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Native pollinator management may be a key to improving fruit set in Tasmanian Mountain Pepper, *Tasmannia lanceolata* (Winteraceae), an emerging spice resource

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ABSTRACT

Tasmanian Mountain Pepper is a new crop that produces a valuable flavoring agent derived from its fruit. However, annual yields are unpredictable and its commercial prospects depend upon a reliable fruit set. As poor pollination is a possible cause, the pollination biology of this dioecious plant was examined in natural and plantation settings using direct observations, sticky traps, gas chromatography, and microscopy. A community of small insects visits the flowers, and the insect species represent at least seven families of small flies, three families of beetles, and various wasps and moths. Pollen grains were observed on the bodies of bibionid flies (Bibionidae), syrphid flies (Syrphidae), and snail-flies (Sciomyzidae). The tetrahedral tetrad pollen grains are unlikely to be suitable for dispersal by wind. Similar volatiles were emitted by both males and females; several ocimene compounds are attractants for insect pollen vectors. The female flowers also emitted indole, a known fly attractant. It is therefore likely that *Tasmannia lanceolata* is dependent on the services of diverse insects, especially Diptera, for promoting fruit set. Plantations should be established in the vicinity of biodiverse native vegetation that supports a wide variety of native flying insects capable of transferring pollen.

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Introduction

The Tasmanian Mountain Pepper, *Tasmannia lanceolata* (Poir.) A.C. Smith, is a small dioecious shrub native to the wet forests of Tasmania and southeastern Australia (Morris 2009). It is a member of the Winteraceae, a basal family of plants distributed widely in the southern hemisphere and of interest for its primitive morphology (Gottsberger, Silberbauer-Gottsberger, and Ehrendorfer 1980; Doyle 2000; Feild and Holbrook, 2000). *Tasmannia lanceolata* produces small, purple-black, bilobed berries harvested by the

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emerging Australian native foods industry which are valued for their polygodial content and are sought after as a spicy flavoring agent in food and drinks (Read and Menary 2001). Currently, most fruits sold are collected from the wild; however, production is highly inconsistent and large variations in annual fruit yields have led producers to trial the development of managed plantations (Wilson, Menary, and Close 2016).

Pollination failure is one possible reason for low fruit yield and has been identified as a limiting factor in fruit production across many plant species (Bierzychudek 1981; Klein et al. 2007; Lee 1988). Its management is a key challenge to the horticultural development and success of a new crop, such as Tasmanian Mountain Pepper. To address this challenge in cultivated populations, however, it is important to first establish an understanding of the pollination ecology of *T. lanceolata* within its natural range. The mechanism of pollen dispersal is previously undocumented for Tasmanian Mountain Pepper, although recent speculation alludes to possible movement by insects (Worth et al. 2010). The related *Drimys confertifolia* has been described as being wind pollinated (Bernardello et al. 2001); however, studies of modern and fossil pollen describe *T. lanceolata* as having very poor pollen dispersal, with pollen only recorded in substantial numbers at sites where plants grow (D'Costa and Kershaw 1997; Dodson et al. 1998; Ladd 1979).

Flowering plants synthesize and emit a fascinating range of volatile organic compounds as part of their interaction with the broader environment (Tholl et al. 2006). Examination of the aromatic profile of organic volatiles emitted from flowers and other floral parts can aid in determining pollination vectors (Dobson 2006; Raguso 2008), as odor profiles can be selective for certain flower visitors. A diverse array of airborne compounds has been demonstrated to attract particular insects and other pollination vectors, such as birds, bats, and other mammals (Dodson et al. 1969; Heinrich and Raven 1972; Raguso and Pellmyr 1998; Rieger and Jakob 1988). Furthermore, the presence of key odors in the headspace of developed flowers can also be a useful reinforcement of the significance of particular pollinators (Raguso 2004). For example, the occurrence of unpleasant fecal or carrion-like odors is often associated with the attraction of pollinating flies (Johnson and Jürgens 2010). Some nectar-free plants even mimic the sexual pheromones of insects to attract vectors to visit their flowers (Brodmann et al. 2009; Schiestl et al. 1999; Stökl et al. 2011); however, male flowers of *T. lanceolata* can produce significant amounts of nectar and pollen, whereas females only produce small amounts of nectar.

Sampson (1981) compared pollen development across the Winteraceae, confirming that synchronous pollen mitosis is present in *T. lanceolata*. Anther and ovule development of three related Australian taxa (*T. insipida, T. glaucifolia*, and *T. stipitata*) was profiled by Prakash, Lim, and Sampson (1992), who identified tetrahedral tetrad pollen grain shapes in all three. The pollen tube

development of *T. insipida* has also been comprehensively studied (Frame 2003), including hand pollination and examination of pollen exudate. However, the pollen structure and pollinating agents of *T. lanceolata* have not been reported previously and is known to vary greatly within the genera of the Winteraceae (Sampson 2007). Pollen morphology can be a key indicator of potential pollinators (Cruden and Lyon 1985; Muller 1979). For example, small-sized smooth pollen grains can be indicative of wind pollination (Baker and Baker 1979), whereas larger pollen grains are associated with dispersal via insects (Lee 1978), such as beetles (Coleoptera) (Jones and Coppedge 1999).

Our study seeks to discover the pollination biology and related plant attributes of Tasmanian Mountain Pepper, including its pollen structure and floral scent composition. A better understanding of these interactions should improve our knowledge about the conditions necessary for maximizing fruit set in a commercial plantation setting.

Materials and methods

Pollen structure and properties

Male and female flowers were taken from clones of *T. lanceolata* selected for their potential as commercial planting stock on the basis of high leaf polygodial yield. Male and female plants had been grown in close proximity within a shade house at the Horticultural Research Centre in Hobart, in prevailing temperatures ranging between 5°C and 25°C. Flowers were dissected and their anthers, stamens, ovules, and pistils examined for pollen grains under an FEI Quanta 600 MLA environmental scanning electron microscope (ESEM) (FEI, Hillsboro, OR, USA). A Carl Zeiss Standard RA microscope (Carl Zeiss AG, Oberkochen, Germany) was used with Nomarski Phase techniques to further examine the structure and size of the pollen grains.

Floral headspace

To sample volatiles emitted by flowers, Multix 25 cm × 38 cm oven bags (McPherson's Consumer Products, Glen Waverley, Victoria, Australia) were placed over plants with large clusters of flowers to concentrate the odors. Solid-phase microextraction (SPME) needles were inserted into the bags and the fibers were exposed for 30 min. To separate volatiles, cuttings from leaves were simultaneously taken, and male and female flowers were collected and placed in oven bags before being extracted by SPME in laboratory conditions.

Gas chromatography was conducted with a Varian CP-3800 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and a Bruker-300 Triple Quadrupole benchtop mass spectrometer (Bruker Corporation, Billerica, MA, USA) on the headspace of male and female flowers (Figures 1–3) using SPME

Figure 1. Small, mature plantation of flowering Tasmanian Native Pepper at Birchs Bay used for sampling of potential pollinating insects. A mix of male and female plants, 1.5–2 m tall, are present in each row. Photo: AL.

Figure 2. Male *Tasmannia lanceolata* flowers. Photo: AL.

needles fitted with a Supelco 50/30 micron DVB/CAR/PDMS fiber (Sigma-Aldrich, St Louis, MO, USA) to extract headspace volatiles. An Agilent DB5-MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used, with high-purity helium as the carrier gas at a flow rate of 1.2 ml min^{-1}. The injector

Figure 3. Female *T. lanceolata* flowers. Photo: AL.

temperature was 270°C, the transfer line temperature was 290°C, and the detector temperature was 220°C. The oven temperature was programmed from 40°C (4 min hold) at 6°C min^{-1} to 80°C, then 8°C min^{-1} to 250°C, and then 25°C min^{-1} to 290°C. Identification of components was based on commercial (NIST) and in-house specialized databases and relative retention indices. The mass spectrometer was operated in electron ionization mode at 70 eV scanning from *m/z* 35 to 350 every 0.2 s. Comparisons were made of the headspace profile of the male and female flowers with known insect attractants in the literature and with an online database of pheromones (El-Sayed 2014).

Flower visitor survey

Insects were documented by a combination of trapping and direct observation of their behavior at flowers. Sticky traps were used to sample the airborne insects active in a mature plantation of varied clonal and geographic origin at Birchs Bay, Tasmania (43°11.29′S, 147°14.64′E, Figure 1).

Yellow styrene boards (10 cm × 12 cm) were coated in Tanglefoot™ adhesive (The Tanglefoot Company, Grand Rapids, MI, USA) and suspended by wire ties for 7 days within the canopy of shrubs in full flower (17–23. ix.2016). A total of 24 boards were dispersed through the plantation in a semi-regular grid to sample a representative cross-section of flowering shrubs of both sexes. Weather conditions ranged from cool and overcast to mild and sunny, typical of the austral spring at this latitude. Trapped insects were examined under the microscope to search for any attached *T. lanceolata* pollen grains.

Timed observation surveys were conducted at the plantation at Birchs Bay 2 weeks apart, at flowering. Plants were surveyed for approximately 3 min every

1.5 m along each plantation row. On the first occasion (23.ix.2016), conditions were mainly sunny and moderately warm (13–15°C) during the survey time (11:00–14:00 AEST), with little to no wind. Male plants were mostly at peak anthesis, although many female plants remained in the late flower bud stage, suggesting either a degree of protandry or possibly a consequence of mixed origins of the original planting stock that was sourced from the wild in the last two decades. During the second survey (6.x.2016), weather conditions were less favorable (windy, alternating between overcast and sunny) but warmer (20°C) during the survey time (10:00–14:00 AEST). It was noted that female plants had largely ceased flowering and were developing fruits, while a larger number of male plants remained in flower. At this locality at least, female flowers were receptive for considerably less time than pollen was available. The preceding temperature records have been sourced from the Australian Bureau of Meteorology (BOM) for the two nearest weather stations to this study site, Grove and Dennes Point (BOM, 2016).

For geographical and elevational comparison, an additional survey was conducted on wild *T. lanceolata* populations at two sites on nearby Mt Wellington, Tasmania (17.xi.2016, 12:00–18:00 AEDT). Weather conditions were warm and calm, with a maximum temperature of 19.3°C at the summit 1280 m (BOM, 2016). Two small male and two large female *T. lanceolata* plants were in peak to late flowering stage at Site A (42°53.52'S, 147°14.08′E, 1000 m). Several individuals at different stages of flowering were identified at Site B (42°53.33′S, 147°13.17′E, 1130 m), with some approaching peak anthesis and others at the budding stage.

Insects were identified to at least family level and allocated to feeding guilds to gain an appreciation of their ecological context. Insect images annotated "AL" are by Adelina Latinovic.

Results

Tasmannia lanceolata flowers featured deciduous sepals and a variable number of yellow or cream narrow oblanceolate petals. Male flowers had numerous stamens and produced copious yellow pollen, shed as tetrads. Flowers of both sexes produced small amounts of nectar.

Anthesis

In the plantation, female flowers were available over a shorter period than male flowers. Observations from a nearby wild population (Mt Wellington, approximately 1000 m) also suggested that female anthesis was shorter and consequently pistillate flowers had a limited time over which they were receptive to male pollen.

A large number of pollen grains were present in the anthers of male flowers (Figure 5) and a few pollen grains were observed by light microscopy on the stigma of the female plants sampled. A tetrahedral tetrad morphology of the pollen grains was confirmed. The pollen was relatively large and approximately 26 μm in diameter (range 25.92–27.9 μm, n = 10), with the outer exine heavily reticulate (Figures 4, 5).

Floral head-space

Gas chromatography of the headspace of male and female flowers revealed a strong similarity in their composition profiles. The volatile monoterpenes *trans*-β-ocimene (IUPAC trans–3,7-dimethyl-1,3,6-octatriene) and *cis*-β-ocimene (IUPAC cis–3,7-dimethyl-1,3,6-octatriene) were found in both cut and

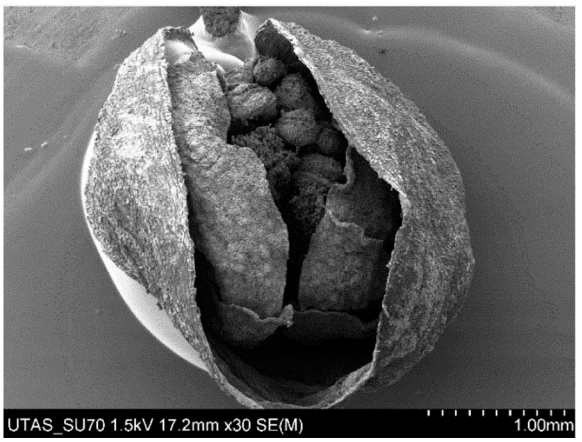

Figure 4. Male flower bud of *T. lanceolata*.

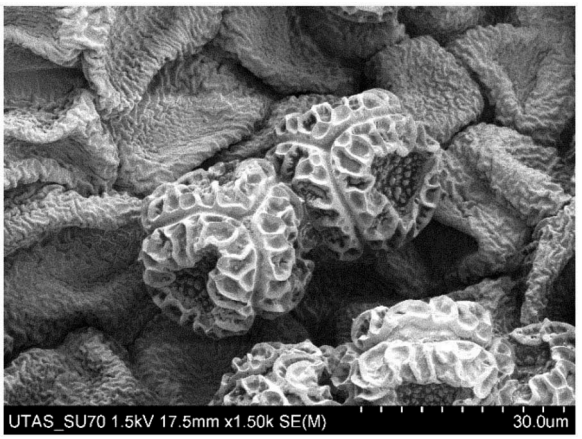

Figure 5. Pollen grains of *T. lanceolata*.

living floral samples of male and female plants, with only traces of these isomers found in cut leaf samples of either sex (Figure 6). Alloocimene (IUPAC 2,6-dimethyl-2,4,6-octatriene) was observed in some flower samples of both sexes, and the common floral fragrance compound indole (IUPAC 1H-indole) was also observed in female flower samples.

Pollen vectors

Numerous native insects, especially flies (Diptera) and microwasps (mainly Chalcidoidea), were detected on the sticky traps (Figure 7, Table 1). A small number of beetles were also trapped near flowers of both sexes at the Birchs Bay site, notably *Atoichus bicolor* (Tenebrionidae) and a small species of *Lebiini* (Carabidae). European honey bees (*Apis mellifera*) selectively visited male flowers in the plantation. A few bees were observed collecting pollen on male flowers across several days in October 2015; however, none was seen to visit female flowers at any time. No native bees were observed or captured, although small members of the Apidae (*Exoneura* spp.) and Halictidae (*Lasioglossum* spp.) would be likely to be common in the immediate area given its proximity to bushland. Native parasitic wasps (Ichneumonidae and

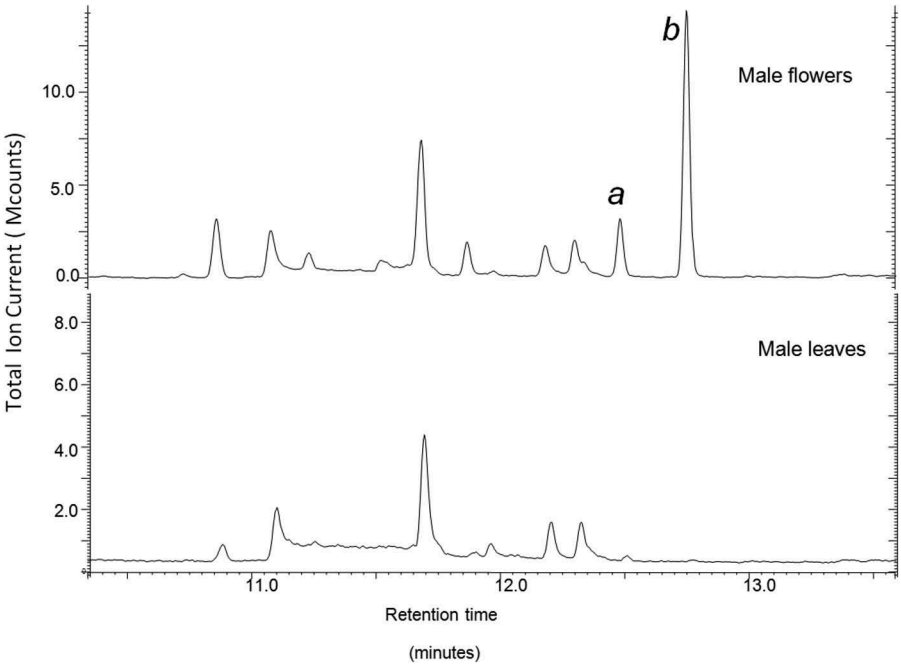

Figure 6. Comparison of GC chromatograms (10.0–13.5 minutes) of male leaves + flowers (above) and male leaves only (below), highlighting the presence of *cis*-β-ocimene (*a*) and *trans*-β-ocimene (*b*) in the floral odor.

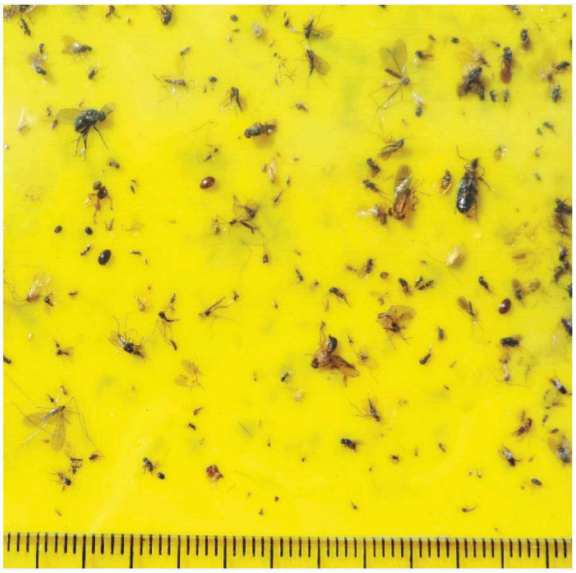

Figure 7. Insects, mainly Diptera, on sticky trap exposed near male flowers of *T. lanceolata*. Scale in mm. Photo: AL.

Table 1. Taxa and guild membership of insects most commonly captured on sticky traps exposed in the canopy of flowering *Tasmannia lanceolata*

Diptera	Coleoptera	Hymenoptera	Other orders
Bibionidae † d	Carabidae (Lebiini) † pr	Bethylidae p	Hemerobiidae pr
Cecidomyidae h	Cleridae † pl, pr	Braconidae † p	Psocoptera f a
Chironomidae † d	Coccinellidae † pr (*Rhizobius, Halyzia*)	Diapriidae † p	Psyllidae h
Chloropidae h	Elateridae † d, pl	Ichneumonidae † p	
Empididae pr	Lathridiidae f	Microwasps p	
Heleomyzidae † p	Phalacridae f, pl		
Keroplatidae f	Scarabaeidae h, pl		
Lauxaniidae † d	Scirtidae d a		
Muscidae † d	Staphylinidae pr		
Mycetophilidae f	Tenebrionidae † d, pl (*Atoichus*)		
Phoridae d			
Platystomatidae f			
Psychodidae d			
Sciaridae f			
Sciomyzidae † p			
Syrphidae † pl, pr			
Tachinidae † p			
Tephritidae s			
Tipulidae † d			

Those also observed on flowers are annotated †. Guilds are as follows: a, algivore; d, detritivore; f, fungivore; h, herbivore; p, predator; pa, parasite; pl, pollen feeder; and s, saprophyte.

Tiphiidae) were also observed on the foliage adjacent female inflorescences, investigating the flowers and buds.

Many of the trapped flies were clothed in fine hairs or bristles suitable to entrap pollen when visiting flowers. *Tasmannia lanceolata* pollen was

positively identified on the leg of a small muscoid fly and on the head of a larger sciomyzid fly (Sciomyzidae) trapped in a female plant canopy. No sciomyzid flies were found on sticky traps placed after flowering had ceased. No pollen grains were found on any of the other insects collected. The medium-sized fly *Tapeigaster argyrospila* (Heteromyzidae) breeds in forest fungi and was present on most traps.

A variety of insect visitors were recorded during both timed observation surveys at the commercial *T. lanceolata* plantation (Figures 8–16). During the first survey (23.ix.2016), four darkling beetles [*Atoichus bicolor* (Tenebrionidae)] dusted in pollen were observed actively wandering between male flowers and on adjacent stems (Figure 10). Blowflies [*Calliphora stygia* (Calliphoridae)] were observed resting on leaves adjacent to male flowers and two small unidentified flies (<2 mm in length) were noted to be resting on female flowers. The results of the second survey (6.x.2016) are presented in Table 2, and it should be noted that outside of the timed survey period, a solitary, introduced bumblebee [*Bombus terrestris* (Apidae)], small unidentified flies, and a hoverfly [*Melangyna viridiceps* (Syrphidae)] were opportunistically observed visiting male flowers. Floral visitors appeared to include a diverse range of Diptera, such as blowflies (Calliphoridae), hoverflies (Syrphidae), stiletto flies (Therevidae), crane flies (Tipulidae), and other small unidentified dipterans. Of the Coleoptera, *Atoichus bicolor* was the only consistently observed floral visitor. Hymenopteran insects at flowers included ants [*Iridomyrmex* sp. (Formicidae)] and a flower wasp (Tiphiidae). Potential pollen or nectar feeders included ladybirds [*Harmonia conformis* (Coccinellidae)], darkling beetles [*Lepispilus sulcicollis* (Tenebrionidae)], and plant bugs [*Pseudopantilius* sp. (Miridae)].

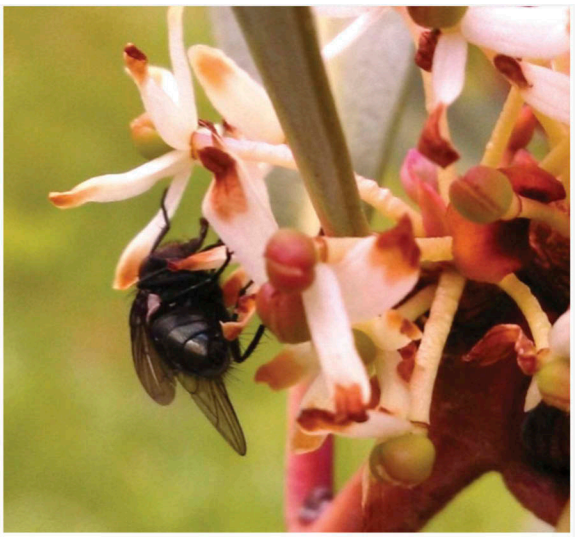

Figure 8. Fly (Muscidae) on female flower. Photo: AL.

Figure 9. Hover fly *Melangyna* (Syrphidae) on male flower. Photo: AL.

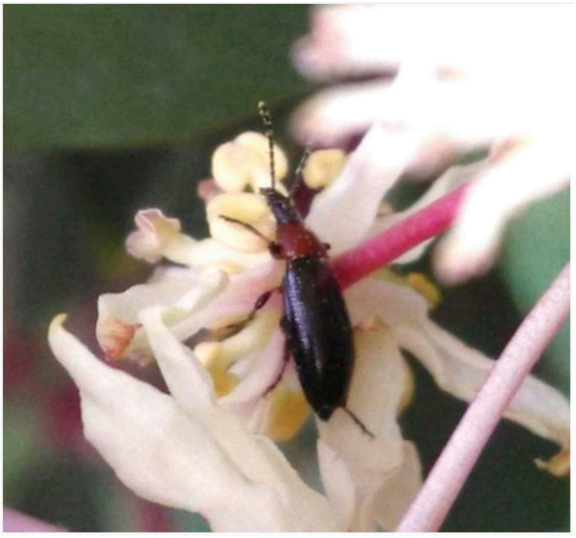

Figure 10. Beetle *Atoichus* (Tenebrionidae) on male flower. Photo: AL.

The survey of wild *T. lanceolata* populations at Site A and Site B on Mt Wellington (17.xi.2016) revealed a variety of insect visitors, especially on female flowers (Table 2), and small flies, wasps, and medium-sized beetles were observed feeding on the nectar (Figures 8–16). Bibionid flies (Bibionidae) at female flowers appeared to be well dusted in yellow pollen (Figure 15). With regard to Lepidoptera, small diurnal tortricid moths were observed flying about female plants (16:30 AEDT) and were noted pausing to feed from pistillate flowers (Figure 14). While a large majority of *T.*

Figure 11. Midge (Chironomidae) on female flower. Photo: AL.

Figure 12. Microwasp (Diapriidae) on female flower. Photo: AL.

lanceolata plants surveyed were dioecious, numbers of monoecious and hermaphrodite flowers were observed in a single clonal selection and could be important for future horticultural development.

Discussion

We have found that pollination in the Tasmanian Mountain Pepper is mediated by a diverse community of native insects and wind pollination is

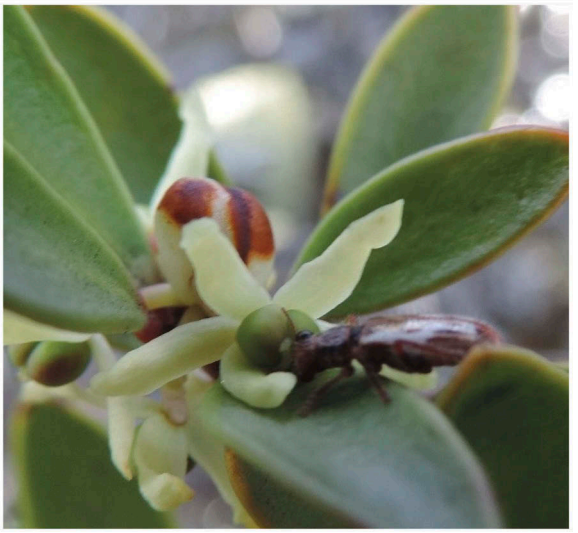

Figure 13. Clerid beetle (Cleridae) at female flower. Photo: AL.

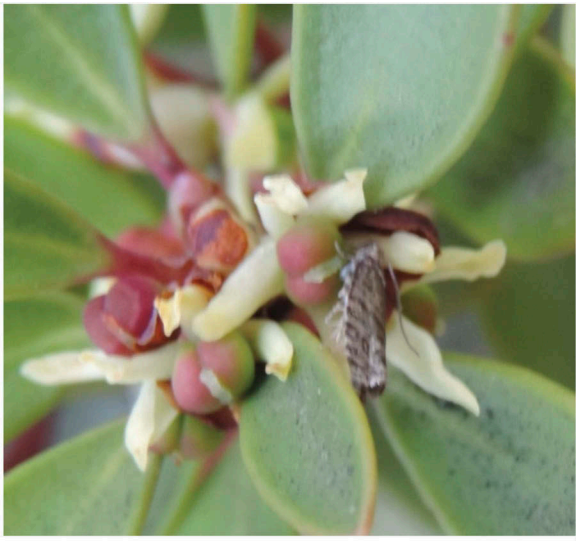

Figure 14. Moth *Epichorista* (Tortricidae) at female flowers. Photo: AL.

very unlikely. Evidence for this accrues from floral and pollen morphology, the profile of headspace volatiles emitted by flowers, and field observations of interactions between the flowers and insects in both plantation and wild settings. Given that male flowers have 20–35 stamens (Morris 2009), pollen production per plant is high and not likely to be a limiting factor in pollination success.

However, the window of opportunity for insect activity is often narrow in the austral spring when poor weather conditions can reduce visitation rates

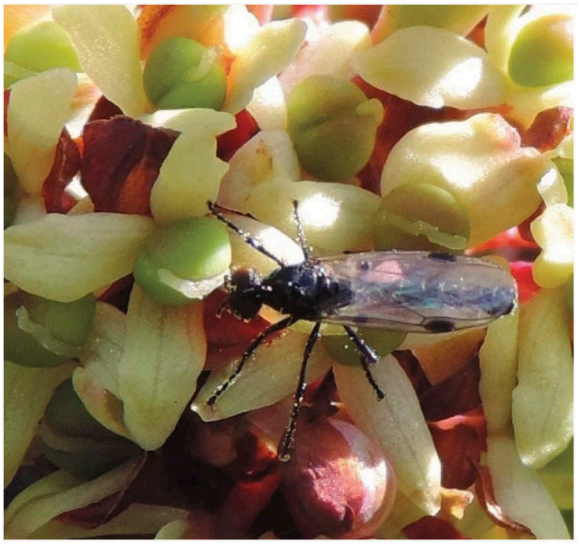

Figure 15. Bibionid fly *Dilophus* (Bibionidae) at female flowers. Photo: AL.

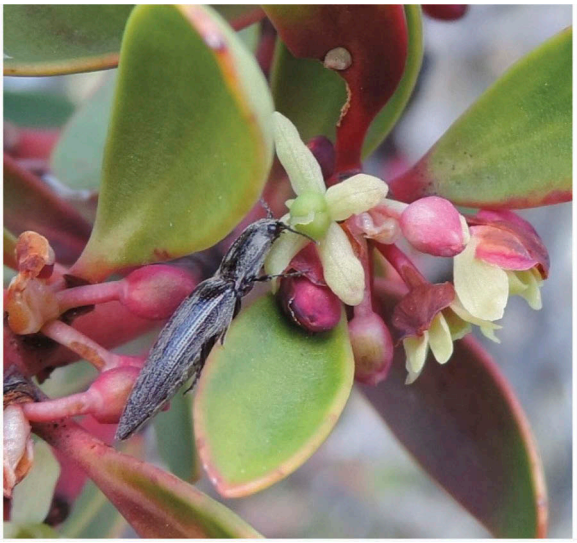

Figure 16. Click beetle (Elateridae) at female flowers. Photo: AL.

at flowers. Honeybees can operate at lower temperatures, but were not seen to visit female flowers, suggesting reliance on native insects for pollen transfer is necessary in plantations.

Pollen structure

Comparisons of ESEM images of pollen grains of *T. lanceolata* with those of *Drimys winteri* (Roland 1971; Sampson 1981), a widely studied member of

Table 2. Insect taxa and their behavior observed on or near male (♂) and female (♀) flowers in the canopy of *Tasmannia lanceolata* in plantation (Birchs Bay) and wild (Mt Wellington) populations

Birchs Bay		
Blowfly (Calliphoridae) actively visiting flowers; resting near flowers		♀
Crane fly (Tipulidae) hovering over flowers		♀
Small flies (<2mm) at flowers		♀
Stiletto fly (Therevidae) investigating flowers		♀
Hoverfly *Melangyna viridiceps* (Syrphidae) at flowers; feeding on pollen	♂	
Ladybird *Harmonia conformis* (Coccinellidae) on leaves adjacent flowers	♂	♀
Darkling beetle *Atoichus bicolor* (Tenebrionidae) on flowers	♂	♀
Darkling beetle *Lepispilus sulcicollis* (Tenebrionidae) on leaves adjacent flowers	♂	♀
Plant bug *Pseudopantilius* sp. (Miridae) on stems adjacent flowers	♂	♀
Ants (Formicidae) on flowers	♂	
Flower wasp (Tiphiidae) on leaves adjacent flowers	♂	♀
Mt Wellington		
Fungus gnats (Sciaridae), hovering about flowers	♂	
Midges (Chironomidae), visiting flowers		♀
Scuttle flies *Megaselia* sp. (Phoridae), resting by flowers		♀
Bibionid flies *Dilophus* sp. (Bibionidae), orienting on flowers dusted in yellow pollen		♀
Midge (Chironomidae), feeding on nectar		♀
Tachinid fly (Tachinidae), inspecting buds and flowers		♀
Unidentified fly (4 mm), inspecting flowers		♀
Microwasps (Hymenoptera), hovering about flowers		♀
Beetle *Lemidia cicatricosa* (Cleridae), crawling about flowers and feeding on nectar		♀
Click beetle (Elateridae), feeding on nectar		♀
Leaf beetle *Arsipoda* sp (Chrysomelidae), crawling about leaves and flowers		♀
Diapriid wasp (Diapriidae), feeding on nectar		♀
Ichneumonid wasps (Ichneumonidae) inspecting buds and flowers		♀
Epichorista moths (Tortricidae), flying about flowers, feeding on nectar		♀

the Winteraceae, revealed strong similarities in shape, with *T. lanceolata* grains being more spherical. The ESEM revealed pollen grains on both male and female floral parts, with particularly large numbers observed on the anthers of the male flowers, suggesting that the sampled clones were producing adequate quantities of pollen.

Floral head-space

Little information exists on the comparison of headspace volatiles of male and female flowers of other dioecious plant species. Percentage sugar content from pollen exudate of the related *T. insipida*, found in eastern New South Wales and Queensland, was found to be similar in both male and female plants (Frame 2003). This supports the results of this study where phytochemistry of the headspace volatiles was very similar in male and female flowers of *T. lanceolata*. Conversely, Ashman et al. (2005) found that scent emission rates of unisexual female flowers were lower than that of hermaphrodite flowers in wild strawberries (*Fragaria virginiana*) and concluded that the aroma produced by the stamens in the hermaphrodite flowers led to pollinators visiting these flowers in

preference. In many other dioecious species, male flowers offer more rewards to pollinators than female flowers (Willson and Jon 1989), to the point that some female flowers have been shown to mimic the properties of male flowers to encourage visitors (Bawa 1980a). One could speculate that the similarities of floral headspace found in male and female *T. lanceolata* flowers could reflect the evolutionary basal nature of the genus, as seen in its less developed forms of water transport (Feild and Holbrook 2000), floral development (Doust and Drinnan 2004), and floral morphology (Doust 2000). However, Jurgens (2009) notes that recent studies have identified a strong floral odor and diversity of floral scent across many extant basal angiosperms and provide evidence for the complex rather than primitive nature of their scent in certain species.

There is evidence of generalization in the floral scent profile of *T. lanceolata*. While odorous compounds were present, no pheromones were identified likely to target particular insect taxon. Monoterpenes dominated the scent profile and ocimenes were present in many floral scents known to attract small flies and some bees (Chen et al. 2012; Dobson 2006). The isomers *trans*-β-ocimene and *cis*-β-ocimene have previously been identified in the leaf and stem bark extract of another member of the Winteraceae, *Drimys brasiliensis* (Ribeiro et al. 2008) and are also found in a wide variety of plants and fruits. Ocimenes commonly exist in nature as mixtures of various isomers and these, as well as the pure compounds, have a pleasant odor, likely to be attractive to insects (Dobson 2006). However, the presence of indole in the female scent profile is noteworthy, as this compound has a fecal smell and is implicated in the attraction of flies to certain flowers (van der Niet, Hansen, and Johnson 2011; Zito, Dötterl, and Sajeva 2015). Consequently, it may be used to deceptively attract flies to female *T. lanceolata* flowers in the absence of a pollen reward.

Pollen vectors

The wide geographical and elevational distribution of *T. lanceolata*, from the far south of Tasmania (latitude 43°S) to the Blue Mountains of central New South Wales (latitude 33°S), including areas of southern Victoria, significantly warmer than montane Tasmania, could implicate either a single widespread pollinator adapted to a wide range of environments or else local communities of pollinators as responsible for pollen transfer. Our observations from both the sea-level and higher altitude sites in Tasmania revealed the recruitment of broadly similar communities of flies, beetles, and wasps and confirm the absence of a single dominant pollinator.

The morphology of the pollen grains, aromatic scent profile of the flowers, presence of nectar, and small stigmatic area of *T. lanceolata* suggested that wind pollination was an unlikely strategy used by this plant. Although wind

is responsible for the pollination of approximately one-third of plant genera containing dioecious species (Culley, Weller, and Sakai 2002; Renner and Ricklefs 1995), anemophily is rare in dioecious plants found in tropical environments and infrequent in more temperate locations, such as Hawaii and New Zealand (Bawa 1980b).

Our discovery of small insects as likely pollinators of *T. lanceolata* is consistent with some observations on Winteraceae elsewhere (Lloyd and Wells 1992; Thien 1980). Chironomid flies (reported as a *Smittia* sp.) are attracted to the stigmatic exudates on *Pseudowintera* in New Zealand (Lloyd and Wells 1992) and the widespread *Smitta aterrima* is also present in Tasmania (Freeman 1961). Sciomyzid flies are endoparasites of snails and occur most commonly in damp environments or at forest margins. Widespread in wet forests both in Tasmania and in SE Australia, the species on which many pollen grains were observed is likely to be a key pollen vector for the plant, possibly alongside the more commonly observed *Tapeigaster argyrospila*. Records of sciomyzid flies at flowers are otherwise scarce globally. Judd (1964) reported three species of sciomyzids feeding on another primitive dicot, the marsh marigold (*Caltha palustris* Ranunculaceae), whereas Hopkins and Carruth (1954) observed sciomyzids at the flowers of *Tamarix* in Arizona, and a single sciomyzid, among other flies, was seen on flowers of *Hamamelis virginiana* in Connecticut (Anderson and Hill 2002). The common syrphid fly (*Melangyna viridiceps*) is an annual immigrant to Tasmania from mainland Australia. Large numbers of these flies arrive in September and undergo a summer breeding cycle (Hill 2013). It is attracted to the white, cream, and yellow flowers of numerous native and introduced plants.

Beetles (Coleoptera) can have an important role in pollinating dioecious plants (Bond 1994) and must be considered a potential vector of *T. lanceolata* pollen. Beetles visit Winteraceae in Papua New Guinea, but were not considered to be a primary pollinator, suggesting a role for other types of insects (Thien 1980). We observed beetles among *T. lanceolata* plants at the Birchs Bay and Mt Wellington sites but not at the Horticultural Research Centre. A variety of insects, including beetles, flies, and thrips (Thysanoptera), are reported to attend the flowers of *Drimys brasiliensis* (Winteraceae) in Brazil (Gottsberger, Silberbauer-Gottsberger, and Ehrendorfer 1980).

The ichneumonid wasps observed in the vicinity of the flowers are possible parasites of moth larvae, which occur on the plant in Tasmania, including a tortricid leaf-tier [*Epiphyas caryotis* (Meyrick)] and a geometrid browser [*Archephanes zalosema* Turner] (McQuillan 1992; Young and McQuillan 2003). The tiphiid flower wasps observed on the shrubs are parasites of soil-dwelling scarab beetle larvae common in the adjacent grasslands.

Finally, the observed foraging behavior of honeybees suggested that they acted to deplete the pollen resource of the male flowers without transferring the pollen to the pistil. The learning capacity of honeybees to recognize

unrewarding flowers (Dukas 1987; Menzel and Erber 1978) might explain why female flowers were avoided by them.

We conclude that *T. lanceolata* is an entomophilous plant that exploits a generalized profile of local insects, notably flies, beetles, and wasps. The odor profiles of the male and female flowers did not yield evidence of a specialist pollinator type, with no discriminating volatiles indicative of a particular vector group detected.

The unreliable fruit set seen in wild populations of *T. lanceolata* may in part reflect the efficacy of the local pollinator guild and variable weather. This could be attributable to competition for pollinators, as *T. lanceolata* flowers in late September–October when much of the sympatric plant community also reaches anthesis. Also pertinent is that the spring climate in montane Tasmania is highly variable year to year, with often extended periods of cold, windy, or overcast conditions prevailing, which can suppress the foraging activity of small flying insects. Similarly, unseasonally warm spring weather (Grose et al. 2014) may hasten anthesis and risk decoupling the synchronicity of insect emergence and flower availability.

This study highlights the importance of maintaining viable populations of native insects, especially flies, in the vicinity of plantations and the need to better understand their ecological requirements. Many of these vectors have specialized life histories as parasites, predators, and fungivores and the retention of biodiverse native vegetation in the vicinity of plantations should therefore be encouraged.

Future experiments could examine how the effect of genotype diversity in a plantation affects fruit production variability and observe geographic and clonal variations in fruit production. While a large majority of the *T. lanceolata* plants surveyed were dioecious, both monoecious and hermaphroditic plants have been observed in a single clonal selection. These could be of commercial importance in the future and would also help minimize the need for male plants that represent reduced productivity for fruit producers.

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