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To prune or not to prune; pruning induced decay in tropical sandalwood

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ABSTRACT

Heartwood rot (a fungal disease) has the potential to significantly reduce the sandalwood oil production of *Santalum album*. With new plantations being established with sandalwood oil as the major product, it is imperative to know the consequences of routine form-pruning in tropical areas. Examining pruning wound responses in 1 and 5-year-old trees at the end of the dry and wet seasons, it was possible to ascertain the best pruning age and season in relation to occlusion. Pathogenic wood fungi were isolated from wounds and fungal spore monitoring was undertaken at the two trial sites at time of pruning. Younger trees had smaller wounds which were quicker to occlude and produced a lower decay rating which decreased over time. Wood inhabiting fungi were isolated from all pruning wounds and identified using the ITS gene region. There was a total of 531 fungal isolates, identified in 75 fungal taxa. Older trees had significantly more fungal species compared to younger trees. Pruning season did not significantly affect occlusion, the amount of resultant decay, nor the total number of fungal species isolated. Endophytes were isolated from every tree, comprising 52% of isolations. Younger trees had more endophytes, but less rot fungi than older trees. Canker fungi were isolated from almost all the older trees. In the older trees, rot fungi were predominant in trees pruned at end of the dry season. Metabarcoding of spores collected in spore traps resulted in 565 operational taxonomic units (OTU's) which were placed in 211 genera and 114 families, with about 55% classified as unknown fungi. About 23% of all reads corresponded to potential pathogens, however, the proportion of spore groups present at time of pruning at each plantation was not indicative of the fungal niches isolated from wounds. This study has shown that it is better to prune *S. album* when they are young to decrease the amount of potential decay. To reduce the risk of heartwood rot fungal diseases entering via the pruning wound, it would be preferable to prune at the beginning of the dry season. This will be applicable to other tropical tree species.

1. Introduction

Santalum album (sandalwood), an obligate wood hemi-parasite, produces a highly valued essential oil from its heartwood (Jones et al., 2007). Within the *Santalum* genus, whilst species produce similar sandalwood oil components, the proportions differ. *S. album* produces the highest levels of alpha and beta santalols with an absence of farnesol and therefore easily meets the ISO standard for the valuable high-end perfume market. This makes this species the most sought-after.

It has long been recognised that the natural *S. album* stands of Timor-Leste and India are insufficient to meet world demand for sandalwood oil. To fill the need, plantations are being established across the natural sandalwood range of the Pacific Rim and in northern Australia (Brand et al., 2012; Subasunghe, 2013). Currently, northern Australia is managing the world's largest area of *S. album* plantations, more than 13,000 ha (Keenan and Parija, 2017; Santanol, 2017).

The oil of sandalwood is a complex mixture of sesquiterpenes that the tree naturally produces in its heartwood to protect the core of its bole against pests and diseases (Jones et al., 2007). Heartwood production begins to initiate, in some trees after 10 years of growth, in the base of tree bole, extending progressively up the bole and into the roots as the tree ages (Rai, 1990). The formation of this heartwood is an active process requiring the redirection of assimilates from growth into the formation of the sesquiterpenes (Celedon and Bohlmann, 2018). Investigation into oil production in managed plots in Western Australia revealed the highest yields were obtained from solid, infection and insect-free trees (Brown et al., 2011). This highlights the need for *S. album* to have protection against diseases in a tropical environment.

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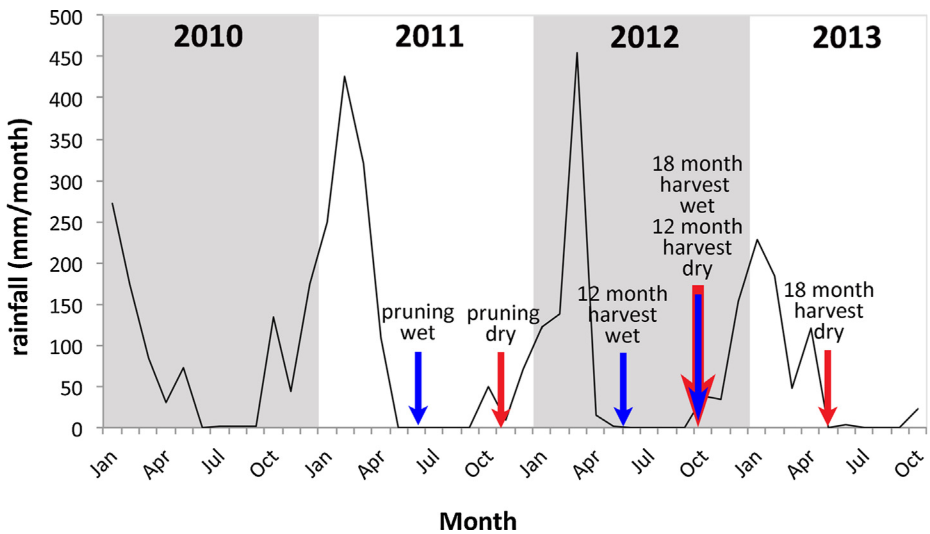

Fig. 1. Monthly rainfall in Kununurra from January 2010 to October 2013 (Bureau of Meteorology). Pruning times (red arrows) and harvest time (blue arrows) of samples from two sandalwood plantations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Plantation sandalwood production is complex with multiple hosts required through the life of a sandalwood tree. Due to the complexities of the parasitic/host relationship, Barbour et al. (2010) found that it was difficult to ascertain if the cause of the sporadic deaths was due to fungal disease or lack of host relationships. Sandalwood seedlings are transplanted to the field with a pot host, and planted with both a short- and long-term host which requires a transition when the fast-growing short-term host dies. The plantations are managed by slashing the grass, form-pruning the sandalwood and, in some cases, mechanical pruning of the short-term hosts which can damage the sandalwood trees in the process. Additionally, sun-scald, due to lack of shading of the sandalwood trunk, can result in bark loss. Any damage to the main trunk of the sandalwood can allow pathogen ingress.

There have been few reports of pests and pathogens affecting *S. album* (Subasunghe, 2013). The tree is designed with an impervious bark and the formation of the protective heartwood as the tree ages. However, disease risk can be immediate in tropical plantations, rather than taking time to build up over 2–3 generations (Barry, 2002; Barbour et al., 2010), so it is imperative that the quantity and quality of the heartwood of this slow growing tree is protected. In the early 1900s, India's sandalwood was severely affected by sandal spike disease which is characterised by witches' broom symptoms and caused by a phytoplasma which was not identified until the 1970s (Khan et al., 2004). Infected trees die within a couple of years after visible symptoms appear (Ghosh et al., 1992). In Western Australia, there has been some erratic damage from a stem girdling moth (McKinnell, 1990) and other pests include termites and stem borers (Remadevi et al., 2005; Subasunghe, 2013). Recently, Barbour et al. (2010) isolated endophytes, cankers and rot fungi from heartwood from *S. album* in Western Australia where fungal infections occurred via pruning wounds. They found that rot in 8 and 15-year-old trees, and estimate while 3–4% of heartwood (areas of oil deposition) could be lost to rots, the hollows left in the centre of the bole provide access for termites and ants. There is little information in the literature regarding disease or damage to sandalwood.

Tree pruning to encourage a single stem is a standard practice in the sandalwood industry; aiming to direct the vigour of the tree towards increasing the size of the valuable sandalwood lower bole. This practice provides a mechanism by which fungal pathogens can potentially have a strong economic impact by reducing the growth of the tree and subsequently reducing heartwood development and oil production. Review of the West Australian industry showed no consensus on when to prune, nor the style of pruning to undertake (flush/close cut or stub cut) (Barbour *pers. comm.*). Plantation form pruning occurred in the first year to remove any double leaders, followed by annual pruning to the age of 4 to produce a clear bole of up to 2.5 m (Kimber, 2010). Wounds are a potential infection site for fungal pathogens, particularly in tropical areas. The warm temperatures and high relative humidity due to flood irrigation encourages tree growth as well as fungal diseases. The proximity of neighbouring host trees can allow for opportunistic cross infection. The time for branch occlusion after wounding is important as the stem wood is exposed to humidity and fungal infections before closure occurs (Shigo, 1989). However, there is very little information on pruning and wound occlusion in tropical trees, most of the literature focuses on conifers (Hein, 2008).

To ensure the sustainability of sandalwood in the Ord River Irrigation Scheme and other tropical areas, efforts to guard against the constant threat of disease are imperative. The aim of this study was to determine best practice commercial pruning by examining the effect of pruning in relation to tree age and pruning season. Recovery of potentially pathogenic fungi from wounds, and the rate of wound closure will determine best practice. Fungal spore monitoring will reveal pathogens present at the time of pruning and allow exploration of prediction of infection risks from the main ecological groups of wood pathogens. It is hypothesised that (a) younger trees will be more resistant to infection, (b) pruning at the end of the wet season will result in more infections, and (c) there will be a relationship between communities of spores present at time of pruning and those associated with wounds.

2. Methods

2.1. Site selection

Two commercial flood irrigated sandalwood plantations in the Ord region of Western Australia were selected for this study. Firstly, 1-year-old trees (established 2010) with 463 *S. album*, 463 *Dalbergia latifolia* long-term hosts and 926 *Sesbania formosa* intermediate hosts per ha. Secondly, 5-year-old trees (established 2007) which had been pruned in its first year with 463 *S. album*, 463 *Milletia pinnata* long term hosts, and 926 *S. formosa* intermediate hosts per ha. Rainfall data from the nearest meteorological station approximately 50 km SE (Kununurra Aero) and pruning times are provided (Fig. 1).

2.2. Current pruning practises

There are no pruning recommendations in the literature for tropical sandalwood. Three growers from the Ord region use form pruning (between March and October) to produce a bole of 1–2.5 m, and they are prepared to remove up to half the canopy to achieve this. The frequency of pruning varied from: 2 prunes in the first year followed by annual pruning