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Floral and reproductive biology of the medicinally significant rainforest tree, *Fontainea picrosperma* (Euphorbiaceae)

Elektra L. Grant[a], Helen M. Wallace[a], Stephen J. Trueman[a], Paul W. Reddell[b], Steven M. Ogbourne[a,*]

[a] Centre for Genetics, Ecology and Physiology, University of the Sunshine Coast, Sippy Downs, Queensland, Australia
[b] EcoBiotics Limited, Yungaburra, Queensland, Australia

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ABSTRACT

Fontainea picrosperma (Euphorbiaceae) is a dioecious rainforest tree from northern Australia that is of commercial interest following the recent discovery of the putative anti-cancer agent, tigilanol tiglate, in its seed. Production of tigilanol tiglate will rely on purification from harvested fruit and therefore an understanding of the reproductive characteristics that determine fruit set of this species is critical. Most rainforest plant species rely exclusively on animal vectors to transport pollen between plants for successful reproduction. Flower traits and phenology can facilitate sexual reproduction by attracting pollinators whereas failure to attract pollinators can result in low fruit set due to pollen limitation. Here, we describe the floral morphology, flowering phenology and reproductive biology of *F. picrosperma.* This species bears small, white, actinomorphic flowers with a shallow receptacle. These floral traits are often associated with generalist insect pollination and are common to other dioecious tropical rainforest flowers. Individual female flowers persisted on the tree for several days longer than individual male flowers. Male panicles contained significantly more flowers than female inflorescences, and male flowers opened sequentially on a panicle whereas female flowers opened almost simultaneously within an inflorescence. *F. picrosperma* was pollen limited, as hand pollinated female flowers produced almost double the final fruit set (39.6 ± 4.4%) of open pollinated flowers (21.3 ± 3.4%). Optimised production of tigilanol tiglate may therefore rely on improving pollen flow from male to female trees.

1. Introduction

Tigilanol tiglate (EBC-46) is a novel compound from the tropical rainforest tree, *Fontainea picrosperma* (Euphorbiaceae) that is being developed as a cancer therapeutic for veterinary and human markets (Boyle et al., 2014; Linkliter et al., 2015). Tigilanol tiglate (Fig. 1) cannot be synthesised easily and so it is manufactured for research and development solely by extraction and purification from the seed of *F. picrosperma*. A reliable and economical supply of the Active Pharmaceutical Ingredient is a key element in the development of a therapeutic agent. Consequently, an understanding of the floral and reproductive biology of *F. picrosperma* is essential for sustainable seed production and commercial production of tigilanol tiglate. *F. picrosperma* is dioecious (i.e. male and female flowers are located on separate trees) but little else is known of the floral and reproductive biology of this tropical rainforest plant.

Most tropical rainforest plants are facultative or obligate outcrossers that rely almost exclusively on animal pollinators for seed production (Ollerton et al., 2011). Dioecious species in particular are not able to produce seed by self-pollination and those that rely on animal pollination must invest resources to attract pollinators (Williams and Adam, 2010). Plant traits likely to be involved in pollinator attraction include flower colour, shape, size, scent and food reward (Barrett and Harder, 1996; Boulter et al., 2006). Dioecious species in tropical rainforests globally display similar flowering strategies to attract animal pollinators (Bawa and Opler, 1975; Renner and Feil, 1993; Adam and Williams, 2001; Queenborough et al., 2007; Gao et al., 2012; Field et al., 2013a,b). These include producing small, actinomorphic, pale-coloured flowers that attract generalist insect pollinators (Machado and Lopes, 2004; Boulter et al., 2006). Male and female flowers have become specialized in dioecious species and generally have different inflorescence structures and flowering patterns within a species (Ainsworth, 2000). Male trees generally flower earlier and for a longer period, and produce twice as many flowers as female trees (Gao et al.,

* Corresponding author.
E-mail addresses: egrant@usc.edu.au (E.L. Grant), hwallace@usc.edu.au (H.M. Wallace), strueman@usc.edu.au (S.J. Trueman), Paul.Reddell@ecobiotics.com.au (P.W. Reddell), sogbourn@usc.edu.au (S.M. Ogbourne).

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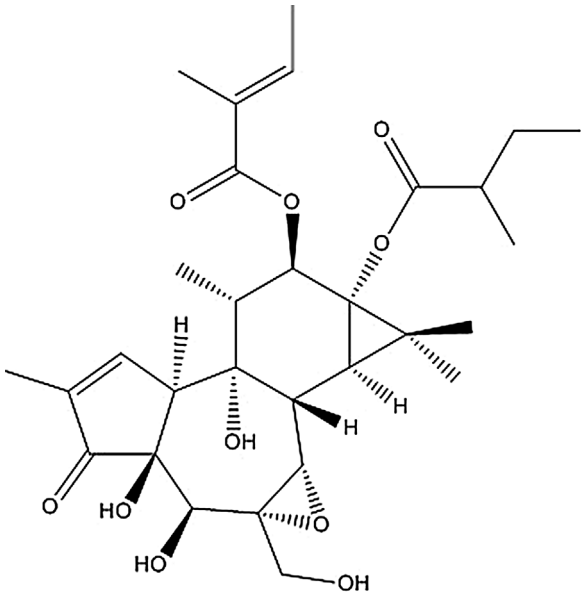

Fig. 1. Chemical structure of the putative cancer therapeutic, tigilanol tiglate, isolated from *Fontainea picrosperma*.

2012). An understanding of a species' floral traits and phenology are necessary to elucidate potential pollinators and understand pollinator behaviour (Barrett and Harder, 1996; Rosas-Guerrero et al., 2014). Failure to attract effective pollinators can result in pollen limitation and reduced seed production (Larson and Barrett, 2000; Ashman et al., 2004) which, in some cases, can reduce whole-plant reproductive output (Trueman, 2013). Dioecious and self-incompatible species generally exhibit lower fecundity than related self-compatible species (Larson and Barrett, 2000; Knight et al., 2005; Aguilar et al., 2006; Davila et al., 2012). Never the less, pollination, fruit set and seed production have not been studied empirically for many dioecious rainforest trees.

In this study, we assessed the floral and reproductive biology of *Fontainea picrosperma*, which is endemic to the wet tropical rainforests of north Queensland, Australia (Agostini et al., 2013; Lamont et al., 2016). We provide the first description of flowering phenology and pollen limitation in this dioecious species.

2. Materials and Methods

2.1. Species and study sites

F. picrosperma is a small dioecious understorey tree found in complex mesophyll and notophyll vine forests (Tracey, 1982) on the Atherton Tablelands of north Queensland, Australia. The species grows on basalt soils between 700 m and 1200 m altitude (Cooper, 2004). Flowering occurs from September to November, occurring later at the higher altitudes. The red drupaceous fruit (up to 3 cm diameter) ripen in December and January and are eaten by cassowaries, musky rat kangaroos and giant white tailed rats (Cooper, 2004).

Data in this study was collected from trees in the natural population (Boonjie, Evelyn Highlands and Malanda; see Lamont et al., 2016), potted nursery trees (grown in 20 L pots under 50% shade in Yungaburra, Queensland) derived from Malanda, and trees in a commercial plantation (grown under 50% shade near Yungaburra, Queensland) also derived from Malanda. Natural stands of *F. picrosperma* often form small but relatively dense (2–10 m inter-tree spacing) and clumped populations with ~50:50 male:female ratios. The potted nursery trees were between 1 m and 2 m in height and placed 1 m apart with a random ~50:50 distribution of male and female trees. Plantation trees were planted with 1.5 m intra-row and 2.5 m inter-row spacing with a random ~50:50 distribution of male and female trees. Investigations were performed during four reproductive seasons in 2011/2012, 2012/2013, 2013/2014 and 2014/2015. We examined floral morphology (inflorescence structure, flower structure, anther morphology and pistil morphology) and flowering phenology (male flower longevity, stigma receptivity, and patterns of flower opening on male and female inflorescences). We also performed controlled pollinations to determine final fruit set and assess whether fruit set was pollen limited. Pollinations were performed using male parents from the same natural population.

2.2. Floral morphology

We examined inflorescence structure in the natural population in 2011. Flowers were counted on a total of 24 male inflorescences across four male trees and 46 female inflorescences across eight female trees. Flowers were counted on 2–8 inflorescences per tree depending on availability. Flower diameter was measured in the commercial plantation in 2014 on 30 flowers from each of three male and three female trees (180 flowers in total). Diameter was measured at the widest part of the corolla of open flowers.

Pollen morphology, anther dehiscence, and stigma morphology were examined by scanning electron microscopy. Open male and female flowers were collected and fixed in 3% glutaraldehyde at room temperature and then stored overnight. The samples were then dehydrated in an aqueous ethanol series of increasing concentration (10, 30, 50, 70, 80, 90 and 100% ethanol), critical point dried (Quorum K850, Quorum Technologies, East Sussex, UK), sputter coated with gold (Quorum Q150T S, Quorum Technologies, East Sussex, UK), and examined using a JSM-6010LA scanning electron microscope (JEOL, Tokyo, Japan).

2.3. Flowering phenology

Individual male flower longevity was observed in the natural population in 2011 on eighteen inflorescences across six male trees (78 flowers in total). Flowers were observed daily to determine the number of days that each individual flower remained open. The number of days from anthesis to abscission was calculated for each flower.

Female flowers were monitored for timing of stigma receptivity in the potted trees in 2012 and in the natural population in 2014. Inflorescences were enclosed in fine mesh bags (0.5 mm × 1.0 mm pore size) to exclude pollen. Individual unopened buds were tagged and monitored daily for anthesis. To test stigma receptivity by peroxide activity, two to four flowers were collected at each age (-1, 0, 1, 3, 5, 7 and 11 d post anthesis) from each of five trees in the natural population, where 0 d post-anthesis represented flowers that had opened within the past 12 h. Stigma receptivity was determined by testing for the presence of peroxide on the stigma immediately after the flower was collected from the tree. Peroxide activity was examined using a Peroxtesmo Ko paper indicator test (Macherey-Nagel, Dueren, Germany) as previously described (Kearns and Inouye 1993), with one drop of deionised water placed on the test paper to increase the effectiveness of the test (Dafni and Maués 1998). To test receptivity by observing fruit set, between seven and 12 panicles were hand pollinated at each age (0, 1-2, 3-4, 6-8, and 9-11 d post anthesis) across six to nine replicate potted trees, with 0 d post-anthesis representing flowers that had opened within the past 12 hours. One panicle on each of five trees was included as a bagged control. Fruit set was observed after 7-9 weeks, when the fruit were ~2 cm in diameter.

We assessed the patterns of flower opening on male and female inflorescences in the natural population and on potted nursery trees in 2011 and 2012. Nine inflorescences across three male trees and two inflorescences from one female tree were observed in the natural population in 2011. In addition, one inflorescence from each of 20 male trees and 20 female trees was observed on potted nursery plants in