spread of wood decay (White and Kile 1993).

Conclusion

The production of exudates, either within specialised kino veins or in less organised wound-associated wood or bark, was consistently observed as part of host response to dry ice, bruising and drill wounding treatments, regardless of colonisation by fungi. The integrity of the vascular cambium appeared to be the key factor in kino vein production, as has been found in previous studies. Any stress, whether physiological, mechanical or chemical, that damages but does not kill the vascular cambium has the potential to cause kino veins to be produced in a susceptible species. Kino veins are not readily induced in E. nitens — unlike other Eucalyptus species — providing some evidence that this species is suitable for the commercial production of kino-free timber. In contrast, as kino veins were readily induced in E. globulus, kino vein defect may be a problem when trees of this species are grown for sawlogs.

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