Industrial Crops & Products 108 (2017) 416–422

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

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

ARTICLE INFO

Keywords:
Cancer
EBC-46
Dioecy
Pollination
Pollen limitation
Tigilanol tiglate

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).

http://dx.doi.org/10.1016/j.indcrop.2017.07.013
Received 28 February 2017; Received in revised form 8 June 2017; Accepted 5 July 2017

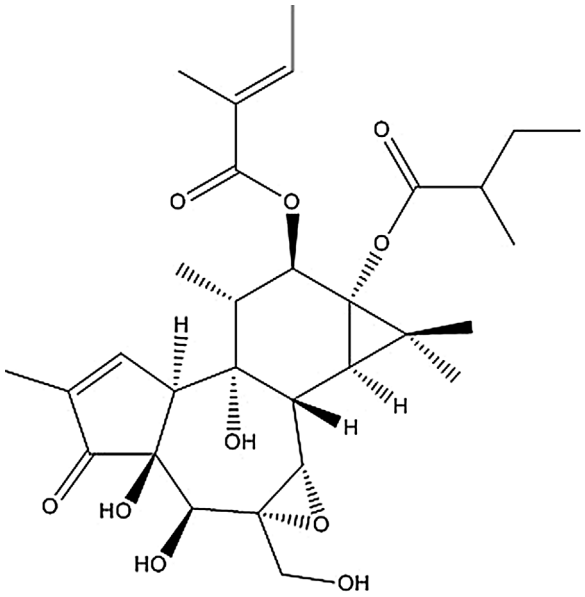

Fig. 1. Chemical structure of the putative cancer therapeutic, tigilanol tiglate, isolated from *Fontainea picrosperma*.

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

2012. The number of open flowers on each inflorescence was counted at the same time each day until all flowers on the inflorescence had opened and the petals had browned and shrivelled.

2.4. Fruit set and pollen limitation

Controlled pollination experiments were conducted over two reproductive seasons in the natural population using 11 female trees in 2012/2013 and ten female trees in 2013/2014. Three to nine inflorescences per tree were selected and assigned randomly to one of three treatments: 'bagged' to exclude flower visitors (n = 126 flowers); 'open' to flower visitors (n = 143 flowers); or 'hand-pollinated' (n = 127 flowers). Any flowers that had already opened were removed from the inflorescence and the remaining unopened flowers were counted. Inflorescences assigned to the 'bagged' treatment were enclosed in fine mesh bags (0.5 mm × 1.0 mm) to exclude pollen. The bags were fastened to the stem with a wooden peg and left in place until all flowers had withered. Inflorescences left 'open' were not enclosed in mesh bags, allowing flower visitors access to flowers. Inflorescences assigned to the 'hand pollinated' treatment were also enclosed in fine mesh bags. Their flowers were hand pollinated within 12 h of anthesis (i.e. 0 d post-anthesis) by brushing the stigma with anthers that had been dried over silica gel for 12 h to assist with pollen release. A hand lens was used to confirm that pollen had been deposited on the stigma. Hand pollination continued until all petals of all flowers in the treatment had browned and shrivelled. Initial fruit set was determined by counting the number of flowers with swelling of the ovary; i.e. at 5 mm diameter (~2 weeks). Final fruit set was counted when fruit were considered ripe; i.e. when they reached 20 mm diameter (~10 weeks). Fruit set was calculated as the percentage of the total number of flowers per inflorescence that formed fruit.

We also assessed the success of pollination treatments by observing pollen grains on the stigma and counting pollen tubes in the style. Three replicate inflorescences per treatment (bagged; open; and hand pollinated) on each of three female trees were selected in either the natural population, commercial plantation or potted trees in each of 2012 and 2013. The flowers in the bagged and hand pollinated treatment groups were either bagged or hand pollinated, as described above. The flowers of all treatments (23 open pollinated, 26 hand pollinated, and 9 bagged flowers) were removed 3 d post-anthesis and placed in fixative (25% glacial acetic acid, 75% ethanol; v,v). Flowers were examined for the presence of pollen grains and pollen tubes using a fluorescence microscope (Leica DM5500B, Leica Microsystems, Wetzlar, Germany) and a confocal microscope (Nikon Eclipse Ti/Nikon C2S, Nikon Inc, New York, USA) after staining with decolourised aniline blue (Shepherd, 2000).

2.5. Statistical analysis

Data comparing the number of flowers on male and female inflorescences were analysed using a two-way ANOVA with tree and sex as fixed factors. Data for flower diameter were log transformed to meet assumptions for normality and analysed using a nested ANOVA with sex and tree (nested within sex) as factors. Tree differences in male flower longevity were tested using a one-way ANOVA with tree as a fixed factor. Data for inflorescence opening duration were initially analysed by two-way ANOVA with site and sex as factors. However, due to significant site × sex interactions, data were then analysed with a one-way ANOVA with sex × site combined as a single factor (i.e. male inflorescences in natural populations; female inflorescences in natural populations; male inflorescences on potted trees; and female inflorescences on potted trees). Stigma receptivity data were analysed by a one-way ANOVA. Controlled pollination and fruit set data were analysed with a two-way ANOVA (treatment × year). Almost no bagged-treatment flowers produced initial fruit (5 out of 126) or final fruit (2 out of 126) and so the bagged treatment was excluded from the analysis. No significant year effects or interactions between treatment and year were detected in the analyses of initial and final fruit set. Tukey's HSD tests were used to assess differences between the means when significant differences among the means were detected by ANOVA.

3. Results

3.1. Floral morphology

F. picrosperma bears mostly terminal inflorescences (Jessup and Guymer, 1985). The inflorescences that bore male flowers were paniculate whereas female flowers were arranged in a flat umbel pattern (Fig. 2A and B). Male inflorescences contained many more flowers (25.8 ± 1.5) than female inflorescences (4.9 ± 0.2) (***$P < 0.001$). There were no significant differences in flower number per inflorescence between trees within a sex ($P = 0.366$). *F. picrosperma* had actinomorphic planar flowers with a shallow receptacle and white coloured petals. Male flowers had five petals with well exposed anthers and an orange staminal disk (Fig. 2C). Female flowers had five petals and the pistil had five deeply bi-lobed stigmas (Fig. 2D). Stigmas were greenish-yellow upon opening but turned yellow and then brown as the flower matured and senesced or was successfully pollinated. The syncarpous gynoecium contained five locules but generally produced a single-seeded fruit following successful pollination.

Flower size was significantly different between male and female trees (***$P < 0.001$) and between trees within each sex (***$P < 0.001$) (Table 1).

Anthers began to dehisce 1 to 2 d after anthesis. Dehiscence occurred via an external stomium; i.e. extrorse dehiscence (Fig. 3A). Pollen grains were spheroidal, relatively small with diameter of approximately 40 μm, and released as a monad. The pollen had an exine ornamentation consisting of clavate elements with triangular sculpturing arranged in a croton-type pattern (Fig. 3B) (after the classification of Punt et al., 2007). This surface pattern is a unique synapomorphy within the subfamily Crotonoideae to which *F. picrosperma* belongs. The stigmatic surface was wet with an exudate, and pollen grains were observed germinating on the wet stigma (Fig. 3B).

3.2. Flowering phenology

Individual male flowers opened for 1 to 2 d (1.6 ± 0.1 d) and no significant within-tree variation in male flower longevity was observed. All stigmas gave a positive result for peroxidase activity, indicating that stigmas were receptive at least 1 d before anthesis until at least 11 d after anthesis (Table 2). However, only 3.7 ± 3.7% of flowers hand pollinated at 0 d post-anthesis and none of the flowers pollinated at 9-11 d post-anthesis set fruit, compared with 76.1 ± 7.6%, 84.9 ± 6.9% and 54.8 ± 8.7% of flowers pollinated at 1-2, 3-4 and 6-8 d post-anthesis, respectively (Table 2). No set fruit was observed from the bagged control flowers in this experiment.

There were no significant differences in inflorescence opening duration in the natural population; however, male inflorescences on potted plants had flowers open for significantly longer than in natural populations (***$P < 0.001$) and the number of days to peak flowering was also longer (***$P < 0.001$) (Table 3; Fig. 4). Male inflorescences in the natural population were open for a maximum of 14 d and the mean peak of flower opening was at 6.1 ± 0.9 d, while female inflorescences were open for 11 d and the mean peak of flower opening was at 6.0 ± 0.0 d (Fig. 4A). Male inflorescences in the potted plants were open for a maximum of 49 d and the mean peak of flower opening was at 14.9 ± 0.7 d, while female inflorescences were open for 12 d and the mean peak of flower opening was at 3.0 ± 0.4 d (Fig. 4B).

Fig. 2. Flower arrangement on inflorescences and flower morphology of *Fontainea picrosperma*. (A) Male inflorescence. (B) Female inflorescence. (C) Male flowers. The open flowers on the left and right have undehisced and dehisced anthers, respectively. (D) Female flower showing the stigma with five double lobes.

Table 1
Diameter of male and female *Fontainea picrosperma* flowers.

Sex	Tree number	Mean ± s.e. diameter (mm)
Male[a]	1	19.8 ± 0.20[a]
	2	19.6 ± 0.19[a]
	3	14.3 ± 0.21[b]
Female[b]	1	18.0 ± 0.18[a]
	2	21.4 ± 0.19[b]
	3	20.2 ± 0.15[c]

Significant differences (ANOVA and Tukey's test, $p < 0.05$, n = 30) are represented by different letters.

3.3. Fruit set and pollen limitation

Hand pollination resulted in higher initial fruit set (82.6 ± 3.9%) than did open pollination (45.6 ± 4.7%) (***P < 0.001, Fig. 5A). Initial fruit set from bagged, unpollinated flowers was very low (3.8 ± 1.5%) (Fig. 5A). This may be the result of rare insect movement through the mesh by particularly small insects such as thrips, although a very low level of apomictic reproduction in this species cannot be ruled out. Hand pollination also resulted in higher final fruit set (39.6 ± 4.4%) than did open pollination (21.3 ± 3.4%) (***P < 0.001, Fig. 5B). Final fruit set from bagged, unpollinated flowers was negligible (1.7 ± 1.2%) (Fig. 5B).

No pollen grains or pollen tubes were observed on or within any flowers from the open or bagged pollination treatments at 3 d after anthesis. Pollen grains and pollen tubes were observed on the stigma and within the style of every hand pollinated flower that was sampled (Fig. 6). We observed 66 ± 9 (n = 12) and 36 ± 9 (n = 14) pollen tubes in the upper style of hand pollinated flowers in 2012 and 2013, respectively. However, the pollen tubes that were observed within the style had not reached the ovules by 3 d after anthesis.

4. Discussion

The floral morphology and flowering phenology of *F. picrosperma* were typical of many dioecious plants found in rainforest communities. Many woody Australian rainforest species possess small flowers that are either white or green with an unspecialised flower structure and a shallow open-access receptacle (Bawa, 1990; Williams and Adam, 1999; Boulter et al., 2010). These common floral traits are often associated with generalist entomophilous pollination (Armstrong and

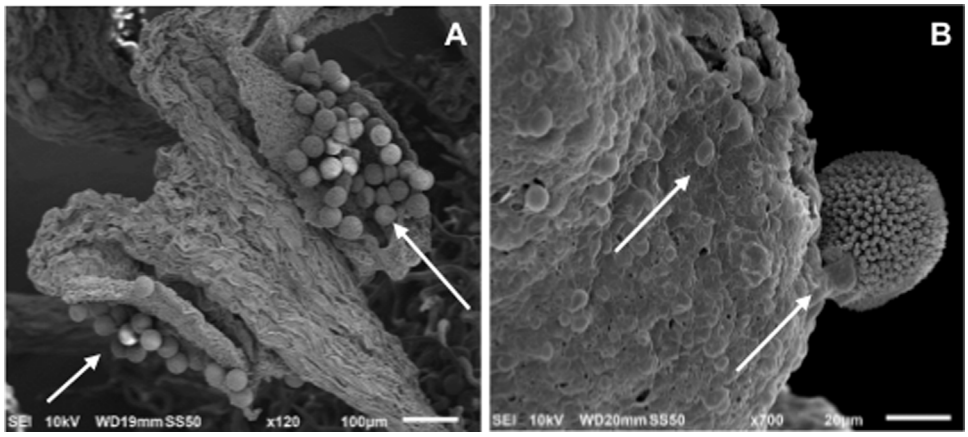

Fig. 3. Scanning electron micrographs of *Fontainea picrosperma* flower parts. (A) Anther. Arrows point to anther dehiscence via longitudinal slits, showing the release of numerous spherical pollen grains. (B) Stigma. Arrows point to stigma exudate and a germinating pollen grain. Note the exine ornamentation consisting of clavate elements with triangular sculpturing arranged in a croton-type pattern, unique to the Euphorbiaceae subfamily Crotonoideae.

Table 2
Timing of receptivity of female *Fontainea picrosperma* flowers as determined by stigma peroxide activity and fruit set after hand pollination.

Technique	Time post-anthesis (d) Flower harvest date						
	-1	0	1	3	5	7	11
Stigma peroxide activity	+	+	+	+	+	+	+
	Hand pollination date						
	0	1–2	3–4	6–8	9–11		
Mean ± s.e. fruit set (%)	3.7 ± 3.7	76.1 ± 7.6^{ab}	84.9 ± 6.9^{a}	54.8 ± 8.7^{b}	0 ± 0		

'+' indicates a positive result for peroxide activity.
Significant differences among three means (ANOVA and Tukey's test, $p < 0.05$, n = 7 trees) are represented by different letters.

Table 3
Duration that individual male and female *Fontainea picrosperma* inflorescences possessed open flowers in the natural population and on potted plants.

Site location and sex	Mean ± s.e. duration (d)
Males from natural population	12.5 ± 0.6^{a} (n = 9)
Females from natural population	10.0 ± 1.0^{a} (n = 2)
Male potted plants	30.9 ± 1.9^{b} (n = 20)
Female potted plants	7.3 ± 0.6^{a} (n = 20)

Significant differences (ANOVA and Tukey's test, $p < 0.05$) are represented by different letters.

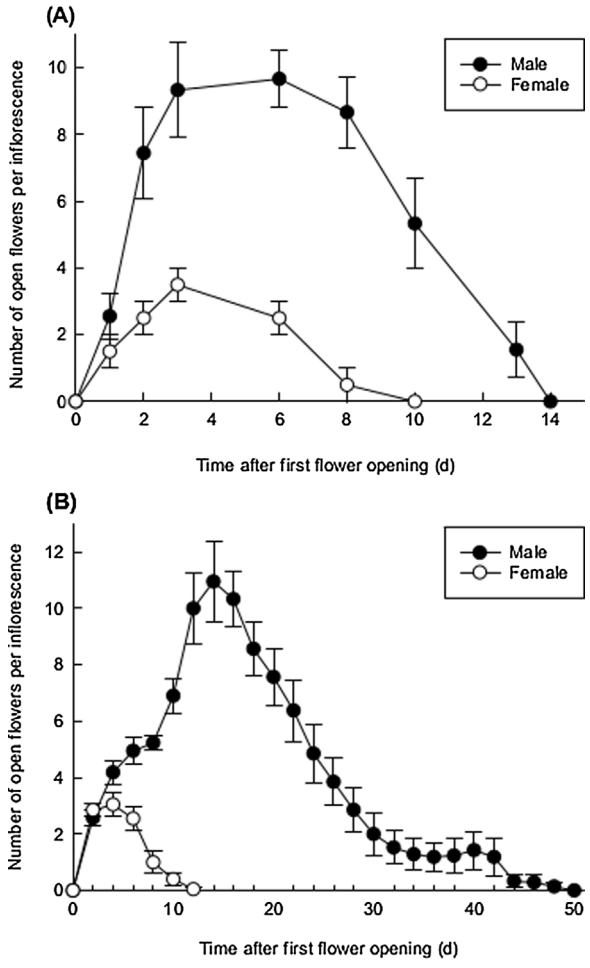

Fig. 4. Patterns of flower opening within male and female *Fontainea picrosperma* inflorescences. Mean (± s.e.) number of open flowers on individual inflorescences over the lifespan of the inflorescence in (A) the natural population (female inflorescences: n = 2; male inflorescences: n = 9) and (B) potted nursery plants (female inflorescences: n = 20; male inflorescences: n = 20).

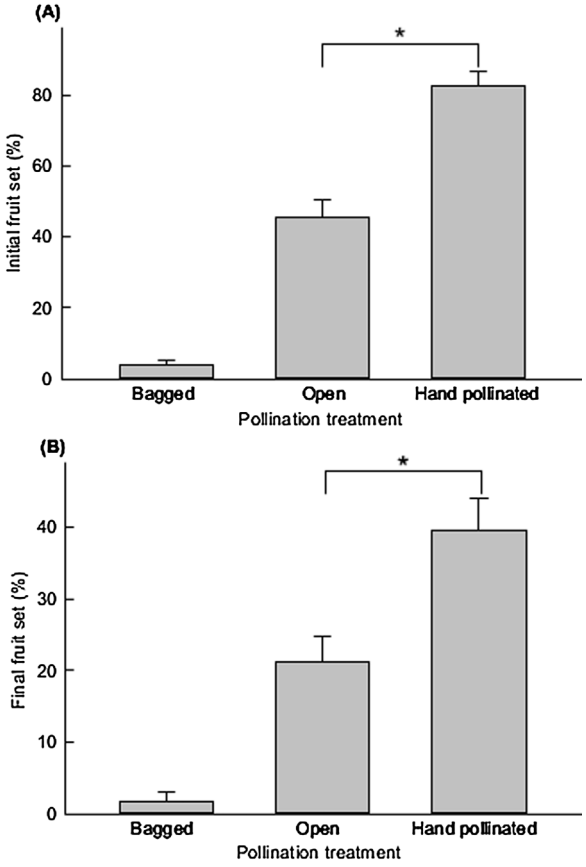

Fig. 5. Fruit set of *Fontainea picrosperma* inflorescences. (A) Initial fruit set and (B) final fruit set (mean ± s.e.) following three different pollination treatments: Bagged flowers were isolated from insect visitors; Open flowers were left open to insect visitors; Hand pollinated flowers were bagged but manually pollinated. * Indicates differences between open and hand pollinated inflorescences were significant (two-way ANOVA, ***P < 0.001, n = 10–11 trees).

Irvine, 1989; Hansman, 2001; Carpenter et al., 2003; Corlett, 2004; Machado and Lopes, 2004; Gross, 2005; Rosas-Guerrero et al., 2014). These features were all evident in *F. picrosperma* and it is therefore likely to be pollinated by insects. Male *F. picrosperma* flowers were clustered together in paniculate inflorescences that contained significantly more flowers than female inflorescences. This may ensure that pollen is available across the entire female flowering period (House, 1993; Barrett and Harder, 1996; Williams and Adam, 2010), provided that male and female trees flower more or less synchronously. Individual flowers within the male inflorescence opened sequentially and individual flowers senesced 1 to 2 days after anthesis, possibly encouraging pollinators to visit more inflorescences within the population (House, 1993; Osunkoya, 1999; Moog et al., 2002; Yamasaki and Sakai, 2013).

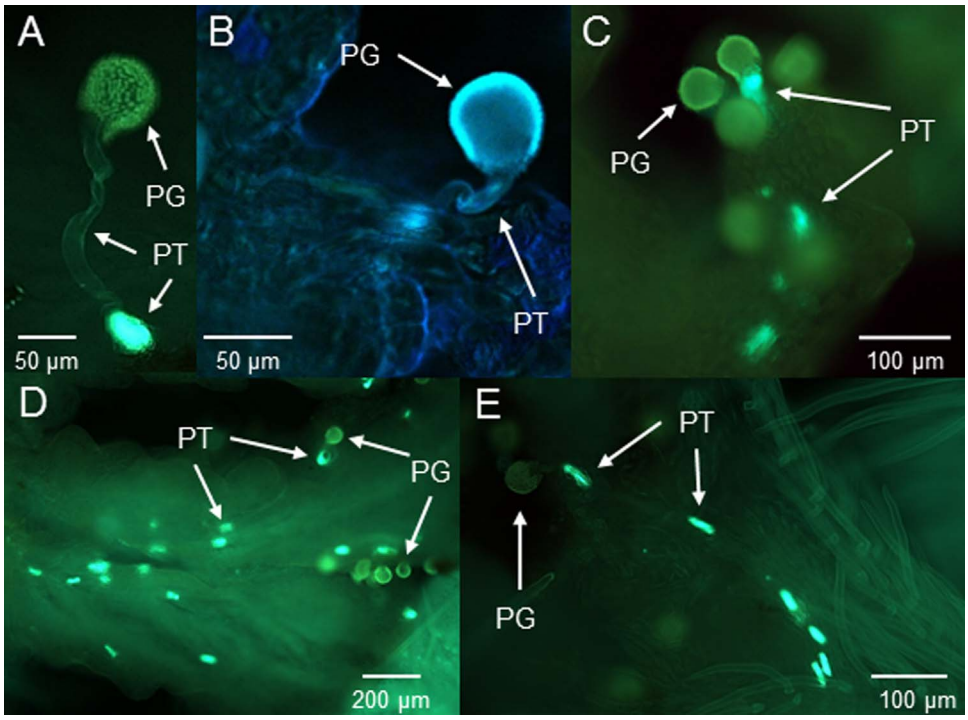

Fig. 6. Pollen grains and pollen tubes of *Fontainea picrosperma*. (A–C) Germinating pollen grains on the stigma and (D–E) pollen grains on the stigma and pollen tubes within the style of hand pollinated flowers. Arrows point to pollen grains (PG) and pollen tubes (PT).

Male and female flower diameters varied by approximately 33% and 17%, respectively, in plantation *F. picrosperma* trees. Flower size is a potentially significant commercial trait as it has been correlated with pollinator visitation (Klinkhamer and van der Lugt, 2004), fruit set (Scorza et al., 1991; Johnson et al., 2011; Wetzstein et al., 2013) and fruit size (Andersson, 1993; Scorza et al., 1991; Rosati et al., 2009; Johnson et al., 2011, 2011; Wetzstein et al., 2013). Relationships between flower diameter and fruit set or fruit size in *F. picrosperma* warrant further research to ensure that fruit yield is maximised through plantation management practices or genotypic selections that optimise flower quality.

No pollen grains or pollen tubes were observed on or within open-pollinated female flowers of *F. picrosperma* after 3 days of flower opening. This was despite stigmas being receptive within the first 2 days after anthesis and despite fruit set in open-pollinated flowers being observed in approximately 20% of flowers. This implies very slow or limited pollen movement in the plantation, and suggests that this species requires an extended period of stigma receptivity to produce a high fruit load. Female *F. picrosperma* flowers remained open for long periods and remained receptive for up to 8 days post-anthesis, suggesting an adaptation to low pollinator activity. While strong phylogenetic constraints operate at the family level to limit the evolution of flower longevity (Kochmer and Handel, 1986; Stratton, 1989), flower longevity can vary at the species level because of low pollination activity (Stratton, 1989; Bawa, 1990; Devaux and Lande, 2010). Final fruit set of *F. picrosperma* was 21.3 ± 3.4% when the flowers were open to insect visitors whereas almost no fruit were produced when insects were excluded from the flowers. This demonstrates that *F. picrosperma* did not reproduce apomictically, at least not at levels that are likely to be reproductively significant, and that individual fecundity relied on attracting foragers to both male and female flowers. Pollen limitation occurs when pollen augmentation increases seed production relative to open pollinated controls (Trueman and Wallace, 1999; Knight et al., 2005). We found that hand pollinating flowers almost doubled fruit set relative to open pollinated flowers (39.6 ± 4.4% *v.* 21.3 ± 3.4%), indicating that fruit set of *F. picrosperma* was limited by pollen transfer under natural pollination conditions. The floral traits of *F. picrosperma* suggested that it was adapted for generalist insect pollination, but we have not yet identified the pollinators and it remains possible that this species has a specialist pollinator. Pollen limitation is common in tropical rainforests (Vamosi et al., 2013) and environmental conditions affect the degree of pollen limitation more strongly than common ancestry (Larson and Barrett, 2000). Although seed production and, therefore, tigilanol tiglate production will depend on insect pollinators or hand pollination to produce fruit, further research is required to determine the potential implications of pollen limitation on the whole-tree yield of seed and tigilanol tiglate in this species. The availability of maternal resources to support seed development may also affect the retention, size and tigilanol tiglate content of fruit. Higher frequencies of fruit set associated with hand pollination may result in compensatory effects that reduce average seed size or seed quality including tigilanol tiglate content.

Reproductive strategies strongly influence the ability of plant species to bear viable seed (Adam and Williams, 2001). Dioecy can be highly beneficial for species by allowing sex-specific resource allocation and promoting out-crossing, but it can also lead to pollen limitation and reduced fruit set (Larson and Barrett, 2000; Knight et al., 2005; Aguilar et al., 2006; Davila et al., 2012). Our results from two reproductive seasons clearly showed that *F. picrosperma* was pollen limited. Pollen limitation may be the result of temporal variability in pollination services, often encountered in rainforests where pollinator abundance and competition from co-flowering species is highly unpredictable (Freeman et al., 1980; Williams and Adam, 2010; Vamosi et al., 2013). However, species that exhibit pollen limitation are vulnerable to habitat fragmentation and loss when individual population sizes are reduced (Kearns et al., 1998; Warburton et al., 2000; Knight et al., 2005; Memmott et al., 2007; Williams and Adam, 2010). Although *F. picrosperma* has persisted through rainforest contraction and expansion in response to glacial-interglacial cycles, it has been affected by anthropogenic habitat fragmentation and so subtle impacts on population genetic diversity are possible due to altered gene flows (Lamont et al., 2016).

In conclusion, *F. picrosperma* displayed floral characteristics, reproductive strategies and pollen limitation that are common amongst woody, dioecious, tropical rainforest species. Seed production for the extraction of tigilanol tiglate could be enhanced by improving pollen

transfer from flowers on male trees to flowers on female trees. This could be achieved by increasing the ratio of male to female trees in plantations. Optimising seed production of *F. picrosperma* could also depend upon improving pollen transfer between male and female trees by managing natural or introduced pollinators and optimising the placement of male trees around female trees. This will rely on a better understanding of gene flow in *F. picrosperma* populations and the identification of flower visitors that contribute most significantly to pollination and fruit set.

Acknowledgements

We thank Tracey McMahon, Anastasia George, Ton Stewart, Daniel Shelley and Katie Roberts for assistance with sample collection and analysis, and the Queensland Department of Environment and Heritage Protection for providing a Scientific Purpose permit. This work was supported by EcoBiotics Ltd and the University of the Sunshine Coast.

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