Reproductive biology of rainforest Rutaceae: floral biology, breeding systems and pollination vectors of *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*

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Abstract: The conservation of plant species requires an understanding of the factors that affect viable seed production, but often these factors are poorly understood. We investigated the reproductive biology of two Australian endemic rainforest species, *Acronychia oblongifolia* (A.Cunn. ex Hook.) Endl. ex Heynh and *Sarcomelicope simplicifolia* (Endl.) T.G.Hartley subsp. *simplicifolia*, with the intent of improving conservation and restoration outcomes.

The floral biology of these species was quantified to provide baseline data and insights into their pollination syndrome. Flower visitor surveys (using both digital recordings and human observations), a manipulative wind pollination experiment, and hand-pollination experiments were carried out to investigate pollination vectors and determine the breeding system.

Acronychia oblongifolia and *Sarcomelicope simplicifolia* subsp. *simplicifolia* were both found to best fit the general entomophily pollination syndrome. All floral visitors were arthropod species (*Acronychia oblongifolia*: 31; Sarcomelicope *simplicifolia* subsp. *simplicifolia*: 47) and fewer than 30% of the floral visitors identified, predominantly Diptera, Hymenoptera and Coleoptera, were regarded as potential pollinators. Failure of simulated wind gusts (40 km h^{-1}) to transport pollen 50 cm indicated anemophily is unlikely for these species. Autonomous and manipulative selfing treatments produced few (*Acronychia oblongifolia*: <3%) or no (*Sarcomelicope simplicifolia* subsp. *simplicifolia*) viable seed, indicating these are predominantly outcrossing species, although fruit and viable seed production were highly variable within and among all other treatments (open to natural pollinators, pollinator exclusion, pollinator exclusion and manipulative outcross, and pollinator exclusion and manipulative selfing). Pre-dispersal seed predation was recorded for both species, at several study sites. Pre-dispersal seed predation and increased distances between compatible individuals caused by habitat fragmentation, are two factors limiting the production of viable seeds for both species.

Key words: Viable seed, entomophily, entomophilous, White Aspen, Bauerella, Yellow-wood, Hard Aspen.

Introduction

Viable seed contains a live embryo that can germinate under appropriate conditions (Bradbeer 1988), and therefore, the reproduction of non-clonal angiosperms depends on the production of viable seed. Many factors, both biotic and abiotic, affect seed set, seed development and seed maturation (Burd 1994; Fenner & Thompson 2005; Chen & Zuo 2019). As a result, viable seed production in angiosperms is highly variable. Some common hypotheses for the factors that prevent viable seed set include inbreeding (Baskin & Baskin 2015), pollen limitation (Stephenson 1981), resource limitation (Lee 1988) and seed predation (Auld 2001; Armstrong 2002).

Over 85% of angiosperms require a biotic pollination vector for outcross seed set (Ollerton *et al.* 2010). Species with variable or poor fruit production, and in turn seed set, are often hypothesized to be pollen limited (Stephenson 1981; Knight *et al.* 2005; Chen & Zuo 2019). An inadequate supply of viable and compatible pollen grains, or a low abundance of biotic pollinators, can cause pollen limitation (Chen & Zuo 2019). However, for many species the ecological interactions needed for seed production are unknown; this includes whether a pollination vector is needed to set seed and, if so, what the pollination vector is (Williams & Adam 2001; Bennet *et al.* 2018). This significant gap in our understanding of the factors that affect viable seed production has impeded conservation and restoration for some angiosperm species (Martyn *et al.* 2009).

Viable seed production is known to be variable in some species, particularly Rutaceae (Auld 2001; Martyn *et al.* 2009). Rutaceae are common and widespread in rainforests of eastern Australia (Mills & Jakeman 1995); however, these rainforests in the Illawarra landscape are now highly fragmented communities, as a result of post-European settlement (Mills 1988; Mills & Jakeman 1995), particularly clearing for agriculture. *Ex situ* conservation is vital to provide insurance against extinction for individual species and populations, and to support *in situ* management and restoration (Sommerville *et al.* 2017). Seed banking is considered the most effective *ex situ* conservation technique (Offord & Meagher 2009); however, seed banking hinges on sourcing and storing viable seed (Martyn Yenson *et al.* 2021). Nevertheless, the factors influencing viable seed production in rainforest Rutaceae are rarely examined (Auld 2001; Armstrong 2002; Martyn *et al.* 2009).

Acronychia oblongifolia and *Sarcomelicope simplicifolia* subsp. *simplicifolia* are Rutaceae species that inhabit temperate and subtropical rainforests of eastern Australia (DPIE 2019a; DPIE 2019b). Both have wide coastal distributions, *Acronychia oblongifolia* from Queensland to Victoria, and *Sarcomelicope simplicifolia* subsp. *simplicifolia* from North Queensland to Mt Dromedary on the NSW South Coast. Seed set is known to be variable in both species, but the causes of this remain unknown (Martyn *et al.* 2009). *Sarcomelicope simplicifolia* subsp. *simplicifolia* is a dioecious tree (Pellow *et al.* 2011), while *Acronychia oblongifolia* produces hermaphroditic flowers (PlantNET

2020). No other aspects of the reproductive biology of these species have previously been studied.

The aim of this study was to investigate the floral biology, pollination vectors and breeding systems to determine the factors contributing to seed production in these two common rainforest species. Specifically, this study aimed to: 1) examine the floral morphology and phenology of *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* to provide insights into the likely pollination syndrome and breeding system; 2) identify floral visitors and, by analysing the foraging behaviour and pollen load of those visitors, identify the likely pollinators; 3) determine whether anemophily plays a role in pollination of these species; 4) confirm the breeding system by quantifying fruit and seed set in response to hand pollination experiments.

Methods

Study sites

This study was conducted between March 2020 and May 2021 on plant populations located in remnant rainforest, restored rainforest and botanic gardens mainly in the Illawarra area south-eastern New South Wales (NSW), Australia. *Acronychia oblongifolia* trees were located at two sites: one restored population at Jerrara Dam Reserve (34°40′19″S, 150°48′22″E) (hereafter Jerrara) and one planted population at the Australian Botanic Garden, Mount Annan (34°04′14″S, 150°46′04″E) (hereafter Mount Annan). The original provenance of the trees at Mount Annan was Boatharbour Nature Reserve, New South Wales. *Sarcomelicope simplicifolia* subsp. *simplicifolia* trees were studied at four sites: three restored populations at Jerrara, Spring Creek Wetland Kiama (34°39′41″S, 150°50′48″E) (hereafter Kiama) and Spring Creek Wetland South Kiama (34°39′48″S, 150°50′40″E) (hereafter South Kiama); and one planted population at Wollongong Botanic Garden (34°24′34″S, 150°52′30″E) (hereafter Wollongong). The trees planted at Wollongong were sourced from natural populations in the Illawarra region (Carl Glaister pers. comm.).

The methods for studying the floral biology, floral visitors and pollination vectors, and the breeding systems followed those described in Lopresti *et al.* (Under Review).

Floral biology

To examine the floral phenology of each species, 20-40 flowers from 2-4 trees were observed daily from floral opening to abscission or fertilisation (following Kearns & Inouye 1993). On each day of observation, the anthers and stigmas of each flower were classed as immature, mature or senesced. The anthers were considered mature when pollen grains were visible when viewed through a hand lens. Stigma maturity was based on the size and colour of the stigma. Stigma receptivity was confirmed by a hydrogen peroxide test (following Kearns & Inouye 1993). For each species, 10 flowers each from individual plants thought

to contain mature stigmas were placed in a petri dish and individually examined under a 40 x dissecting microscope. Hydrogen peroxide (3% v/v) was dropped onto each stigma and observed for 60 seconds. The formation of bubbles on the stigma was considered an indication of receptivity. The first day that the androecium (male) and gynoecium (female) appeared mature, the duration of maturity, and the total anthesis period were recorded for each species.

Pollen morphology and exine characteristics were described as outlined by Halbritter *et al.* (2018) to gain insight into potential pollination vectors, and an indication of breeding system. Pollen characteristics described included: the shape (both equatorial (E) and polar (P) views); the length of the polar axis compared to the equatorial diameter (the P/E ratio); the dispersal unit of pollen grains; and the ornamentation on the aperture and exine regions. Stamens from three flowers of each target species were examined under a scanning electron microscope (JEOL JSM-6490LA, Japan) on the day of collection (*Acronychia oblongifolia*) or following a brief period of refrigerated storage (*Sarcomelicope simplicifolia* subsp. *simplicifolia*). Samples were mounted on a metal stub using conductive double-sided carbon tape and sputter coated with 20 nm gold using an Edwards AUTO 306 Sputter Coater (Edwards Australia, Yatala, Queensland). Imaging was completed in high vacuum mode at 10 mm working distance using SEI/BSE imaging at 15 KV operating voltage and with a spot size setting of 45.

Nectar production was measured to determine whether flowers provided rewards to potential pollinators. Prior to quantifying nectar production, inflorescences that housed at least five buds near to opening were selected haphazardly and bagged using a hard plastic inner layer of coarse mesh (Saxon Gutter Guard) covered with polyorganza fabric (15 cm x 35 cm) to prevent biotic visitors extracting nectar prior to the flowers opening. For *Acronychia oblongifolia*, nectar was extracted from 10 flowers with a mature androecium and 10 flowers with a mature gynoecium at Mount Annan. For *Sarcomelicope simplicifolia* subsp. *simplicifolia*, nectar was extracted from five flowers with a mature androecium from staminate trees, and five flowers with a mature gynoecium from pistillate trees, at each of Wollongong, Jerrara, Kiama and South Kiama. Nectar was extracted into a 1 µl or 5 µl microcapillary tube and the volume withdrawn was determined by calculating the proportion of the column that was filled (in accordance with Morrant *et al.* 2009). Nectar produced in flowers with a mature androecium and a mature gynoecium were compared using a t-test on untransformed data (data were normally distributed). Due to the small sample size, data were pooled across sites.

Floral visitors and biotic pollination vectors

Any fauna species that contacted a flower were regarded as floral visitors. A combination of diurnal and nocturnal human observations, video recordings, time lapse photography (TLP), and nocturnal infrared motion sensor imaging were used to observe floral visitors. Sampling was conducted on 2-6 trees at 2-4 sites for each species (Table S1 details the survey effort). Observations were done on sunny days with

no or light wind speeds (gusts did not exceed 20 km h^{-1}), and temperatures ranged between 17°C and 32°C.

Diurnal human observations and video recordings were conducted during the day (10:00-15:00). TLP was conducted from first light to last light, to capture species that may forage outside the 10:00–15:00 period. Infrared cameras were recording between last light and first light, and nocturnal human observations were undertaken for three hours commencing after last light. Sampling was done on the outermost part of canopy containing high floral densities, and on flowers containing mature reproductive structures, for all sampling methods.

Human observations were done on six fixed sections (50 cm x 50 cm) of canopy on each tree. Floral visitors were opportunistically captured following their foraging bout and euthanized. Captured specimens were identified and their pollen load was analyzed under a stereo microscope (40 x magnification).

Digital video recordings (DVR, 1080p resolution) of inflorescences containing a high proportion of open flowers were obtained using GoPro Hero4 Session CHDHS-101 digital cameras (GoPro Australia Pty Ltd) attached to a tripod. Up to six DVRs were simultaneously recording for a two-hour period on each day that observations were done. Each camera had a 15 cm x 30 cm field of view.

Time-lapse photographs (TLP) were captured on Brinno TLC200 Pro HDR cameras (Brinno Incorporated, Taiwan). Over three non-consecutive days, three time-lapse cameras were set to capture an image every two seconds between sunrise and sunset (at four study sites). The field of view of each camera captured a 15 cm x 30 cm area of canopy. TLP was employed to guide the survey effort for DVR and diurnal human observations.

Nocturnal human observations were conducted over three non-consecutive nights at one (Jerrara: *Acronychia oblongifolia*) or three (Jerrara, Kiama, South Kiama: *Sarcomelicope simplicifolia* subsp. *simplifcifolia*) study sites. Sampling was conducted in one 3 h block each night, starting at last light. A battery-operated head torch was used to scan floral patches for 5 seconds (following Hermansen *et al.* 2014). Two observers scanned the canopy every 55 seconds, and each hour a new tree was surveyed. *Acronychia oblongifolia* trees flowered asynchronously, so scans were done on the most suitable tree each night (suitability based on individuals with high floral density). Nocturnal human observations were not carried out at Wollongong or Mount Annan due to evening access restrictions.

Nocturnal infrared camera recordings (ICR) were captured on Bushnell Trophy Cam HD Essential E3 Trial motion sensor cameras. Cameras were set to record a single inflorescence (camera field of view 15 cm x 30 cm) from last light to first light. Over three nights, three cameras were set at both Mount Annan and Jerrara for *Acronychia oblongifolia* sampling. For *Sarcomelicope simplicifolia* susbp. *simplicifolia*, two nocturnal cameras were set to record over two nights at each of the four study sites. ICR was employed to guide the survey effort for nocturnal human observations.

Floral visitors that either carried pollen of the target species on their body or contacted the stigma while foraging were classed as potential pollinators (following Kearns & Inouye 1993; Dafni 1992). To distinguish floral visitors from potential pollinators for species detected by DVR, contact with the stigma was recorded for all floral visitors. Pollen presence for captured individuals was examined under a stereo microscope (40 x magnification). Specimens carrying pollen were then examined under a scanning electron microscope (SEM) (JEOL JSM-6490LA, Japan) to examine whether the pollen was that of the target Rutaceae species. Specimens were mounted as previously described for scanning electron microscopy.

To determine whether there was a dominant pollinator taxon, the percentage of sampling intervals in which a potential pollinator of a given taxon was present (hereafter referred to as 'presence') was compared among species for each of the observational methods that a potential pollinator was detected by: human observations and DVR. Potential pollinator presence was standardized by the duration of the sampling interval (5 minutes for human observations and 2 hours for DVR). 'Presence' provided an estimate of the visitation frequency for each potential pollinator observed under each survey method.

To examine whether the presence differed among potential pollinators, a one-way analysis of variance (Kruskal and Wallis 1952) was applied for human observation and DVR data as normality and equal variance assumptions were not met for transformed data. A Dunn All Pairs for Joint Ranks test was undertaken when a significant difference arose. Data were pooled across sites for all of these analyses as pollinator species differed among sites. All data were analysed using the statistical software JMP Pro 15.

Wind pollination

To examine whether wind may be a pollination vector, the pedicels of flowers that contained visible pollen (viewed through a hand lens) were taped to a rod so that the anthers were exposed. A fan was used to generate wind speeds (5 km h^{-1} and 40 km h^{-1}) blowing onto each flower for 10 seconds. A 9 cm Petri dish smeared with Vaseline was placed behind each open flower to capture any dislodged pollen. Wind speeds were measured using a handheld anemometer (Kestrel® 3500, accuracy ± 3%) and were selected based on below and above average wind speeds within the Illawarra region (recorded at Bellambi AWS from 1997-2010, weather station ID 068228 , BOM 2023). To determine the maximum distance pollen could travel, petri dishes were placed at 5 cm, 10 cm or 50 cm behind the open flowers. This design yielded six wind speed x distance treatment combinations: (1) 40 km h^{-1} x 5 cm, (2) 40 km h^{-1} x 10 cm, (3) 40 km h^{-1} x 50 cm, (4) 5 km h^{-1} x 5 cm, (5) 5 km h^{-1} x 10 cm and (6) 5 km h^{-1} x 50 cm. Pollen traps for treatments one, two, four and five comprised a single Petri dish, whereas those for treatments three and six comprised four Petri dishes arranged in a square to increase the surface area available for pollen capture. To determine whether pollen grains were present on the traps, the pollen traps were observed under a light microscope (100 x magnification). Treatments were replicated 10 times each for *Acronychia oblongifolia*, using flowers sourced from one tree at Mount Annan and one tree at Jerrara, and 15 times each for *Sarcomelicope simplicifolia* subsp. *simplicifolia*, using flowers sourced from one tree at each of Jerrara, South Kiama and Kiama.

A two-factor ANOVA was conducted to determine the effect of wind speed and distance on the percentage of replicates bearing pollen. Treatments five and six were excluded from this analysis to maintain an orthogonal design, as there was no pollen on any plates for treatment five, and as a result treatment six was not applied. Data were arcsine-transformed to ensure homogeneity of variance and normality assumptions were met.

Breeding Systems

A series of pollination experiments were conducted for both species to determine their breeding systems. Inflorescences containing at least five buds were haphazardly selected and subjected to one of five randomly applied treatments: (1) manipulative outcross, (2) autonomous selfing, (3) manipulative selfing, (4) open control (no bag), or (5) procedural control (partial bag). Inflorescences were selected on the same day and open flowers, or very young buds were removed so that flowers developed synchronously within and among the treatments. Polyorganza bags (15 cm x 35 cm) were used to exclude floral visitors throughout the experiment.

Manipulative outcrossing involved bagging an inflorescence in the bud phase, and then hand pollinating each flower as the stigma became receptive, with pollen sourced from a donor tree located at least 10 m away but within the same population. Manipulative selfing was treated the same way, but with pollen sourced from the same tree. Both manipulative treatments were done over three consecutive days to maximise the likelihood of viable pollen being deposited on a receptive stigma. Autonomous selfing involved bagging an inflorescence in the bud phase without manipulating the flowers. Control inflorescences were open to natural pollinators and not manipulated. The procedural control had three openings cut into each bag (10 cm x 5 cm) which tested whether the presence of a bag affected experimental outcomes by allowing biotic pollinators to visit the flowers. Bags that had signs of insect activity, or where the bag tore during the experimental period were excluded from the analysis.

To hand pollinate *Acronychia oblongifolia* flowers, 8 to 16 filaments that held visible pollen (viewed through a hand lens) were removed with forceps from one or two flowers that were not assigned a pollination treatment. Anther heads were brushed onto the receptive stigma of each flower that was assigned a hand pollination treatment. To hand pollinate *Sarcomelicope simplicifolia* subsp. *simplicifolia*, two staminate flowers that were not assigned to a pollination treatment and held visible pollen (viewed through a hand lens) were removed and anthers were brushed onto each stigma that required hand pollination until pollen was visible on the stigma's surface.

Treatments were applied and replicated five (*Acronychia oblongifolia*) or ten (*Sarcomelicope simplicifolia* subsp. *simplicifolia*) times per tree. The reproductive success for all inflorescences was monitored, from pollination through to the maturation of fruit. Early fruit development was based on the ovary swelling, after the petals and stamens had senesced. Fruit maturity was based on the size and the colour of the fruits. Mature fruits were harvested and examined for viable seed. Reproductive success was classed as the percentage of flowers that developed fruit (early fruit development), and the quantity of viable seeds produced per treatment.

Fruits and seeds were dissected and examined under a stereo microscope (40x magnification) to determine the number of seeds per fruit and the proportion of filled seeds for all treatments. A 'filled' seed housed an embryo and endosperm filling the entire space and an 'empty' seed lacked both embryo and endosperm. A 'partially filled' seed contained an embryo, but the endosperm did not fill the entire space enclosed by the seed coat; these seeds are potentially viable. Seeds were also classed as 'not predated' or 'predated' based on signs of pre-dispersal seed predators (e.g., frass or an exit hole in the seed coat).

To determine the reproductive success for each species, the percentages of flowers that produced fruits were compared among treatments using a one-way ANOVA. For both plant species, data were pooled from all sites and trees, and arcsine transformed if needed. A Tukey HSD analysis was applied when a significant difference arose. For both plant species, a separate analysis was undertaken to examine whether spatial factors influenced fruit production; site was included as a factor and a subset of the data was used to ensure a balanced design. For *Acronychia oblongifolia*, a two-way ANOVA (tree, treatment) was conducted on the two plants at Jerrara that all treatments were applied to. For *Sarcomelicope simplicifolia* subsp. *simplicifolia*, a three-factor nested ANOVA was carried out on three individuals from each site. 'Plant' and 'site' were considered random factors and 'plant' was nested in 'site'. A t-test was undertaken to compare fruit set between the open control and procedural control to examine whether bagging affected the results for both species.

Results

Floral biology

The sequence of floral development and maturity of reproductive structures indicated *Acronychia oblongifolia* is protandrous and confirmed that *Sarcomelicope simplicifolia* subsp. *simplicifolia* is dioecious (Figure 1). For both species, the androecium was mature and usually dehisced within one day of floral opening. Pollen was dispersed on average 2 (± 0.07 SE) or 3 (± 0.05) days after floral opening for *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*, respectively. For *Sarcomelicope simplicifolia* subsp. *simplicifolia*, pistillate flowers contained a mature gynoecium upon opening and remained receptive for on average 5 (± 0.01) days; staminate flowers dehisced on average 4 (± 0.4) days after opening, while pistillate flowers dehisced on average 7 (± 0.5) days after opening.

For *Acronychia oblongifolia*, the stigma became receptive on average 5 (± 0.1) days after floral opening and remained receptive for on average 4 (± 0.1) days. Pollen was dispersed prior to stigma receptivity in 17 of the 25 *Acronychia oblongifolia* flowers observed. The anthesis period was on average 12 (± 0.14) days.

Individual *Acronychia oblongifolia* trees flowered for approximately 6 weeks. Pistillate *Sarcomelicope simplicifolia* subsp. *simplicifolia* trees flowered for approximately 4 weeks, while staminate *Sarcomelicope simplicifolia* subsp. *simplicifolia* trees typically produced flowers for up to 9 weeks.

Figure 1. *Acronychia oblongifolia* flowers housing (a) mature androecium and immature gynoecium and (b) old androecium and mature gynoecium, and *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers showing (c) a staminate flower with mature stamens and (d) a pistillate flower with a receptive stigma. Scale bar = 1 mm.

Based on SEM observations, the external morphology of pollen grains across the plant species was indistinguishable, apart from variation in the grain size. All pollen grains were presented as discrete single units (monads) and were elliptical in overall shape. Based on the P/E ratio, the pollen shape was prolate. From polar view, the pollen grains were three-lobed circular and isopolar. Exine ornamentation was heterobrochate. Each grain contained three equidistant apertures with two distinct aperture types: endoaperture present (colporus aperture type) or endoaperture absent (colpus aperture type). Based on the size classes presented in PalDat (2020), *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* pollen grains may be considered 'medium' sized (approximately 40 μm and 30 μm diameters, respectively).

Acronychia oblongifolia flowers with a mature androecium produced significantly more nectar (twice the volume) than flowers with a mature gynoecium ($t_{(1, 38)}$=5.740, p=0.028). Overall, the volume of nectar produced in *Acronychia oblongifolia* flowers did not exceed 0.23 μl. *Sarcomelicope*

simplicifolia subsp. *simplicifolia* staminate flowers produced significantly less nectar than pistillate flowers ($t_{(1, 39)}$=12.454, p=0.001). Overall, the volume of nectar produced in *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers did not exceed 0.4 µl (mean pistillate volume), while staminate flowers produced less than half that volume of nectar.

Floral visitors and biotic pollination vectors

Floral visitors were diverse (31 species for *Acronychia oblongifolia*; 47 species for *Sarcomelicope simplicifolia* subsp. *simplicifolia*) and consisted only of arthropods (Table S2). For *Acronychia oblongifolia*, Diptera was the most species rich taxon found visiting (20 species), followed by Coleoptera (4 species), Hymenoptera (3 species) and Lepidoptera (3 species). Species richness was greater at Jerrara (14 species) than Mount Annan (10 species), and only three species, all Diptera, were detected at both sites (Calliphoridae species 2, *Dichaetomyia* and *Melangyna* species). For *Sarcomelicope simplicifolia* subsp. *simplicifolia*, Diptera was the most species rich taxon found visiting (35 species), followed by Hymenoptera (9 species), Coleoptera (2 species), Hemiptera (1 species) and Arachnida (1 species). Species richness was greatest at Jerrara (28 species), and 19% of species were detected at more than one site. Four species were detected visiting *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers at three sites: *Calliphora* (species 2) and *Apis mellifera* Linnaeus, 1758 were both detected at Kiama, South Kiama and Jerrara, while the *Melangyna* species was detected at Kiama, South Kiama and Wollongong. Species richness of floral visitors was greatest at Jerrara (28 species), with more than double the number of species detected at any other site. No nocturnal visitors were detected by human observation or infrared video recording for either plant species.

Nine of the 31 floral visitors contacted the stigma or carried pollen and, therefore, are potential pollinators (hereafter referred to as pollinators) of *Acronychia oblongifolia* (Table S2). Across both sites, two species carried pollen and contacted the stigma as they foraged (the flies: *Dichaetomyia* species and an unidentified Calliphoridae). Additionally, four species contacted the stigma while foraging: a hoverfly (*Melangyna* species), a fly (*Pogonortalis* species), a beetle (*Luperini* species) and a butterfly (*Heteronympha mirifica* Butler, 1866). Three of the captured species carried pollen on their bodies: a fly (*Chrysomya rufifacies* Macquart, 1844) and two beetles (and an unidentified Elmidae and *Chauliognathus* species).

Eleven of the 47 floral visitors contacted the stigma or carried pollen of *Sarcomelicope simplicifolia* subsp. *simplicifolia* and therefore are potential pollinators (hereafter referred to as pollinators) (Table S2). Across all sites, two species contacted the stigma while foraging and carried pollen on their bodies: a bee (*Apis mellifera*) and a fly (*Calliphora* species 2). Additionally, two flies contacted the stigma while foraging (*Calliphora* species 3 and the unidentified species 31). Seven of the captured species carried pollen on their bodies: three flies (*Dichaetomyia* species, Muscidae species 9 and

Tephritidae species 19), two native bees (*Exoneura* species and *Hylaeus* species), a beetle (Chrysomelid species 1) and an ant (Dolichoderinae species).

For *Acronychia oblongifolia*, the presence analysis (percentage of sampling intervals where a pollinator was present) revealed variable trends in visitation frequencies of pollinators across both sampling methods. Fly species of two taxa, *Dichaetomyia* and Calliphoridae, were the most frequent pollinators detected by human observations, present in significantly more sampling intervals than any other pollinator (x^2_5 =127.772 p<0.0001) (Figure 2a). These fly species were observed in 27% of sampling intervals, while all other pollinators detected by human observations were present in fewer than 2% of sampling intervals. Two flies, *Dichaetomyia* and *Pogonortalis* species, and a beetle, *Luperini* species, were all present in the greatest number of sampling intervals detected on DVR (16%) (Figure 2b); although pollinator presence detected on DVR did not significantly differ among species (x^2_5=3.817 p=0.576). TLP revealed few floral visitors foraged outside the 10:00-15:00 survey period (Figure S1) and all floral visitors were Dipteran species.
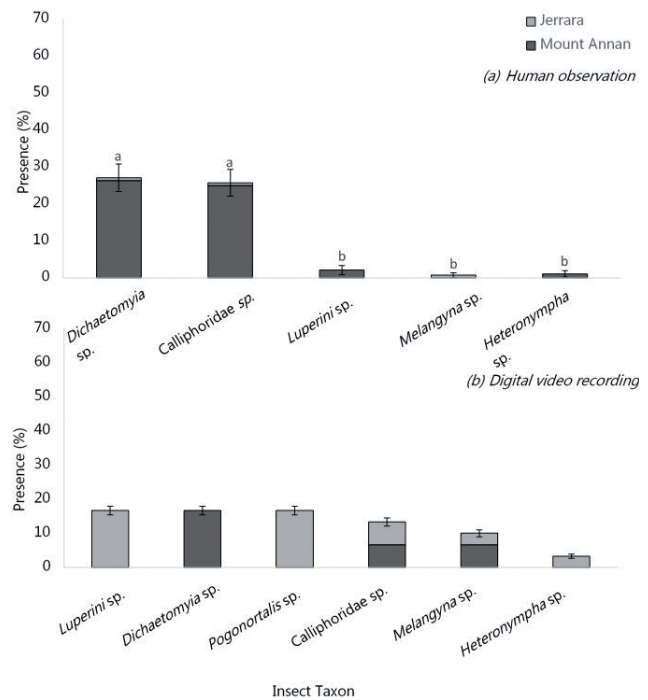

Figure 2. Potential pollinators of *Acronychia oblongifolia* as detected by (a) human observations for 5-minute intervals (n=144), and (b) digital video recordings for 2 hours (n=30). Note that different methods detected different pollinators. Data presented are means ± SE; bars with different letters are significantly different, according to Dunn All Pairs for Joint Ranks test after Kruskal-Wallis analysis.

The presence analysis of *Sarcomelicope simplicifolia* subsp. *simplicifolia* pollinators revealed the fly, *Calliphora* (species 2), and the bee, *Apis mellifera*, to be the most frequent floral visitors across both sampling methods. Human observations showed *Calliphora* (species 2) to be detected in a significantly greater number of sampling intervals than any other taxa (18%) (x^2_{10}=213.112, p<0.001; Figure 3a). *Calliphora*

(species 2) was also the most frequent floral visitor detected by DVR; present in significantly more sampling intervals than the other Dipteran species (species 31 and Muscidae species 9) ($F_{(3, 108)}$=5.648, p=0.001; Figure 3b). All other pollinators were infrequent floral visitors, present in less than 8% of sampling intervals, for both sampling methods (Figure 3). TLP detected some floral visitors active outside the 10:00-15:00 survey period but the suite of species did not differ; nine Dipterans and one bee (*Apis mellifera*) were recorded foraging earlier in the morning or later in the afternoon (active between 07:40 and 16:15) (Figure S2).
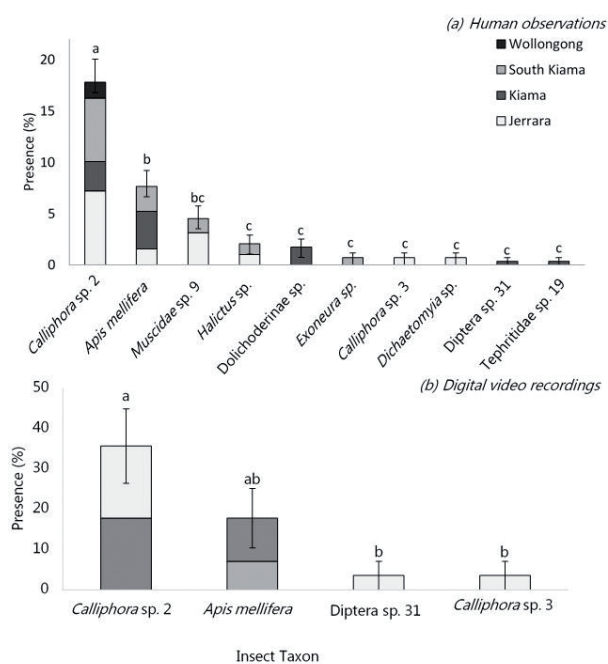
Figure 3. Potential pollinators of *Sarcomelicope simplicifolia* subsp. *simplicifolia*, as detected by (a) human observations for 5 minute intervals (n=288), and (b) digital video recordings for 2 hours (n=28). Note that different methods detected different pollinators. Data presented are means (± SE); bars with different letters are significantly different, according to (a) Dunn All Pairs for Joint Ranks test or (b) Tukey HSD analysis, after Kruksal-Wallis analysis or ANOVA, respectively.

Wind pollination

No pollen grains were detected on the *Acronychia oblongifolia* pollen traps, regardless of windspeed or plate distance; indeed, through visual inspection there were no signs that pollen had been dislodged from the anthers during the experiment.

Wind dislodged pollen of *Sarcomelicope simplicifolia* subsp. *simplicifolia*, but the grains did not travel far (≤10 cm) (Figure 4). Pollen presence differed significantly among some treatments ($F_{(3,66)}$=3.611, p=0.018) as strong winds (40 km h⁻¹) dislodged pollen more frequently than light wind speeds (5 km h⁻¹) (Figure 4). Pollen was transported short distances (5 cm) in both strong (40 km h⁻¹) and light (5 km h⁻¹) winds, although only with strong wind was pollen dispersed further than 5 cm. Treatment six (5 km h⁻¹ x 50 cm) was not applied due to time constraints and as no pollen was detected in treatments three or five (40 km h⁻¹ x 50 cm and 5 km h⁻¹ x 10 cm).
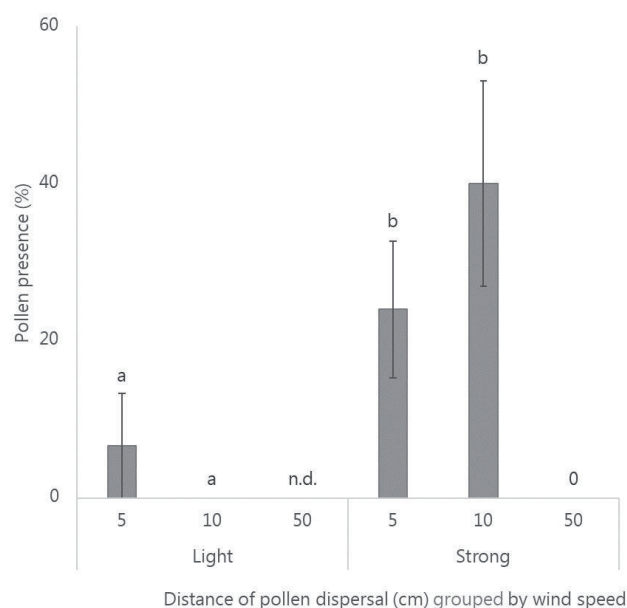

Figure 4. Mean (±SE) percent of plates with pollen of *Sarcomelicope simplicifolia* subsp. *simplicifolia* after exposure to one of six manipulative wind pollination experiments. Light (5 km h⁻¹) and strong (40 km h⁻¹) wind speeds were simulated, with pollen traps 5 cm, 10 cm or 50 cm behind one open flower. Bars with different letters are significantly different, according to two-way ANOVA. Note the 50 cm treatments were excluded from statistical analysis. n.d. indicates no data for that treatment.

Breeding systems

Fruit production did not differ significantly between the partial bag and open treatment with no bag for either plant species (*Acronychia oblongifolia*: $t_{(1, 27)}$=0.477, p = 0.496; *Sarcomelicope simplicifolia* subsp. *simplicifolia* $t_{(1, 81)}$=0.149, p = 0.701) indicating that bags have no substantial effect on fruit production and, therefore, open controls could be legitimately compared to treatments with bags.

Acronychia oblongifolia

Fruit was produced from all treatments except manipulative selfing; however less than 10% of flowers produced fruit overall (Figure 5). Fruit production varied significantly among treatments ($F_{(3, 121)}$=6.099, p=0.007; Figure 5a), with flowers that received experimental pollen from a different tree (manipulative outcross) producing more than four times as many fruits as those subjected to any other treatment (Figure 5a).

The balanced analysis comparing fruit set between two trees at Jerrara demonstrated that fruit set differed significantly between trees, but this was dependent on treatment ($F_{(3, 32)}$=3.743, p=0.002). Fruit production where pollen was sourced from a different tree (manipulative outcross) varied between trees, with flowers from tree three producing nearly ten times as much fruit as tree five (Figure 5b).
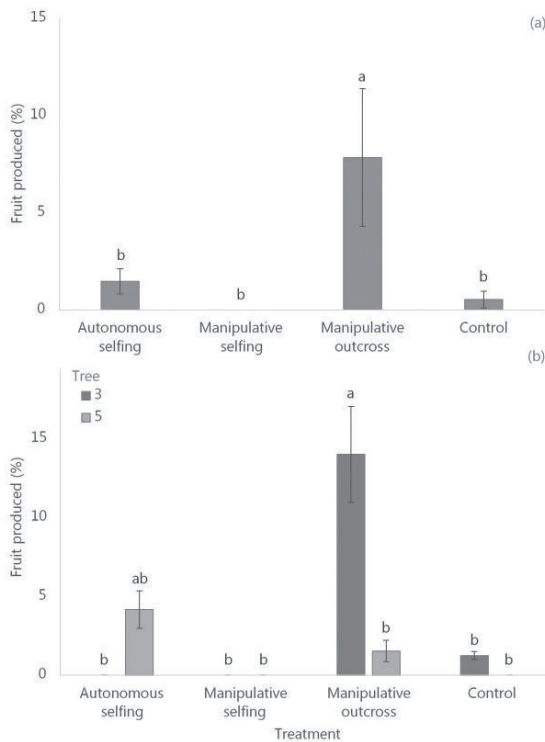
Figure 5. Mean (±SE) percentage of *Acronychia oblongifolia* flowers that produced fruit following pollination treatments: (a) data for seven plants and two sites are combined (autonomous selfing n=35; manipulative selfing n=20; manipulative outcross n=15; and open n=30); (b) plants located at Jerrara are included as a factor in a balanced design (n=5). For each panel, bars with difference letters are significantly different, according to Tukey HSD analysis after ANOVA.

Fruits and seeds were harvested from the outcross and autonomous selfing treatments, or no treatment, and of the seeds collected, 41%, 47% and 47% respectively were filled and therefore likely viable. Almost half (47%) of 19 seeds sourced from the autonomous selfing treatment were likely viable. However, this treatment also yielded the highest proportion of partially filled seeds (50%) compared with the outcross treatment or inflorescences that were not exposed to a treatment (13% and 2% respectively). Overall, 33% of 91 seeds were empty and not viable. Seeds from both Jerrara and Mount Annan were predated, with obvious small holes visible or frass within the seed, however <5% of seed had signs of insect predation.

Sarcomelicope simplicifolia subsp. *simplicifolia*

On average, 23% of flowers produced fruit, but fruit production varied significantly among treatments ($F_{(2, 250)}$=38.777, p<0.001; Figure 6a). Flowers that received experimental pollen from a different tree (manipulative outcross) produced the greatest abundance of fruit, more than double that of any other treatment (Figure 6a). Nearly 20% of flowers exposed to natural pollinators (open control) produced fruit, while no fruit was produced when pollinators were excluded from flowers (Figure 6a).

On average, more fruit was produced at Kiama (27%) than Wollongong (10%), but fruit production was highly variable among trees at both sites, and these differences depended on treatments (Table 1; Figure 6b). Trees one and three produced six times more fruit than tree two at Kiama; and significantly more fruit was produced on trees one and three at Kiama than any tree at Wollongong (Figure 6b). Fruit set in flowers exposed to natural pollinators (open control) was also highly variable among trees and between the two sites. The trees at Wollongong all produced fruit in low quantities, with less than 10% of flowers fruiting, while more than 60% of flowers on tree three at Kiama fruited (Figure 6b). Importantly, outcomes were consistent in terms of the source of pollen, with no fruit set on any tree when pollinators were excluded from the flowers.
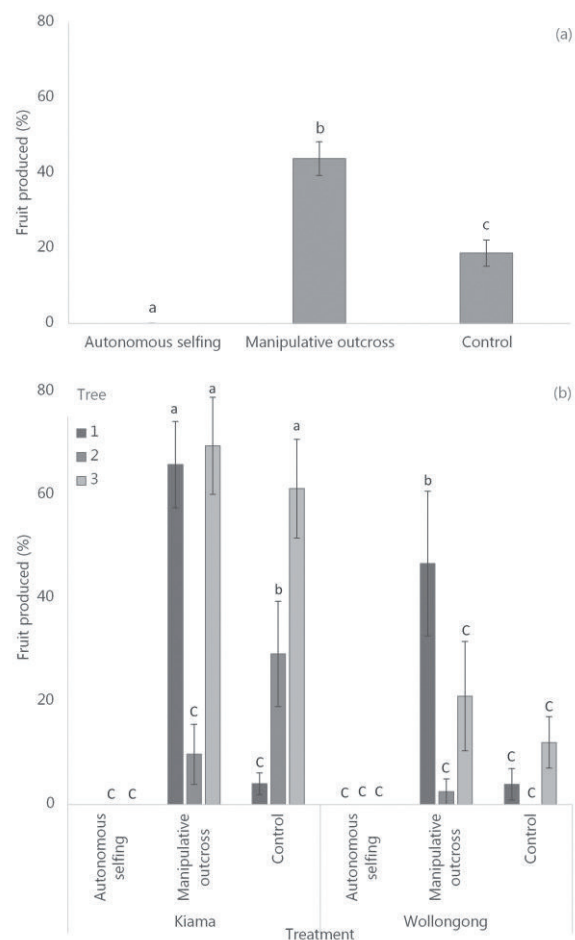

Figure 6. Mean percent (±SE) of *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers that produced fruit following pollination treatments: (a) all treatments on all eight plants (outcross n=89; autonomous selfing n=73; control n=89) and (b) a subset of data where three plants at two site were analysed in a nested three-way ANOVA (n=10). For each panel, bars with different letters are significantly different, according to Tukey HSD analysis after ANOVA.

The seed dissection results indicated that seed viability was variable between sites and treatments; however, 45% of seeds were filled and thus viable (n=810) (Table 2). Merely 16% of seeds collected were empty, while 39% of seeds were partially filled and potentially capable of germination. Insect predation was confirmed within seed collected from Kiama,

South Kiama and Wollongong; but overall, only 3% of seeds collected were predated (Table 2).

Table 1. Analysis of fruit production on each of three *Sarcomelicope simplicifolia* subsp. *simplicifolia* plants at two sites (Wollongong and Kiama). Plant and site were considered random factors and plant was nested within site. Treatment was a fixed factor. Data were arcsine transformed prior to analysis. [*] Depicts significant interaction.

Source	d.f.	d.f. for F	MS	F	P
Site: S	1	1, P(S)	7.905	11.523	0.027*
Plant(site): P(S)	4	4, E	0.686	14.596	< .00001*
Treatment: TM	2	2, P(S) x TM	2.203	3.448	0.083214
S x TM	1	1, P(S) x TM	2.203	3.448	0.083214
P(S) x TM	8	8, E	0.639	13.596	< .00001*
Error: E	161		0.047		

Table 2. Viability of seeds of *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* produced during the manipulative pollination experiments. The number of fruits harvested, and the number of seeds, from each treatment at each site is detailed. The proportion of seeds that were filled, partially filled or empty is provided. The proportion of seeds from each treatment that had signs of insect predation is detailed. The total number of seeds in each seed class (filled, partial filled, empty) is presented for each experimental treatment. The total number of predated seeds is presented as a percentage of the total number of seeds examined below the respective treatment. Shrubs were located at The Australian Botanic Gardens Mount Annan [Mount Annan], Jerrara Dam Reserve [Jerrara], Spring Creek Wetland South [South Kiama], Spring Creek Wetland Kiama [Kiama], and Wollongong Botanic Gardens [Wollongong]. [–] indicates no data obtained.

Treatment	Site	No. fruit	No. seeds	% Filled	% Partial filled	% Empty	% Predated
Acronychia oblongifolia							
Outcross	Jerrara	12	39	41	13	46	0
	Mount Annan	0	0	-	-	-	-
	Total			41	13	46	0
Autonomous selfing	Jerrara	3	4	0	100	0	0
	Mount Annan	4	15	93	0	7	7
	Total			47	50	4	4
No treatment	Jerrara	1	1	0	0	100	0
	Mount Annan	4	32	94	3	3	0
	Total			47	2	51	0
Sarcomelicope simplicifolia subsp. *simplicifolia*							
Outcross	Jerrara	2	3	0	100	0	0
	Kiama	38	305	70	12	18	5
	South Kiama	28	157	9	70	21	1
	Wollongong	33	79	52	27	22	0
	Total			33	52	15	2
Open control	Jerrara	0	0	0	-	-	-
	Kiama	28	172	60	14	26	3
	South Kiama	19	32	3	56	41	9
	Wollongong	0	0	0	-	-	-
	Total			32	35	34	6
Procedural control	Jerrara	1	3	33	66	1	0
	Kiama	15	58	78	9	14	10
	South Kiama	0	0	0	-	-	-
	Wollongong	1	1	100	0	0	0
	Total			70	25	5	3

Discussion

Floral biology

The floral biology of the study species indicated that both fit best into the general entomophilous pollination syndrome. Initial observations of floral phenology found *Acronychia oblongifolia* to be protandrous and *Sarcomelicope simplicifolia* subsp. *simplicifolia* to be dioecious. Protandrous species are typically self-incompatible, while dioecious species are self-incompatible by definition, indicating that both plants likely require a pollen vector for seed set (Bertin & Newman 1993). The highly ornamented pollen walls of both species indicated that they are unlikely to utilise an abiotic pollination vector (Halbritter *et al.* 2018); the heterobrochate reticulum on the exine walls, in particular, is a key indicator of insect pollination (Walker 1976; Sannier 2009) and, specifically, of pollination by beetles and bees (Faegri & van der Pijl 1979). Nectar, the primary floral reward for many pollinators (Kearns & Inouye 1993), was produced by both species, providing further evidence for biotic pollination (Dafni 1992), while the small, white flowers indicated that these two plants are most likely pollinated by a range of insect species (Faegri and van der Pijl 1979).

Pollination vectors

Pollinator assemblages for *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* were rich across all study sites indicating pollinator scarcity is unlikely to inhibit viable seed production in these species. Diptera (flies) were dominant pollinators of both species; insects in this group are known to be significant pollinators of rainforest plants, both in Australia (Williams & Adam 1994) and globally (Barth 1991; Vázquez & Simberloff 2002; Smith-Ramírez *et al.* 2005). This is likely a result of their global distribution and morphological traits (e.g., numerous setae and large bodies) enabling a large pollen carrying capacity (Cook *et al.* 2020). Hymenoptera (bees and wasps) were also frequently detected visiting *Sarcomelicope simplicifolia* subsp. *simplicifolia* and thus, are most likely common pollinators of this species.

A suite of floral visitors identified in our study could be mistaken for pollinators if floral visitation was used as the metric for potential pollinators. Historically, some studies on pollination vectors of Rutaceae have only undertaken observations of floral visitors and not quantified their pollen load (e.g., Armstrong 2002; Sgolastra *et al.* 2016; Pradhan & Devy 2019). Less than one-third of the floral visitors we identified were classed as pollinators, based on foraging behaviour or pollen load analyses (*Acronychia oblongifolia*: 23%; *Sarcomelicope simplicifolia* subsp. *simplicifolia*: 29%). King *et al.* (2013) demonstrated the importance of examining pollen deposition on the stigma and quantifying pollen load to distinguish a floral visitor from a pollinator. However, the same species were rarely detected by both DVR and human observation in our study, preventing validation of potential pollinators using the combined analysis. Future studies should aim to quantify the pollen load of visitors and use a combined analysis of pollen load and pollen deposition on the stigma to confirm whether the species classed as likely pollinators are true pollinators.

Given that no pollen was transported in the *Acronychia oblongifolia* wind pollination experiment, it can be concluded that wind is not a likely pollination vector for this species. Field observations showed pollen of this species to be generally sticky, and SEM observations of the pollen found the exine wall to contain heterobrochate structures, traits that are not typical of species relying on an abiotic pollination vector (Dafni 1992; Walker 1976). This result supports the common view that rainforest plants are not wind pollinated (Bawa & Crisp 1980; Williams & Adam 1994). However, as pollen was dislodged in the *Sarcomelicope simplicifolia* subsp. *simplicifolia* simulations, wind may be a vector, particularly if male and female trees are closely situated. Storms and strong wind gusts would be needed to transport pollen to a pistillate tree, and pollination would be unlikely unless the staminate and pistillate trees were in close proximity. In the populations examined, *Sarcomelicope simplicifolia* subsp. *simplicifolia* trees were adjacent at only two sites, but the canopies never overlapped. This demonstrates wind pollination is possible, but a rare event in the populations studied.

Breeding systems

The results from the breeding system experiment for both species highlight the necessity of biotic pollinators for seed set. For both species, the small percentage of fruit produced from flowers exposed to natural pollinators, compared with those exposed to manually transferred pollen from a different tree, suggests pollen limitation has inhibited fruit production in the populations examined. In rainforests, pollen limitation may result from temporal variation in pollinator abundance and behaviour (Freeman *et al.* 1980; Williams & Adam 2010; Vamosi *et al.* 2013). Extreme weather events, including drought, are known to influence insect behaviour and abundance within rainforest communities (Itioka & Yamauti 2004; Gutiérrez-Fonseca *et al.* 2020). The Sydney Basin Bioregion was classed as being in a drought recovery state throughout the study (DPIE 2020), following the 2019-2020 extreme drought event. To account for the potential effects of this drought on pollinator abundance and behaviour, the reproductive success of both Rutaceae should be examined over an extended period that incorporates multiple seasons.

There is evidence of rainforest Rutaceae producing malfunctioning pollen grains (Armstrong 2002), and pollen viability in some rainforest Rutaceae is known to be low (Auld 2001). The production of non-viable pollen grains could be a contributing factor to the small percentage of flowers exposed to natural pollinators that fruited for both species. Malformed pollen grains were not detected during analysis of pollen ultrastructure; however, no assessment of pollen viability was made. Given the abundance and diversity of pollinators observed visiting these species, short-lived pollen may be one explanation for the poor fruit set. Staining pollen of varying ages would help to determine how long the pollen of these species remains viable (Kearns

& Inouye 1993), and whether short-lived pollen is a factor limiting reproductive success.

Fruit production for both study species varied among trees and sites for some treatments; for example, fruit production from flowers that received experimental outcross pollen varied significantly among trees for *Acronychia oblongifolia*. This spatial variation may indicate that environmental factors have influenced pollination processes and fruit development in these populations. Environmental factors, such as low nutrient availability, can directly reduce fruit production and therefore the reproductive success of rainforest plants (Zagt 1997; Teixeira *et al.* 2006). In a similar study conducted by Adams and Williams (2001), *Acronychia imperforata* F.Muell. failed to develop fruit in any treatment and it was concluded that fruiting success in this species may vary as a consequence of fluctuating resource availability. A greenhouse-based experiment comparing viable seed production after plants are exposed to environmental stress (such as low water or nutrient availability) would aid in understanding the degree to which resource limitation is impacting fruit production.

The level of genetic diversity within each of the populations used in this study may also have influenced fruit production. Jerrara is a restored rainforest community, with *Acronychia oblongifolia* individuals propagated from cuttings from a neighbouring population (D. Black pers. comm.). Therefore, it is possible that the genotypic diversity of some of the *Acronychia oblongifolia* populations examined is limited, thus inhibiting seed production. Similarly, for *Sarcomelicope simplicifolia* subsp. *simplicifolia*, fruit production was significantly less at Wollongong compared with Jerrara. The population at Wollongong, which was situated within a Botanic Garden, consisted of cultivated plants and there is potential that low genetic diversity contributed to poor seed set.

Poor gene flow is one hypothesis for the factors preventing viable seed production in rainforest Rutaceae (Martyn *et al.* 2009). Within the Sydney Basin Bioregion, rainforest habitat is restricted to small pockets now fragmented by clearing for European agriculture (Mills & Jackeman 1995). This fragmentation has potentially increased the distance between conspecific individuals, and in turn, decreased the chance of viable pollen transfer between sexually compatible individuals, which can reduce fecundity in obligate outcrossing species. This reduced fitness can lead to reduced diversity in the population and in turn, inbreeding depression. Both *Acronychia* and *Sarcomelicope* in the Illawarra are both well within their species latitudinal limits, nor are there any major distributional disjunctions evident at the continental scale (Australasian Virtual Herbarium). A genetic analysis of the populations studied would confirm whether inbreeding is impeding viable seed production for *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*.

Finally, evidence of seed predation was apparent for both *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* at several populations, indicating pre-dispersal seed predation is another factor contributing to non-viable seed production in these species. No fruit harvested had a predator present; but a *Megastigmus* (Ichneumonidae) wasp was reared from a single *Zieria granulata* (Rutaceae) fruit from Jerrara, in a study conducted in parallel to the present one (Lopresti *et al.* Under Review). The size and shape of the exit hole in the *Zieria granulata* seed was comparable to the predated *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* seed. There is potential, then, that Ichneumonid wasps are the seed predators for the Rutaceae in this study, but confirmation of the predatory species is required. Importantly, this result indicates that bagging developing fruit to exclude predators may help to improve seed yield for collection.

It should be noted that rainforest habitat within the Sydney Basin Bioregion was restricted before European settlement (Mills 1988) and as a result, low seed production may be a natural state for these plants. Variable seed set may not be an issue for longer-lived trees, provided at least one instance of successful reproduction and establishment occurs within each individual's lifespan. For relatively short-lived trees like *Acronychia oblongifolia* (Floyd 1990), and for tree species suffering reduced life-span due to pressure from climate change (such as with some Australian tropical rainforest Rutaceae, see Bauman *et al.* 2022), this window of opportunity may not be wide enough to sustain the population. This is of particular relevance if land clearing and habitat fragmentation persist, further increasing the distance between conspecific individuals.

Conclusion

This study highlights the importance of insect pollinators for viable seed set in two rainforest Rutaceae species: *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*. Pollen limitation and seed predation was shown to inhibit viable seed production for both species, but this was not the sole limitation. Spatial variation in fruit production indicates that additional factors such as population size, abiotic conditions or poor gene flow among populations are also contributing to non-viable seed production in both *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia*. This study is the first to combine an investigation of the floral biology, pollination vectors and breeding system to examine the factors contributing to viable seed production for these species.

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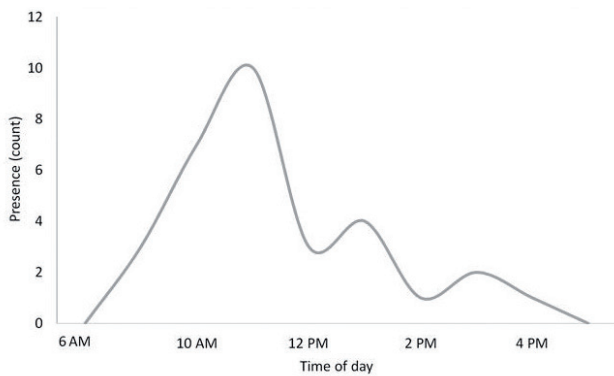
Appendix - Supplementary Material

Figure S1. Count of floral visitors foraging on *Acronychia oblongifolia* flowers throughout the day, detected on time-lapse photography cameras recording between sunrise and sunset. Three cameras were set to record on three non-consecutive days at Jerrara (n=108 h).
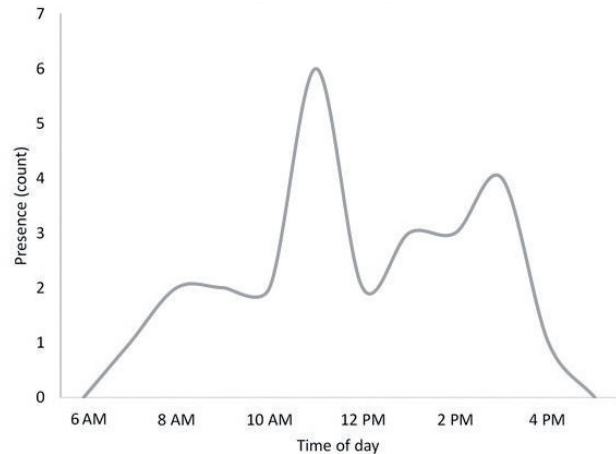

Figure S2. Count of floral visitors foraging on *Sarcomelicope simplicifolia* subsp. *simplicifolia* flowers throughout the day, detected on time-lapse photography cameras. Data is pooled across each of four sites, where at each site three cameras were set to record between sunrise and sunset on the same day (n=144 h).

Table S1. Survey effort: total time spent observing floral visitors at each study site and the number of trees observed for each study species, using each of four methods: digital video recordings [DVR], time-lapse photography [TLP], human observations (diurnal and nocturnal) [HO] and nocturnal infrared camera recordings [ICR].

Study Species	Site	No. trees	DVR (h)	TLP (h)	HO (diurnal) (h)	HO (nocturnal) (h)	ICR (nocturnal) (h)
Acronychia oblongifolia	Jerrara	4	30	0	6	9	108
	Mount Annan	2	30	108	6	0	108
Sarcomelicope simplicifolia subsp. *simplicifolia*	Jerrara	2	14	36	6	3	48
	Kiama	4	14	36	6	3	48
	South Kiama	2	14	36	6	3	48
	Wollongong	6	14	36	6	0	48

Table S2. Insects observed contacting flowers of *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* at each study site. Insects were detected by human observation [Direct] or digital video recordings [DVR] or both methods [Both]. Insects were opportunistically captured following their foraging bout and found to carry pollen of the target species [Yes] or not [No]. The percentage of individuals of each species that contacted the stigma while foraging (for species detected on DVR) is detailed. The stigma was not visible in all recordings and these were excluded from this analysis. 'Stigma contact' is the percentage of flowers contacted where the stigma was visible for each species (n is the number of individuals observed). Species that contacted the stigma or carried pollen of the target plant species were considered potential pollinators [*]. [-] Indicates no data. The number of study sites where each species was observed is detailed. Note *Acronychia oblongifolia* and *Sarcomelicope simplicifolia* subsp. *simplicifolia* datasets are independent and unidentified floral visitors observed on each plant species are unique (e.g. "Diptera species 4" observed on *Acronychia oblongifolia* is a different species to "Diptera species 4" observed on *Sarcomelicope simplicifolia* subsp. *simplicifolia*).

Class	Family	Taxon	Insect	Site	Method detected	No. detected on DVR (stigma present)	No. individuals captured	Carrying Pollen	Stigma contact (%) (n)
Acronychia oblongifolia									
Coleoptera	Cantharidae	*Chauliognathus* sp.*	Beetle	1	Direct	0	3	Yes	-
Coleoptera	Chrysomelidae	Luperini sp.*	Beetle	1	Both	3(2)	1	No	100(2)
Coleoptera	Elmidae	Species 1*	Beetle	1	Direct	0	1	Yes	-
Coleoptera	Lycidae	*Trichalus* sp.	Beetle	1	Direct	0	1	No	-

Class	Family	Taxon	Insect	Site	Method detected	No. detected on DVR (stigma present)	No. individuals captured	Carrying Pollen	Stigma contact (%) (n)
Diptera	Calliphoridae	*Chrysomya rufifacies* (Macquart, 1843) *	Fly	1	Both	0	1	Yes	-
Diptera	Calliphoridae	Species 2*	Fly	2	Both	3(3)	2	Yes	100(3)
Diptera	Muscidae	*Dichaetomyia* sp.*	Fly	2	Both	8(7)	4	Yes	100(7)
Diptera	Syrphidae	*Melangyna* sp.*	Hover fly	2	DVR	4(4)	0	-	100(4)
Diptera	Unknown	Species 3	Fly	1	Direct	0	1	-	-
Diptera	Unknown	Species 4	Fly	1	Direct	0	5	No	-
Diptera	Unknown	Species 5	Fly	1	Direct	0	1	No	-
Diptera	Unknown	Species 6	Fly	1	Direct	0	1	No	-
Diptera	Unknown	Species 7	Fly	1	Direct	0	1	No	-
Diptera	Unknown	Species 8-18	Fly	1	Direct	0	1	No	-
Diptera	Platystomatidae	*Pogonortalis* sp.*	Fly	1	Both	15(4)	1	No	100(4)
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758	Honey bee	1	Direct	0	0	-	-
Hymenoptera	Colletidae	*Hylaeus* sp.	Bee	1	Direct	0	1	No	-
Hymenoptera	Formicidae	Dolichoderinae sp.	Ant	1	Both	67(51)	19	No	0.08(51)
Lepidoptera	Erebidae	*Amata* sp.	Tiger moth	1	Direct	0	2	No	-
Lepidoptera	Nymphalidae	*Heteronympha mirifica* (Butler, 1866)*	Butterfly	1	DVR	1(1)	0	-	100(1)
Lepidoptera	Unknown	Species 19*	Moth	1	DVR	1(0)	0	-	0
Sarcomelicope simplicifolia* subsp. *simplicifolia									
Arachnid	Thomisidae	*Sidymella* sp.	Spider	1	Direct	0	4	No	-
Coleoptera	Chrysomelidae	Species 1*	Beetle	1	Direct	0	1	Yes	-
Coleoptera	Coccinellidae	Species 2	Lady beetle	1	Direct	0	1	No	-
Diptera	Calliphoridae	*Calliphora dubia* (Norris 1959)	Fly	1	Direct	0	1	No	-
Diptera	Calliphoridae	*Calliphora* sp. 2*	Fly	3	Both	29(9)	32	Yes	90(9)
Diptera	Calliphoridae	*Calliphora* sp. 3*	Fly	2	Both	1(1)	1	No	100(1)
Diptera	Calliphoridae	*Calliphora* sp. 4	Fly	1	DVR	1(0)	0	-	-
Diptera	Cecidomyiidae	Species 3	Fly	1	Direct	0	1	No	-
Diptera	Chloropidae	Species 4	Fly	2	Both	1(0)	7	No	-
Diptera	Chloropidae	Species 5	Fly	1	Direct	0	2	No	-
Diptera	Chloropidae	Species 6	Fly	1	Direct	0	2	No	-
Diptera	Chloropidae	Species 7	Fly	2	Direct	0	2	No	-
Diptera	Culicidae	Species 8	Mosquito	1	DVR	1(1)	0	-	0(1)
Diptera	Muscidae	*Dichaetomyia* sp.*	Fly	1	Direct	0	5	Yes	-
Diptera	Muscidae	Species 9*	Fly	1	Direct	0	1	Yes	-
Diptera	Muscidae	Species 10	Fly	1	Direct	0	1	No	-
Diptera	Muscidae	Species 11	Fly	1	Direct	0	1	No	-
Diptera	Muscidae	Species 12	Fly	1	Direct	0	1	No	-
Diptera	Platystomatidae	Species 13	Fly	2	Direct	0	2	No	-
Diptera	Sciaridae	Species 14	Fly	1	Direct	0	1	No	-

Class	Family	Taxon	Insect	Site	Method detected	No. detected on DVR (stigma present)	No. individuals captured	Carrying Pollen	Stigma contact (%) (n)
Diptera	Sciaridae	Species 15	Fly	1	Direct	0	1	No	-
Diptera	Syrphidae	*Melangyna sp.* *	Hover fly	3	Both	15(0)	6	No	-
Diptera	Tachinidae	Species 16	Fly	2	Direct	0	6	No	-
Diptera	Tachinidae	Species 17	Fly	1	Both	2(0)	2	No	-
Diptera	Tachinidae	Species 18	Fly	2	Direct	0	7	No	-
Diptera	Tephritidae	Species 19*	Fly	1	Direct	0	1	Yes	-
Diptera	Tachinidae	Species 20	Fly	1	Direct	0	1	No	-
Diptera	Unknown	Species 21-30	Fly	3	Direct	0	10	No	-
Diptera	Unknown	Species 31*	Fly	1	Both	2(2)	1	No	100(2)
Diptera	Unknown	Species 32	Fly	1	Direct	3(0)	1	No	-
Hemiptera	Unknown	Species 33	Bug	1	Direct	0	1	No	-
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758*	Honey bee	3	Both	9(4)	10	Yes	100(4)
Hymenoptera	Apidae	*Exoneura* sp.*	Bee	1	Direct	0	3	Yes	-
Hymenoptera	Colletidae	*Hylaeus* sp.*	Bee	1	Direct	0	2	Yes	-
Hymenoptera	Formicidae	Dolichoderinae sp.*	Ant	1	Direct	0	7	Yes	-
Hymenoptera	Formicidae	Species 34	Flying ant	1	Direct	1(0)	1	No	-
Hymenoptera	Halictidae	*Halictus* sp.	Bee	1	Direct	0	1	No	-
Hymenoptera	Halictidae	*Seladonia* sp.	Bee	1	Direct	0	1	No	-
Hymenoptera	Unknown	Species 35	Bee	1	Direct	0	1	No	-
Hymenoptera	Unknown	Species 36	Bee	1	Direct	0	1	No	-