Nectar Variation in Aotearoa New Zealand Tree Species

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Nectar Variation in Aotearoa New Zealand Tree Species

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Abstract

In this thesis, I investigated whether and how the nectar of New Zealand flowering trees varies within and among species and across regions. For this, I quantitatively assessed interspecific variation in floral (size, weight, shape, and colour) and nectar traits (volume, concentration, and chemical composition) across 50 woody species in New Zealand. Sampled species included, but were not limited to, those associated with honey production and those susceptible to Myrtle Rust, such as the Myrtaceae *Leptospermum scoparium* (mānuka), *Kunzea ericoides* (kānuka), *Lophomyrtus* spp. (rama rama), *Metrosideros* spp. (pōhutukawa, rāta), and *Knightia excelsa* (rewarewa). I also examined the impact of climate on nectar traits using a subset of eight common native tree species sampled from five coastal regions. Additionally, the study investigated the Removal-Enhanced Nectar Replenishment (RENR) response of *Vitex lucens* Kirk (pūriri, Lamiaceae), an endemic bird-pollinated tree, to frequent two-hourly nectar removal.

Sampling comprised approximately 10,500 flowers from 436 trees across all six climate regions of New Zealand, covering both main islands (35-45° S / 170-177° E), and measuring the diameter at breast height (DBH) of these trees. Most flowers (10,000 flowers from 428 trees) were analysed for the interspecific study (Chapter 4), with a subset of these (4,276 flowers from 164 trees) used in the inter-regional climate study (Chapter 3), and 120 pūriri flowers from 8 trees examined in the nectar removal study (Chapter 2).

For all studies, nectar was extracted using micropipettes; flower size was measured with digital callipers; flower and nectar weight were determined using a scale; flower colour by applying picture analysis software; nectar concentration was assessed with a refractometer; and nectar chemical composition was analysed using High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS).
Data were analysed using a comprehensive suite of statistical techniques, including linear regression, Spearman rank and Pearson’s correlations, and generalised additive models (GAMs) and generalised additive mixed models (GAMMs), to identify correlations, detect multicollinearity, and elucidate multivariate trends. Additionally, a range of tests, including t-tests, Wilcoxon rank-sum tests, Tukey's Honestly Significant Difference (HSD) tests, Dunn's tests (with Bonferroni correction), and Kruskal-Wallis tests, as well as Analysis of

Variance (ANOVA), were employed to determine covariances and assess statistical significance among groups. Lastly, I tested traits for phylogenetic signals using Pagel's lambda.

Flower traits ranged from 3–879 mg in fresh weight and 2–67 mm in size, and they secreted 1–82 μL of nectar containing 0.01–54% solubles. I identified 62 distinct nectar components, comprising 25 sugars and 37 non-carbohydrate compounds. On average, nectar solubles consisted of 97% carbohydrates and 3% non-carbohydrates, including six alkaloids, sixteen amino acids, eleven phenolics, and four vitamers. Only 8% of the 50 tested species produced sucrose-rich nectar, defined as nectar in which sucrose comprised more than 50% of total solubles. Species exhibited unique nectar sugar profiles, containing between 4 and 25 sugar types.

In the RENR study (Chapter 2), *V. lucens* flowers exhibited a neutral response to frequent nectar removal: total nectar volumes from flowers sampled five times at two-hour intervals were similar to those from control flowers sampled once after ten hours. Interestingly, the replenishment patterns of frequently sampled flowers followed a diurnal rhythm that correlated with changes in Vapour Pressure Deficit (VPD).

The inter-regional study (Chapter 3) highlighted that nectar and floral trait variation among regions was significant for all but one species, underscoring the highly species-specific nature of climate-trait relationships. Climate factors each accounted for approximately 30-80% of the regional variation in plant traits for most species.

The analysis of a substantial interspecific dataset (Chapter 4) revealed positive correlations between nectar volume, concentration, and alkaloid content with floral traits such as size, weight, shape (especially those more difficult to access, such as tube- and flag-shaped flowers), and colour (notably yellow, orange, or red). In contrast, levels of hexasaccharides were negatively correlated with these floral characteristics, with higher concentrations found in smaller, white, green, or purple flowers of shapes that are more accessible (e.g. dish-shaped). Hence, some nectar traits aligned with the species' pollination syndrome. Moreover, significant phylogenetic signals were observed; for example, nectar from Fabid and Campanulid taxa had higher sucrose concentrations than other clades, whereas nectar from Myrtales and Lamiales was characterised by higher glucose content than other clades.

I found that nectar variation is highly complex and species-specific, shaped by phylogenetic relationships, climate and water status. These findings offer new insight into the factors driving nectar composition across species. The results can support large-scale estimates of nectar availability, with applications in honey production and habitat conservation. In addition, the identified nectar profiles provide a foundation for comparison with honey chemistry to develop new authenticity markers for high-value honeys.

For future studies, I propose conducting simultaneous nectar removal experiments across multiple species under controlled environmental conditions to assess whether phylogenetic relationships influence species-specific responses to nectar removal. Particular attention should be given to the type of nectary vascularisation and secretion mechanism, as well as to potential effects of flower colour and shape on nectar production concerning heat management. In field-based RENR studies, I recommend that nectar sampling be carried out by multiple researchers simultaneously, as replenishment is highly time-sensitive. Additionally, sampling should be paired with pollinator observations, and the analysis should incorporate information on the diurnal activity patterns of key pollinators.

Similarly, studies on inter-regional differences in nectar composition should account for local pollinator communities and investigate additional potential drivers of variation, including the species' nectary type, vascularisation, secretion mechanism, plant water status, soil nutrient availability, and phylogenetic relationships. To build a detailed picture of regional nectar variation, I recommend using large sample sizes per species per region, with a focus on closely related species known for their high nectar production.
Further research into interspecific nectar variation should explore whether the observed phylogenetic patterns persist across broader taxonomic and ecological contexts, and whether species-specific nectar profiles are consistently shaped by nectary structure and function.

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Firstly, I thank Associate Professors Michael Clearwater, Sebastian Leuzinger, and Dr Sarah Richardson for supervising my thesis. I extend special thanks to the Ministry of Business, Innovation and Employment – Hikina Whakatutuki – for awarding me the Endeavour Doctoral Scholarship, part of the '*Building Resilience and Provenance into an Authentic Māori Honey Industry Research Programme*'. I also thank Landcare Research – Manaaki Whenua, the Department of Conservation, and the New Zealand City Councils for facilitating the smooth process of flower sampling nationwide. The positive and friendly interactions during permit applications served as great motivation, resulting in nectar samples from 55 species and over 10,000 flowers. This achievement would not have been possible without the selfless assistance of supportive fellow students, friends and family members who went above and beyond to help me, reaching even the most distant yet crucial flowers. Special thanks for logistics, safety and field assistance to my husband Leo van Delden, Joshua Neale, Jem Benoy, Sara Rafiei, Dr Fabian Döweler, Berta Greulich, Mike Gwyther, Danielle Le Lievre, and Mayasa and Zumu. I further acknowledge Dr Stevie Noe, Dr Megan Grainger, and especially the postgraduate students at the Department of Chemistry, University of Waikato, for their valuable advice on HPLC analysis. Additionally, I thank Dr John van Klink and Nigel Joyce for their tremendous support in processing LC-MS samples at Plant and Food Research, Lincoln, and for organising laboratory access for processing flowers at Plant & Food, Dunedin. Lastly, I am grateful to the Fleet and the School of Science office team at the University of Waikato for their assistance in organising transport and ensuring my safety during my 17,000 km sampling journey.

Preface

I conceptualised the research outlined herein at the Schools of Science facilities at the University of Waikato (UoW) and the Auckland University of Technology (AUT). Experiments and investigations were carried out at the premises of the UoW, Plant and Food Research (Dunedin; Lincoln), the Botanical Gardens of Auckland and Dunedin, Landcare Research (Lincoln), and across Council and Department of Conservation (DOC) parks and reserves throughout New Zealand. My supervisory panel, consisting of Assoc. Prof. Dr Michael Clearwater (chief supervisor), Dr Sarah Richardson, and Assoc. Prof. Dr Sebastian Leuzinger provided revisions to the final monograph and consulted with me on experimental design, interpreting results, and statistics. Prof. Martin Bader also advised on the latter. All experimental work, except for LC-MS sample processing and a small percentage of flowers measured under my guidance by assistants, and data analyses were exclusively conducted by me. LC-MS samples were processed collaboratively with Dr John van Klink and Nigel Joyce, Plant and Food Research, Lincoln.

This thesis is presented as a monograph comprising five chapters:

- **Chapter 1:** A general introduction;
- **Chapter 2 - 4:** Research chapters, and
- **Chapter 5:** A general discussion.

    - Chapter 2 is currently being prepared for submission as an original research article under the working title *Neutral Nectar Removal-Enhanced Replenishment Response in the Bird-Pollinated New Zealand Tree* Vitex lucens *Kirk (Lamiaceae)*.

    - Chapter 3 is in the final stages of review with *Frontiers in Plant Science*, submitted as an original research article titled *Nectar Traits of New Zealand Trees Vary Across Climatic Zones*.

    - Chapter 4 will serve as the basis for multiple original research publications reflecting the breadth of the study's findings.

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1. Introduction

1.1 What is Nectar and How Did It Evolve?

Recent research indicates that 90% of flowering plant species are animal-pollinated (Tong et al., 2023). For these angiosperms, flowering and transfer of pollen are crucial for reproduction and a major event through which they interact with other organisms (Willmer, 2011). In return, most of these plants provide floral nectar as a key reward, a sugary exudate mostly containing sucrose, glucose and fructose, which is commonly secreted by nectaries (Pimienta-Barrios et al., 2002; Erbar & Leins, 2010; Cota-Sánchez et al., 2013; Farré-Armengol et al., 2013). For the plant, nectar production comes with a high demand for photosynthates (Southwick, 1984) and energetic investment in secretory tissue production (Pyke, 1991; Pacini et al., 2003). The allocation of resources to nectar production entails a trade-off in growth, reproductive success, and seed production (Zimmerman & Pyke, 1988; Pyke, 1991; Pyke & Ren, 2023).

Despite the increasing interest in and knowledge of floral nectar, our understanding of its evolution remains limited (Abrahamczyk et al., 2017). Fossil evidence of nectaries dates back to the mid to late Cretaceous period (Crepet et al., 1991; Friis et al., 2011). A hypothesis by Nepi et al. (2009) suggests possible support for nectar secretion early in angiosperm evolution. The emergence of enveloped ovules (angiospermy) is thought to have led to the disappearance of sugary exudates, such as the ‘pollination drop’ - a sugary secretion mainly excreted from gymnosperms serving as a landing site for pollen. The initial role of the pollination drop might have functioned as a form of reward for early pollinators, primarily insects (Lloyd & Wells, 1992; Endress, 1994). With insects already adapted to feeding on sugary liquids, the appearance of angiosperms likely exerted strong selective pressure, potentially driving the development of a new sugary exudate in flowers: nectar (Erbar, 2014). Despite the convergent evolution of nectaries within angiosperms (e.g. Hobbahn et al., 2013), nectaries are not exclusive to flowers. Extra-floral nectaries have been described for Polypodiophyta (ferns), gymnosperms and angiosperms, and can be found on petioles, leaves, stems, and bracts (Nicolson & Thornburg, 2007).

1.2 Nectaries

Nectaries are specialised nectar-secreting plant structures which can vary in histology, shape, location and function (Bernardello, 2007). Classifying nectary types has been attempted multiple times from different perspectives. However, none of these classifications can universally capture their full diversity.

1.2.1 Structural Classification

Nectaries can be classified based on their basic structure: structural and non-structural, with structural nectaries recognised as regularly secreting nectar and being anatomically sharply localised. Structural nectaries have an epidermis with or without stomata and trichomes, where nectar is released to the exterior; specialised parenchyma producing or storing nectar solutes, and a vascular bundle conveying water and nutrients to the parenchyma (Nepi, 2007). Non-structural nectaries secrete nectar only sporadically and are non-differentiated areas (after Zimmermann, 1932).

1.2.2 Topographical Classification

Nectaries can be distinguished based on their topography, being either ‘floral’ (FN, on reproductive structures) or ‘extrafloral’ (EFN, on strictly vegetative structures), and on their location and arrangement within the various floral and vegetative organs. Interestingly, both types can coexist within the same plant (Elias et al., 1975; Beardsell et al., 1989; Thomas & Dave, 1992; Freitas et al., 2001).

After Nicolson et al. (2007), the nectar of both FN and EFN share similar chemical composition and function, primarily serving as an attractant or reward for animals. FN nectaries facilitate pollen transfer by animals such as insects, birds, and mammals, with nectar secretion lasting from a few hours to several days. In general, the volume of nectar produced correlates with the total volume of nectary parenchyma tissue, ranging from less than 1 µL to a few mL. FN nectar composition and viscosity vary widely among species, with this variation often correlated with the identity of the nectar consumer (Nicolson et al., 2007).

In contrast, EFNs primarily attract ants with their sugary exudate, which, in turn, provide defence against herbivores. EFNs can secrete a few µL of nectar per day for extended periods, ranging from a few days to several months. The composition of the exudate is less variable, as it predominantly caters to ants (Nicolson et al., 2007).

Further to the FN/EFN-model, others have been proposed that categorise nectaries based on histological structure and the mechanism of nectar secretion to the plant surface (Vogel, 1977), on the anatomy and ultrastructure of the nectaries and their mode of secretion (Fahn, 1979a), and the fate of the organs where the secretory tissue is located (e.g. Smets, 1986, 1988).

Floral Nectary Locations

This thesis focused on nectar exuded from floral nectaries, which can vary in location on the plant's flower structures (Vogel, 1997, 1998a, b, c). They can be directly at the organ's surface, form a protrusion, or be deeply embedded (Fahn, 1979a & 1988). In evolutionary terms, this high variation reflects the broad diversity of their foraging pollinators (Wist & Davis, 2006 & 2008; Nepi & Stpiczyńska, 2008), as well as the phylogenetic diversity of the plants that produce them. Nectaries can be found on/in/between either the sexual organs of a flower (gynoecium, androecium) or on the non-sexual structures (sepals, petals, tepals, hypanthia).

Among the plant families investigated in this thesis, the location of nectaries mainly correlates with the following structures, depicted in Figure 1 (from Bernardello, 2007), with '*n*' representing the number of species studied in this thesis with this specific nectary type, where a species may have multiple nectar-secreting structures within one plant:

a) Ovarian (*n* = 19) – in Pittosporaceae, Griseliniaceae (Erbar & Leins, 2010), Loganiaceae, Gesneriaceae, Lamiaceae, Plantaginaceae, Scrophulariaceae (Cronquist, 1981), Primulaceae (Vogel, 1986, 1997), Rousseaceae (Lundberg & Bremer, 2003), Elaeocarpaceae (Matthews & Endress, 2002);

b) Intrastaminal (*n* = 13) – in Fabaceae (Vogel, 1997; Horner et al., 2003), Cunoniaceae (Matthews & Endress, 2002), Proteaceae (Douglas & Tucker, 1996; Rao, 1967), and Rutaceae (Cronquist, 1981);

c) Hypanthial (*n* = 10) – in Myrtaceae and Onagraceae (Cronquist, 1981; Ronse De Craene, 2010);

d) Staminal/staminodes (*n* = 5) – in Winteraceae (although described as 'secretion' and not 'nectar'; Thien, 1980; Gottsberger, 1988), Violaceae (Erbar & Leins, 2010), and Corynocarpaceae (Matthews & Endress, 2004);

e) Sepal/Tepal (*n* = 4) – in Asparagaceae and Arecaceae (Bernardello, 2007; Erbar & Leins, 2010).

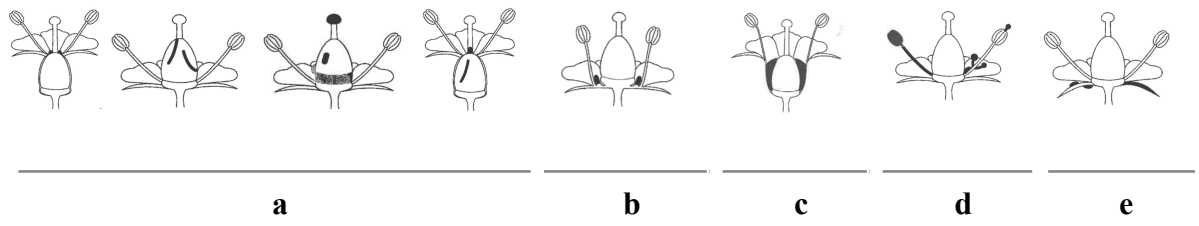

Figure 1: The variety of nectary locations (shown as black-shaded structures) in a hypothetical flower, limited to the taxa studied in this thesis, which excludes nectaries within nectar spurs. Locations include: a) ovarian, b) intrastaminal, c) hypanthial, d) staminal, and e) sepal/tepal (drawings from Bernardello, 2007).

1.2.3 How is Nectar Secreted?

Floral nectaries have independently evolved numerous times, resulting in a diverse array of mechanisms for nectar secretion, including apoplastic, merocrine, holocrine and eccrine secretion (Bernardello, 2007; Heil, 2011; Roy et al., 2017), the latter of which has been best understood. Likely, the merocrine and eccrine mechanisms operate at the same time, carrying different substances (Chatt et al., 2021).

In the model of apoplastic nectar secretion, metabolites in phloem sap move around parenchyma cells to the nectary surface (Kram & Carter, 2009; Vassilyev, 2010). However, this mechanism fails to explain the distinct differences in chemical composition typically observed between nectar and phloem sap, or the common occurrence of large starch stores in floral nectaries before anthesis (Liu & Thornburg, 2012; Lüttge, 2013).

During merocrine (also known as granulocrine) secretion, metabolites are thought to travel to secretory cells near the nectary surface via plasmodesmata (Fahn, 1979a, b; Pacini & Nepi, 2007). After accumulation into vesicles derived from the endoplasmic reticulum and Golgi apparatus, these metabolites are released through fusion of the vesicles with the plasma membrane. While supported by ultrastructural studies, this theory has yet to be experimentally confirmed (Fahn, 1979a, b).

Holocrine nectar secretion seems rare as it has been described for only a few species (Horner et al., 2003; Nepi, 2007; Vesprini et al., 2008). It involves nectary parenchyma cell apoptosis followed by rupture of the plasma membrane and the overlying cuticle (Wunnachit et al., 1992).

In eccrine nectar secretion sugars are transported across the nectary parenchyma's plasma membrane via specialised membrane pores or transporters (Lin et al., 2014). Nectar sugars first enter the nectary parenchyma from the phloem via a symplastic or apoplastic route, where they may be stored as starch in amyloplasts or amylochromoplasts. Subsequent starch

degradation into sucrose facilitates nectar secretion during anthesis (Horner et al., 2007; Ren et al., 2007; Lin et al., 2014), which may be supplemented by the simultaneous import of additional sugars from the phloem (Solhaug et al., 2019). Sucrose (S) was long believed to be hydrolysed exclusively by β-fructofuranosidase, commonly known as invertase (Lüttge, 1977; Baker & Baker, 1983; Minami et al., 2021; Figure 2), into equal parts of fructose (F) and glucose (G), both hexose sugars, to a 1S:(1F+1G) ratio ('Baker's ratio' from here on).

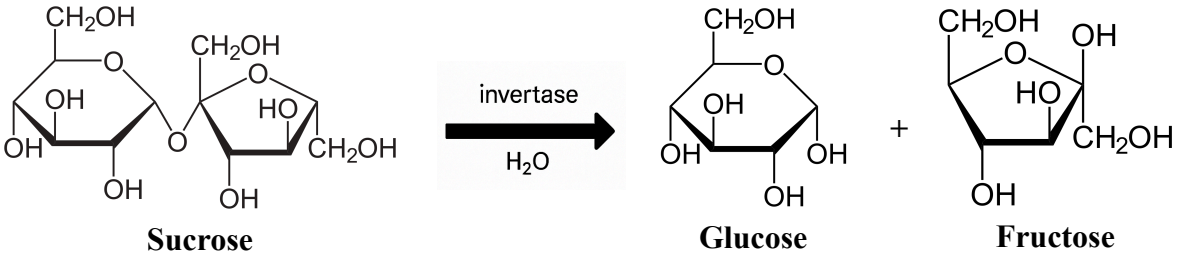

Figure 2: Invertase catalyses the irreversible hydrolysis of sucrose into glucose and fructose in a 1:1 ratio of hexoses. (Adapted from Minami et al., 2021).

However, more recent research in the hexose-rich model organism *Arabidopsis* has shown that sucrose hydrolysis is primarily driven by the activity of cell wall invertase 4 (CWINV4) (Kram & Carter, 2009; Ruhlmann et al., 2010; Lin et al., 2014; Minami et al., 2021; Figure 3).

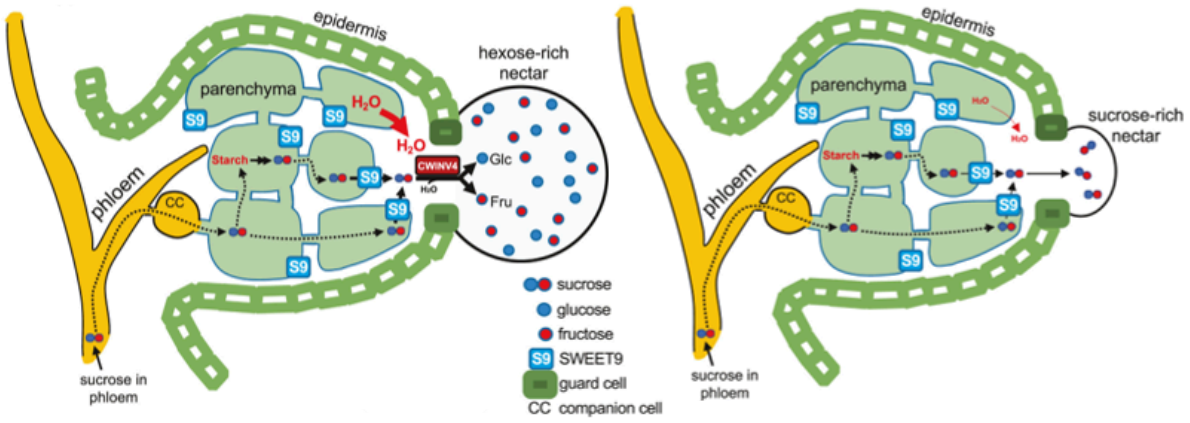

Figure 3: Simplified model of sucrose hydrolysis and nectar secretion in the nectary of the wild-type (left) and mutant (right, on the example of the model organism *Brassica napus*) with cell wall invertase 4 (CWINV4) only active in a) (Figure from Minami et al., 2021).

Minami et al. (2021) supported this hypothesis by showing that silencing the gene controlling CWINV4 activity (in another hexose-rich model organism, *Brassica napus*) leads to the secretion of sucrose-rich nectar, as hydrolysis does not take place (Figure 3, right). Hence, in

a wild-type flower with active CWINV4 (Figure 3, left), the nectary receives sucrose or other sugars from the phloem, passes it through the parenchyma and stores it as starch. During anthesis, starch breaks down and is re-synthesised into sucrose, facilitated by sucrose-phosphate synthase and other enzymes. Subsequently, sucrose is transported to the extracellular space by the sucrose uniporter SWEET9 (S9) on the plasma membrane in a concentration-dependent process. Then cell wall invertase (CWINV4) breaks down sucrose into hexoses (Ruhlmann et al., 2010), creating a more negative osmotic potential and promoting the diffusion of water. Lastly, this forms a nectar droplet, which exits through open stomata (Roy et al., 2017). When CWINV4 activity is absent (Figure 3, right), the sucrose exported by SWEET9 remains unhydrolysed in the apoplast. This situation increases the sucrose gradient and decreases the osmotic gradient, hindering water flow out of nectary parenchyma cells and resulting in a smaller, sucrose-rich nectar droplet (model based on Kram & Carter, 2009; Lin et al., 2014).

However, the F:G ratio often deviates significantly from the expected 1S : (1F + 1G) in many species as hexoses often undergo further biochemical pathways and microbial digestion (Wenzler et al., 2008), which alters the Baker's ratio (Herrera et al., 2008). Moreover, it is also hypothesised that hexose ratios could be altered by hexose transporters, which move F and G in or out of the nectary parenchyma (Lin et al., 2014).

Conversely, many nectaries are green, containing chloroplasts rather than amyloplasts, which are likely to photosynthesise their sugars and typically do not accumulate starch before or after anthesis (Davis et al., 1986; Davis et al., 1988; O'Brien et al., 1996; Vesprini et al., 1999; Vezza et al., 2006; Nepi, 2007; Clearwater et al., 2021).

1.3 What is Nectar Composed of?

Nectar is a sugar-rich fluid mainly containing the three sugars sucrose [S; a disaccharide, $C_{12}H_{22}O_{11}$; ~342 g/mol], glucose (G), fructose (F) [both hexoses, $C_6H_{12}O_6$; ~ 180 g/mol] and water next to minor amounts of other sugars, amino acids, vitamins and secondary metabolites. Total SFG concentrations vary from 7 - 70% w/w, with S : F : G ratios used to classify nectar based on its dominant sugar (Baker & Baker, 1983). Moreover, nectar may contain proteins, ions, terpenoids, volatiles (e.g. benzyl acetone), lipids, cytoplasmic remnants and microorganisms (Nicolson & Thornburg, 2007; Brandenburg et al., 2009; Raguso, 2004; Boughton, 1981; Mittelbach et al., 2015), which are not part of this thesis but are briefly reviewed here.

1.3.1 Carbohydrates

Besides sucrose, possible nectar disaccharides are cellobiose, maltose, lactose and alpha-trehalose. Within the monosaccharides, next to F and G, nectar can also contain a) hexoses – rhamnose, galactose, mannose, sorbose, and myoinositol; b) polyols (sugar alcohols, ~ 182 g/mol; $C_6H_{14}O_6$) – mannitol and sorbitol; c) pentoses (~ 150 g/mol; $C_5H_{10}O_5$) – xylose, arabinose, and ribose; d) the tetrose erythrose, and e) the triose (~ 90 g/mol, $C_3H_6O_3$) dihydroxyacetone (DHA). Common nectar oligosaccharides are part of the Raffinose-Family (RFO, 504 - 828 g/mol, $C_{18\text{-}30}$-structure), including pentasaccharides, such as verbascose (828 g/mol, $C_{30}H_{52}O_{26}$), tetrasaccharides like stachyose (~ 666 g/mol, $C_{24}H_{42}O_{21}$), and trisaccharides, e.g. raffinose and melizitose (~ 504 g/mol, $C_{18}H_{32}O_{16}$) (Nicolson & Thornburg, 2007).

1.3.2 Non-Carbohydrates

More recently, advanced analysis methods like Liquid Chromatography - Mass Spectrometry (LC-MS) revealed the presence in nectar of proteins, amino acids, vitamins and secondary metabolites such as alkaloids and phenolics, often with bioactive activity.

Proteins (nectarins) can alter nectar chemistry and inhibit microbial growth (Roy et al., 2017). Amino acids appear to influence the taste of the nectar (Gardener & Gillman, 2002), its nutritional value (Erhardt & Mevi-Schütz, 2005), and are the most abundant nectar solutes after sugars (Petanidou et al., 2006). Alkaloids repel nectar robbers (often insects). They can reduce the pathogen load in the plant's main pollinator as some animals (particularly birds) can have a tolerance towards the alkaloid's toxicity (Clinch et al., 1972; McDougal et al., 2015; Gunasekaran et al., 2021). Dark, bitter-tasting nectar in Aloes has been associated with

generalist passerines, functioning as a filter for flower visitors (Johnson et al., 2006). Phenolics, especially flavonoids, have raised particular interest in nectar research over the past decade based on their medicinal properties, particularly their antimicrobial activity (Yilmaz & Karadeniz, 2014; Smallfield et al., 2018; Viteri et al., 2021; Sotelo-Lara et al., 2023). Some phenolics, as well as a number of other types of compounds present in nectar, including ascorbate (Vitamin C), can have antioxidant properties that are involved in nectar homeostasis (Carter & Thornburg, 2004).

1.3.4 Water

The overall nectar concentration depends on both the secretion mechanism and the floral microclimate and may be greatly affected by evaporation in flower shapes with exposed nectaries, such as in dish shaped flowers, compared to flowers with gullet or tube shapes, particularly when temperatures are elevated (Corbet, 2003). Conversely, nectar water levels increase with relative humidity (e.g. Bertsch, 1983).

Based on anatomical studies of nectary vascularisation, water derives from the phloem alone (about 48% of species studied), the xylem and the phloem (about 12 %) or the nectaries are not vascularised at all, depending on the nectary structure and species (Frei, 1955; Davis et al., 1986, 1988). As concentration determines the nectar’s viscosity, it plays an important role in the feeding responses of animals (Nicolson & Thornburg, 2007). Besides balancing the nectar's viscosity, the water in nectar may serve as an essential reward for pollinators in dry conditions.

1.4 Does Nectar Vary Within Individuals and Among Populations and Species?

1.4.1 Nectar is an Extremely Variable Plant Trait

Nectar composition and concentration varies with flower (Baker & Baker, 1983) and nectary morphology (Pacini & Nepi, 2007; Willmer, 2011), genetics (Pacini et al., 2003), and pollination syndrome (Cruden et al., 1983; Pleasants, 1983; Gill, 1988). It dynamically responds to the microenvironment, the season (Pacini et al., 2003), the time of day (Baker & Baker, 1983), the flower's age (Gottsberger et al., 1990), and the microbiome (Mittelbach, 2015; Bohórquez, 2018). Variation in nectar volume, concentration and composition can occur between single flowers on the same individual, within an individual at different times (see 1.4 and Chapter 2), between individuals within populations, between populations (see 1.4 and Chapter 3), and between species (see 1.4 and Chapter 4) (Baker & Baker, 1983).

1.4.2 Do Regional Climate Differences Affect Species-Specific Nectar Traits?

Nectar is an extremely variable trait, often varying between flowers and sampling times within plants (e.g. Noe et al., 2019). However, some nectar traits may vary within species based on the influence of environmental factors, such as air temperature, radiation, humidity, precipitation, groundwater access and soil fertility (Shuel, 1975; Cruden et al., 1983; Pleasants, 1983; Faegri & van der Pijl, 2013).

However, studies on the variability of nectar production by individual species at regional or national scales—where climatic differences are pronounced—are scarce. A New Zealand study by Noe et al. (2019) analyzed how mānuka (*Leptospermum scoparium*) floral nectar traits varied among different locations, individual plants, and branches, exploring the influence of both plant and environmental factors on this variation. They found significant differences in nectar traits across locations, yet the most substantial variation (40 - 70%) occurred from one plant to another within the same site. The observed variation among neighboring wild plants highlights the complex interplay between genotype, environmental conditions, and flowering timing.

1.4.3 How Do Plants Respond to Nectar Removal?

The phenomenon of how nectar varies daily when frequently removed by pollinator visits, remains underexplored (Farkas et al., 2012; Luo et al., 2014). These experiments are termed 'Removal-Enhanced Nectar Replenishment' or 'RENR' studies (Chapter 2). The term 'removal-enhanced' is slightly misleading, implying solely a positive response to nectar removal, although the 'enhanced' refers to the removal's manual or anthropogenic nature. However, the plant's response to frequent nectar removal can also be 'reduced' (negative), or 'consistent' (neutral) compared to unmanipulated controls (Galetto et al., 1994, 1997; Torres & Galetto, 1998; McDade & Weeks, 2004; Nicolson & Nepi, 2005; Carlson, 2007; Ornelas et al., 2007; Bobrowiec & Oliveira, 2012; Veiga Blanco et al., 2013; Luo et al., 2014). A positive response indicates that a frequently emptied flower secretes more nectar than unmanipulated control plants within the same given timeframe and under the same environmental conditions.

Bird-pollinated species often exhibit positive replenishing responses (Torres & Galetto, 1998; Nicolson & Nepi, 2005; Ornelas et al., 2007; Bobrowiec & Oliveira, 2012; Luo et al., 2014). Concomitant to a positive nectar replenishment response is the secretion of more diluted nectar to balance the demand of large pollinators against the photosynthetic supply. While less concentrated, this nectar may temporarily satisfy birds and encourage their return. However, this may only stay effective until the pollinator's sugar intake is insufficient to maintain energy balance (Fleming et al., 2008). The plant may also face limitations in supplying additional water indefinitely and might eventually cease secretion to maintain overall water balance efficiently (Kim et al., 2011; Willmer, 2011).

Having a closer look at the level of a plant individual, changes in humidity throughout the day can cause a characteristic diurnal pattern of change in nectar concentrations (Pleasants, 1983). Based on the hydrophilic nature of nectar, nectar concentration can decrease if atmospheric water is absorbed. Alternatively, drier air causes nectar water to evaporate, increasing concentration (Corbet, 2003) Extreme temperatures can cause flowers to reduce nectar production (Willmer, 2011; Descamps et al., 2021). The plant's water status may cause the nectar to be reabsorbed, allocating additional water to be redistributed to the flower's sexual organs and preventing thermal damage (Patiño & Grace, 2002; Roddy, 2019). Nectar resorption is claimed to be a strategy for resource recovery, reclaiming invested materials in nectar production. Despite typically occurring in ageing flowers, nectar resorption can also coincide with nectar secretion within flowers in anthesis. Viewing nectar production as a unified process involving both secretion and resorption allows for the modulation of these

phases, ensuring nectar concentration within a suitable range for pollinators, establishing 'nectar homeostasis' (Nepi & Stpiczyńska, 2008). This holistic perspective on nectar dynamics underscores the complexity of plant-pollinator interactions. Yet, intriguingly, plant responses to nectar removal among New Zealand tree species remain underexplored. In contrast, non-woody herbaceous species such as *Phormium tenax* (harakeke, New Zealand flax) have been studied for their nectar replenishment responses, notably in a pollinator-focused dissertation (Murphy, 1996). However, as my thesis focused exclusively on woody species, Murphy's study is of general interest but not directly comparable to my research.

1.5 How Do Floral Traits Relate to Nectar?

As previously discussed, floral characteristics and plant-pollinator interactions play significant roles in nectar variation in addition to the influence of abiotic factors. The subsequent section outlines key floral traits, including size, shape, and colouration, as well as general and specific insights into pollinators, crucial for comprehending variations in nectar. Chapters 3 and 4 will more closely test the idea that visual floral traits (and phylogeny) like size, colour, and shape can predict nectar traits like sugar amount, composition, volume and concentration.

1.5.1 Do Visual Floral Traits Signal Quantity and Quality Of Nectar?

Visual floral traits, like shape, size, and colour, likely evolved as signals to reveal concealed or access-limited rewards (Fenster et al., 2006). Some pollinators, especially vertebrates and hymenopterans, can learn to associate floral signals with rewards, enhancing constancy on more rewarding flowers. This collective integration of rewards and cues is crucial at the community level, where co-flowering species compete for pollinators (Ortiz et al., 2021).

A recent broad interspecific study on almost 100 Mediterranean nectar-producing species showed that nectar sucrose content, as well as flower colour and size, displayed no discernible phylogenetic signal, indicating adaptive evolution in response to varying conditions in the pollinator community. However, flower size was significantly correlated with nectar quantities and flower colour (chromatic contrast) was correlated with nectar concentration, whereas no associations were found between flower shape and rewards (Ortiz et al., 2021).

For a signal to effectively encourage pollinators to return, it must be honest—that is, it should accurately reflect the quantity or quality of the reward (Armbruster et al., 2005; Knauer & Schiestl, 2015). In cases where nectar secretion ceases in older flowers, the integrity of the floral signal may temporarily diminish in many species or is enhanced by a visual change of the signal. For instance, New Zealand's *Fuchsia excorticata* flowers change mid-anthesis from green-purple to pink, with flowers holding significantly more nectar before their colour change (Delph & Lively, 1985). Despite occasional mismatches between floral signals and rewards, plants offering more abundant rewards maintain honest signals, facilitating pollinator recognition and memory. For example, large corollas, which are hard to access, signal great nectar rewards (Ortiz et al., 2021).

Flower Shape

The level of a flower's accessibility is a simple way to categorise flower shapes more objectively (Faegri & van der Pijl, 1979; Figure 4). It can be expressed in self-defined numerical values that represent a determined hierarchy. This is essential for statistical testing to reveal relationships between flower shape and nectar characteristics (as applied in Chapter 4). According to Faegri and van der Pijl's proposed generalised hierarchy—from highly to hardly accessible (with actual accessibility ultimately depending on the specific pollinator's size)—flower shapes in my thesis are categorised as follows (Figure 4):

1. 'dish-/bowl-shaped',
2. 'head-/brush-shaped',
3. 'bell-/funnel-shaped',
4. 'gullet-shaped',
5. 'flag-shaped', and
6. 'tube-shaped'.

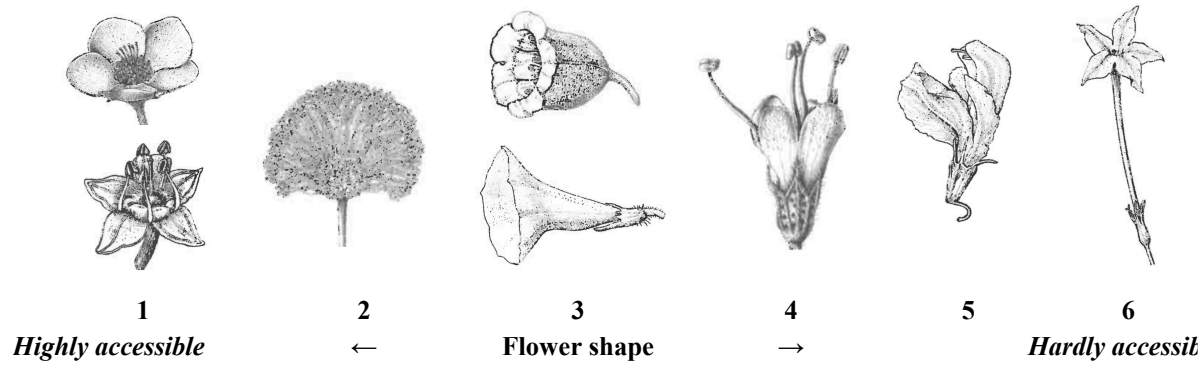

Figure 4: Flower shape categories from highly to hardly accessible, with 1) dish- or bowl-shaped, 2) head- or brush-shaped, 3) bell- or funnel-shaped, 4) gullet-shaped, 5) flag-shaped, 6) tube-shaped flowers (from Faegri & van der Pijl, 1979).

Flower Colour

Flower colour is a crucial trait through which plants communicate information about their identity and location to potential visitors (Streinzer et al., 2021). It is a psychophysical phenomenon dependent on how the observer's visual system impacts the perceived light spectrum (Kelber & Osorio, 2010; Skorupski & Chittka, 2011). Literature on colour vision in pollinators reveals a diverse array of adaptations in flower colouration, with specific colours aligning with the visual capabilities and preferences of different pollinator species (Willmer, 2011).

For instance, bees, major angiosperm pollinators, exhibit trichromatic colour vision with photoreceptor sensitivity in the ultraviolet (UV), blue, and green regions (Peitsch et al., 1992; Briscoe & Chittka, 2001). Flowers showing these colours have higher than average reward quantities and are mostly pollinated by bees (Menzel & Shmida, 1993; Giurfa et al., 1995). Red flowers prevail in habitats rich in birds, as they are drawn to the red flower's superior visibility from a distance (Crosswhite & Crosswhite, 1982). While bees can distinguish and visit red flowers (Rodríguez-Gironés & Santamaría, 2004), difficulty arises in detecting them, given the poor contrast against green foliage in the background (Willmer, 2011). Yellow flowers are typically linked to unspecialised species, pivotal in attracting and rewarding various pollinator species (Willmer, 1990). Alternatively, floral colouration in some species may be unrelated to pollinator selection pressure and instead be the result of pleiotropic effects within pigmentation biosynthetic pathways (Armbruster, 1993; Schemske & Bierzychudek, 2007; Smith et al., 2008). For instance, floral variation in anthocyanin content, responsible for blue, red and purple tissue colouring, may be influenced by various environmental stresses (Strauss & Whittall, 2006). This phenomenon is likely attributed to the role of flavonoids, the biochemical precursors of red anthocyanin pigments (Harborne, 1967), in buffering plants against extremes of light and heat (Holton & Cornish, 1995; Chalker-Scott, 1999; Hoch et al., 2001; Coberly & Rausher, 2003).

1.5.2 How Do Pollinators Relate To Floral Traits?

The visual diversity observed flower shape, size and colour is captivating and raises intriguing questions about evolution. Since the propositions of Kölreuter (1761) and Sprengel (1793; both translated in Lloyd & Barrett, 1996) suggested that the primary function of animal-pollinated flowers is to attract animals, the dynamics of plant-pollinator relationships have become a focal point of extensive research (reviewed in Fenster et al., 2004).

Whereas pollen is consumed by pollinators and their larvae to foster growth, nectar provides a quickly digestible energy source, especially to cater for the high demands of flying pollinators (Winter & von Helverson, 1998). Pollinators often prefer specific floral characteristics, such as size, shape and colouration (Müller, 1883; Knuth, 1906; Baker, 1963; Grant, 1965; Ollerton, 1996; Waser, 1998).

Through repeated visits, pollinators develop learned preferences based on experiences, with innate preferences existing among pollinator groups (Giurfa et al., 1995; Lunau & Maier, 1995; Goyret, 2008; Streinzer et al., 2019). Innate preferences, while often overridden by learned experiences, may aid naïve individuals in efficiently locating rewarding food sources.

Generally, the level of nectar volume correlates with the pollinator's size and energetic requirements (Faegri & van der Pijl, 2013). Pollinator selectivity can lead to reproductive isolation, driving the evolution of divergent floral morphologies (Hodges & Arnold, 1994; Bradshaw & Schemske, 2003; Ippolito et al., 2004). However, there is ongoing debate regarding the general predictive power of floral traits in determining pollinator types (Waser et al., 1996; Ollerton, 1998). Nonetheless, floral traits can undergo evolutionary diversification for various reasons, potentially decoupling their evolution from pollinator-mediated selection (Strauss & Whittall, 2006). A significant challenge lies in assessing the relative importance of pollinators in the evolutionary processes shaping floral traits (Cooley et al., 2008).

Flowers that attract similar groups of pollinators often develop converging characteristics, allowing them to be categorised into 'syndromes.' The most frequently mentioned are the entomophilous (insect-pollinated) and ornithophilous (bird-pollinated) syndromes (Castro & Robertson, 1997). The 'entomophilous syndrome' defines insect-pollinated plants with small scented flowers, usually white, cream, purple, green or yellow. They secrete small amounts of highly concentrated nectar (Faegri & van der Pijl, 2013). In comparison, flowers of the 'ornithophilous syndrome' are bird-pollinated, large, brightly coloured (red, orange, yellow), hardly scented but contain large volumes of low concentrated nectar (Faegri & van der Pijl,

2013). Lastly, some flowers show no particular pollinator-associated syndrome as they are pollinated by diverse guilds (Herrera, 1996).

Pollination Syndromes in New Zealand

The entomophilous syndrome is prevalent among New Zealand tree species (Cockayne, 1928; Godley, 1979), whereas the ornithophilous syndrome is comparatively less frequent (Castro & Robertson, 1997). Many important pollinators in other countries, like long-tongued bees and hawkmoths, are missing in New Zealand (Heine, 1937; Godley, 1979; Webb & Kelly, 1993). Researchers argue that this role has been filled by Diptera (flies) (Thomson, 1927; Heine, 1937), reptiles (Whitaker, 1987), bats (Daniel, 1976; Ecroyd, 1996) or birds (Godley, 1979; Delph & Lively, 1989).

Presumably, many New Zealand flowers coevolved with short proboscis flies, as New Zealand flowers show few characteristics adapted to heavy insects with long mouthparts, such as bumble bees (Nicolson & Thornburg, 2007). However, the flowers of some New Zealand species are robust enough to support the weight of small vertebrates and are known to be visited by reptiles, bats, and birds (Whitaker, 1987; O'Donnell & Dilks, 1994; Castro & Robertson, 1997; Arkins et al., 1999; Newstrom & Robertson, 2005; McCartney et al., 2007; Pattemore, 2011). Birds have been observed feeding on species traditionally categorised as entomophilous, including *Carpodetus serratus*, *Elaeocarpus dentatus*, *Leptospermum scoparium*, *Pseudopanax* spp., and *Weinmannia racemosa*. These plants arrange their flowers in inflorescences that provide sufficient physical support for a bird's weight (O'Donnell & Dilks, 1994).

However, the dietary needs of most vertebrates are primarily met by the abundant nectar of New Zealand's relatively few ornithophilous species. These plants typically feature large, tubular flowers with bright colouration, such as *Alepis flavida*, *Alseuosmia* spp., *Fuchsia excorticata*, *Clianthus* spp., *Phormium tenax*, *Rhabdothamnus solandri*, *Sophora* spp., and *Vitex lucens* (Whitaker, 1987; O'Donnell & Dilks, 1994; Ladley et al., 1997; Newstrom & Robertson, 2005; McCartney et al., 2007; Merrett et al., 2007; Anderson et al., 2011). Moreover, the red-coloured, brush-shaped flowers arranged in large inflorescences of most *Metrosideros* spp. are favoured by the native short-tailed bat (*Mystacina tuberculata*), many lizard species, and nectar-feeding birds (Whitaker, 1987; O'Donnell & Dilks, 1994; McCartney et al., 2007).

1.6 Studied Taxa

To obtain information on nectar production by New Zealand forest tree species, I studied 50 (+ 1 variant) species (Table 1), with 47 (+1) native and 3 exotic to New Zealand (*Paraserianthes lophanta* and *Cordyline stricta* from Australia, and *Robinia pseudoacacia* from North America; naturalised in New Zealand since 1870; New Zealand Plant Conservation Network, 2023), selected based on their wide distribution, abundance and basal area in National Vegetation Survey vegetation plot data, at a national scale, and on previously reported nectar production (Martin, 1961; Castro & Robertson, 1997; GBIF.org, 2018; Landcare Research, 2018a, b, c, d).

Of the 48 native species examined, 36 exhibited floral traits characteristic of the entomophilous syndrome, while 16 displayed traits indicative of the ornithophilous syndrome. However, it's important to note that a species' pollinator syndrome doesn't always align with its pollinator guild, as multiple pollinator guilds visit many species.

The sampled species represent a diverse phylogenetic range, spanning 15 orders, 21 families, and 31 genera. Most genera are represented by a single species, except for *Sophora* (6 spp.), *Metrosideros* (4 spp. + 1 variety), *Pittosporum* (5 spp.), *Cordyline, Lophomyrtus*, and *Melicytus* (3 spp. each), and *Griselinia, Kunzea*, and *Pseudopanax* (2 spp. each), as detailed in Table 1. These species include 44 core eudicots (27 rosids and 17 asterids) and six basal species (one magnolid, four monocots, and one basal eudicot).

Table 1: Species and their phylogenetic relationships and abbreviations within the following chapters, with non-native species listed under the column 'exotic'.

GROUP	CLASS	SUBCLASS	CLADE	SUBCLADE	ORDER	FAMILY	GENUS	SPECIES	Abbreviation	CHAPTER 2	CHAPTER 3	CHAPTER 4	exotic
ANGIOSPERMS	BASAL	MAGNOLIIDS			CANNELALES	WINTERACEAE	*PSEUDOWINTERA*	*COLORATA*	PSE COL			X	
CORE	MONOCOTS		COMME-LINIDS		ARECALES	ARECACEAE	*RHOPALOSTYLIS*	*SAPINDRA*	RHO SAP			X	
			LILIOIDS		ASPARAGALES	ASPARAGACEAE	*CORDYLINE*	*AUSTRALIS*	COR AUS		X	X	
								BANKSII	COR BAN			X	
								STRICTA	COR STR			X	X
	EUDICOTS	BASAL			PROTEALES	PROTEACEAE	*KNIGHTIA*	*EXCELSA*	KNI EXC			X	
		CORE	ROSIDS	MALVIDS	SAPINDALES	RUTACEAE	*MELICOPE*	*TERNATA*	MEL TER			X	
					MYRTALES	ONAGRACEAE	*FUCHSIA*	*EXCELSA*	FUC EXC		X	X	
						MYRTACEAE	*LOPHOMYRTUS*	*OBCORDATA*	LOP OBC			X	
								BULLATA	LOP BUL			X	
							KUNZEA	*ERICALES*	KUN ERI			X	
							LEPTOSPERMUM	*SCOPARIUM*	LEP SCO		X	X	
							METROSIDEROS	*EXCELSA*	MET EXC		X	X	
								EXCELSA AUREUS	MET E AUR			X	
								FULGENS	MET FUL			X	
								COLENSOI	MET COL			X	
								UMBELLATA	MET UMB			X	
				FABIDS	CUCURBITALES	CORYNOCARPACEAE	*CORYNOCARPUS*	*LAEVIGATUS*	COR LAE			X	
					OXALIDALES	CUNONIACEAE	*WEINMANNIA*	*RACEMOSA*	WEI RAC			X	
						ELAEOCARPACEAE	*ARISTOTELIA*	*SERRATA*	ARI SER			X	
							ELAEOCARPUS	*DENTATUS*	ELA DEN			X	
					MALPHIGIALES	VIOLACEAE	*MELICYTUS*	*LANCEOLATUS*	MEL LAN			X	
								NOVAE ZEALANDIAE	MEL NOV			X	
								RAMIFLORUS	MEL RAM			X	
					FABALES	FABACEAE	*CARMICHAELIS*	*AUSTRALIS*	CAR AUS			X	
							CLIANTHUS	*MAXIMUS*	CLI MAX			X	
							ROBINIA	*PSEUDOACACIA*	ROB PSE			X	X
							PARASERIANTHES	*LOPHANTA*	PAR LOP			X	X
							SOPHORA	*CHATHAMICA*	SOP CHA			X	
								GODLEYI	SOP GOD			X	
								LONGICARINATA	SOP LON			X	
								MICROPHYLLA	SOP MIC		X	X	
								PROSTATA	SOP PRO			X	
								TETRAPTERA	SOP TET			X	
			ASTERIDS	LAMIDS	GENTIALES	LOGANIACEAE	*GENIOSTOMA*	*RUPESTRE*	GEN RUP			X	
					LAMIALES	SCROPHULARIACEAE	*MYOPORUM*	*LAETUM*	MYO LAE			X	
						PLANTAGINA	*VERONICA*	*DIOSMIFOLIUM*	VER DIO			X	
						GESNERIACEAE	*RHABDOTHAMNUS*	*SOLANDRII*	RHA SOL			X	
						LAMIACEAE	*VITEX*	*LUCENS*	VIT LUC	X		X	
				CAMPANULIDS	APIALES	PITTOSPORACEAE	*PITTOSPORUM*	*COLENSOI*	PIT COL			X	
								CRASSIFOLIUM	PIT CRA		X	X	
								EUGENIOIDES	PIT EUG		X	X	
								RALPHII	PIT RAL			X	
								TENUIFOLIUM	PIT TEN		X	X	
						GRISELINIACEAE	*GRISELINIA*	*LITTORALIS*	GRI LIT			X	
								LUCIDA	GRI LUC			X	
						ARALIACEAE	*PSEUDOPANAX*	*ARBOREUS*	PSE ARB			X	
								CRASSIFOLIUM	PSE CRA			X	
					ASTERALES	ROUSSEACEAE	*CARPODETUS*	*SERRATUS*	CAR SER			X	
						ARGOPHYLLACEAE	*COROKIA*	*COTONEASTER*	COR COT			X	
				ASTERIDS	ERICALES	PRIMULACEAE	*MYRSINE*	*AUSTRALIS*	MYR AUS			X	

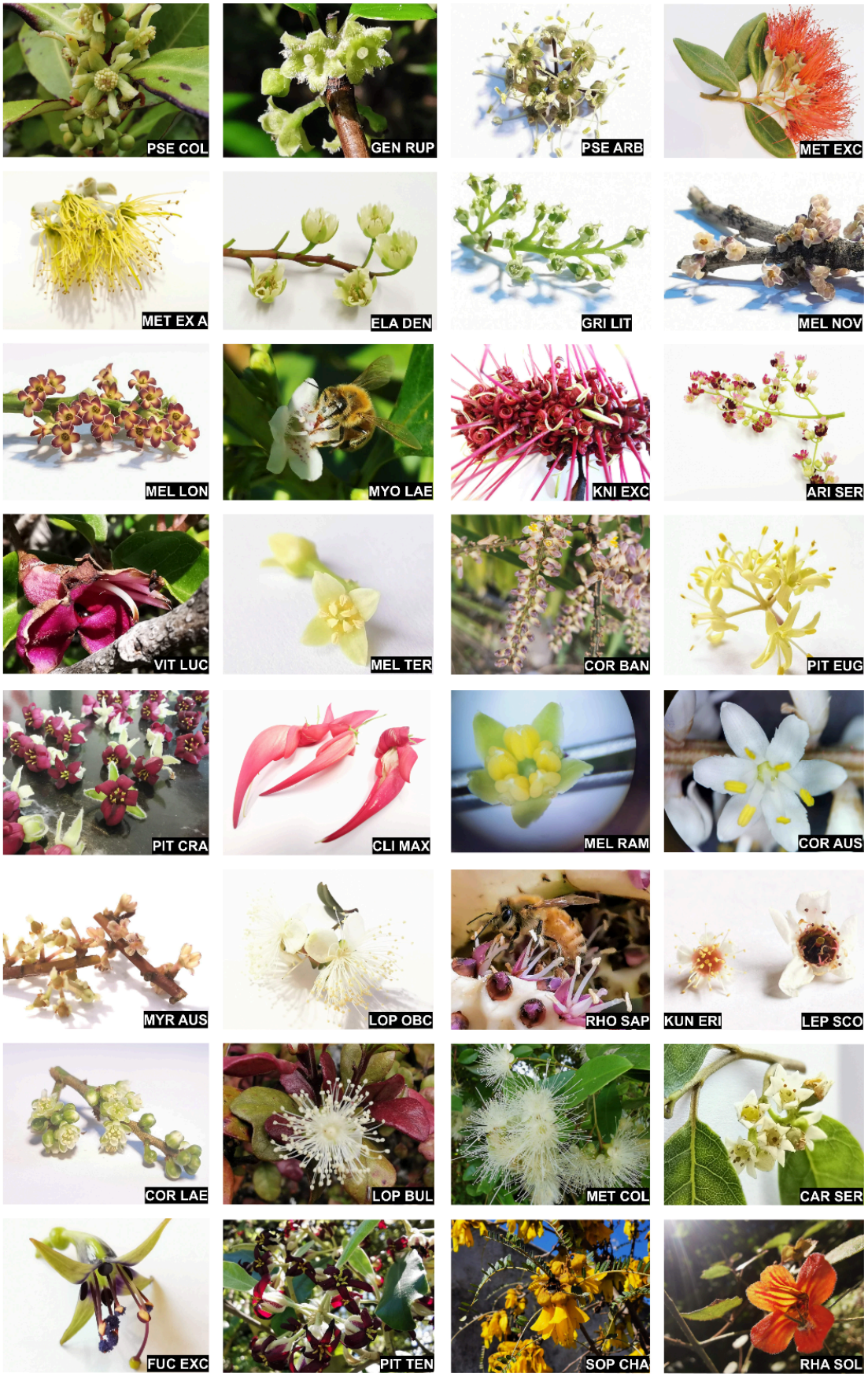

Figure 5: Selection of sampled species (own photographs, with key to abbreviations in Table 1) showing a wide variety of visual floral traits.

1.7 How to Assess Floral Nectar Production Rates

To assess a flower's nectar production, visually fresh-appearing flowers in peak anthesis are typically shielded from pollinator access and left for 24 hours. This protection can be achieved in a controlled environment like a greenhouse or using mesh-like bags to cover inflorescences in the field, allowing continuous airflow. The amount of nectar collected after 24 hours is called the 'nectar production rate (NPR)' (e.g. Pleasants, 1983). I distinguish between two NPR methods in this thesis:

1. In the 'ecological' NPR method, flowers are bagged as found regardless of whether they already contain any nectar or not, with the nectar that is already present referred to as a 'standing crop'. The last pollinator visit, evaporation, and reabsorption effects all influence the standing crop (Baker & Baker, 1983; Burquez & Corbet, 1991). The 'ecological' NPR sums the amount of naturally present nectar (at the time of bagging) and the amount of replenished nectar within the subsequent 24-hour period. During the refill period, the flower may secrete additional nectar to compensate for previous visits, maintain the current level (when homeostasis is present), or reabsorb nectar (Burquez & Corbet, 1991).

Chapters 3 and 4 use the ecological method for practical reasons and because I was more interested in comparisons between sites and species than absolute nectar yields.

2. In the 'physiological' NPR method, the standing crop is initially removed before flowers are bagged. The physiological NPR exclusively reflects only the amount of replenished nectar after a single (simulated) pollinator visit. This method risks potential tissue damage or nectar contamination due to non-sterile equipment. Conversely, nectar accumulated ecologically from unmanipulated flowers may be just as, or even more, contaminated. Flower visitors can introduce foreign pollen and microorganisms or get trapped while attempting to access the nectar (e.g. Mittelbach et al., 2015).

Chapter 2 uses the second method because it is focused on the physiology of nectar replenishment and, therefore, requires precise data on nectar yield.

The advantage of the 'ecological' NPR method is the reduced risk of tissue damage and the efficient processing on a large scale (Chapters 3 and 4), as flowers do not require handling (while removing a standing crop) while still on the tree, and because I was more interested in comparisons between sites and species, rather than absolute nectar yields.

1.8 Objectives and Hypotheses

This thesis investigates factors influencing nectar variation in New Zealand trees through three progressively broader studies (Figure 7), spanning from temporal variation within an individual species (Chapter 2), to variation between regional populations of eight species (Chapter 3), and to nationwide variation between many species (Chapter 4).

In Chapter 2, I explore the diurnal variation of nectar traits within a single species, simulating frequent pollinator visits to observe how flowers respond to frequent removals under fluctuating hourly microclimate conditions. Chapter 3 focuses on inter-regional variation in nectar production and composition in response to local climatic conditions. Finally, in Chapter 4, I delve into variations in nectar traits between numerous species on a broader scale, investigating the role of visual floral traits and phylogenetic relationships in driving such variations. The last chapter encompasses a comprehensive analysis of both nectar production and composition.

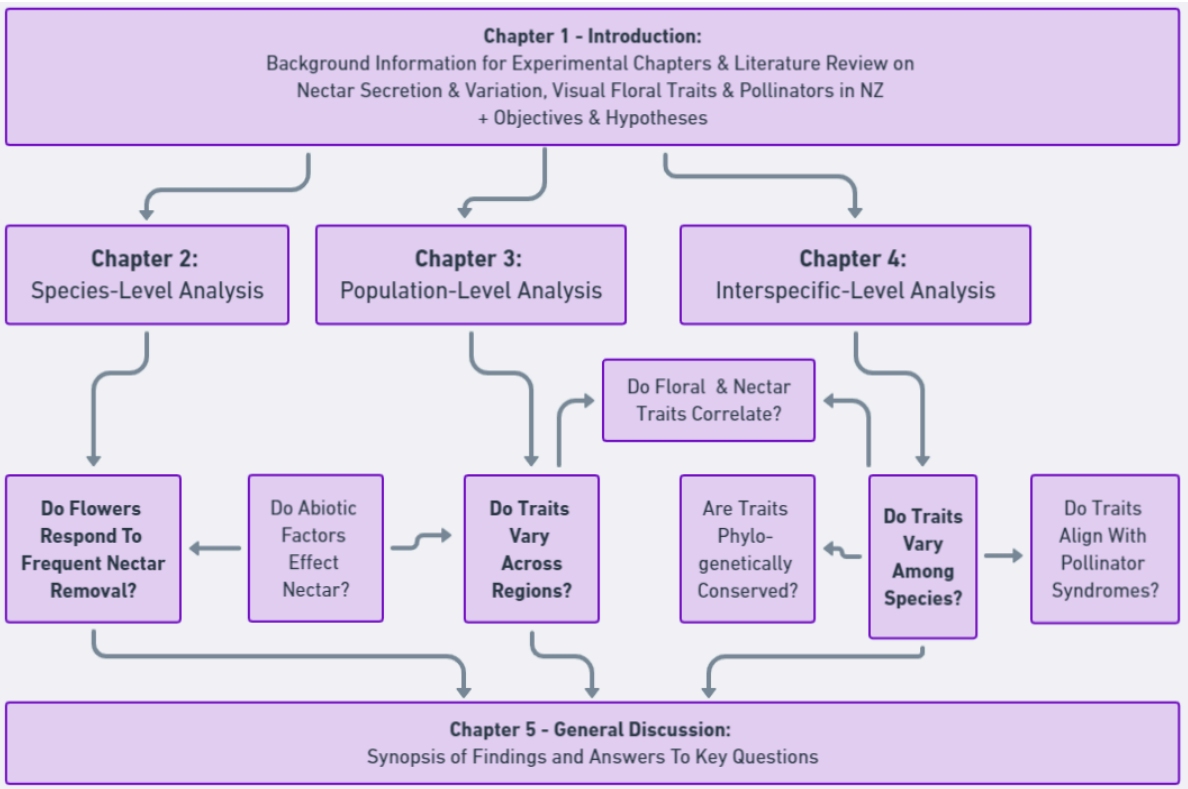

Figure 7: Thesis structure with key questions.

The Research Objectives included:

- Investigate the responses of nectar replenishment to frequent removal manipulations and determine the extent of influence by diurnal microclimate variations.

- Identify regional variation in nectar traits within species and determine the drivers, considering environmental (climatic) and floral traits.

- Identify interspecific variation in nectar and elucidate its drivers, including floral and phylogenetic factors.

Chapter 2 – Nectar Composition and Neutral Replenishment Response to Frequent Removal in the Bird-Pollinated Tree *Vitex lucens* Kirk (Lamiaceae)

This experiment investigated the nectar replenishment responses of the endemic bird-pollinated tree species *Vitex lucens* to frequent removal manipulations simulating regular pollinator visitations. I hypothesised that *V. lucen*s flowers would initially respond positively to frequent nectar removal, aligning with observations in other ornithophilous species. However, I posited that flowers would eventually cease nectar secretion and therefore doubled the experimental timeframe used by Luo et al. (2014), which motivated the research to test the hypothesis explicitly. Additionally, I hypothesised that *V. lucens* nectar component levels would fluctuate over the 24-hour experimental period, with the plant adjusting its sugar balance to cope with increased nectar demand.

Chapter 3 – Regional Nectar Variation in New Zealand Trees Across Climate Zones

This study quantified variation in floral traits within eight species across coastal climate zones in New Zealand. I hypothesised that nectar properties would vary among zones according to local climate conditions. Specifically, I anticipated that nectar volumes would be highest in regions with high humidity, given the hydrophilic nature of nectar. Additionally, I expected nectar concentrations to peak in the driest regions, characterised by limited precipitation and higher evaporation rates. Finally, I predicted that nectar sugar mass, flower fresh mass, and flower size would be the greatest in regions with the highest sunshine hours that support more productive photosynthesis, fostering plant growth and sugar production.

Chapter 4 – Interspecific Nectar Variation in New Zealand Tree Species

In this experiment, I investigated interspecific variation in nectar traits across 50 New Zealand tree species. The primary objective was to identify species-specific nectar traits, including volume, concentration, and the specific chemical composition of solubles. I tested whether closely related taxa exhibit similarities and whether visual floral signals, such as 'colour' (using photo software to calculate averaged HEX colour values) and flower size indicate the presence of specific nectar traits appealing to the species' preferred pollinator. I predicted that floral traits would be phylogenetically conserved. Furthermore, I was interested in whether nectar traits were related to easily measurable morphological floral traits, such as flower size and fresh weight.

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2. Nectar Composition & Removal-Enhanced Nectar Replenishment Response (RENR) in the Bird-Pollinated New Zealand Tree *Vitex lucens* Kirk (Lamiaceae)

Abstract

This Removal-Enhanced Nectar Replenishment (RENR) study examined the nectar composition of *Vitex lucens* Kirk (pūriri, Lamiaceae) in its warm natural habitat in Auckland, New Zealand, and assessed whether the plant responds to frequent nectar removal with a surplus (positive response), deficit (negative), or no change (neutral) in nectar production, compared to flowers sampled only once at the end of the day (controls). I hypothesised that the species would respond positively to frequent nectar removal, as bird pollination in other species has previously been linked to enhanced nectar secretion.

To do this, I first measured the ecological standing crop of nectar (from unbagged flowers) at sunrise by sampling 120 flowers across eight trees, then bagged flowers between subsequent nectar collections. Half the flowers were drained every two hours after that, whereas the remaining (controls) were drained only once in the late afternoon or at sunset.

To determine nectar replenishment amounts, their concentrations and carbohydrate compositions, nectar samples were weighed and analysed via refractometer and High-Performance Liquid Chromatography (HPLC). Data from two-hourly removed nectar were compared against time and vapour pressure deficit (VPD) using generalised additive mixed models (GAMM). I compared the cumulative amounts of nectar removed every two hours against controls using t-tests or Wilcoxon tests.

Over a 10-hour winter day, nectar secretion never ceased. Two-hourly drained flowers replenished 8.3 mg of nectar containing 6% solubles, comprising 83% monosaccharides, 14% disaccharides, 3% oligosaccharides, and traces of 16 other components on average. *V. lucens* flowers responded neutrally to frequent nectar removal: Cumulative nectar and sugar amounts

of the 10-hour and the 7-hour replenishment experiments showed no significant differences between frequently drained and control flowers (t- or Wilcoxon tests, all $P > 0.05$).
Interestingly, two-hour nectar refill volumes in frequently drained flowers exhibited a distinct diurnal pattern, with peaks in the morning and again in the evening, and a midday decline. VPD had a significant, negative cubic effect on most tested response variables (GAMM, $P = 0.03 - 0.001$; $R^2 > 0.12 - 0.36$), but not nectar concentration, sucrose-to-hexose ratio, and total sucrose, suggesting that the plant's water status influences nectar secretion throughout the day. In conclusion, the RENR response of the studied New Zealand bird-pollinated tree species appears to be driven more by the plant's water status than by the simulated increased demand from potential pollinators. I had to reject my hypothesis that this bird-pollinated species would exhibit a positive refill response. These findings suggest that the refill response is not determined by the species' pollination system, but rather reflects a species-specific physiological trait.

My results emphasise the species-specific nature of nectar removal-response types in plants and highlight the importance of monitoring environmental conditions while conducting RENR experiments. It would be interesting for future studies to determine whether phylogenetic relationships drive these species-specific nectar removal response types. Hence, I suggest conducting future RENR studies on several closely related species simultaneously.

2.1 Introduction

Nectar responds dynamically to the surrounding microenvironment, the time of the day and its removal (Baker & Baker, 1983; Pleasants, 1983). Plants can respond to repeated nectar removal with an overall surplus (positive response), deficit (negative response), or no change (neutral) in nectar production, relative to the amount accumulated in unvisited flowers over the same timeframe. Bird-pollinated species often elicit a positive replenishment response, producing increasingly diluted nectar with every additional removal (Luo et al., 2014). Across species of different pollination syndromes, positive responses were also observed by Torres and Galetto (1998), Nicolson & Nepi (2005), Ornelas et al. (2007), Bobrowiec & Oliveira (2012), and Amorim et al. (2013), negative responses by Galetto et al. (1994, 1997), McDade & Weeks (2004), and Carlson (2007), and neutral responses by Luo et al. (2014) and Veiga Blanco et al. (2013).

Most removal-enhanced nectar replenishment (RENR) experiments do not exceed monitoring periods of more than six hours (Farkas et al., 2012; Luo et al., 2014), which does not capture the natural activity periods of most pollinators (Corbet, 1978; Collins & Briffa, 1983; Alqarni, 2014; Macías-Macías, 2016). For example, bees and bumblebees forage for 11-16 hours under optimal conditions, with activity peaks before noon and late afternoon. Nectar-feeding birds are active throughout most of the photoperiod, exhibiting activity peaks similar to those of bees, as observed in the Australian brown honeyeater (*Lichmera indistincta*; Collins & Briffa, 1983). The lowered activity during the warmest and driest hours of the day, following solar noon, could be driven either by the extreme microclimatic conditions or reduced nectar supply during those periods. Nectar-feeding birds alter their foraging efforts in response to nectar quantity and energy content (Gill & Wolf, 1977; Feinsinger & Colwell, 1978; Hainsworth & Wolf, 1983), and insects like *Bombus* spp. (bumble bees; Rao et al., 2017) *Apis mellifera* (honey bee) can remember the location and success rate of past foraging efforts and apply this experience in future foraging flights (Reinhard et al., 2004; Luo et al., 2014).

It can be argued that the plant's fitness may benefit from producing more dilute nectar under frequent visitation, as sugar represents a more costly resource than water. Although the nectar may be less concentrated, it may temporarily satiate birds but keep them 'hungry and loyal' and encouraged to return. Offering too much energy decreases pollinator movement between

flowers, while too little leads to their abandoning a flower (Willmer & Stone, 2004). Moreover, the plant may eventually reach a point where it can no longer invest water for nectar production to balance tissue hydration (Patiño & Grace, 2002; Roddy, 2019).

The half-day RENR study by Luo et al. (2014) motivated me to apply their approach to a bird-pollinated New Zealand species and go one step further by doubling the experimental timeframe. For this, I chose the large pink-flowering pūriri tree (*Vitex lucens* Kirk, Lamiaceae, Chapter 1 Figure 5 M), as it secretes ample nectar amounts and shows high resilience towards handling, which I observed in preliminary studies. The tree bears flowers all year round but peaks in July and August, secreting golden yellow nectar (de Lange, 2021), collected by birds and (Petrie, 1905; Bergquist, 1987; Rasch & Craig, 1988; Anderson, 2003) potentially also by bats.

I also aimed to investigate whether sucrose levels in *V. lucens* nectar would be higher in flowers sampled every two hours compared to control flowers sampled once after 7 or 10 hours. Sucrose in nectar is hydrolysed into equal parts fructose and glucose by the enzyme cell wall invertase 4 (‘CWIN4’; Minami et al., 2021). Accordingly, I anticipated that nectar in control flowers, having remained in the flower three to five times longer than in frequently drained flowers, would exhibit reduced sucrose concentrations.

Objectives and Hypotheses

I had five aims:

i) First (preliminary study), I calculated the average daily nectar production of *Vitex lucens* flowers from various New Zealand regions to assess the plant's daily nectar replenishment following a single removal event.

ii) Second (Removal-Enhanced Nectar Replenishment 'RENR' study), I determined whether frequent nectar removal from pūriri flowers triggers a positive replenishment response, as often observed in other ornithophilous species. The extended experimental timeframe, simulating long-term high pollinator demand, also allowed me to test whether nectar replenishment can cease.

iii) Third, I evaluated whether *V. lucens* nectar contains components besides the common three sugars, sucrose, fructose, and glucose.

iv) Fourth, I determined whether any of the nectar components fluctuated in their concentrations throughout the experimental day.

v) Fifth, I tested whether cumulative sucrose amounts of frequently-drained *V. lucens* flowers were higher than in control flowers, as sucrose in the nectar of control flowers has three to five times longer (controls: 6 - 10 hours, frequently-drained: 2 hours) to hydrolyse into hexoses.

2.2 Materials and Methods

2.2.1 Study Sites

Preliminary Study

To prepare for the Auckland-based RENR study and establish a baseline for average nectar production of *V. lucens* at a national scale, I conducted a preliminary study in September and October of 2019 and 2020. Nectar was sampled from 311 bagged (24 hours) pūriri flowers across 18 trees in seven coastal regions (see Table 1).

Table 1: Sampling sites across New Zealand's North Island (Northland, Auckland, Hawke's Bay, Taranaki, and Wellington) and South Island (Nelson-Tasman & Marlborough, and West Coast), climate conditions and sampling amounts from the preliminary study on flowers of 18 *Vitex lucens* trees, with MSH = mean annual sunshine hours, MAT = mean annual air temperature, MAR = mean annual rainfall, MRH = mean relative humidity.

REGION	LATITUDE & LONGITUDE	CLIMATE ZONE	MSH h/a	MAT ℃	MAR mm/a	MRH %	*TREES n*	*FLOWERS n*
Northland	35.0° S, 173.5° E	Northern North Island	1992	15.6	1392	85	3	50
Auckland	36.8° S, 174.7° E		2062	15.6	1119	81	2	25
Hawke's Bay	39.5° S, 176.8° E	Eastern North Island	2265	14.6	786	71	2	26
Taranaki	39.0° S, 174.0° E	South-West North Island	2197	14.0	1683	85	3	52
Wellington	41.2° S, 174.7° E		2110	12.8	1249	79	3	48
Nelson-Tasman & Marlborough	41.2° S, 173.2° E	Northern South Island	2472	12.7	959	82	3	60
West Coast	42.4° S, 171.1° E	Western South Island	1725	12.2	2425	85	2	50

Sampling sites covered six New Zealand (NZ) climate zones (35° - 42° South), ranging from the subtropical Northland on NZ's North Island to the temperate Otago on the southern end of the South Island, and from the extremely wet South Island west coast to dry regions on the east coasts of both islands. Across regions, annual sunshine hours (MSH) ranged between 1725 to 2472, mean air temperature (MAT) between 12.2 to 15.6℃, rainfall (MAR) between 786 to 2425 mm, relative humidity (MRH) between 71 - 85%, and a vapour pressure deficit (VPD) between 0.21 - 0.48 kPa, with the Auckland region (also my RENR study site) reaching a mean of 0.34 kPa (based on 30-year norms, 1981 - 2010, of each sample region, NIWA, 2018).

The Auckland samples (n = 2) were collected at 9 a.m. on the 10th of October 2020 after the 24-hour bagging period. The environmental conditions during this period comprised a mean temperature of 16.8℃ (15 - 19.9, range) and a mean RH of 86.6% (64 - 95, range)(Auckland North Shore Electronic Weather Station, Number A64776, Agent 37852 - National Institute of Water and Atmospheric Research, NIWA, 36.7482° S, 174.71377° E, elevation 64 m), resulting in a mean VPD of 0.27 kPa (0.09 - 0.84, range).

Removal-Enhanced Nectar Replenishment (RENR) Study

For the RENR study, I sampled at two Auckland sites on two winter days (Site 1: 21st of August 2020, Birkenhead Reserve, 36.793289° S, 174.723899° E; Site 2: 4th of September 2020, Wenderholm Forest, Upper Waiwera; 36.537362° S, 174.711391° E), about an hour after sunrise until sunset (8 - 9 a.m. - 5 - 6 p.m.), when *V. lucens* trees were in peak flowering season. Sites were selected to have neighbouring trees of similar size, flowering state and sun exposure. RENR sites shared the same weather station (Auckland North Shore Electronic Weather Station, Number A64776, Agent 37852 - National Institute of Water and Atmospheric Research, NIWA, 36.7482 °S, 174.71377 °E, elevation 64 m). Across both sites, the 10-hour experimental day (8 a.m. - 6 p.m.) showed a mean hourly temperature ranging between 8.0 - 16.5℃, a mean hourly relative humidity of 54-94%, and a mean hourly VPD of 0.06 - 0.86 kPa (averaging 0.54 kPa), with their maxima around 3 p.m. Within the 24-hour bagging period before the experiment, both sites combined showed a mean temperature of 8℃ (1.6 - 13.7, range) and a mean RH of 76% (50 - 98, range).

To contextualise the VPD ranges that the sampled flowers experienced during the day, I calculated daily (8 a.m. - 6 p.m.) extremes and means for the sites for 2020. Monthly averaged daily extremes ranged from 0.05 to 1.65 kPa, and monthly averaged daily means between 0.33 and 1.22 kPa.

2.2.2 Sampling

For the preliminary study, I scoped for pūriri trees that the public had sighted (iNaturalist, 2019). Visually fresh flowers in peak anthesis—the stage at which they are fully open and reproductively active, typically marked by the exposure of stamens and/or pistils, pollen release, or nectar secretion—were protected with synthetic organza bags to prevent pollinator

access for 24 hours. Present nectar crops were not removed before bagging. Tree diameter at breast height (DBH) was measured for each individual. I removed the bagged inflorescences from the tree the following day and processed them a few hours later under field laboratory conditions. I removed the nectar from two trees per site in Auckland, the West Coast, Nelson-Tasman, and Napier, and three trees per site in Northland, Wellington and Taranaki. For this study and the RENR study, cumulative nectar per refill period was pooled for each tree (n = 18) into a pre-weighed 1.5 mL vial (Eppendorf, Germany), which was kept cool during the experiment and frozen immediately afterwards.

For the RENR study, I selected and numbered ten flowers from four trees the day before each experiment. On the morning of the experiment, I initially removed all standing nectar crops to reset volumes to zero and bagged inflorescences afterwards and between each subsequent drain. Standing nectar crops were weighed to serve as a baseline for comparison. Two trees were resampled every two hours until sunset, summing up to five individual refill intervals and respective nectar samples per tree. The other two trees (controls) were resampled only once—after ~ 7 hours (morning to afternoon refill period) or ~ 10 hours (morning to sunset). The 7-hour interval was chosen to assess whether the plant's daily nectar production aligns with typical pollinator activity patterns, which peak in the morning and again approximately six to seven hours later in the afternoon, as noted earlier. This would indicate whether nectar is sufficiently replenished by the afternoon to make a second visit worthwhile following a morning depletion. The 10-hour interval corresponded to the photoperiod duration on the experimental days. After the final nectar extraction, I removed the flowers from the inflorescences, stored them in zip-lock bags, and weighed them later that night.

I repeated the experiment two weeks later at Site 2, using 20 flowers per tree (n = 80). Two trees were resampled every two hours from morning until evening, yielding four refill intervals and corresponding nectar samples per tree. The control trees were resampled only once—after 7 hours (morning to afternoon refill period) and after approximately 8.5 hours (morning to sunset), with the initial drain delayed to 9 a.m. due to the need to replace previously marked flowers that had fallen off.

In total, I collected 26 nectar samples (Site 1: n = 14; Site 2: n = 12). For Site 2, values for the fifth, non-measured refill interval were estimated based on proportional values observed at

Site 1. Among the 26 samples, one vial contained no nectar, as all 20 sampled flowers from that tree were empty during the 12–14-hour interval. Since the entire inflorescence, rather than a single flower, was devoid of nectar during the early afternoon interval, yet resumed secretion afterwards, I attributed the absence to the plant's response to the experimental treatment rather than to potential tissue damage caused by manual handling. Consequently, I included these data by assigning a value of '0' to absolute variables (nectar, total sugar, and component amounts) and an 'NA' value to relative variables like nectar concentration and Baker's ratio [sucrose:(fructose+glucose)].

2.2.3 Measurements

Nectar samples were weighed individually using a precision balance (MS 120, Mettler Toledo, Switzerland; readability 0.001 g; accuracy ± 1 mg), and nectar weight was calculated by subtracting the weight of the corresponding empty vial. To calculate a mean nectar concentration as % sugar (w/v), I used a digital handheld pocket refractometer (Atago PAL-1, Japan; readability 0.1° Brix ~ 0.1 % sugar, accuracy ± 0.2%) to take three Brix measurements per sample. I refrained from converting nectar weight into volume units to avoid introducing slight inaccuracies based on the given value of inaccuracy of the refractometer. To determine the mean fresh weight of a single flower, I first measured the total weight of the sampled 10 - 20 flowers per tree on the same balance and divided the value by the number of flowers weighed. For the RENR study, I did not scale nectar data to the site's average flower weight (based on the 80 or 120 RENR flowers sampled) because linear regression analysis of preliminary data revealed no correlation between a tree's pooled nectar weight and its average flower fresh weight across 18 *V. lucens* individuals ($R^2 = 0.005$, $y = 5.19\times10^{-3}x + 23.7$, $n = 18$).

I used High-Performance Liquid Chromatography (HPLC) to separate nectar carbohydrate compounds. Nectar solutions (50 - 200 µL) were transferred into 0.3 mL clear PP short screw micro-vials (9 mm thread, 11.6*32 mm with inserts from interlab.co.nz, NZ) and loaded into a cooled (10 ± 5°C) Alliance Waters WAT034282 autosampler. The Alliance system was operated at a high limit pressure of 4000 PSI, an operating pressure of ~ 250 PSI, and a flow rate of 0.4 mL/min. Separation was performed using the Shodex KS-801 sugar column (8.0 x 300 mm), the Shodex KS-G HPLC guard column (7 µm, 6 x 50 mm), and the PDA 410

(Waters). The columns were heated to 65°C, and the internal temperature detector (RI) to 40°C. HPLC grade water (type 1), prepared from a Barnstead E-Pure Water System (18.2 MΩ cm) and purified in situ, was used as the mobile phase and wet prime solvent. The Alliance system needle was washed with 5% MeOH (methanol 100%, HPLC grade) after each sample run of 35 minutes and reloaded with an injection volume of 10 µL.

To monitor and rinse the system, I intermittently injected samples of a standard solution containing the three most common nectar sugar types (sucrose, fructose, glucose) and purified water using Empower 3 software (Waters). The software automatically calculated peak areas and retention times (RT). I matched the 22 detected components against standard solutions of 21 common sugar types (90 - 829 g/mol) based on RT to identify them.
For this, I prepared stock solutions for sucrose, fructose, glucose, beta-cyclodextrin (cyclo-heptaamylose; purity ≥ 98%), maltose (grade 1, pur. ≥ 97%), alpha-lactose (monohydrate, beta-anomer content 2.6%), D-mannose, myoinositol, D-galactose (pur. ≥ 97%), xylose (pur. ≥ 99.0 %), L-rhamnose monohydrate, D(-)-ribose, stachyose hydrate (purity ≥ 98%) (purchased from SIGMA-ALDRICH CO., USA); cellobiose, L(+)-arabinose, L(-)-sorbose, sorbitol (purchased from BDH Chemicals Ltd Poole England); D(+)-mannitol (Riedel-de Haen AG, Germany); D(+)-melezitose (SIGMA Chemie GmbH, Germany); raffinose (Chelsea sugar factory, NZ), and verbascose (pur. > 95%, purchased from Megazyme, USA).

For fructose and glucose, 2500 mg of each sugar was dissolved in a 50-mL volumetric flask containing 50 mL type 1 water, resulting in a stock solution of 50 mg/mL, which was then further diluted by 1:1 to obtain three additional solutions with concentrations of 25, 12.5, and 6.25 mg/mL. I repeated this method with 300 mg of sucrose to obtain 6, 3, 1.5, and 0.75 mg/mL solutions. For all other sugar types, 400 mg of each was dissolved in an 8-mL volumetric flask containing 4 mL type 1 water to obtain stock solutions of 100 mg/mL, which I further diluted to 50, 25, and 5 mg/mL per sugar type. I considered components elementary to pūriri's nectar composition when they occurred in at least 50% of the 18 analysed samples. Ten of the 22 detected components met this criterion. For this publication, I focused on the six most abundant components, which exceeded concentrations above 0.5%.

I calibrated detected peak areas using calibration curves to determine the most accurate amount of each of the chosen six components within my nectar samples. These were constructed by plotting the concentration of the analytes (*x*) against the peak areas of the analytes (*y*) by linear regression analysis (see Table 2).

Table 2: Regression equations for nectar components for component calibration (based on $n = 4$ standard solutions per component; Regression equations of the two unknown compounds, the Oligosaccharide and Unknown 5, are estimates based on applying a response factor ratio commonly used to determine unknown compounds).

SUGAR TYPE	STANDARD SOLUTIONS	CALIBRATION RANGE (mg/mL)	REGRESSION EQUATION, with *y* =	R^2
Oligosaccharide	-	-	1.7E+06x - 746164	-
Melezitose	4	5 - 100	1.77E+06x - 50032	1
Sucrose	4	1.7 - 6.1	1.65E+06*x + 19064	1
Glucose	4	6.4 - 48.1	1.67E+06*x + 4810	1
Unknown 5	-	-	1.67E+06x + 34130	-
Fructose	4	6.7 - 48.2	1.65E+06*x + 45596	1

I had to use another approach to calibrate the two unknown components. This involved comparing the peak area or height of the unknown compound to that of a known compound with similar properties already being analysed and for which the concentration is known. The ratio of the unknown compound's peak area or height to that of a known compound is called the 'response factor'. The response factor can then be used to estimate the concentration of the unknown compound. Finally, I used the calibrated component amounts to determine their concentrations. After assessing a discrepancy of 3.3% between the calculated (HPLC) and measured (refractometer) nectar concentration values, I continued using the calculated values in all analyses.

2.2.4 Statistical Analysis

For the preliminary study, I used linear regression to detect dependencies between floral traits, nectar and tree diameter (Table 3). For the RENR study, I tested my response variables, including nectar, total sugar, fructose (F), glucose (G), sucrose (S), melezitose (M), oligosaccharides (O), and Unknown 5 (U5) mass, nectar concentration, and Baker's ratio [S:(F+G)], for normality with Shapiro-Wilk tests and histograms. Differences between

treatment groups and study sites of normally distributed data were tested with parametric tests (t-tests, One-Way ANOVA) and non-normally distributed data with non-parametric tests (Wilcoxon tests; Kruskal-Wallis tests, see Table 6).

For the RENR study, to identify to what degree the time of the day and VPD explained variance in my response variables, I used a generalised additive mixed modelling (GAMM) approach with the R packages 'mgcv', 'statmod', 'dplyr', and 'tweedie.' The Tweedie distribution is a flexible statistical distribution that can model data with a mixture of continuous and discrete outcomes, making it particularly suitable for nectar parameter analysis where the data may include a large proportion of zeros (e.g., no nectar production) and continuous positive values (e.g., varying amounts of nectar production). This distribution allows for the modelling of the mean and variance of nectar traits as functions of predictors while accommodating the skewed nature of nectar data. I applied the 'log' link function to transform my poisson- or gamma-distributed data and three knots to smooth the predictors 'time' and 'VPD' to avoid overfitting based on my small dataset. I pooled data across my two study sites as there were no significant differences in amounts of frequently sampled nectar between sites (Wilcoxon test, W = 44, $P = 0.68$) or sugar (t-test, t = 5.9157, DF = 19, $P < 0.001$, see Table 6). I defined individual trees as replicates that fit GAMMs without specifying a random term and tested their fit visually using Q-Q plots. I conducted all statistical analyses using R versions 3.6.1 - 4.3.1 (R Team, 2020 - 23).

2.3 Results

2.3.1 Preliminary Study

On average, pūriri flowers measured 28 mm in length and weighed 0.4 g (see Table 3 for standard deviation and ranges). This fresh weight included an average of 26 µL of nectar, with a soluble's concentration of 18% (w/v), equivalent to approximately 4.7 mg of solubles. Hence, each flower contained around 6.3% nectar by weight.

Table 3: Floral traits [mean and standard deviation (SD), range, *n*] of *V. lucens* trees (*n* = 18), with DBH = tree diameter at breast height; Baker's ratio = [sucrose : (fructose+glucose)]; SFG fraction = percentage of the total nectar solubles content represented by the sum of sucrose (S), fructose (F), and glucose (G).

	NECTAR CONCENTRATION (%)	FLOWER LENGTH (mm)	FLOWER WEIGHT (g)	NECTAR VOLUME (µL)	DBH (cm)	BAKER'S RATIO	SFG-FRACTION (%)
mean ± SD	19 ± 8	28 ± 4	0.37 ± 0.1	26 ± 7	88 ± 85	0.2 ± 0.2	94 ± 9
range	9 - 36	16 - 38	0.24 - 0.56	17 - 39	5 - 300	0.01 - 0.66	60 - 98
n	18 batches of 17 flowers	289 flowers	18 batches of 24 flowers	18 batches of 17 flowers	18 trees	18 batches of 17 flowers	18 batches of 17 flowers

Linear regression analysis revealed significant ($P < 0.05$) negative relationships between nectar concentration and flower length ($R^2 = 0.386$) and flower weight ($R^2 = 0.21$, see Table 4 and Figure 1), not considering the potential influence of site effects. Hence, smaller and lighter flowers contained more viscous nectar. Other tested pairs of floral traits were non-significant ($R^2 < 0.06$, see Table 4).

Table 4: Linear regression equations for relationships between floral traits (*n* = 311 flowers) from 18 individuals of *V. lucens* sampled from seven populations around New Zealand, with bold R^2-values denoting statistical significance, $P < 0.05$.

	REGRESSION EQUATIONS				R^2			
	Flower Lengths	**Flower Weight**	**Nectar Volume**	**DBH**	**Flower Lengths**	**Flower Weight**	**Nectar Volume**	**DBH**
Nectar Concentration	-1.37*x+57.6	-46*x+35.7	-0.0328*x+19.5	7.59E-03*x+17.6	**0.386 ***	**0.276 ***	0.001	0.005
Nectar Volume	0.401*x+14.2	5.19E-03*x+23.7	-	-2.48E-03*x+25.9	0.050	0.005	-	0.001

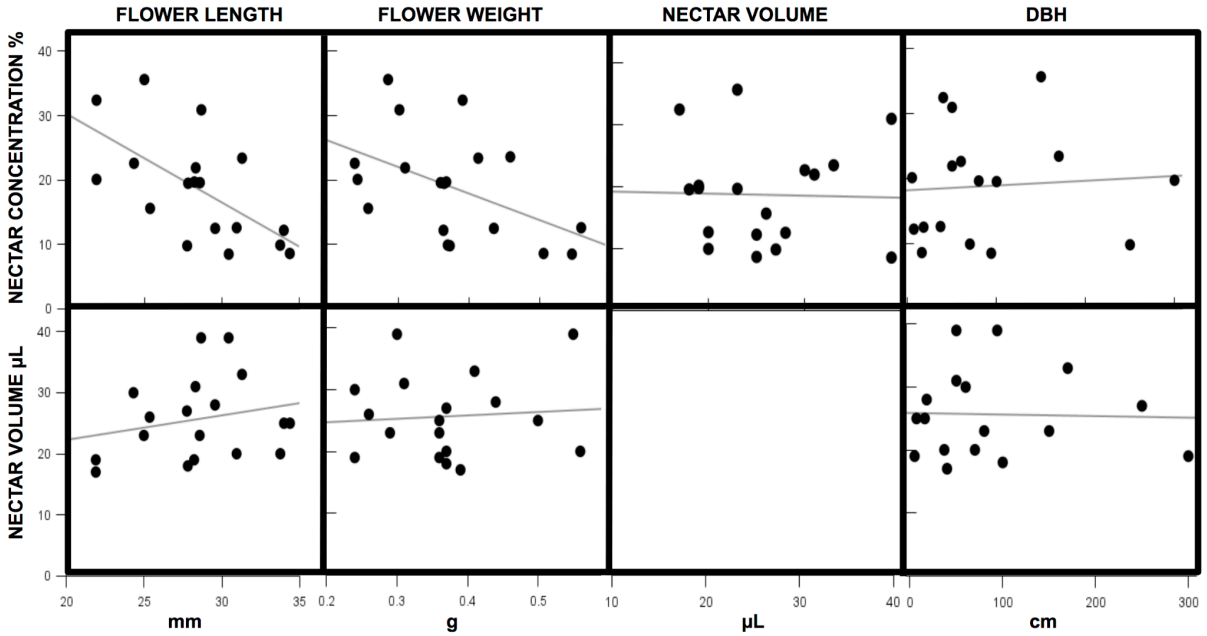


Figure 1: Linear regression graphs showing correlations between floral and tree traits, with only flower length ($R^2 = 0.386$) and weight ($R^2 = 0.21$) showing statistically significant correlations with nectar volume and concentration (others: $R^2 < 0.06$).

Nectar Composition

I identified 22 components (see Table 5), with molecular weights ranging from 90 to >830 g/mol, eluting between 9 and 34.5 minutes. Sugars accounted for approximately 99.1% of the composition, while non-carbohydrates contributed 0.9%. Pūriri nectar predominantly comprises the common nectar sugars: sucrose (S), glucose (G), and fructose (F) (often referred to as the 'SFG-fraction'), constituting 94% of solubles. The Baker's ratio (S/[F+G], after Baker and Baker, 1983) averages 0.2. Notably, fructose and glucose comprise 82% (~ 3.8 mg) of all solubles, defining pūriri's nectar as fructose-glucose dominant. Among the identified carbohydrates were three oligosaccharides (verbascose, raffinose and melezitose (M)), two disaccharides (sucrose and maltose), and four monosaccharides (glucose, fructose, arabinose, and dihydroxyacetone, DHA).

Additionally, *V. lucens* nectar contained 13 unidentified components, designated sequentially based on their HPLC retention time (RT), e.g., 'Unknown 1' or 'U1'. Among them, six are likely oligosaccharides (U0 - 4), one is a monosaccharide (U6.5), and two components are potentially amino acids (U6 & 7). Notably, U5 (RT = 20.1 min) eluted between maltose (18.9 min) and glucose (22.4) and closest to my lactose standard (19.2 min), suggesting it might be

a disaccharide, such as trehalose (standard not tested), found in the nectar of other plants (e.g. Akšić et al., 2015). Alternatively, it might be a secondary metabolite, such as the alkaloid matrine (248 g/mol) found in plant tissues and floral nectar across various angiosperm families (Adler, 2000; McDougal et al., 2015). The classification of the remaining three components (U5.5, U5.75, and U8) could not be determined.

Table 5: *V. lucens* nectar components identified via HPLC and their chemical traits, with RT = retention time of component elution, MW = molecular weight, RENR study = components with > 0.5% (bold), Standard = mean RT of component matched with RT of sugar type standard.

COMPONENT	CONCENTRATION	RENR STUDY	RT	MW	CARBOHYDRATE	COMPONENT	CHEMICAL FORMULA	VERIFIED
	(% of solubles)	(> 0.5%)	(min)	(~ g/mol)				by
Unknown 0	0.010		9.0	≥ 829	-	Oligosaccharide?	C30 +	-
Unknown 0.5	0.010		11.0	≥ 829	-	Oligosaccharide?	C30 +	-
Unknown 1	0.020		12.0	≥ 829	-	Oligosaccharide?	C30 +	-
Unknown 2	0.110		13.3	≥ 829	-	Oligosaccharide?	C30 +	-
Unknown 3	**1.600**	**X**	**14.0**	**≥ 829**	-	**Oligosaccharide?**	C30 +	-
Unknown 4	0.270		15.1	≥ 829	-	Oligosaccharide?	C30 +	-
Verbascose	0.005		15.7	828	X	Pentasaccharide	C30H52O26	Standard
Raffinose	0.060		16.2	504	X	Trisaccharide	C18H32O16	Standard
Melezitose	**0.570**	**X**	**17.1**	**504**	**X**	**Trisaccharide**	**C18H32O16**	**Standard**
Sucrose	**13.760**	**X**	**18.7**	**342**	**X**	**Disaccharide**	**C12H22O11**	**Standard**
Maltose	0.030		18.9	342	X	Disaccharide	C12H22O11	Standard
Unknown 5	**1.290**	**X**	**20.1**	**180 - 342**	-	**Saccharide or Alkaloid**	**C6 - C12**	-
Glucose	**41.290**	**X**	**22.4**	**180**	**X**	**Monosaccharide**	**C6H12O6**	**Standard**
Fructose	**40.520**	**X**	**24.0**	**180**	**X**	**Monosaccharide**	**C6H12O6**	**Standard**
Arabinose	0.170		25.5	150	X	**Monosaccharide**	**C5H10O5**	**Standard**
Unknown 5.5	0.020		26.5	90 - 150	-	-	-	-
Unknown 5.75	0.200		27.4	90 - 150	-	-	-	-
Unknown 6	0.020		29.2	90 - 150	-	Amino Acid (GLU)	C5H10N2O3	-
Unknown 6.5	0.040		30.5	90 - 150	-	Monosaccharide?	C4H8O4	-
Unknown 7	0.010		31.5	90 - 150	-	Amino Acid	C3H7NO3	-
DHA	0.010		33.0	90	X	Monosaccharide	C3H6O3	Standard
Unknown 8	0.001		34.5	90	-	-	-	-

2.3.2 RENR Study

In the primary RENR study, I focused on analysing the six major components that constituted 99% of the solubles due to their concentrations exceeding 0.5%: S, F, G, M, U3, and U5 (see Table 5). In comparison to the retention time (15.7 min) of my largest available sugar molecule, the pentasaccharide verbascose (~ 829 g/mol M.W.), the component U3 eluted earlier (14 min), suggesting it might be an oligosaccharide. Therefore, I refer to U3 as 'oligosaccharide' or 'O' hereafter. The identity of U5 remains uncertain based on the 21 tested sugar standards; as a result, I have retained the initial identification name but classified it as a disaccharide for this publication.

Standing Crop

The flowers of my RENR study had a mean fresh weight of 335 ± 75 mg (nectar removed, $n_{flowers}$ = 120, n_{trees} = 8), a nectar standing crop (unbagged) of 29 ± 16 µL containing 17 ± 7% of solubles (~ 6 mg). The weight of an unsampled flower (362 ± 80 mg) contained ~ 7.5% of nectar on average. The solubles comprised about 76% of monosaccharides (F & G), 21.8% of disaccharides (S & U5), and 1.8% of oligosaccharides (M & O). In particular, F and G accounted for 38% each, S for 19, U5 for 2.8, O for 1.5, and M for 0.3% on average. The 'SFG-fraction' clearly dominated the soluble composition with a mean concentration of 95% and a Baker's ratio of 0.27 ± 0.20. The nectar properties of the standing crop samples fell within the value range I previously observed for pūriri flowers in my preliminary study.

Cumulative Nectar Replenishment

During the 10-hour refill experiment, frequently sampled trees (n = 4) accumulated 8.8 ± 2 µL of nectar, containing 1.3 ± 0.6 mg of sugar based on a concentration of 14 ± 4% on average. The sugar composition included approximately 45% G (505 µg) , 41% F (468 µg), 12% S (153 µg), 1.8% O (20 µg), 0.6% U5 (6 µg; present in n = 2), and 0.2% M (3 µg), resulting in a mean Baker's ratio of 0.12 ± 0.20. Consequently, monosaccharides accounted for about 86%, disaccharides for 12.6%, and oligosaccharides for 2% of the soluble fraction. The change in Baker's ratio between the standing crop and frequently sampled flowers reflects the lower sucrose content in frequently sampled nectar, with sucrose amounts decreasing over

time—from an initial morning drain ratio of 1:5.5 [S:(F+G)], to 1:8.9 in the late afternoon, followed by a slight increase to 1:6.6 during the final refill period.

Control trees sampled once after 10 hours (n = 4) secreted 11.5 µL of nectar containing 1.6 mg of sugar, with an average concentration of 14.5%. The soluble fraction consisted of 41% G, 39% F, 18% S, 1.4% O, 0.7% U5 (present in n = 3), and 0.3% M, resulting in a mean Baker's ratio of 0.23 ± 0.05. Monosaccharides constituted about 80%, disaccharides 18.7%, and oligosaccharides 1.7%.

During the 7-hour refill period, when flowers replenished nectar from early morning to mid-afternoon hours when VPD-values peaked, frequently drained trees produced 6.1 ± 1.2 mg nectar comprising 0.9 ± 0.3 mg sugar (n = 4). Control flowers produced 7.7 ± 1.6 mg nectar with 1.5 ± 0.8 mg sugar (n = 4).

Cumulative nectar and sugar amounts of the 10-hour and the 7-hour replenishment experiments showed no significant differences between frequently drained and control flowers (t- or Wilcoxon tests, all $P > 0.05$; see Table 6; Figure 2).

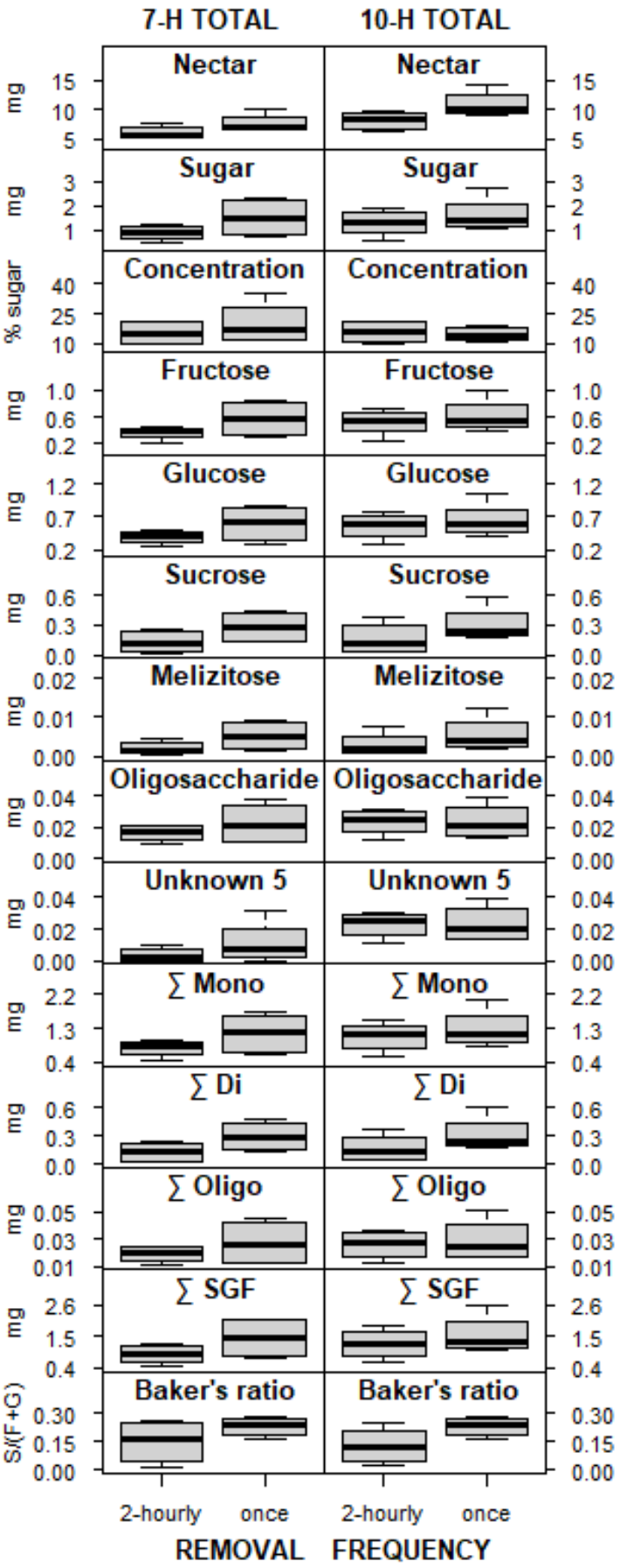


Figure 2:

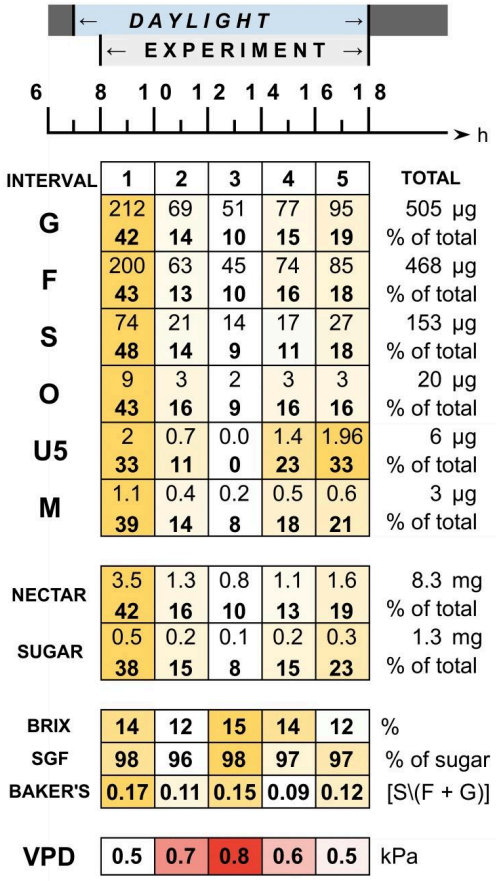
Cumulative nectar properties showed no significant differences between my treatment groups of 2-hourly sampled trees and trees that were sampled only once after 7 hours or 10 hours ($P > 0.05$), with ∑ Mono = total mono-saccharides, ∑ Di = total disaccharides, ∑ Oligo = total oligosaccharides, ∑ SGF = summed amounts of sucrose, glucose, and fructose, Baker's ratio = sucrose/(fructose + glucose).

Table 6: Statistical parameters of t- (test type 't') and Wilcoxon (test type 'W') tests comparing mean sums of frequently [2-hourly; mean (frequent)] and one-time-only [mean (once)] sampled flowers, with each group $n = 4$.

	Shapiro *P*	Mean (frequent)	Mean (once)	CI Lower	CI Upper	Test Type	t- or W Value	DF	*P*
Refill				**10 hours**					
Nectar	0.2	0.9	1.5	-1.8	0.6	t	-1.4	4	0.2
Sugar	0.3	8.3	11.1	-6.4	0.8	t	-1.9	5	0.1
Brix	0.1	15.4	14.5	-8.1	9.9	t	0.3	5	0.8
G	0.8	0.6	0.7	-0.5	0.3	t	-0.5	6	0.6
F	0.7	0.5	0.6	-0.5	0.3	t	-0.6	6	0.6
S	0.4	0.2	0.3	-0.4	0.2	t	-1.2	6	0.3
O	0.6	0.02	0.02	-0.02	0.02	t	-0.05	5	1
U5	0.02	-	-	-	-	W	6		0.7
M	0.1	0.003	0.01	-0.01	0.004	t	-0.9	5	0.4
∑ Monosaccharides	0.8	1.1	1.3	-1.0	0.6	t	-0.6	6	0.6
∑ Disaccharides	0.3	0.2	0.3	-0.5	0.2	t	-1.2	6	0.3
∑ Oligosaccharides	0.5	0.03	0.03	-0.03	0.02	t	-0.3	5	0.8
∑ SFG	0.6	1.2	1.6	-1.5	0.8	t	-0.7	6	0.5
Baker's Ratio	0.4	0.13	0.23	-0.3	0.0	t	1.8	5	0.1
Refill				**7 hours**					
Nectar	0.3	6.1	7.7	-4.2	0.9	t	-1.6	6	0.2
Sugar	0.1	0.9	1.5	-1.8	0.6	t	-1.4	4	0.2
Brix	0.1	15.2	19.8	-21	11.7	t	-0.7	5	0.5
G	0.2	0.4	0.6	-0.6	0.2	t	-1.3	4	0.3
F	0.2	0.4	0.6	-0.6	0.2	t	-1.4	4	0.2
S	0.7	0.1	0.3	-0.4	0.1	t	-1.5	6	0.2
O	0.2	0.02	0.02	-0.03	0.02	t	-0.7	4	0.5
U5	0.01	-	-	-	-	W	5		0.5
M	0.1	0.002	0.005	-0.01	0.003	t	-1.5	4	0.2
∑ Monosaccharides	0.2	0.8	1.2	-1.3	0.4	t	-1.4	4	0.2
∑ Disaccharides	0.7	0.1	0.3	-0.4	0.1	t	-1.5	5	0.2
∑ Oligosaccharides	0.1	0.02	0.03	0.0	0.0	t	-0.9	4	0.4
∑ SFG	0.1	0.9	1.4	-1.6	0.5	t	-1.4	4	0.2
Baker's Ratio	0.1	0.15	0.23	-0.3	0.1	t	-1.3	4	0.3

Frequent Nectar Replenishment

During each 2-hour refill interval (n_{trees} = 8, n_{nectar} = 20, $n_{intervals}$ = 5, refill time = 8 a.m. - 6 p.m., refill period = 10 h), each flower replenished between 0.8 - 3.7 µL of nectar and 0.1 - 0.5 mg of sugar on average, including one empty nectar sample. Nectar concentrations (n_{nectar} = 19) ranged between 12 - 15% (see Table 7). The SFG fraction comprised 96 - 98% of total solubles per refill interval. Nectar, total sugar, individual component amounts, and the Baker's ratio peaked in the first refill interval (8 - 10 h). This early morning production accounted for 42% of nectar, 38% of sugar, and 41 ± 4% of each component's total refill production (see Figure 3).



INTERVAL	1	2	3	4	5	TOTAL
G	212	69	51	77	95	505 µg
	42	14	10	15	19	% of total
F	200	63	45	74	85	468 µg
	43	13	10	16	18	% of total
S	74	21	14	17	27	153 µg
	48	14	9	11	18	% of total
O	9	3	2	3	3	20 µg
	43	16	9	16	16	% of total
U5	2	0.7	0.0	1.4	1.96	6 µg
	33	11	0	23	33	% of total
M	1.1	0.4	0.2	0.5	0.6	3 µg
	39	14	8	18	21	% of total
NECTAR	3.5	1.3	0.8	1.1	1.6	8.3 mg
	42	16	10	13	19	% of total
SUGAR	0.5	0.2	0.1	0.2	0.3	1.3 mg
	38	15	8	15	23	% of total
BRIX	14	12	15	14	12	%
SGF	98	96	98	97	97	% of sugar
BAKER'S	0.17	0.11	0.15	0.09	0.12	[S\(F + G)]
VPD	0.5	0.7	0.8	0.6	0.5	kPa

Figure 3: Heatmap showing individual mean measurements of nectar properties (continuous colour scale from white to golden yellow, with highest values golden yellow) and vapour pressure deficit (VPD) values (white to red, with highest values red), with Nectar = nectar mass, Sugar = sugar mass, Brix = nectar concentration (1% ~ 1° Brix), 'SGF' = relative abundance of sucrose, glucose and fructose within total sugar, Baker's = unitless ratio of sucrose to fructose and glucose.

The third refill interval (12 - 14 h) which exhibited the lowest secretion rates, averaging only 8% of the daily component and sugar and 10% of the total nectar production. Surprisingly, the last refill interval showed an unexpected rise in production, comprising about a fifth of the total day production. Nectar exhibited its highest mean concentration (Brix) in the early afternoon (12 - 14 h) and its lowest before noon (10 - 12 h). Nectar volume varied significantly between refill intervals (Kruskal-Wallis, $P = 0.026$), with a notable difference between 8 - 10 a.m. and 12 - 2 p.m. (Dunn's test with Bonferroni correction: $Z = 3.09$, $P = 0.01$). In contrast, no other nectar traits showed significant variation across intervals: one-way ANOVAs for normally distributed variables yielded F-values of 0.21 - 3.72 ($P = 0.07$ - 0.65), and Kruskal-Wallis tests for non-normally distributed traits ('Baker's', 'S', and 'U5') returned P-values between 0.62 and 0.84.

Caption for Table 7 on the next page:

Table 7: Environmental conditions and nectar properties of samples. The standing crop was removed at 8 a.m. on the day of the experiment. The elapsed time since the flowers were presumably fully depleted by pollinators was estimated by dividing the initial standing crop (~ 27 mg) by the species' standard nectar production rate (~ 1 mg/hour). Nectar volumes for standing crop samples were calculated using a mean specific density of 1.055 g/L (**). Simplified data represents 10-hour nectar replenishment, averaging control flower data over 10 and 7 hours, with 7-hour data extrapolated to 10 hours. Data for frequently drained flowers was averaged over 10-hour (from five 2-hour intervals) and 8-hour (from four 2-hour intervals) periods, with 8-hour data similarly extrapolated to 10 hours.

			unmanipulated			*manipulated*								
			STANDING CROP		CONTROLS	REFILL		INTERVALS					VALUE RANGE	
			TOTAL	hourly	TOTAL	hourly	TOTAL	1	2	3	4	5	of	
			mean ± SD	mean	mean ± SD	mean	mean ± SD	mean ± SD					INTERVALS	SAMPLES
CONDITIONS														
	Refill time (am/pm)		~5 pm - 8 am*		8 am - 6 pm	8 am - 6 pm		8 - 10	10 - 12	12 - 14	14 - 16	16 - 18	8 am - 6 pm	
	Refill interval (h)		27.21	1	10	1	5 x 2	2	2	2	2	2	10	10
	Daylight (h)		13.21	0.5	10	10	10	2	2	2	2	2	10	10
	mean VPD (kPa)		0.3 ± 0.3	0.3	0.6 ± 0.1	0.6	0.6 ± 0.1	0.5 ± 0.3	0.7 ± 0.3	0.8 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.5 - 0.8	0.2 - 1.0
	Flower + nectar weight (mg)		362 ± 80	336	±	336	338 ± 66	338 ± 66	336 ± 67	335 ± 67	336 ± 67	336 ± 67	335 - 338	283 - 425
	Flower - nectar weight (mg)		335 ± 75	335	±	335	335 ± 75	335 ± 75	335 ± 75	335 ± 75	335 ± 75	335 ± 75	335	283 - 423
NECTAR PROPERTIES														
	Samples (n)		4	4	4	20	20	4	4	4	4	4	4	20
	Total nectar (mg)		27 ± 14	1.0	11 ± 2	0.83	8.3 ± 2	3.5 ± 0.9	1.3 ± 0.4	0.8 ± 0.7	1.1 ± 0.5	1.6 ± 0.5	0.8 - 3.5	0 - 4.2
	Total nectar volume (µL) **		29 ± 16	1.08	12 ± 2	0.88	8.8 ± 2	3.7 ± 0.9	1.4 ± 0.4	0.8 ± 0.7	1.1 ± 0.5	1.8 ± 0.8	0.8 - 3.7	0 - 4.4
	Total sugar (mg)		6 ± 5	0.2	1.6 ± 0.7	0.13	1.3 ± 0.6	0.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.1 - 0.5	0 - 0.7
	Concentration (%)		17 ± 7	21	14 ± 4	16	14 ± 4	14 ± 5	12 ± 8	15 ± 6	14 ± 8	12 ± 3	12 - 15	2.7 - 22
	COMPONENTS	MASS (µg)												
			Glucose	1989 ± 1414	73	659 ± 272	50	505 ± 155	212 ± 51	69 ± 50	51 ± 36	77 ± 49	95 ± 33	51 - 212	0 - 284
			Fructose	2021 ± 1447	74	628 ± 253	47	468 ± 148	200 ± 51	63 ± 46	45 ± 33	74 ± 47	85 ± 31	45 - 200	0 - 269
			Sucrose	1461 ± 1450	54	308 ± 181	15	153 ± 150	74 ± 74	21 ± 30	14 ± 16	17 ± 25	27 ± 38	14 - 74	0 - 172
			Oligosaccharide	71 ± 45	2.6	24 ± 12	2	20 ± 6	9 ± 2.6	3 ± 1.8	2 ± 1.5	3 ± 1.8	3 ± 0.8	2 - 9	0 - 12
			Unknown 5	126 ± 66	5	14 ± 18	0.6	6 ± 9	2.0 ± 2	0.7 ± 1.4	0 ± 0	1.4 ± 2.7	1.96 ± 4	0 - 2	0 - 8
			Melizitose	23 ± 23	0.8	6 ± 4	0.3	3 ± 3	1.1 ± 1.1	0.4 ± 0.4	0.2 ± 0.2	0.5 ± 0.6	0.6 ± 0.7	0.2 - 1.1	0 - 3
			Baker's ratio	0.27 ± 0.2	0.4	0.23 ± 0.05	0.2	0.12 ± 0.2	0.17 ± 0.1	0.11 ± 0.1	0.15 ± 0.1	0.09 ± 0.1	0.12 ± 0.2	0.09 - 0.17	0.004 - 0.35
		CONCENTRATION (%)												
			Glucose	38 ± 5	35	41 ± 3	39	45 ± 4	43 ± 5	46 ± 4	45 ± 5	46 ± 4	47 ± 6	43 - 47	38 - 51
			Fructose	38 ± 4	36	39 ± 2	36	41 ± 4	41 ± 6	41 ± 3	41 ± 4	44 ± 4	41 ± 5	41 - 44	34 - 48
			Sucrose	19 ± 11	26	18 ± 3	12	12 ± 8	13 ± 11	9 ± 8	12 ± 10	7 ± 8	9 ± 11	7 - 13	0.4 - 26
			Oligosaccharide	1.5 ± 0.7	1.2	1.4 ± 0.3	2	1.8 ± 0.3	1.8 ± 0.3	2.5 ± 0.8	1.7 ± 0.5	2.2 ± 0.9	1.7 ± 0.6	1.7 - 2.5	0.9 - 3.3
			Unknown 5	2.8 ± 1.2	2.2	0.7 ± 0.7	0.5	0.6 ± 0.8	0.5 ± 0.5	0.8 ± 1.6	0 ± 0	0.6 ± 1.2	0.8 ± 1.6	0.0 - 0.8	0 - 3.2
			Melizitose	0.3 ± 0.2	0.4	0.3 ± 0.1	0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	0.2 - 0.3	0 - 0.5
			S + G + F	95 ± 1	96	98 ± 1	87	97 ± 1	98 ± 0	96 ± 2	98 ± 0	97 ± 1	97 ± 2	96 - 98	93 - 99

* 6 h of the prior day until 8 h on the experiment day; based on an estimated mean refill period (~27.21 hours); calculation based on ratio of sampled standing crop and the species' standard hourly nectar production rate of 1 mg

** based on mean specific density of 1.055 g/L

Modelling

For the GAMM models, the predictor 'time' resulted in a significant cubic polynomial (see Figure 4) for five of my response variables, with minima occurring in the early afternoon (P < 0.001 - 0.05; R^2 > 0.2 - 0.9; see Table 8).

Table 8: Parameters of the generalised additive mixed models (GAMMs) for the tested nectar traits, employing a 'Tweedie' distribution, with Nectar = nectar amount, Sugar = sugar amount, Conc. = nectar concentration, F = fructose, G = glucose, S = sucrose, M = melezitose, O = oligosaccharide, U5 = unknown 5. *[The Tweedie distribution is a flexible statistical distribution that can model data with a mixture of continuous and discrete outcomes, making it particularly suitable for nectar parameter analysis where the data may include a large proportion of zeros (e.g., no nectar production) and continuous positive values (e.g., varying amounts of nectar production). This distribution allows for the modelling of the mean and variance of nectar traits as functions of predictors while accommodating the skewed nature of nectar data.]*

	NECTAR	SUGAR	CONC.	F	G	S	M	O	U5	BAKER'S
n	20	20	19	20	20	20	20	20	10	20
	TIME									
Tweedie	1.0	1.4	2.0	1.3	1.3	1.8	1.7	1.2	1.1	1.5
Estimate	0.4	-1.5	2.7	4.5	4.6	3.3	-0.6	1.4	0.8	-2.2
Standard Error	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.4	0.2
t	4	-12	26	38	40	12	-2	13	2	-10
Pr(>|t|)	***	***	***	***	***	***	*	***	.	***
df	**2.0**	**1.9**	1.0	**1.9**	**1.9**	1.7	1.8	**2.0**	1.7	1.0
F	**21**	**10**	0	**11**	**11**	3	2	**12**	1	1
P	***	**	n.s.	**	**	n.s.	n.s.	***	n.s.	n.s.
R^2	**0.71**	**0.52**	-0.05	**0.56**	**0.57**	0.17	0.08	**0.59**	0.07	0.01
Scale Estimate	**0.2**	**0.1**	0.2	**5.3**	**5.8**	2.7	0.9	**0.7**	2.4	0.3
	VPD									
Tweedie	1.0	1.3	2.0	1.2	1.2	1.8	1.7	1.2	1.4	1.5
Estimate	0.4	-1.5	2.7	4.5	4.6	3.4	-0.6	1.4	0.5	-2.2
Standard Error	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.4	0.2
t	4	-11	26	36	37	12	-3	11	1	-10
Pr(>|t|)	**	***	***	***	***	***	*	***	n.s.	***
df	**1.6**	**1.4**	1.0	**1.0**	**1.0**	1.0	**1.0**	**1.0**	**1.0**	1.0
F	**15**	**12**	0	**14**	**13**	3	**5**	**12**	**6**	0
P	**	**	n.s.	**	**	n.s.	*	**	*	n.s.
R^2	**0.53**	**0.45**	-0.05	**0.42**	**0.41**	0.07	**0.11**	**0.37**	**0.18**	-0.05
Scale Estimate	**0.3**	**0.1**	0.2	**9.4**	**9.8**	2.9	**0.8**	**0.9**	**1.7**	0.3

However, 'time' was not a significant smoothing term for the other five response variables ('concentration', 'Baker's ratio', sucrose, melezitose, and U5). In my fitted GAMM models, VPD had a significant, negative cubic effect on seven response variables, but not 'concentration', 'Baker's ratio', and sucrose (P = 0.03 - 0.001; R^2 > 0.12 - 0.36). Consequently, pūriri secretes less nectar and sugar during the hotter and drier hours of the day. The non-significant responses observed for sucrose, melezitose, and U5 with the predictors are likely due to the small sample size.

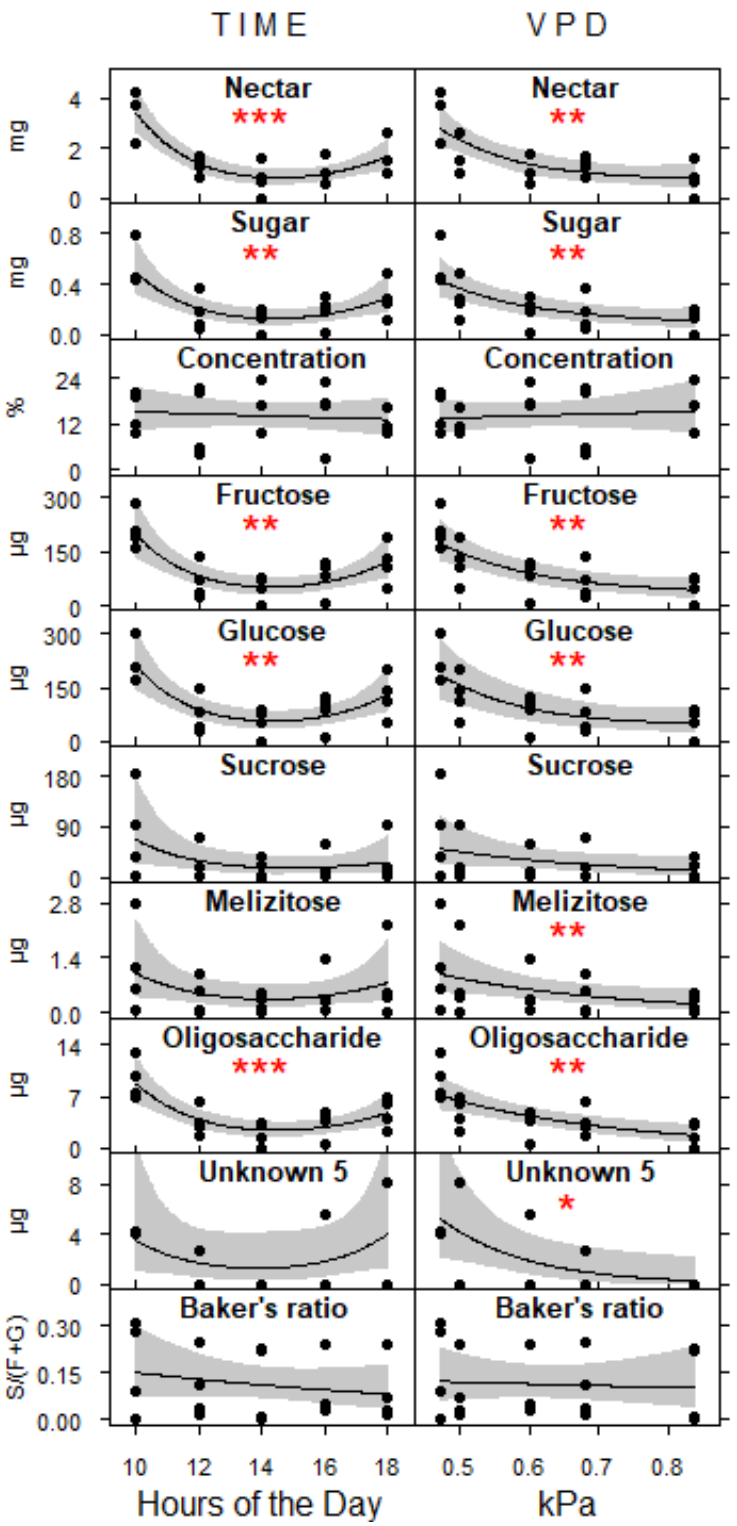


Figure 4: Fitted generalised additive mixed models (GAMMs) of nectar variables against the predictors 'time' and 'vapour pressure deficit' (VPD). Red asterisks indicate significance, with '*' for $P < 0.05$, '**' for $P < 0.01$, and '***' for $P < 0.001$.

2.4 Discussion

2.4.1 Cumulative Nectar Replenishment

My experiments with *V. lucens* revealed a neutral replenishment response to frequent removal. Frequently drained flowers produced nectar of comparable quantity and quality to those that were drained only once (controls). Contrary to my hypothesis, frequently drained flowers did not exhibit significantly higher sucrose levels—and thus no increase in Baker's ratio—compared to controls. I had expected elevated sucrose levels, as the newly produced nectar in frequently drained flowers had only a fifth of the time to hydrolyse into equal parts fructose and glucose, compared to controls which had a full ten hours of refill time.

Luo et al. (2014) suggested that removal-enhanced replenishment of nectar is more likely to be observed in flower species adapted to large insect visitors. Nine out of eleven species in their study responded positively to frequent nectar removal, with 77% of these being primarily insect-pollinated. Species attracting birds and bees displayed positive RENR, although lower than those in exclusively insect-pollinated species. Only *Castilleja linarifolia* (Orobanchaceae) showed a strongly positive RENR among exclusively bird-pollinated species. In contrast, *Ipomopsis aggregata* (Polemoniaceae) and *Aquilegia elegantula* (Ranunculaceae) exhibited a neutral RENR response, aligning with previous studies (Pleasants, 1983) and my findings for *Vitex lucens* (see Figure 5).

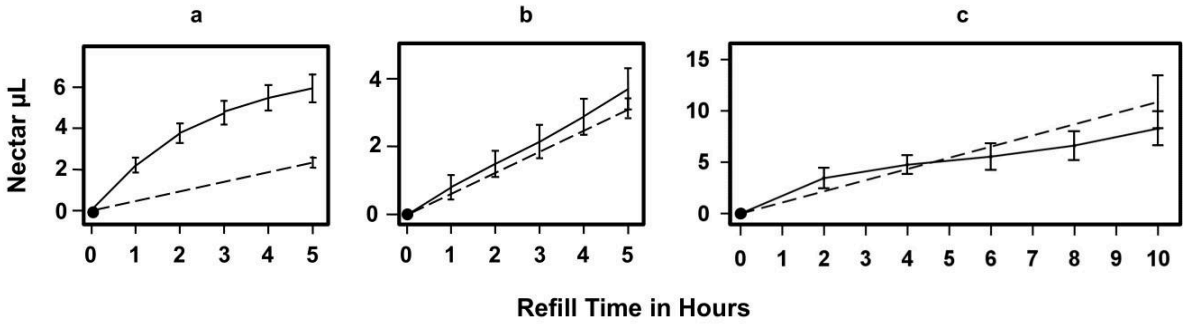


Figure 5: Nectar removal-response types in bird-pollinated species: a) positive in *Castilleja linarifolia*, b) neutral in *Ipomopsis aggregata* (a & b - redrawn figures from Luo et al., 2014), c) neutral in *Vitex lucens;* solid lines = RENR, dashed lines = controls.

However, since bird-pollinated species can also exhibit negative (e.g., Gill, 1988; Bernardello et al., 1994; Rivera et al., 1996; McDade & Weeks, 2004; Carlson, 2007) and positive responses (e.g. Lara & Ornelas, 2001; Nicolson & Nepi, 2005; Ordano & Ornelas, 2005; Luo et al., 2014), pollinator guilds cannot easily be correlated with a specific removal-response type. I endorse Luo et al.'s suggestion to explore removal-response patterns across diverse plant lineages, incorporating within-lineage variations. Including lineages with bird- and insect-adapted flowers could be most profitable, making it an interesting phylogenetically constrained study.

2.4.2 Frequent Nectar Replenishment

I identified a negative correlation between vapour pressure deficit (VPD) and nectar replenishment amounts of *Vitex lucens* flowers throughout the day, confirming the findings of Fairhurst et al. (2021). Both nectar volume and sugar amounts correlated with VPD. In particular, nectar secretion peaked in the mornings and evenings and troughed during the driest time of the day around 3 p.m., with ten flowers of one particular inflorescence ceasing secretion completely. Interestingly, the early morning nectar production was significantly higher than all other intervals. With decreasing VPD in the late afternoon, flowers resumed production. However, mean nectar concentrations remained stable, suggesting that high VPD conditions inhibited the entire nectar secretion mechanism instead of causing increased viscosity due to reduced water movement into nectar, or increased water loss through evaporation, as I initially assumed during fieldwork.

Numerous studies have documented diurnal changes in nectar volume across various plant species (Collins & Clow, 1978; Ford, 1979; Ford & Pursey, 1982; Paton, 1982; Collins & Newland, 1986). Typically, nectar volume peaks at dawn and is rapidly depleted over the next few hours, reaching low or negligible levels that persist throughout the day. Despite my flowers exhibiting higher nectar secretion rates during the cooler evening hours, my results were consistent with this pattern.

Furthermore, sampling my specimens during two winter days exposed them to the lower end of the VPD spectrum (with a 10-hour mean of 0.54 kPa) recorded throughout the year (ranging from 0.33 to 1.22 kPa). *Vitex lucens*' peak flowering is in winter when VPD is

lowest and nectar production is potentially greater. For year-round flowering species like *V. lucens*, studying nectar properties throughout the year would be valuable for determining how climate and flowering intensity relate to individual flower nectar properties.

Effect of Shoot Water Relations

One possible explanation for reduced nectar secretion in drier conditions is the impact of VPD on shoot water relations and exudation mechanisms. Assuming the flowers are hydraulically (xylem) connected to the shoots, the main driver of variation in shoot (and flower) water potential will be leaf transpiration, as they have a much larger surface area than flowers. Eccrine nectar secretion is hypothesised to involve the diffusion of sucrose through the SWEET9 uniporter into the apoplastic space (Minami et al., 2021). Hydrolysis by invertases will double the osmotic effect of this accumulation of extracellular solute, causing water to also move into the space, possibly facilitated by aquaporin proteins, resulting in nectar 'secretion'. If shoot water potential decreases significantly during the day, the diffusive movement of water may be slowed down, potentially also slowing the accumulation of nectar in the apoplastic space, the volume flux of nectar to the cuticle surface, and the entire secretion process, including the movement of sugars (Lin et al., 2014). The role of tissue-water relations in nectar secretion has not been described.

Although flowers contribute minimally to carbon assimilation and have a shorter lifespan (days to weeks) compared to leaves (weeks to months), they can transpire significant amounts of water (Roddy & Dawson, 2011; Teixido & Valladares, 2014). A consistent water supply is crucial throughout various stages of floral development, including bud expansion, flower opening, nectar production, and the maintenance of turgor in floral organs (Mohan Ram & Rao, 1984; Patiño & Grace, 2002; Tsukaguchi et al., 2003; van Doorn & van Meeteren, 2003; Galen, 2005).

Pūriri flowers might have higher cuticle leakiness than their thick, glossy leaves, suggesting low resilience against dehydration. Hence, uncontrolled water loss from potentially leaky flowers could enhance diurnal reductions in flower water potential, showing that water stress can reduce nectar volume (Bourbia et al., 2020; Kuppler & Kotowska, 2021). Since measuring flower water potential was not within the scope of this study, I lack information

on how pūriri flowers manage water loss. Under drought stress, pūriri flowers might redirect nectar-designated water to regulate temperature, protecting their sexual organs from thermal damage and potentially affecting fertilisation and seed development (Patino & Grace, 2002; Roddy, 2019). To confirm this, data on the species' hydraulic anatomy, specifically whether these long-lived flowers (4 days to weeks after Primack, 1985) are supplied by xylem, is essential. However, a recent study by Zhang et al. (2017) suggests that the xylem primarily supplies long-lived flowers, enabling them to maintain their water status more effectively.

Additional research is needed to generalise this observed trend where long-lived flowers are xylem-supplied while short-lived ones rely on the phloem. Floral longevity also increases with decreasing temperature, but not flower size (Song et al., 2022).

Plant-Pollinator Relations

The rapid morning refill suggests a consequence of the effect of shoot water relations on the nectar secretion process. It could be argued that the pollinators have adapted to forage at dawn and dusk because nectar flow is more favourable. After being depleted at sunset, a solitary flower replenishes approximately 14 µL of nectar overnight, containing 12.5% sugar. Regarding energy yield, each flower supplies 290 - 430 kJ, meeting a significant portion of the daily energy requirements for New Zealand honeyeaters (Castro & Robertson, 1997). However, birds forage throughout the day, indicating competition between pollinators or that many flowers are empty or contaminated.

The observed low nectar presentation in the early afternoon poses the risk of repelling pollinators, especially considering birds tend to shift their focus based on resource availability (Bednekoff & Houston, 1994; Mac Nally, 1994a, b). To investigate whether this is true for the local pollinators of *V. lucens,* their visits must be monitored.

The increase in nectar production by *V. lucens* at dawn, along with continued secretion throughout the night, may suggest that its nectar is accessible to and potentially foraged by larger nocturnal pollinators such as bats. Although native bats have not been observed feeding on *V. lucens*, their visitation is plausible, given that the lesser short-tailed bat (*Mystacina tuberculata*) has been documented feeding on other large, pink-flowering native species such as *Clianthus maximus* and *Metrosideros* spp. (McCartney et al., 2007).

Monitoring pollinator activity during or prior to RENR experiments would provide a more comprehensive understanding of nectar replenishment and the various factors influencing it. Future studies could explore the feasibility of 24-hour RENR experiments on flowers of species known to attract nocturnal pollinators such as bats and moths, potentially offering deeper insights into diurnal and nocturnal nectar dynamics. However, practical limitations—such as the need for artificial lighting to remove nectar after sunset—may affect results by altering the natural light environment and influencing floral responses.

Interestingly, while Luo et al.'s and my manipulated flowers showed a decreasing trend in nectar refilling within the first five hours, my flowers gradually refilled progressively more nectar with each refill interval after that, which I now would hypothesise for Luo et al.'s flowers if they had been sampled for an extended period. Although they started their sampling comparatively late (early afternoon instead of my morning), flowers were sampled on a summer day (approx. 1 - 6 p.m.), which would have provided additional hours of daylight. Luo et al. acknowledged that nectar replenishment could vary with time of day, evaporation, and possible reabsorption but could not conduct longer monitoring times due to the broad scope of their survey.

My results shed light on diurnal replenishment dynamics, highlighting the influence of VPD rather than the mechanical stress expected with pollinator probing. Despite my monitoring phase being twice as long as the 5-hour study by Luo et al. (2014), my flowers consistently produced nectar after being emptied. When probed with micropipette tips, pūriri flowers displayed remarkable resilience, making them an appropriate choice for simulating mechanical stress akin to a bird's beak. As pūriri flowers are long-lived structures, they require being highly resilient to mechanical damage. Given their lower construction costs, it would be intriguing to investigate whether short-lived flowers exhibit lower resilience. Physical damage to certain flowers may not significantly impact the plant, as a new flower naturally replaces the damaged one the following day, potentially facilitating uninterrupted pollination, unlike in plants with long-lived flowers.

2.4.3 Conclusion

My study underscores the species-specific nature of nectar removal responses, with replenishment amounts varying throughout the day due to changes in VPD. I propose analysing RENR across various species to explore whether response types are phylogenetically conserved. Additionally, a deeper understanding of how VPD influences flower water relations and nectar production would be beneficial. I advocate for an interdisciplinary approach, integrating habitat-specific pollinator observations into RENR experiments. This integration contextualises nectar mechanisms within a broader ecological framework.

Standardised collection protocols are essential, encompassing full-day observations during daylight hours after removing the nectar standing crop 24 hours before sampling. Moreover, trees need to be sampled simultaneously, requiring field assistance. This approach enhances interspecific comparisons within the research community, ensuring more reliable results.
Shoot water relations might significantly affect nectar flow, which suggests that nectar production is probably quite vulnerable to drought or heat stress (Kuppler & Kotowska, 2021).

Future RENR studies may benefit from comparing closely related species with similar floral traits—such as dark pink to purple, bell-shaped *Pittosporum* species—under controlled conditions. Such comparative approaches could deepen our understanding of nectar replenishment dynamics and contribute to a reevaluation of commonly held assumptions regarding plant responses to environmental stressors.

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3. Regional Nectar Trait Variation in New Zealand Trees Across Climate Zones

Abstract

This study explores the impact of regional location on nectar traits across eight native tree species in New Zealand. Flowers were collected from seven regions spanning both main islands (37 - 45° S / 170 - 177° E). I collected 4276 flowers and 2240 µL of nectar from 164 trees. Flower size ranged from 4 - 54 mm, fresh mass from 4 - 1116 mg, nectar volume from 0.3 - 72 µL, concentration from 0.4 - 53° Brix, sugar mass from 0.01 - 13 mg, and tree diameter at breast height (DBH) varied from 2 - 160 cm.

Samples were analysed for regional variation in nectar (volume, concentration, sugar mass), which was attributed to climate, DBH, and floral traits (flower fresh mass and size). I conducted further sub-analysis using pooled species data to analyse general trends across all species.

Nectar and floral traits (plant traits) varied among regions in all species, and relationships between traits were highly species-specific. Nectar from South Island climate zones comprised the highest sugar amounts in most species (67%). Generalised additive mixed models were used to model plant traits as a function of regional variables, revealing significant correlations between nectar traits and DBH in a single species and between plant traits and climate factors in 87.5% of species. Climate factors explain 18 - 84% of regional plant trait variation, with annual sunshine hours and rainfall amounts showing the strongest effects.

Interspecific sub-analyses revealed that the combination of climate factors and DBH accounted for 22 - 36.5% of plant trait variation. In conclusion, although no uniform pattern across species was evident, I observed specific trends: in sunnier regions, nectar volumes tend to be lower and flowers larger, while nectar concentrations are typically higher in drier areas.

For a more comprehensive understanding of nectar variation, I recommend further research incorporating other potential predictors, such as soil type and phylogenetic relationships.

3.1 Introduction

Abiotic regional differences exert various pressures on local plant populations, leading to genotypic and phenotypic variation, evidenced, for example, by changes in flower size (Domínguez et al., 1998; Galen, 1999; Herrera, 2005; Hattori et al., 2015; Garcia et al., 2021). Interspecific variability in nectar volume and composition among plant species is well documented. However, intraspecific variation in nectar characteristics remains less understood (Lanza et al., 1995).

Specific nectar characteristics may vary within species due to environmental factors such as air temperature, sunlight exposure, humidity, rainfall, access to groundwater, and soil nutrient levels (Shuel, 1952; Cruden et al., 1983; Pleasants, 1983; Boose, 1997; Faegri & van der Pijl, 2013; Descamps et al., 2021), which also represent key drivers of genetic differentiation (Napier et al., 2023). For example, a recent broad study on nectar availability across a 314,400 ha Australian region (Hawkins et al., 2018) showed that nectar volume was strongly correlated with primary productivity in the past year, average yearly solar radiation, topographic moisture, and rainfall in the past six months.

A South American study (Chalcoff et al., 2006) on nectar traits in nine populations of *Embothrium coccineum,* a self-incompatible endemic forest tree, found regional divergence in nectar composition. These were related to differing bird-pollinator assemblages across the compared regions. However, a large amount of variation was also found in nectar traits among intraregional populations of *E. coccineum* that could be related to environmental variation, local selective factors and genetic drift. The authors emphasised that the large intraspecific variation in nectar sugar composition showed the underlying potential for evolutionary change in nectar traits under different selective pressures (cf. Schluter, 2000), which merited further investigation.

Intraspecific variation in nectar properties has also been related to the effects of evaporation and plant water status, e.g. in *Silphium perfoliatum* (Mueller et al., 2020), *Leptospermum scoparium* (Clearwater et al., 2018) or *Epilobium angustifolium* (Bertsch, 1983; Carrol et al., 2001). Conversely, these environmental factors can also have little effect on other species, such as *Ipomopsis longiflora* (Villarreal & Freeman, 1990).

Other studies mention the positive effect of rainfall on nectar volumes the day prior to nectar sampling (Keasar et al., 2008; Wyatt et al., 1992). Wyatt et al. performed a watering experiment, involving the addition of the equivalent of a 10-cm rain to their *Asclepias syriaca*

plot. After watering, nectar volumes and sucrose amounts were increased approximately twofold. A study by Leiss & Klinkhamer (2005) on different genotypes of *Echium vulgare* showed that variation in nectar volumes has a heritable component. Environmental conditions, specifically water availability, significantly influenced nectar production in *Echium vulgare*. Increased water availability increased nectar production more markedly in genetically low-producing lines, illustrating a genotype-by-environment interaction. Furthermore, high-nectar lines displayed greater root mass and resilience to drought, maintaining higher nectar production under dry conditions. Field observations also revealed that while additional watering increased nectar production, this effect was consistent across genetic lines.

Similarly, studies in New Zealand—such as those on mānuka (*Leptospermum scoparium*) and mistletoes (Loranthaceae)—suggest that both environmental and phylogenetic factors may contribute to regional variation in nectar traits (Ladley et al., 1997; Williams, 2012). However, research to date in New Zealand has often focused on the contribution of nectar to pollinator energetics, incorporating a limited number of plant species and sites (Whitaker, 1987; Rasch & Craig, 1988; Butz Huryn, 1995; Castro & Robertson, 1997; Ladley et al., 1997; Murphy & Kelly, 2003), or has relied on estimated rather than measured nectar values (Ausseil et al., 2018). One reason for the lack of information about regional variation in nectar production by individual species is the difficulty of gathering nectar samples. The accurate sampling of nectar, considering its dynamic nature and the multitude of influencing floral factors, presents significant challenges for research (Cruden et al., 1983; Gottsberger et al., 1990; Petit et al., 2011; Faegri & van der Pijl, 2013).

Given these challenges, my study aims to dissect the influence of various abiotic factors on the nectar traits of native New Zealand tree species, leveraging the country's diverse climatic zones, ranging from warm temperate in the north to cooler temperate in the south, influenced by a wide latitudinal range and complex geography (Kidson, 2000; NIWA, 2018). This unique setting allows one to explore nectar trait variability across different environmental conditions.

Drawing on prior studies that demonstrate the impact of environmental factors on nectar traits, I questioned to what extent common New Zealand species vary in their nectar-related responses across different climatic regions. Understanding this variation could help scale nectar production across landscapes. It also raises important questions about the adequacy of using species averages to predict nectar abundance and composition, and whether it is crucial to include environmental influences in these predictive models.

Objectives and Hypotheses

My study seeks to elucidate the influence of climate, flower fresh mass, flower size, and tree diameter on nectar variation in common tree species spanning diverse coastal climate zones in New Zealand.

I hypothesised that:

i) Correlations between floral and nectar traits (summarised under 'plant traits') and

ii) between climate drivers and plant traits will be uniform/consistent across species.

a) Nectar volumes will be highest in regions with high humidity because of the hydrophilic nature of nectar;

b) Nectar concentrations will be highest in the driest regions, specifically those on the east coasts of both islands, where limited precipitation and consequent higher evaporation rates are prevalent;

c) Nectar sugar mass, flower fresh mass and flower size will be highest in regions with the highest sunshine hours, supporting photosynthesis and the growth of plants with larger, sugar-rich flowers.

3.2 Materials and Methods

3.2.1 Study Sites

Sampling was conducted across seven New Zealand regions between 37 - 45° S and 170 - 177° E. Based on insufficient sample sizes, I excluded samples from the Western South Island region (see Table & Figure 1). Across the sample regions, the annual mean air temperature (MAT) ranges from 11.1 - 15.6℃, annual mean relative humidity (MRH) from 71-85%, annual sunshine hours (MSH) from 1681 - 2472 h, and mean additive rainfall (MAR) from 594 - 1683 mm (based on 30-year norms, 1981 - 2010, of each sample region, NIWA, 2018). Sample regions fell within five of the six climate regions identified by Kidson (2000; Fig. 1, circled numbers).

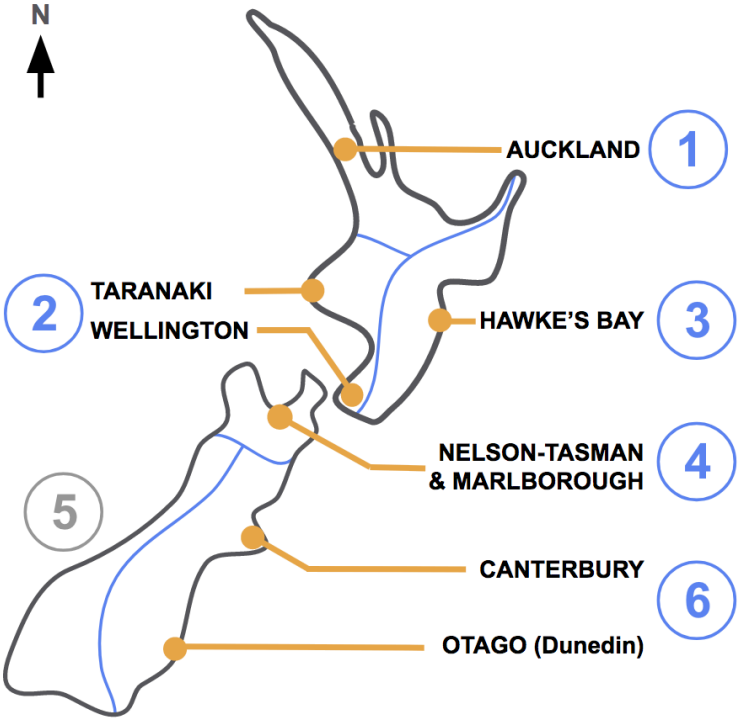


Figure 1: Seven sample regions (orange dots) within five of the six New Zealand climate regions (blue-circled numbers) after Kidson (2000), with Zone 1 = Northern North Island, Zone 2 = South-West North Island, Zone 3 = Eastern North Island, Zone 4 = Northern South Island, Zone 5 = Western South Island (excluded from this study), Zone 6 = Eastern South Island.

Table 1: Overview of sample regions, climatic 30-year norms (NIWA, 2018), and sampling details, with 'MSH' = mean annual sunshine hours, 'MAT' = mean annual air temperature (℃), 'MAR' = mean annual rainfall in mm/a, 'MRH' = mean annual relative humidity in %, 'Flowers | emptied' = number of flowers sampled for nectar, 'Flowers | measured' =number of flowers measured for size, 'Flowers | weighed' = number of flowers weighed for fresh mass).

SAMPLE REGION AND CLIMATE								SAMPLING AMOUNTS				
Region	**Climate Zone**	**MAR**	**MAT**	**MRH**	**MSH**	**LAT**	**LON**	**Trees**	**Flowers**			**Nectar**
		mm	**℃**	**%**	**h**	**° S**	**° E**	***n***	**emptied**	**measured**	**weighed**	**µL**
Auckland ('A')	Northern North Island	1119	15.6	81	2062	36.8	174.7	22	395	398	366	5000
Taranaki ('T')	South-West North Island	1683	14.0	85	2197	39.0	174.0	21	466	382	618	6147
Wellington ('W')	South-West North Island	1249	12.8	79	2110	41.2	174.7	24	410	414	560	5132
Hawke's Bay ('H')	Eastern North Island	786	14.6	71	2265	39.5	176.8	23	432	423	551	5877
Nelson-Tasman & Marlborough ('N')	Northern South Island	959	12.7	82	2472	41.2	173.2	26	471	483	563	6373
Canterbury ('C')	Eastern South Island	594	11.6	81	2143	43.5	172.6	24	428	410	628	4578
Otago (Dunedin, 'D')	Eastern South Island	968	11.1	85	1681	45.8	170.5	24	620	489	990	11334

3.2.2 Species

Based on their broad distribution across New Zealand (Martin, 1961), I chose to study eight common species (Figure 2). The selected species included three Asterids: karo (*Pittosporum crassifolium* Banks & Sol.; henceforth abbreviated as 'PIT CRA', Pittosporaceae), kōhūhū (*P. tenuifolium* Gaertn., 'PIT TEN'), and tarata (*P. eugenioides* A Cunn.; 'PIT EUG'). Additionally, I sampled four Rosids, including three members of the Myrtales: kōtukutuku (*Fuchsia excorticata* L. f; 'FUC EXC'; Onagraceae), pōhutukawa (*Metrosideros excelsa* A Cunn. ex G Don; 'MET EXC'; Myrtaceae), and mānuka (*Leptospermum scoparium* J R Forst. & G Forst.; 'LEP SCO'; Myrtaceae), and one member of the Fabales: kōwhai (*Sophora microphylla* Aiton; 'SOP MIC'; Fabaceae). Last, I analysed tī kōuka (*Cordyline australis* Forster; 'COR AUS'; Asparagaceae), representing monocotyledons.

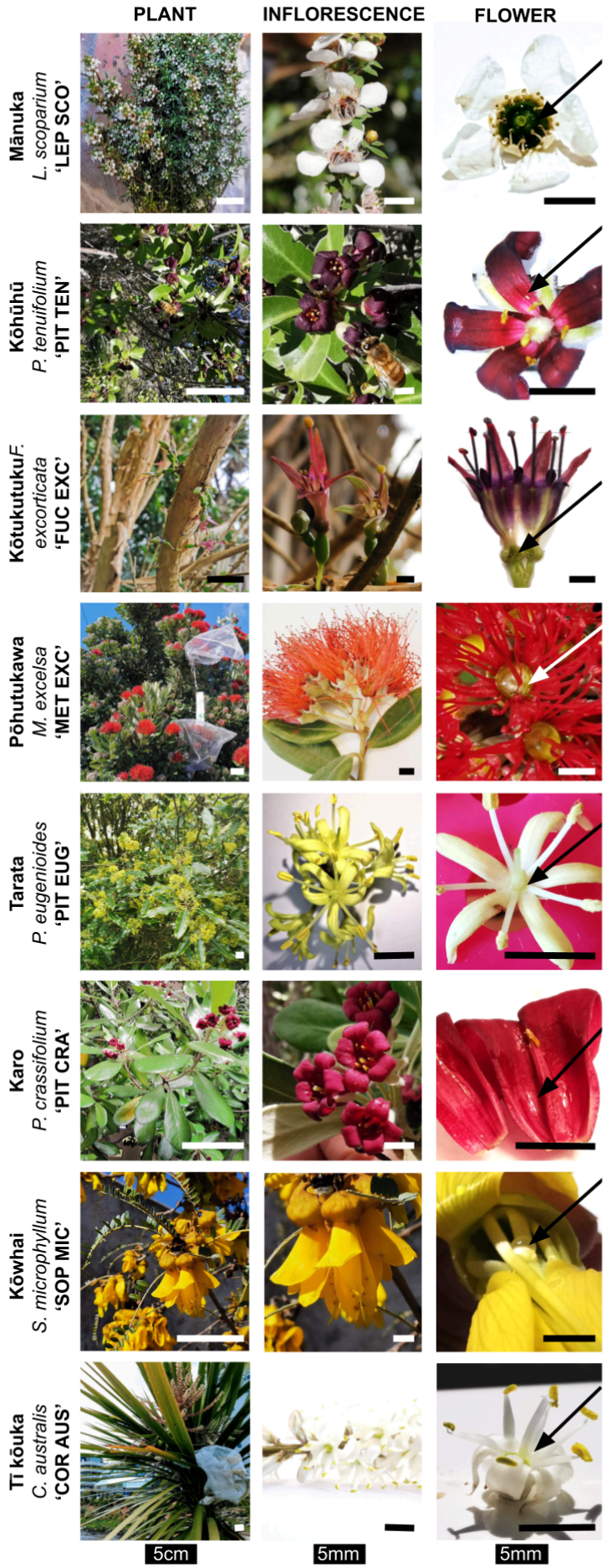


Figure 2:

Photographs of the eight sampled species (rows), with columns showing (1) overall appearance, (2) the entire flower or inflorescence, and (3) a close-up of nectar secretion. Arrows indicate the location of nectar within the flower. All images are original (own photographs), and scale bars represent flower size, with bar lengths explained below each column.

3.2.3 Sampling

I sampled an average of three trees per species at each study site, totalling 164 trees. The sampled individuals were predominantly naturally occurring wild plants growing in parks and forest reserves. Some individuals may have been planted, but ornamental cultivars were excluded. During each species' peak anthesis period (iNaturalist, 2018 - 2020; Newstrom-Lloyd, 2013) in the spring and summer of 2019 and 2020, I selected 20 - 40 fully opened flowers from each tree, totalling 340 - 889 flowers per species and 4276 flowers overall (see Table 1).

To collect nectar, I covered flowers with transparent synthetic organza bags, with transparent rain shields when necessary, to exclude nectar feeders, allowing the nectar to accumulate for 24 hours. The following day, I cut the flowers from the plants and sealed the branch ends in humid paper tissue. After collecting all bagged inflorescences across several sub-sites in the morning, I removed the nectar, measured the maximum dimensions of each flower, and weighed the flowers immediately after collection. First, I randomly removed 20 - 40 suitable flowers and extracted their nectar using micropipettes. I worked on a hydrophobic plastic sheet to collect water runoff when rinsing particular flowers. Nectar was pooled into a pre-weighed 1.5 mL vial (Eppendorf, Germany) assigned to the respective tree. I collected pure nectar for species with larger flowers, such as MET EXC, SOP MIC, and FUC EXC. I rinsed flowers with 5 - 20 µL of distilled water for species with smaller flowers to collect their smaller nectar volumes (Morrant et al., 2009). Nectar mass was measured as the difference in mass between empty and filled vials using a balance (MS120, Mettler Toledo, Switzerland). To calculate the net nectar volume of rinsed flower samples, I adjusted for the volume of water added by subtracting it from the total mass of the vial. Nectar concentrations were measured in ° Brix using a digital refractometer (Atago PAL-1 3810, Japan, accuracy ± 0.1) with 20 - 100 µL of nectar or nectar solution and corrected for any water added to rinsed samples. Concentration values were used to calculate specific gravity and convert nectar mass to volume units. Any remaining nectar was frozen for future analysis.

Mean flower mass was determined by weighing batches of 20 - 100 flowers per tree using the same balance as nectar mass. Flower size was measured with digital callipers. The dimension was defined for each species: corolla diameter was measured for LEP SCO and COR AUS, and corolla length for SOP MIC, FUC EXC, and *Pittosporum* species. The flower size of MET EXC is the total longitudinal length of the flower, summing the lengths of the capsule and the mean lengths of 20 measured filaments per inflorescence.

3.2.4 Statistical Analysis

To evaluate relationships among and between plant traits, trait values for each species were scaled to a 0–100 range. Plant traits included *i*) floral traits, such as flower fresh mass and flower size; *ii*) nectar traits, such as volume, sugar amount, and concentration; and *iii*) tree diameter at breast height (DBH). Initially, relationships between response variables were examined using linear regression analysis on a pooled dataset encompassing all species to investigate the uniformity and consistency of intraspecific relationships among plant traits across species. Species-specific analyses followed this.

First, each trait underwent normality testing using Shapiro-Wilk tests and histograms to examine regional variation within and across species. Based on the normality results, traits were analysed using either parametric tests (One-Way ANOVA with Tukey's Honestly Significant Difference, HSD) or non-parametric tests (Kruskal-Wallis with Dunn's test). Due to the lack of uniformity detected across species, the pooled dataset was refined by excluding species showing no regional variation within the respective trait, after which my analysis was repeated.

To assess the impact of climate and geographical explanatory variables (mean annual sunshine hours – MSH; mean annual additive rainfall amounts – MAR; mean annual air temperature – MAT; mean annual relative humidity – MRH; latitude; and longitude) on intraspecific plant trait variation, strong correlations among predictor variables were initially identified using Pearson’s correlation analyses. Strong correlations between latitude, longitude, and climate variables led to the exclusion of geographical variables from the generalised additive mixed modelling (GAMM) to prevent multicollinearity. GAM modelling explored the relationship between climate variables and plant traits, treating response variables as functions of the predictors and refining models to retain only significant predictors. GAMM was conducted on each species individually and on the reduced pooled dataset.

Mean values are given as mean±SD (standard deviation) throughout the text. All statistical analyses were performed using the R packages 'MASS', 'mgcv', 'statmod', 'dplyr', ‘ggplot2’, ‘png’, ‘grid’, ‘latex2exp’, ‘dunn.test’ and 'tweedie' within the R versions 3.6.3 - 4.3.1 (R Core Team, 2020 - 2023).

3.3 Results

I collected 474 ± 51 flowers per study site from 164 trees and extracted 2,240 µL of nectar from 3,222 individual flowers. Across species, fresh flowers had an average mass of 0.2 ± 0.25 g, a size of 18 ± 14 mm, and produced 14 ± 15 µL of nectar, containing 2 ± 3 mg of sugar based on concentrations of 13 ± 11 °Brix. Sampled trees had a mean diameter at breast height (DBH) of 35 ± 31 cm (see Table 3 and S1 in Supplementary Materials).

3.3.1 Linear Relationships between Plant Traits

Relationships between Plant Traits across Species

Linear regression analyses across all species of data scaled by species revealed that nectar sugar content and concentration, and sugar and volume were positively related (Table 2 & Figure 3). However, nectar concentration and volume were not significantly related. Significant linear models relating these variables were anticipated because sugar quantities derive directly from concentration and volume. However, within species, variation in total nectar sugar was more clearly related to variation in concentration than volume, and concentration declined as volume increased. Additionally, linear regression identified positive relationships between flower mass and nectar volume and between flower mass and size, albeit with a relatively weak goodness of fit for these models.

Table 2: Statistical parameters of linear regression analysis for detecting relations among plant traits, with n.s. = not significant.

	NECTAR CONCENTRATION	NECTAR VOLUME	FLOWER WEIGHT	FLOWER SIZE
Nectar Sugar	$P < 0.001$ $R^2 = 0.46$ F = 140	$P < 0.001$ $R^2 = 0.18$ F = 36	n.s.	n.s.
Nectar Concentration		n.s.	n.s.	n.s.
Nectar Volume			$P < 0.001$ $R^2 = 0.06$ F = 12	n.s.
Flower Weight				$P < 0.001$ $R^2 = 0.08$ F = 14

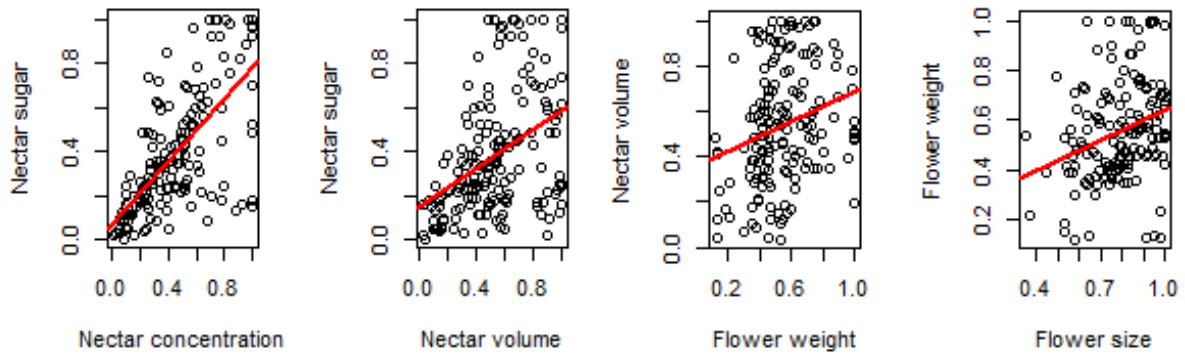


Figure 3: Significant linear relationships between plant traits (interspecific pooled data, scaled by species).

Relationships between Plant Traits within Species

Repeating the linear regression analysis of standardised plant traits individually by species revealed more diverse results among species, except for more concentrated nectar consistently containing larger sugar amounts (lm, $P < 0.001$, $R^2 = 0.175$ - 0.76, with two SOP MIC outliers removed; Figure 4).

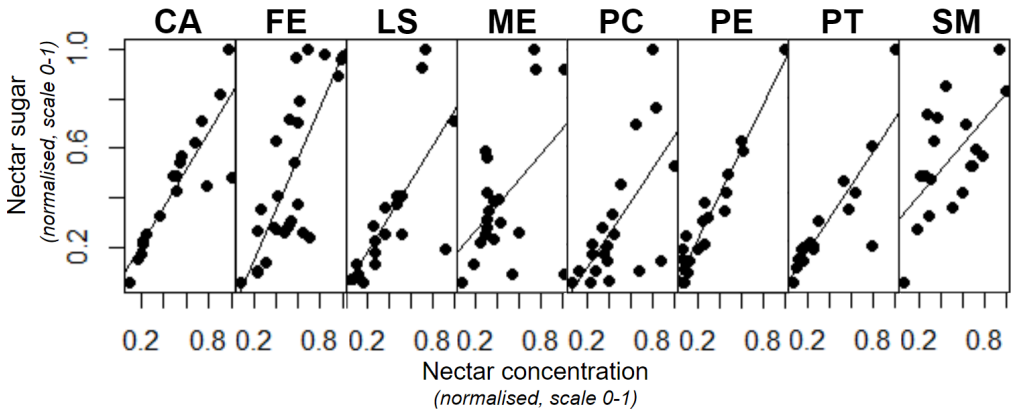


Figure 4: Scatterplots of statistically significant linear relationships between nectar sugar and concentration within species, with CA = *Cordyline australis*, FE = *Fuchsia excorticata*, LS = *Leptospermum scoparium*, ME = *Metrosideros excelsa*, PC = *Pittosporum crassifolium*, PE = *Pittosporum eugenioides*, PT = *Pittosporum tenuifolium*, and SM = *Sophora microphylla*.

Furthermore, for four species (FUC EXC, MET EXC, PIT CRA, and SOP MIC), greater nectar volumes were also associated with more sugar (lm, $P < 0.01$, $R^2 = 0.35$ - 0.7; Figure 5) and higher (FUC EXC) or lower (SOP MIC and PIT TEN) nectar concentration (lm, $P < 0.05$; $R^2 = 0.1$ - 0.4; Figure 6). Trees of COR AUS and PIT CRA with lower DBH produced nectar with higher sugar amounts (lm, $P < 0.001$, $R^2 = 0.5$ - 0.6; Figure 6).

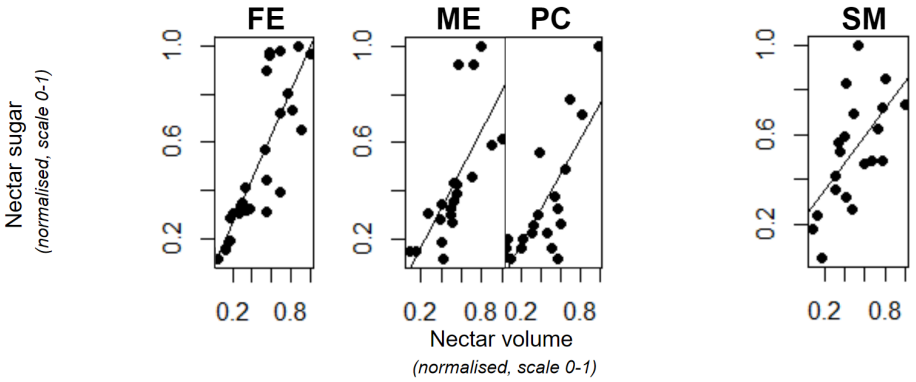


Figure 5: Scatterplots of statistically significant linear relationships between nectar sugar and volume within species, with FE = *Fuchsia excorticata*, ME = *Metrosideros excelsa*, PC = *Pittosporum crassifolium*, and SM = *Sophora microphylla*.

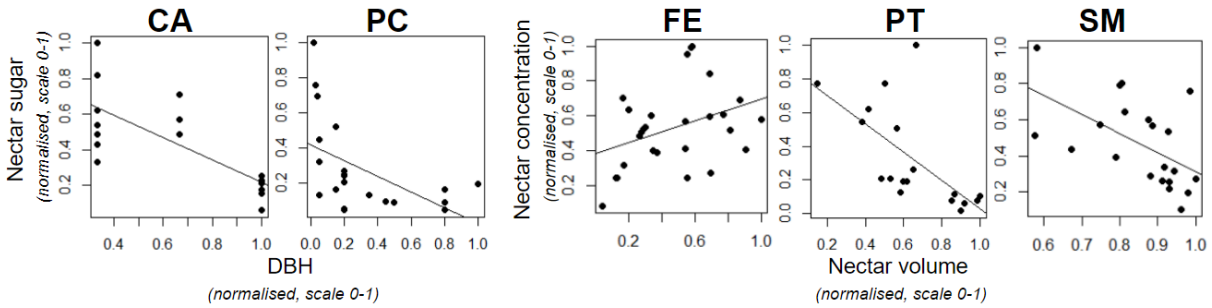


Figure 6: Scatterplots of statistically significant linear relationships between nectar sugar and DBH, and between nectar concentration and volume within species, with CA = *Cordyline australis*, FE = *Fuchsia excorticata*, PC = *Pittosporum crassifolium*, PT = *Pittosporum tenuifolium*, and SM = *Sophora microphylla*.

Smaller LEP SCO flowers secreted more nectar volume (lm, $P = 0.01$, $R^2 = 0.27$, Figure 7). Heavier FUC EXC and heavier and larger SOP MIC flowers yielded more nectar volume (lm, $P < 0.05$, $R^2 = 0.2$ - 0.32, Figure 7), and larger SOP MIC flowers also exhibited lower nectar concentration (lm, $P < 0.01$, $R^2 = 0.27$, Figure 7).

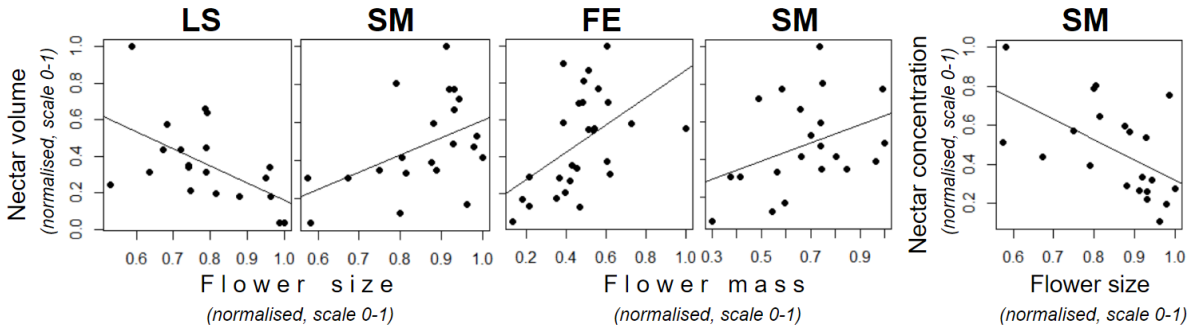


Figure 7: Scatterplots of statistically significant linear relationships between nectar volume and flower size and mass, and between nectar concentration and flower size within species, with FE = Fuchsia excorticata, LS = Leptospermum scoparium, and SM = Sophora microphylla.

SOP MIC and MET EXC were the only individual species in which flower size showed a significant linear relationship with flower mass, though only weakly in SOP MIC (SOP MIC: lm, $P = 0.01$, $R^2 = 0.24$, ME: $P < 0.001$, $R^2 = 0.76$, Figure 8).

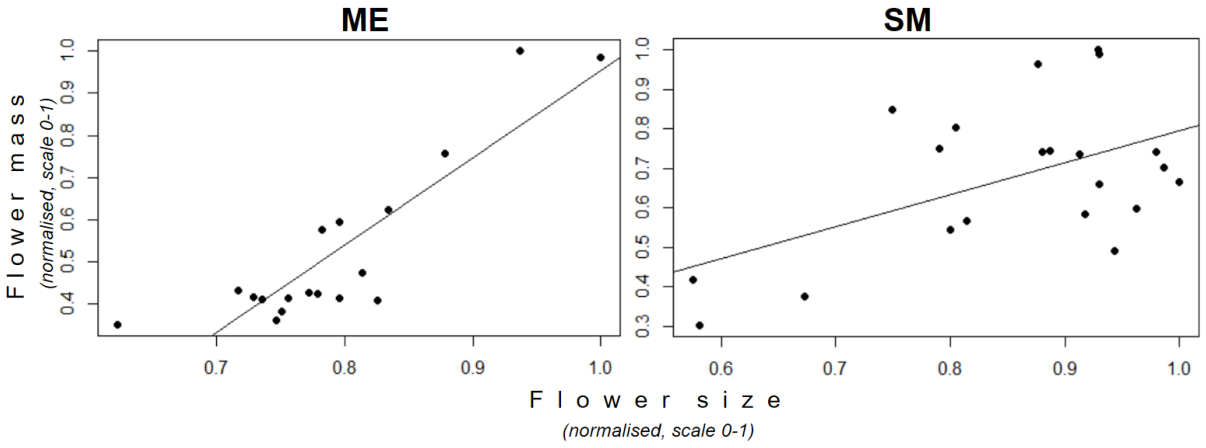


Figure 8: Scatterplots of statistically significant linear relationships between flower size and mass within species, with ME = *Metrosideros excelsa*, and SM = *Sophora microphylla*.

Variation of Plant Traits within Species across Regions

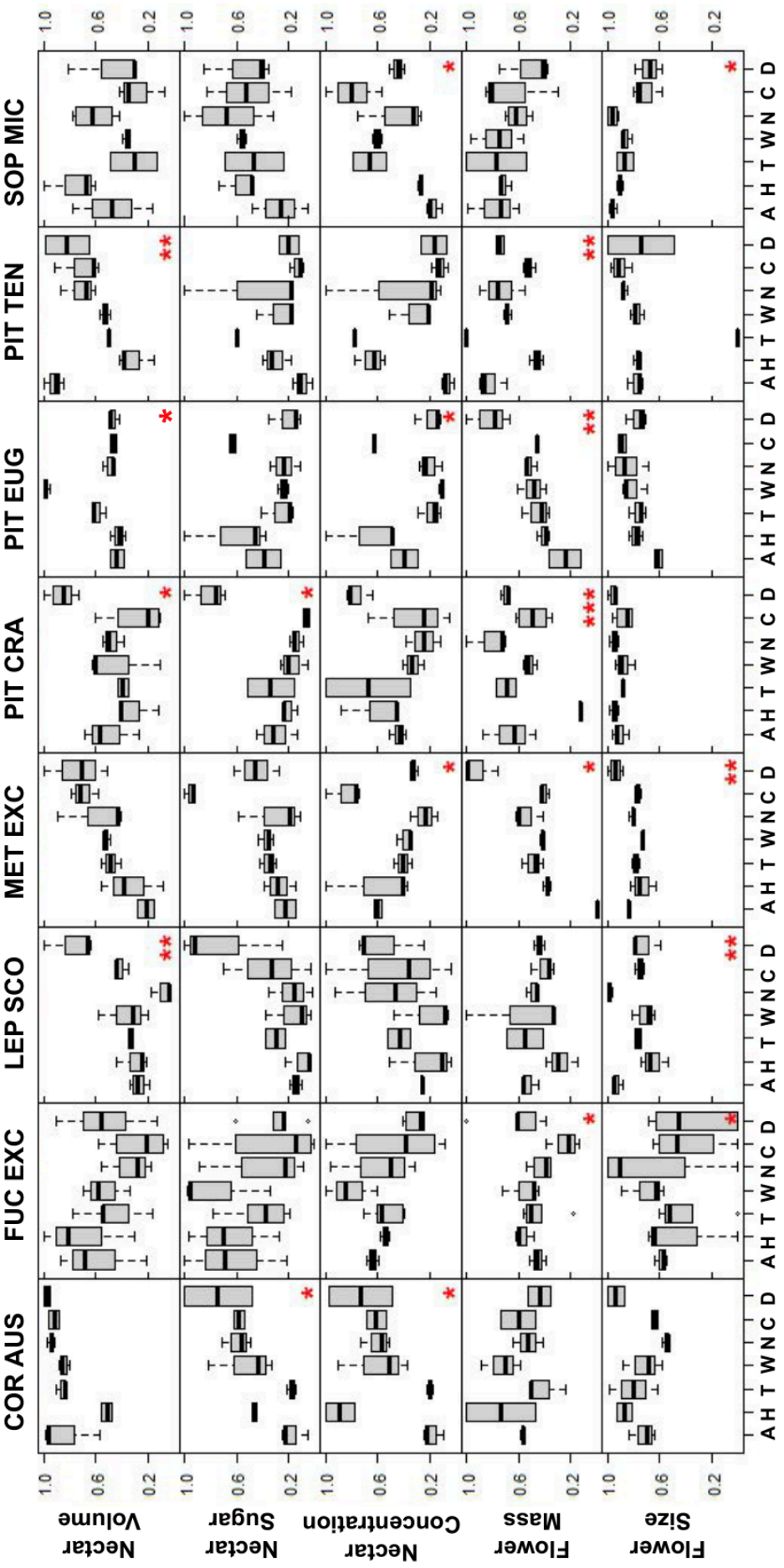
All species exhibited significant regional plant trait variation in two to three out of the five traits included (Table 3, Figures 9 & 10). Across sample regions, sugar amounts significantly varied only in two species, nectar volumes, concentrations and flower size in four, and flower mass in five out of eight species. Therefore, total sugar varies significantly among sample regions in the least number of species and flower mass in the most (Figure 10). In all species, except COR AUS, FUC EXC and LEP SCO, nectar volume and concentration varied inversely across sample regions (Figure 9), leading to similar total sugar amounts, explaining the low number of species with varying sugar amounts per region and the low goodness of fit of my linear regression models between these plant traits mentioned above. Furthermore, flower mass and size tend to vary with each other across sample regions (Figure 9). However, this relationship was only statistically significant for MET EXC. I could not identify a general visual trend between flower mass or size and nectar traits across sample regions based on the data displayed in Figure 9. Additionally, the majority of nectar sugar (75%) and flower size (100%) maxima by species were observed within South Island sample regions, despite this part of the country being represented by one less sample region ($n = 3$ versus $n = 4$ for North Island). 65% of all ranked trait maxima were observed in the south, with 5% in Auckland (37° S) and 37.5% in Dunedin (45° S).

Table 3: Overview of species-specific value ranges across individuals. Red values indicate significant trait variation across sample regions, and the *P*-values of ANOVA (A) and Kruskal-Wallis (KW) tests are below these means, with the species: CA = *Cordyline australis*, FE = *Fuchsia excorticata*, LS = *Leptospermum scoparium*, ME = *Metrosideros excelsa*, PC = *Pittosporum crassifolium*, PE = *Pittosporum eugenioides*, PT = *Pittosporum tenuifolium*, and SM = *Sophora microphylla*.

		TREES		NECTAR				FLOWERS									
Species		DBH (cm)	trees n	nectar samples n	sugar/ flower (mg)	concentration (Brix)	nectar/ flower (uL)	fresh mass (mg)	size (mm)	n / nectar sample n	nectar removed from n n	n weighted / tree sum	n measured/ tree n	Traits varying within species n	Traits varying within species % / all
CA	range:	5-15	18	18	0.03-0.44	1-12	2-4	8-34	5-9	15-20	345	20-50 440	16-33	2	40
	mean ± SD:	10±5			0.2±0.1	6±3	3±1	19±6	7±1	19±2		24±10	20±3		
	P - value				0.02 (A)	<0.01 (A)									
FE	range:	3-110	27	27	0.04-7.11	2-25	2-47	68-512	10-28	5-44	492	6-50 633	11-38	2	40
	mean ± SD:	47±33			3.3±2.4	13±6	23±13	240±91	18±5	18±10		23±13	19±6		
	P - value							0.01 (A)	0.01 (KW)						
LS	range:	2-50	20	20	0.01-0.6	1-15	0-9	13-86	9-17	20-40	460	19-40 519	18-40	2	40
	mean ± SD:	16±16			0.2±0.2	6±4	3±2	40±15	14±2	23±7		26±9	22±6		
	P - value						<0.01 (A)		<0.01 (A)						
ME	range:	15-70	20	20	1.4-12	7-51	4-47	163-463	25-41	20-40	510	17-188 889	9-40	3	60
	mean ± SD:	36±22			5.1±3.1	24±12	25±11	243±92	33±3	26±8		49±53	23±9		
	P - value					0.03 (KW)		0.03 (KW)	<0.01 (KW)						
PC	range:	2-100	21	21	0.1-3	1-26	2-16	19-146	8-10	8-30	396	8-40 441	7-20	3	60
	mean ± SD:	31±30			0.8±0.8	12±7	7±4	82±34	9±1	19±5		21±8	16±5		
	P - value				0.03 (KW)		0.04 (A)	<0.001 (A)							
PE	range:	10-100	19	19	0.1-0.7	2-25	2-6	4-33	8-14	15-20	370	15-40 469	11-22	3	60
	mean ± SD:	44±27			0.2±0.1	8±6	3±1	16±6	11±2	19±2		25±9	18±3		
	P - value					0.03 (KW)	0.04 (KW)	<0.01 (A)							
PT	range:	5-127	18	18	0.02-1	0-27	1-6	40-97	4-9	10-20	258	10-40 340	10-28	2	40
	mean ± SD:	38±26			0.3±0.2	9±8	4±1	66±17	7±1	14±4		19±10	14±5		
	P - value						<0.01 (A)	<0.01 (A)							
SM	range:	8-160	21	21	0.7-13	6-53	5-72	338-1116	31-54	10-30	391	10-60 545	10-60	2	40
	mean ± SD:	52±45			7±3	25±13	34±18	756±213	46±7	19±5		26±13	19±10		
	P - value					<0.01 (A)			<0.01 (KW)						
Species varying across sites	n				2	4	4	5	4						
	% / all				25	50	50	62.5	50				range:	25-63	40-60

Caption for Table 9 on the next page:

Figure 9: Standardised trait variation (rows) of the eight analysed species (columns) across regions: Data presented in boxplots with black bars indicating region median values. Regions are ordered by latitude from left to right (north to south), with 'A' = Auckland, 'H' = Hawke's Bay, 'T' = Taranaki, 'W' = Wellington, 'N' = Nelson-Tasman/Marlborough, 'C' = Canterbury, 'D' = Dunedin. Red asterisks denote significant differences across sites: '*' = $P < 0.05$, '**' = $P < 0.01$, and '***' = $P < 0.001$.


COR AUS
FUC EXC
LEP SCO
MET EXC
PIT CRA
PIT EUG
PIT TEN
SOP MIC
A H T W N C D
1.0
0.6
0.2
Nectar Volume
Nectar Sugar
Nectar Concentration
Flower Mass
Flower Size

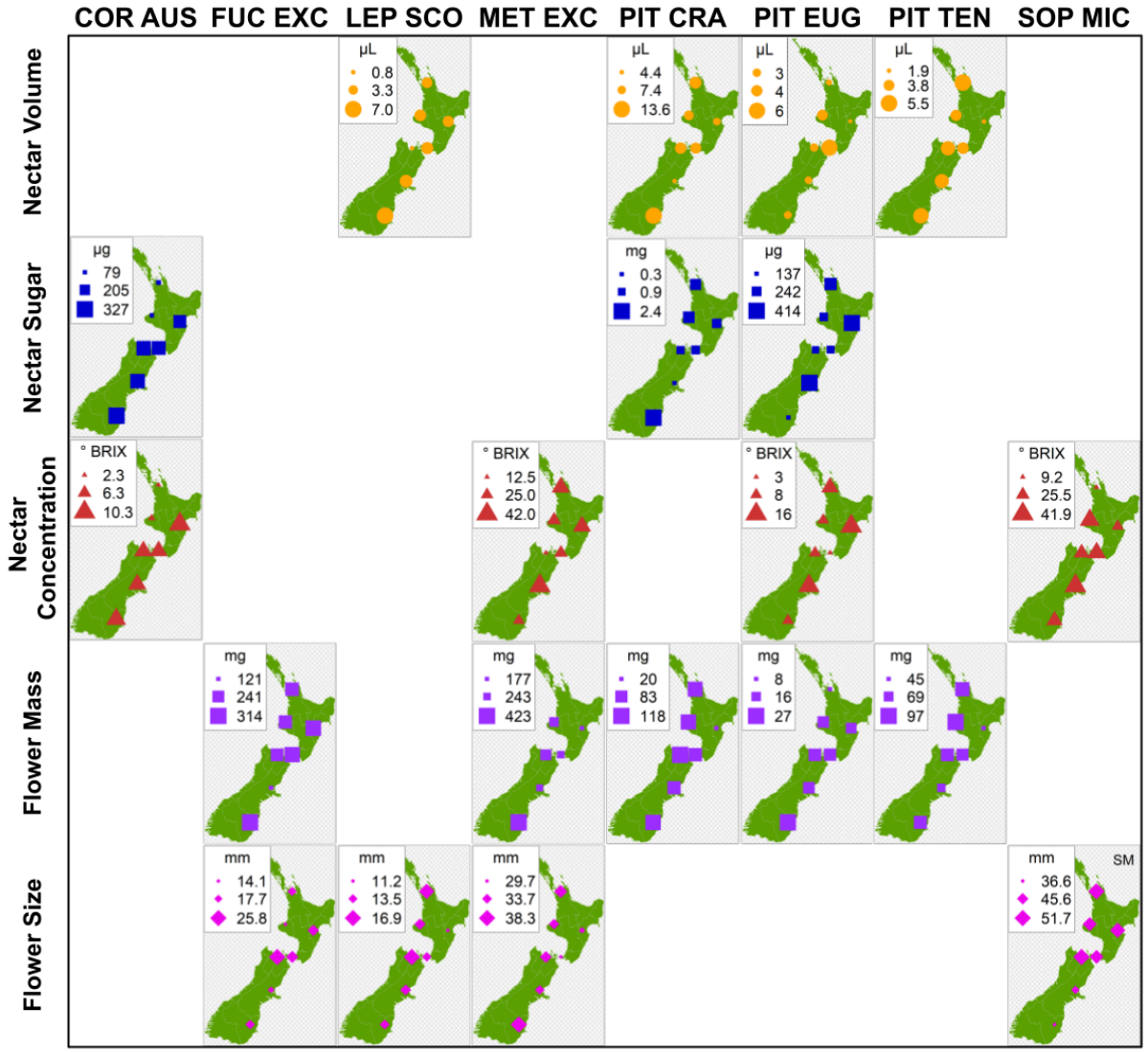


Figure 10: Significant regional variations ($P < 0.05$, ANOVA or Kruskal-Wallis test) in plant traits (rows) within the analysed species (columns), with symbol size representing the mean value for each region.

3.3.2 Climate Drivers of Plant Trait Variation

To streamline the selection of variables for subsequent generalised additive mixed modelling (GAMM), I assessed the correlations among the environmental variables: mean annual rainfall (MAR), mean annual air temperature (MAT), mean annual relative humidity (MRH), mean annual sunshine hours (MSH), latitude and longitude (Table 4) using Pearson's correlation test. Significant linear correlations emerged between latitude, longitude and MAT, longitude and MRH, and latitude and longitude. Therefore, the chosen explanatory variables for the GAM analysis were MAR, MAT, MRH, and MSH.

Table 4: Pearson's correlation matrix showing correlation coefficients and *P*-values for the correlations between environmental variables. For variable abbreviations, see Table 1.

		MAR	MAT	MRH	MSH	Latitude
Pearson's Correlation Coefficients	**MAT**	0.343				
	MRH	0.436	-0.409			
	MSH	-0.004	0.359	-0.363		
	Latitude	-0.426	**-0.968**	0.306	-0.491	
	Longitude	0.108	**0.799**	**-0.797**	0.563	**-0.779**
***P*-values**	**MAT**	0.451				
	MRH	0.329	0.362			
	MSH	0.993	0.429	0.424		
	Latitude	0.340	**0.000**	0.505	0.263	
	Longitude	0.818	**0.031**	**0.032**	0.188	**0.039**

Correlations between Plant Traits and Environmental Factors within Species

Across all species, climate emerged as a key driver of trait variation, with MAR and MSH being the most influential factors (Figure 11, Table 5). In general, nectar volume tended to decrease, and nectar concentration increased in response to variables associated with evaporation or water availability (decreased MAR, increased MSH, or decreased MSH), although these effects varied with species. The climate responses of flower size and mass were even more species-dependent.

The type and extent of climate's influence varied among species, with climate variables explaining variations in two to three traits across species, with deviances between 18% and 84%. Specifically, MAR affected all traits except flower size in at least one species. Similarly, depending on the species, MSH influenced all traits except nectar concentration, MAT impacted total sugar, nectar concentration, and flower size; MRH affected nectar volume, concentration, and flower mass. DBH only influenced nectar sugar in one species.

The direction of regional climate effects was also variable: For nectar volume in four species, MAR had a positive effect, while MRH and MSH had negative impacts, with deviances from 18% to 64%. Nectar sugar in two species was negatively influenced by MAR, MAT, and DBH, with deviances between 51% and 80%. Four species showed nectar concentration variations due to MAR, MRH (negative), or MAT (variable), with deviances from 29% to 58%. Flower mass in five species was influenced negatively by MAR or MSH or variably by

MRH, with deviances between 54% and 85%. Lastly, flower size in four species was negatively affected by MAR or variably by MSH, with deviances ranging from 44% to 63%.

Among species, sunnier habitats typically resulted in larger flowers for species FUC EXC but smaller ones for SOP MIC, MET EXC, and COR AUS. Warmer environments were linked to reduced sugar and concentration in COR AUS nectar, decreased MET EXC nectar volume, and more concentrated nectar in SOP MIC. However, air temperature did not directly influence flower mass or size. In rainier environments, COR AUS nectar contained less sugar, ME nectar was less concentrated, PIT EUG flowers were more voluminous but lighter, PIT TEN flowers were lighter, and SOP MIC flowers were larger. More humid conditions generally increased COR AUS nectar volume but decreased it in PIT TEN, and led to less concentrated nectar in both COR AUS and PIT EUG. Additionally, PIT CRA flowers from trees with smaller diameters at breast height (DBH) contain higher total sugar amounts.

Table 5: Statistical parameters of GAMM analysis on regional variation in plant traits and their correlations with tested environmental factors.

	NECTAR VOLUME	NECTAR SUGAR	NECTAR CON-CENTRATION	FLOWER MASS	FLOWER SIZE	*n*
COR AUS	-	R^2 = 0.514, MAT *P* = 0.006, MAR *P* = 0.017, Tweedie(2), log	R^2 = 0.58, MAT *P* < 0.001, MRH *P* < 0.001, Tweedie(2), log	-	-	18
FUC EXC	-	-	-	-	R^2 = 0.437, MSH *P* = 0.027, Tweedie(2), log	20
LEP SCO	R^2. = 0.643, MSH *P* < 0.001, Tweedie(2), log	-	-	-	-	20
MET EXC	-	-	R^2 = 0.372, MAR *P* = 0.047, Tweedie(1.8), log	R^2 = 0.847, MSH *P* < 0.001, MRH *P* = 0.02, Tweedie(2), log, n = 18	R^2 = 0.559, MSH *P* = 0.001, Tweedie(2), log	20
PIT CRA	R^2 = 0.424, MSH *P* = 0.04, Tweedie(1.8), log	R^2 = 0.799, MSH *P* = 0.019, DBH *P* = 0.003, Tweedie(2), log	-	R^2 = 0.641, MRH *P* < 0.001, Tweedie(2), log	-	21
PIT EUG	R^2 = 0.183, MAR *P* = 0.05, Tweedie(2), log	-	R^2 = 0.324, MRH P = 0.017, Tweedie(2), log	R^2 = 0.538, MAT *P* < 0.001, Tweedie(2), log	-	19
PIT TEN	R^2 = 0.437, MRH *P* = 0.007, Gamma, inverse	-	-	R^2 = 0.596, MAR *P* = 0.003, MRH *P* = 0.005, Gamma, inverse	-	18
SOP MIC	-	-	R^2 = 0.297, MAT *P* = 0.008, Gamma, inverse	-	R^2 = 0.626, MSH *P* = 0.002, MAT *P* = 0.001, Gamma, inverse	21
Range R^2	**0.2 - 0.6**	**0.5 - 0.8**	**0.3 - 0.6**	**0.5 - 0.8**	**0.4 - 0.6**	
MAR	**X**	**X**	**X**	**X**		
MAT		**X**	**X**	**X**	**X**	
MRH	**X**		**X**	**X**		
MSH	**X**	**X**		**X**	**X**	
DBH		**X**				

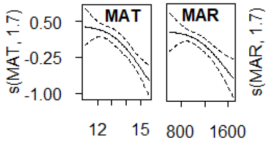
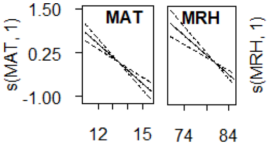
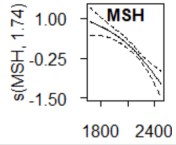
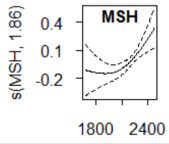
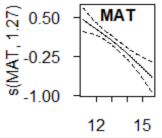
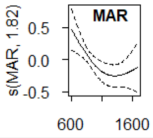
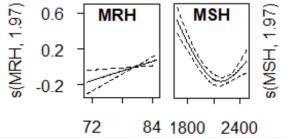
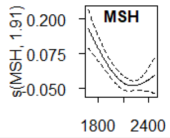
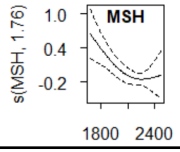
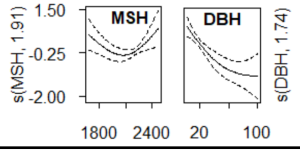
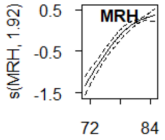
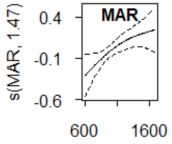
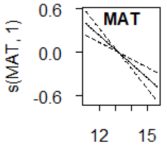
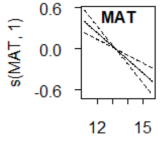
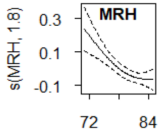
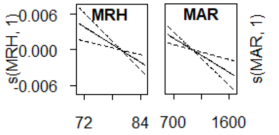
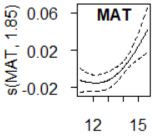
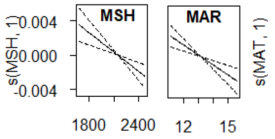
	NECTAR VOLUME	NECTAR SUGAR	NECTAR CONCENTRATION	FLOWER MASS	FLOWER SIZE
COR AUS	*No significant regional variation*			*No significant regional variation*	*No significant regional variation*
FUC EXC	*No significant regional variation*	*No significant regional variation*	*No significant regional variation*	*No significant regional variation*	*Regional variation, with none of the predictors able to explain this.*
LEP SCO		*No significant regional variation*	*No significant regional variation*	*Regional variation, with none of the predictors able to explain this.*	
MET EXC		*No significant regional variation*			
PIT CRA			*No significant regional variation*		*No significant regional variation*
PIT EUG		*No significant regional variation*			*No significant regional variation*
PIT TEN		*No significant regional variation*	*No significant regional variation*		*No significant regional variation*
SOP MIC	*No significant regional variation*	*No significant regional variation*		*No significant regional variation*	

Figure 11: Significant intraspecific trait variations explained by climate factors and diameter at breast height (DBH), derived from the best-fitted generalised additive mixed models (GAMM).

Correlations between Plant Traits and Environmental Factors across Species

Based on scaled and pooled species data (n = 164), only the plant traits flower mass (Kruskal-Wallis test, P = 0.04, χ^2 = 13.2) and size (KW, P < 0.01, χ^2 = 17.3) varied significantly between regions. In particular, flowers from Nelson were larger than all others. Flowers from Nelson, Wellington and Dunedin were heavier than those from Hawke's Bay and Christchurch (both floral traits: Dunn's test P = 0.01 - 0.0006). MSH explained these regional variations very weakly (GAMM, flower size: P = 0.04, R^2 = 0.018; flower mass: P < 0.01, R^2 = 0.05). Based on these weak results, I investigated whether a reduced dataset leads to stronger effect sizes, considering only a subset of species that previously showed significant regional variation (see Figure 11 and Table 2).

The reduced dataset for 'nectar sugar' comprised two species (COR AUS, PIT CRA; n = 38), 'nectar concentration' (COR AUS, MET EXC, PIT EUG, SOP MIC) and 'nectar volume' (LEP SCO, *Pittosporum* spp.) each four species (n = 78), 'flower mass' included five species (FUC EXC, ME, *Pittosporum* spp., n = 105), and the 'flower size' subset was limited to four species (FUC EXC, LEP SCO, MET EXC, SOP MIC, n = 88). All subsets, except for nectar sugar, demonstrated significant regional variations across species (Table 6, Figure 12).

Table 6: Kruskal-Wallis and GAMM analysis results for the effects of region and climate on normalised plant traits, pooled for the species where significant regional differences were detected.

	n	SPECIES	KRUSKAL-WALLIS TEST	DUNN'S TEST *(with Bonferroni correction)*	GAMM
Nectar Sugar	57	COR AUS, PIT CRA	n.s.	-	-
Nectar Concentration	78	COR AUS, MET EXC, PIT EUG, SOP MIC	P = 0.001, χ^2 = 22	P < 0.02	R^2 = 0.23, P < 0.01
Nectar Volume	78	LEP SCO, *Pittosporum* spp.	P < 0.01, χ^2 = 17.8	P < 0.01	R^2 = 0.14, P < 0.001
Flower Mass	105	FUC EXC, MET EXC, *Pittosporum* spp.	P < 0.001, χ^2 = 29.4	P < 0.01	R^2 = 0.22, P < 0.05 for MRH, P < 0.01 for MSH,
Flower Size	88	FUC EXC, LEP SCO, MET EXC, SOP MIC	P = 0.001, χ^2 = 22.6	P < 0.01	n.s.

I detected a general trend of higher nectar concentrations in Canterbury and Hawke's Bay, nectar volumes and flower masses in Dunedin, and larger flower sizes in Nelson. The lower nectar concentrations in Canterbury and Hawke's Bay could partly be attributed to the

regions' lower MAR, located on New Zealand's East Coast. In contrast, the higher nectar volumes and flower masses in Dunedin could be partially explained by the region's lower MSH and, for flower mass additionally, the higher MRH, indicative of this southerly located sample region within my study. However, I could not identify any predictors that sufficiently explained the regional variance in flower size.

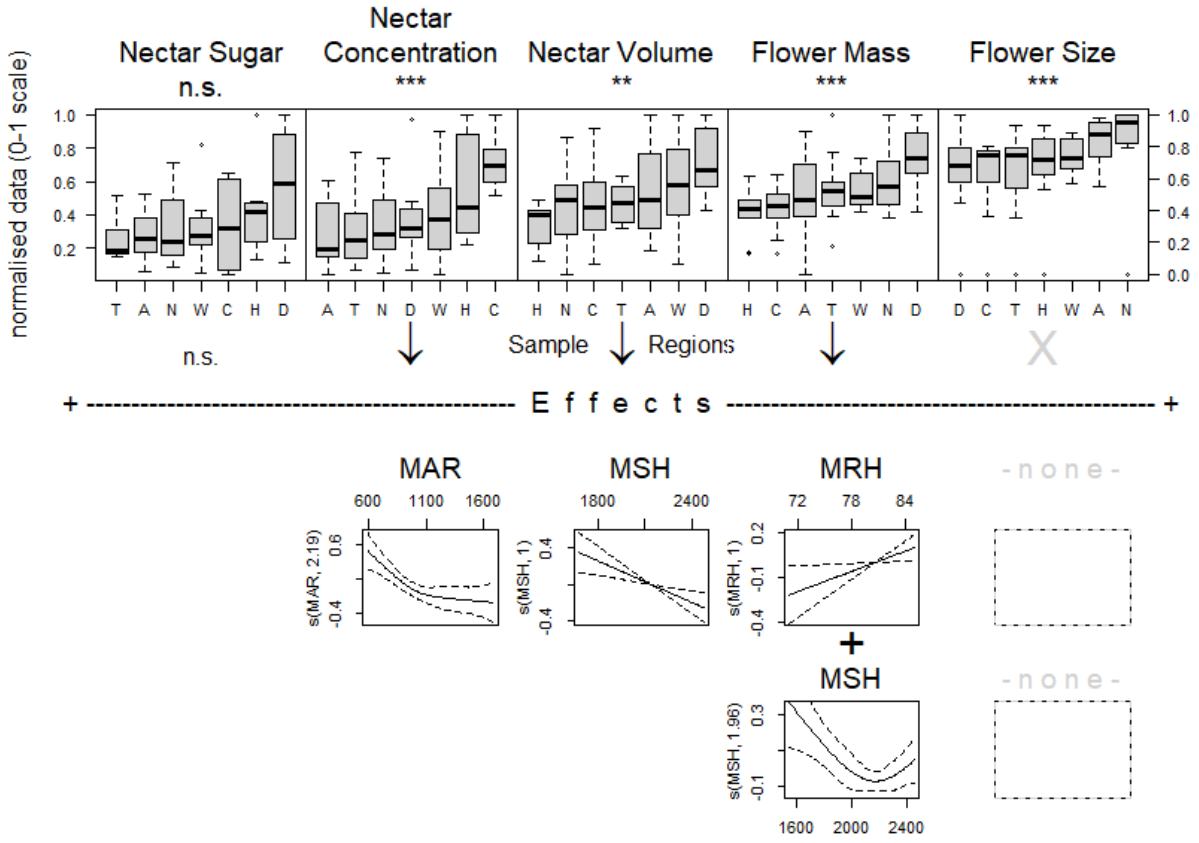


Figure 12: Variation in scaled, pooled subset data across sites, considering only species exhibiting significant differences for each plant trait, with data for boxplots (top of the figure) and generalised additive models (GAMs; bottom of the figure, with GAMs shown only for significantly varying plant traits across sample regions) for sugar mass of the species *Cordyline australis* and *Pittosporum crassifolium*; for nectar concentration of *Cordyline australis*, *Metrosideros excelsa*, *Pittosporum eugenioides* and *Sophora microphylla*; for nectar volume of *Leptospermum scoparium* and all *Pittosporum*; for flower mass of *Fuchsia excorticata*, *Metrosideros excelsa* and all *Pittosporum*; and flower size of *Fuchsia excorticata*, *Leptospermum scoparium*, *Metrosideros excelsa*, and *Sophora microphylla*. Sample regions are labelled as in Figure 9, with each boxplot's sample region order ranked by the *y* variable. The (partial) effects explain 14 - 23% of the variation. Asterisks below plant traits denote significance levels of the covariance: '*' signifies $P < 0.05$, '**' indicates $P < 0.01$, and '***' denotes $P < 0.001$.

3.4 Discussion

In general, there were positive correlations between plant traits of my native New Zealand species. Across species, larger and heavier flowers tended to produce higher volumes of nectar containing higher amounts of sugars. Nectar concentration was an important predictor of total nectar sugars, more so than nectar volume, suggesting that some of the observed intraspecific variation in nectar properties was related to the effects of evaporation and plant water status. This has previously also been described for other species, such as *Silphium perfoliatum* (Mueller et al., 2020), *Leptospermum scoparium* (Clearwater et al., 2018) or *Epilobium angustifolium* (Bertsch, 1983), but has not been shown to play a role in other species, such as *Ipomopsis longiflora* (Villarreal & Freeman, 1990). Whilst nectar volume and total sugar amounts were also correlated with flower size or mass across species (Chapter 4), nectar concentration increased with nectar volume at the interspecific level, indicating that evolutionary differences were more important at the species level. Although there were some consistent correlations between traits within species, not all of these relationships were observed within every species. Consequently, my first hypothesis was not supported, as it posited that correlations between plant traits would follow a uniform pattern within all species. For example, significant correlations between most pairs of traits were specific to individual species: Higher nectar volumes containing more sugar were only observed in half of the tested species (*Fuchsia excorticata*, *Metrosideros excelsa*, *Sophora microphylla*, and *Pittosporum crassifolium*). Variation between species in trait relationships across individuals and sites may be related to differences in their nectar secretion process, flower structure, or evolutionary relationship with pollinators (Vesprini et al., 1999; Pacini et al., 2003; Thompson et al., 2017).

For example, a positive correlation between nectar volume and total sugars may align with the preferences and nutritional needs of the pollinators of the species in which this relationship was significant – birds, as also described for plant species adapted to specialised birds in Western Central Africa (Janeček et al., 2021). Although *Pittosporum crassifolium* (karo) is classified as an entomophilous species, it also attracts bird visits. It can adequately fulfil the energetic requirements of even the largest New Zealand honeyeater, the tūi (*Prosthemadera novaeseelandiae*) (Castro & Robertson, 1997). Interestingly, my data showed that smaller trees of this species produced nectar containing higher total sugar amounts. Ontogenetic changes in flower number and carbon allocation to reproduction associated with tree size could result in related variations in individual flower size or nectar

per flower (Ehlers & Olesen, 2004; Noe et al., 2019). Karo's sister taxon, *P. tenuifolium,* was the only species in which higher nectar volumes were less concentrated. This may be an adaptation to their preferred pollinators, moths, which rely on lower viscosities to efficiently absorb nectar with their proboscis (Wardle, 2011).

The lack of a strong correlation between scaled flower size and mass across species may be explained by flower mass reflecting not only size but also the thickness and tissue density of structures. This could be an adaptation to benefit floral longevity by conserving water in thicker and heavier petals, which do not necessarily need to be longer. These traits, such as more layers of petal cells or a thicker mesophyll (Guo et al., 2023), may contribute to floral longevity. A lack of correlation in some species could also be because the measured dimension for flower size captures the main driver of flower mass better in some species than others, and because flower size and shape vary more in some species than others. I can conclude that flower mass and size are less important drivers of nectar variables within species than among species.

My second hypothesis, proposing uniform relationships between climate drivers and plant traits, was only partially supported. Instead, my study revealed species-specific correlations between plant traits and climate factors in New Zealand tree species. Climate factors explained regional variation in different plant traits across species, but the results varied in their degree of dependence and the direction of influence, as also described for other species by Plos et al. (2023). For instance, sugar amounts correlated positively with annual rainfall in *P. eugenioides* but negatively in *Cordyline australis*. Regional variation in annual rainfall amounts only affected a single species' nectar volume (positively), *P. eugenioides*, a correlation also observed in other species (e.g. Keasar et al., 2008; Bertazzini & Forlani, 2016).

Similarly, the hypotheses *ii*a and *ii*b, suggesting higher nectar volumes of lower concentration in humid regions, were generally supported, but did not show a universal pattern within individual species. I detected a general trend of higher nectar volumes in the highly humid (85% mean annual relative humidity, MRH) Dunedin. However, among individual species and in the pooled model, elevated nectar volumes were most often associated with lower mean annual sunshine hours (MSH), rather than higher regional MRH. Within individual species, Dunedin had the highest nectar volumes for 50% of species. However, 25% of species had their highest nectar volumes in the region with the lowest relative humidity (72%

MRH), Hawke's Bay. Additionally, I found generally higher nectar concentrations in New Zealand's drier regions, Canterbury and Hawke's Bay, with higher nectar concentrations associated with lower MRH and mean annual rainfall (MAR).

An Australian study by Hawkins et al. (2018) also identified regional variations in nectar volumes, with solar radiation and precipitation differences as key drivers. These disparities might arise from species or population-specific differences in compensatory mechanisms mitigating water stress effects (Iqbal et al., 2012; Balducci et al., 2016; Teixido et al., 2022), possibly due to past selection pressures induced by drought (Hawkins et al., 2018). However, the impact of drought, here particularly referring to low MAR and MRH (soil moisture was not investigated), on nectar volume and concentration varies across studies, with possible negative (Descamps et al., 2021), positive (Suni et al., 2020), or neutral (Phillips et al., 2018) responses to water limitations.

Hypothesis *ii*c proposed that nectar sugar mass, flower fresh mass, and size would be highest in regions with abundant sunshine. Again, this hypothesis could only be partly confirmed. In the sunniest region, Nelson-Tasman/Marlborough, the presence of the largest flowers supported my hypothesis. Previous genetic studies have demonstrated that variations in corolla length, which I categorised as 'flower size' for tube- and bell-shaped flowers, within populations can be heritable. Genetic differences among individuals can account for the variation in corolla length (Galen, 1999). Therefore, it would be intriguing to investigate whether the differences in flower size observed among my analysed species across regions are also genetically influenced. Genetic variation is likely an underlying source of variation not accounted for in this study. I am unaware of how much of the observed spatial or climate model variation, or lack thereof, is driven by location versus genetic differentiation between populations. Similarly, my analysis of the influence of climate on nectar and flower traits relied on the use of climate norms and would not have captured the effects of short-term variation in meteorological conditions around the time of sampling. However, sugar quantities hardly varied across sites, and variation in flower fresh mass was more closely linked to relative humidity than sunshine, with Dunedin consistently hosting the heaviest flowers. This unexpected pattern might stem from accelerated desiccation effects in sunnier regions, leading to uncontrolled water loss from flowers (Bourbia et al., 2020) and, hence, reduced fresh mass.

Conclusion

The findings highlight the complex interaction between nectar traits and climate, illuminating the diverse nature of these ecological dynamics. While a uniform pattern across species was not apparent, certain trends were evident: in sunnier regions, nectar volumes are generally lower and flower sizes larger (as in Plos et al., 2023), whereas nectar concentrations tended to be higher in drier areas.

Among all the traits, and across all species, nectar sugar amounts varied the least between sites and in response to climate, confirming findings by Noe et al. (2019) on *Leptospermum scoparium*. Hence, the energetic value of nectar, arguably the most fundamental measure of its importance for pollinators, is more predictable across sites, based on flower number and species alone, than the remaining four related environmental variables tested.

Climate might affect pollinator behaviour by influencing variables like flower size, nectar volume and concentration, as shown elsewhere (Tian et al., 2022). However, it will have less effect on the energetic value of the nectar available to them per flower.

To comprehensively understand the implications of these intricate patterns, further research is essential to unveil the underlying mechanisms driving them. I recommend additional analyses considering factors such as soil nutrient availability and phylogenetic relationships among species to better comprehend other contributing drivers for nectar variation.

My observations will benefit the honey industry and conservation efforts, as practitioners can apply simple field methods like measuring the lengths of a flower or its mass to estimate the nectar sugar yield potential of certain species.

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Supplementary Material

Table S1: Raw data on collected species (following pages).

Species	Short	Site	Date	Tree	DBH	mean sugar/ flower	Nectar concentration	mean nectar/ flower	total nectar	mean flower mass	mean flower size	emptied	weighted	measured
			(M/Y)	ID	(cm)	(mg)	(Brix)	(uL)	(uL)	(mg)	(mm)	n	n	n
Cordyline australis	CA	A	10/2020	AD	15	0.112	2.9	3.96	79	20	6.36	20	20	19
Cordyline australis	CA	A	10/2020	AE	15	0.026	1.1	2.29	46	19	5.83	20	20	21
Cordyline australis	CA	A	10/2020	AF	15	0.099	2.6	3.89	78	19	7.66	20	20	20
Cordyline australis	CA	CH	11/2020	CHA2	5	0.238	6.3	3.87	77	16	6.06	20	20	19
Cordyline australis	CA	CH	11/2020	CHZ	5	0.274	8.0	3.54	71	25	5.65	20	20	19
Cordyline australis	CA	D	11/2020	DG2	5	0.214	5.7	3.83	77	12	7.96	20	20	20
Cordyline australis	CA	D	11/2020	DH2	5	0.440	11.4	4.03	81	18	9.18	20	20	20
Cordyline australis	CA	H	10/2020	NAG2	NA	0.197	9.1	2.23	45	16	7.42	20	20	20
Cordyline australis	CA	H	10/2020	NAH2	NA	0.213	11.7	1.91	38	34	8.54	20	20	20
Cordyline australis	CA	N	11/2020	ND2	10	0.250	6.6	3.90	78	22	5.36	20	20	17
Cordyline australis	CA	N	11/2020	NE2	10	0.312	8.6	3.76	75	14	4.95	20	20	21
Cordyline australis	CA	N	11/2020	NF2	10	0.215	6.0	3.67	73	18	4.84	20	20	20
Cordyline australis	CA	T	12/2019	NPY	15	0.076	2.3	3.35	67	17	5.62	20	20	18
Cordyline australis	CA	T	12/2019	NPZ	15	0.067	2.0	3.35	67	8	9.02	20	20	20
Cordyline australis	CA	T	12/2019	NPA1	15	0.093	2.6	3.64	73	17	7.32	20	20	20
Cordyline australis	CA	W	11/2019	W14	5	0.145	4.3	3.44	52	24	5.34	15	40	22
Cordyline australis	CA	W	11/2019	W15	5	0.361	10.6	3.56	53	20	8.12	15	50	16
Cordyline australis	CA	W	11/2019	W16	5	0.189	6.0	3.22	48	30	6.31	15	50	33
Fuchsia excorticata	FE	A	10/2020	AP	20	4.860	14.9	32.62	652	236	17.50	20	20	20
Fuchsia excorticata	FE	A	10/2020	AQ	100	7.110	17.2	41.33	827	263	15.65	20	40	38
Fuchsia excorticata	FE	A	10/2020	AR	NA	1.550	15.9	9.74	195	201	15.05	20	20	20
Fuchsia excorticata	FE	CH	11/2019	C1	34	0.370	6.0	6.14	129	109	10.12	21	50	20
Fuchsia excorticata	FE	CH	11/2019	C2	19	0.040	2.0	2.02	22	68	17.78	11	30	13
Fuchsia excorticata	FE	CH	11/2019	C3	35	6.910	25.0	27.64	166	198	15.61	6	13	16
Fuchsia excorticata	FE	CH	11/2019	C5	35	1.780	13.0	13.68	68	109	NA	5	50	NA
Fuchsia excorticata	FE	D	9/2020	DA	100	4.330	10.1	42.92	858	198	12.35	20	20	20
Fuchsia excorticata	FE	D	9/2020	DB	100	2.240	6.8	32.96	659	312	18.76	20	20	18
Fuchsia excorticata	FE	D	9/2020	DC	110	1.580	6.0	26.29	920	512	17.41	35	35	20
Fuchsia excorticata	FE	D	9/2020	DA2B	100	1.700	9.7	17.56	351	310	NA	20	10	NA
Fuchsia excorticata	FE	D	9/2020	DC2B	70	0.350	6.0	5.90	148	239	NA	25	25	NA
Fuchsia excorticata	FE	H	10/2020	NAV	20	6.830	14.4	47.42	474	309	18.98	10	15	16
Fuchsia excorticata	FE	H	10/2020	NAW	40	4.980	12.9	38.58	231	249	NA	6	6	NA
Fuchsia excorticata	FE	H	10/2020	NAX	50	1.910	13.3	14.37	273	316	17.44	19	19	19
Fuchsia excorticata	FE	N	10/2019	NEL2	3	6.290	23.9	26.30	158	277	27.52	6	6	23
Fuchsia excorticata	FE	N	10/2019	NEL4	3	1.540	12.1	12.69	254	215	27.37	20	20	33
Fuchsia excorticata	FE	N	10/2019	NEL6	3	0.640	7.9	8.15	65	180	22.68	8	8	21
Fuchsia excorticata	FE	N	10/2019	NEL23	na	1.700	12.7	13.35	587	186	NA	44	44	NA
Fuchsia excorticata	FE	T	11/2020	NPJ	40	2.670	10.3	25.88	699	261	16.73	27	27	13
Fuchsia excorticata	FE	T	11/2020	NPO	20	5.550	15.2	36.52	475	287	15.48	13	13	11
Fuchsia excorticata	FE	T	11/2020	NPP	40	3.650	14.2	25.67	282	275	9.64	11	11	21
Fuchsia excorticata	FE	T	11/2020	NPQ	50	1.400	17.6	7.94	238	92	14.49	30	30	13

Fuchsia excorticata	FE	T	11/2020	NPR	60	1.660	10.0	16.57	249	219	NA	15	15	NA
Fuchsia excorticata	FE	W	11/2019	W17	52	6.800	24.8	27.41	548	371	17.19	20	36	20
Fuchsia excorticata	FE	W	9/2020	WP	30	6.940	21.1	32.88	296	247	24.45	9	10	11
Fuchsia excorticata	FE	W	9/2020	WQ	50	2.400	15.0	15.99	496	232	15.76	31	40	20
Leptospermum scoparium	LS	A	10/2020	AG	20	0.090	3.7	2.62	52	39	16.35	20	19	19
Leptospermum scoparium	LS	A	10/2020	AH	20	0.060	3.9	1.68	34	48	15.13	20	20	20
Leptospermum scoparium	LS	A	10/2020	AI	20	0.120	3.8	3.15	63	48	16.50	20	20	19
Leptospermum scoparium	LS	CH	11/2020	CHF2	2	0.440	14.6	3.18	64	29	12.76	20	20	18
Leptospermum scoparium	LS	CH	11/2020	CHG2	2	0.210	5.2	4.13	83	43	13.56	20	20	20
Leptospermum scoparium	LS	CH	11/2020	CHH2	2	0.020	0.5	4.06	81	32	12.40	20	20	21
Leptospermum scoparium	LS	D	01/2020	D11	10	0.630	10.8	6.12	245	39	13.54	40	40	40
Leptospermum scoparium	LS	D	01/2020	D12	5	0.580	10.3	5.90	236	35	13.60	40	40	40
Leptospermum scoparium	LS	D	01/2020	D13	2	0.160	3.5	9.25	370	41	10.11	40	20	20
Leptospermum scoparium	LS	H	12/2020	NAO	15	0.140	7.5	1.96	39	34	12.85	20	20	19
Leptospermum scoparium	LS	H	12/2020	NAY2	20	0.030	1.6	2.26	45	13	9.13	20	20	20
Leptospermum scoparium	LS	H	12/2020	NAZ2	20	0.020	0.5	4.06	81	26	11.58	20	20	18
Leptospermum scoparium	LS	N	9/2020	NX	50	0.010	2.2	0.33	7	46	17.20	20	40	20
Leptospermum scoparium	LS	N	9/2020	NY	50	0.100	13.6	0.37	7	39	16.96	20	40	20
Leptospermum scoparium	LS	N	9/2020	NZ	50	0.220	6.8	1.67	33	40	16.56	20	40	20
Leptospermum scoparium	LS	T	11/2020	NPA5	10	0.240	7.6	3.25	65	59	12.77	20	20	19
Leptospermum scoparium	LS	T	11/2020	NPA6	10	0.140	5.1	2.91	58	36	13.56	20	20	20
Leptospermum scoparium	LS	W	12/2020	WR	5	0.020	0.9	2.91	58	29	10.94	20	20	20
Leptospermum scoparium	LS	W	12/2020	WS	5	0.060	1.3	5.33	107	29	11.77	20	20	20
Leptospermum scoparium	LS	W	12/2020	WT	5	0.240	6.9	1.82	36	86	14.03	20	40	20
Metrosideros excelsa	ME	A	12/2019	A32	70	1.828	29.0	7.00	280	NA	34.20	40	NA	33
Metrosideros excelsa	ME	A	12/2019	A31	70	3.694	32.0	13.00	260	NA	34.32	20	NA	38
Metrosideros excelsa	ME	CH	11/2020	CHC2	20	11.144	51.0	27.00	540	168	30.47	20	20	11
Metrosideros excelsa	ME	CH	11/2020	CHD2	20	12.040	38.0	37.00	740	198	31.53	20	20	9
Metrosideros excelsa	ME	CH	11/2020	CHE2	20	11.107	39.0	34.00	680	196	31.80	20	20	20
Metrosideros excelsa	ME	D	01/2020	D8	60	7.406	17.0	47.00	1880	350	35.84	40	100	40
Metrosideros excelsa	ME	D	01/2020	D9	25	3.265	15.0	24.00	960	463	38.25	40	188	40
Metrosideros excelsa	ME	D	01/2020	D10	25	5.498	18.0	33.00	1320	456	40.82	40	172	40
Metrosideros excelsa	ME	H	12/2020	NAS2	30	4.704	19.0	26.00	520	189	33.70	20	20	18
Metrosideros excelsa	ME	H	12/2020	NAT2	30	1.799	51.0	4.00	80	177	30.64	20	20	17
Metrosideros excelsa	ME	H	12/2020	NAU2	30	3.416	21.0	18.00	360	163	25.41	20	20	20
Metrosideros excelsa	ME	N	11/2020	NG2	15	2.282	12.0	19.00	380	289	34.05	20	20	20
Metrosideros excelsa	ME	N	11/2020	NH2	15	7.065	18.0	42.00	840	275	32.49	20	20	20
Metrosideros excelsa	ME	N	11/2020	NI2	15	1.413	7.0	20.00	400	192	32.49	20	17	21
Metrosideros excelsa	ME	T	12/2019	NP9	70	5.094	21.0	26.00	780	219	33.20	30	45	20
Metrosideros excelsa	ME	T	12/2019	NP10	70	4.146	24.0	19.00	570	267	31.93	30	78	19
Metrosideros excelsa	ME	T	12/2019	NP11	70	3.616	17.0	23.00	690	192	30.87	30	69	20
Metrosideros excelsa	ME	W	12/2020	WJ2	20	3.895	18.0	23.00	460	200	29.26	20	20	19
Metrosideros excelsa	ME	W	12/2020	WI2	20	4.278	18.0	25.00	500	191	30.02	20	20	20
Metrosideros excelsa	ME	W	12/2020	WG2	20	5.221	23.0	25.00	500	193	29.73	20	20	20
Pittosporum crassifolium	PC	A	10/2019	A1	5	0.971	11.1	9.10	173	127	8.07	19	19	13
Pittosporum crassifolium	PC	A	10/2019	A2	5	0.396	9.9	4.20	38	68	8.87	9	9	7

Pittosporum crassifolium	PC	A	10/2019	A3	5	1.359	13.1	10.90	87	93	9.20	8	8	8
Pittosporum crassifolium	PC	CH	11/2019	C14	50	0.264	17.4	1.60	32	51	7.79	20	15	13
Pittosporum crassifolium	PC	CH	9/2020	CHJ	45	0.284	7.1	4.10	82	91	9.28	20	20	20
Pittosporum crassifolium	PC	CH	9/2020	CHK	20	0.129	5.8	2.30	46	83	8.50	20	20	20
Pittosporum crassifolium	PC	CH	9/2020	CHL	80	0.133	1.4	9.60	192	62	7.79	20	20	14
Pittosporum crassifolium	PC	D	9/2020	DO	4	2.104	16.6	13.50	270	99	9.05	20	20	19
Pittosporum crassifolium	PC	D	9/2020	DP	3	2.297	21.6	11.60	232	99	9.61	20	20	16
Pittosporum crassifolium	PC	D	9/2020	DQ	2	3.037	20.8	15.90	318	108	9.02	20	20	15
Pittosporum crassifolium	PC	H	10/2020	NAD	20	0.739	11.5	6.70	134	20	9.13	20	20	20
Pittosporum crassifolium	PC	H	10/2020	NAE	35	0.397	22.7	1.90	38	19	9.46	20	20	20
Pittosporum crassifolium	PC	H	10/2020	NAF	20	0.726	11.6	6.50	130	19	8.78	20	20	20
Pittosporum crassifolium	PC	N	9/2020	NN	80	0.483	6.2	7.90	158	146	9.13	20	40	20
Pittosporum crassifolium	PC	N	9/2020	NO	100	0.578	10.0	6.00	120	102	9.46	20	25	20
Pittosporum crassifolium	PC	N	9/2020	NP	80	0.264	3.1	8.60	172	106	8.78	20	15	20
Pittosporum crassifolium	PC	T	09/2019	NP2	15	0.488	9.0	5.60	168	112	8.48	30	30	10
Pittosporum crassifolium	PC	T	09/2019	NP3	15	1.576	25.8	6.80	68	90	8.42	10	30	8
Pittosporum crassifolium	PC	W	9/2020	WD	20	0.156	10.4	1.60	32	77	9.02	20	10	10
Pittosporum crassifolium	PC	W	9/2020	WE	20	0.808	8.7	9.60	192	67	7.57	20	40	20
Pittosporum crassifolium	PC	W	9/2020	WF	20	0.616	6.3	10.00	200	83	8.61	20	20	20
Pittosporum eugenioides	PE	A	10/2020	AA	20	0.346	12.3	3.00	60	4	8.26	20	20	20
Pittosporum eugenioides	PE	A	10/2020	AB	20	0.169	7.1	2.40	48	12	9.17	20	20	22
Pittosporum eugenioides	PE	CH	9/2020	CHE	40	0.402	15.7	2.70	54	15	12.32	20	40	20
Pittosporum eugenioides	PE	CH	9/2020	CHF	30	0.427	15.1	3.00	60	15	13.06	20	40	20
Pittosporum eugenioides	PE	D	9/2020	DE	10	0.100	3.4	3.00	60	33	10.62	20	20	11
Pittosporum eugenioides	PE	D	9/2020	DF	80	0.235	7.7	3.10	62	26	12.25	20	20	15
Pittosporum eugenioides	PE	D	9/2020	DG	40	0.074	2.9	2.60	52	22	10.06	20	20	13
Pittosporum eugenioides	PE	H	10/2020	NAR	10	0.252	11.7	2.30	46	13	10.39	20	39	20
Pittosporum eugenioides	PE	H	10/2020	NAS	100	0.658	24.6	3.00	60	12	11.12	20	40	20
Pittosporum eugenioides	PE	H	10/2020	NAT	35	0.300	12.0	2.60	52	15	12.00	20	40	20
Pittosporum eugenioides	PE	N	10/2019	NEL16	53	0.228	6.9	3.40	68	15	9.71	20	20	20
Pittosporum eugenioides	PE	N	10/2019	NEL17	35	0.074	2.6	2.90	58	18	14.28	20	20	19
Pittosporum eugenioides	PE	N	10/2019	NEL18	24	0.158	5.7	2.80	56	18	12.38	20	20	20
Pittosporum eugenioides	PE	T	11/2020	NPG	90	0.274	7.1	3.90	78	14	11.86	20	20	20
Pittosporum eugenioides	PE	T	11/2020	NPH	70	0.109	2.9	3.80	76	12	10.16	20	20	20
Pittosporum eugenioides	PE	T	11/2020	NPI	50	0.127	4.0	3.20	64	19	10.58	20	20	20
Pittosporum eugenioides	PE	W	10/2019	W4	33	0.189	3.1	6.10	122	20	12.61	20	20	18
Pittosporum eugenioides	PE	W	10/2019	W5	24	0.133	2.3	5.90	89	13	9.91	15	15	15
Pittosporum eugenioides	PE	W	10/2019	W6	75	0.158	2.6	6.20	93	16	12.18	15	15	15
Pittosporum tenuifolium	PT	A	10/2019	A9	5	0.158	2.7	6.00	60	67	6.51	10	17	10
Pittosporum tenuifolium	PT	A	10/2019	A11	5	0.024	0.4	5.40	54	84	7.55	10	10	10
Pittosporum tenuifolium	PT	A	10/2019	A20	45	0.106	2.1	5.10	51	87	6.69	10	15	10
Pittosporum tenuifolium	PT	CH	11/2020	C13	49	0.087	1.6	5.50	83	46	7.21	15	15	15
Pittosporum tenuifolium	PT	CH	11/2020	C15	50	0.183	5.0	3.70	56	51	8.68	15	15	14
Pittosporum tenuifolium	PT	CH	11/2020	C16	20	0.112	3.3	3.50	53	55	8.18	15	30	28
Pittosporum tenuifolium	PT	D	9/2020	DR	15	0.117	2.0	5.90	118	69	8.92	20	20	16
Pittosporum tenuifolium	PT	D	9/2020	DT	25	0.268	7.0	3.90	78	75	4.38	20	20	20

Pittosporum tenuifolium	PT	H	10/2020	NAA	30	0.170	20.5	0.90	18	50	6.77	20	40	20
Pittosporum tenuifolium	PT	H	10/2020	NAB	30	0.320	14.5	2.30	46	44	7.15	20	40	19
Pittosporum tenuifolium	PT	H	10/2020	NAC	50	0.383	16.5	2.50	50	40	6.57	20	35	20
Pittosporum tenuifolium	PT	N	10/2019	NEL9	43	0.961	26.5	4.00	44	87	7.90	11	11	11
Pittosporum tenuifolium	PT	N	10/2019	NEL10	38	0.176	5.0	3.60	36	53	7.55	10	10	10
Pittosporum tenuifolium	PT	N	10/2019	NEL11	33	0.161	3.1	5.20	62	74	7.86	12	12	12
Pittosporum tenuifolium	PT	T	11/2020	NPK	40	0.570	20.5	3.00	60	97	NA	20	20	NA
Pittosporum tenuifolium	PT	W	10/2019	W1	127.1	0.159	5.5	2.90	29	69	6.42	10	10	10
Pittosporum tenuifolium	PT	W	10/2019	W2	40.9	0.174	5.5	3.20	32	64	7.03	10	10	10
Pittosporum tenuifolium	PT	W	10/2019	W3	40	0.434	13.5	3.40	34	67	7.28	10	10	10
Sophora microphylla	SM	A	10/2019	A6	55	6.264	11.7	56.00	1064	1105	49.98	19	19	11
Sophora microphylla	SM	A	10/2019	A7	8	0.660	5.5	12.00	120	666	51.72	10	10	10
Sophora microphylla	SM	A	10/2019	A8	105	3.443	10.4	34.00	680	827	52.65	20	20	10
Sophora microphylla	SM	CH	9/2020	CHB	60	10.753	42.5	30.00	600	895	43.26	20	40	20
Sophora microphylla	SM	CH	9/2020	CHC	80	6.794	30.4	25.00	500	947	40.26	20	40	20
Sophora microphylla	SM	CH	9/2020	CHD	15	2.332	53.0	5.00	100	338	31.24	20	30	20
Sophora microphylla	SM	D	9/2020	DU	160	5.352	27.1	22.00	440	465	30.94	20	40	10
Sophora microphylla	SM	D	9/2020	DV	140	10.961	20.7	58.00	1160	837	42.50	20	40	19
Sophora microphylla	SM	D	9/2020	DW	130	4.585	23.1	22.00	440	418	36.18	20	40	17
Sophora microphylla	SM	H	10/2019	N1	37	9.484	14.0	72.00	1440	821	49.06	20	20	20
Sophora microphylla	SM	H	10/2020	N2	34	6.092	15.2	43.00	860	827	47.31	20	20	20
Sophora microphylla	SM	H	10/2020	N3	20	6.263	13.7	48.00	816	737	50.01	17	17	17
Sophora microphylla	SM	N	10/2019	NEL12	10	12.912	40.1	38.00	570	783	53.04	15	15	15
Sophora microphylla	SM	N	10/2019	NEL26	30	4.118	14.5	30.00	450	741	53.76	15	20	20
Sophora microphylla	SM	N	10/2019	NEL27	30	9.315	17.8	56.00	840	652	49.35	15	20	20
Sophora microphylla	SM	N	10/2019	NEL28	30	8.120	16.8	52.00	780	546	50.69	15	20	20
Sophora microphylla	SM	T	09/2019	NP1	15	3.131	41.9	9.00	270	608	42.99	30	60	60
Sophora microphylla	SM	T	09/2019	NP4	15	8.930	28.4	35.00	1050	1116	49.93	30	30	30
Sophora microphylla	SM	W	10/2019	W8	62	7.671	31.7	28.00	420	1076	47.12	15	14	15
Sophora microphylla	SM	W	10/2019	W9	39	6.777	30.1	25.00	375	830	47.68	15	16	15
Sophora microphylla	SM	W	10/2019	W10	23.4	7.270	34.1	24.00	360	632	43.76	15	14	15

4. Interspecific Nectar Variation in New Zealand Trees

Abstract

I quantified interspecific nectar and floral trait variation across 50 woody New Zealand species. I determined species-specific nectar traits (volume, concentration, and chemical composition) and floral traits (flower size, weight, shape, colour, and pollination syndrome) based on ~ 10,000 flowers from 428 individual trees sampled across the country.

I used floral characteristics and phylogenetic relationships to explain interspecific variation in nectar traits. For this, I weighed flowers and their nectar, measured flower size using digital callipers, nectar concentration via refractometer, and flower colour by applying picture analysis software, and identified nectar chemical composition through High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS). I analysed my data using linear regression, generalised additive models (GAMs), Spearman's correlation tests, one-way ANOVA and Kruskal-Wallis tests (including Dunn's and Tukey's HSD post hoc tests), as well as analyses of phylogenetic signals (Pagel's lambda).

Flowers secreted 1 - 82 µL nectar containing 0.01-54% solubles, ranging between 3-879 mg fresh weight and 2 - 67 mm size. I identified 62 distinct nectar components, including 25 sugars (72 - 99.9% of solubles), comprising 12 hexa-, a single penta-, two tri-, three di-, seven monosaccharides, and 37 non-carbohydrate compounds (0.04 - 8.1%), including six alkaloids (ALK), 16 amino acids (AA), 15 bioactive compounds, including 11 phenolics (PHE), and four vitamers (VIT).

Spearman rank correlation analyses and GAM indicated positive correlations between nectar volume, total concentration, solubles, and ALK content with the tested floral traits. Conversely, the quantities of component types and hexasaccharides correlated negatively with these floral traits, with higher levels detected in small flowers of highly accessible shapes and white, green or purple colouration.

Analysis indicated that the species' pollinator syndrome partly drove interspecific variation in nectar and floral traits. New Zealand bird-pollinated flowers were significantly larger, of less accessible shapes (flag & gullet vs. dish, brush or bell/funnel), and more brightly coloured (yellow, orange, red vs. white, green). They produced more concentrated nectar richer in ALK

but with fewer hexasaccharides than insect-pollinated flowers. However, nectar concentration was lower in entomophilous species, in contrast to observations from other floras, a possible adaptation to NZ's unique assemblage of invertebrate pollinators.

Many floral and nectar traits showed strong phylogenetic signals. For instance, Fabid and Campanulid nectar had elevated sucrose levels, and the nectar of Myrtales and Lamiales was rich in glucose. Malvid nectar was rich in penta- and monosaccharides, *Pseudopanax* in hexa- and trisaccharides, Fabid and *Lophomyrtus* in AA, Fabales in ALK, and *Pittosporum* in PHE.

My findings contribute significantly to understanding nectar composition and its driving factors. Future nectar research may explore whether the observed phylogenetic signal trends hold across a broader framework.

4.1 Introduction

While interspecific variation in nectar traits has been recognised for decades (e.g., Shuel, 1952), research on New Zealand (NZ) plants has remained limited. Existing studies often focus on pollinator energetics, typically involving a restricted number of species and specific sites (e.g., Whitaker, 1987; Rasch & Craig, 1988; Butz Huryn, 1995; Castro & Robertson, 1997; Ladley et al., 1997; Murphy & Kelly, 2003), or rely on estimated values rather than direct measurements (Ausseil et al., 2018), except for a handful of studies on *Leptospermum scoparium* and a single publication on alkaloids (ALK) in *Sophora microphylla* nectar (Clinch et al., 1972). The exception is the broad interest in the nectar of NZ honey-related species, such as mānuka *Leptospermum scoparium*, pōhutukawa *Metrosideros excelsa*, kānuka *Kunzea ericoides*, rewarewa *Knightia excelsa*, southern rātā *Metrosideros umbellata*, kāmahi *Weinmannia racemosa* and tāwari *Ixerba brexioides* (e.g. Schmidt-Adam, 1999; Senanayake, 2006; Goss, 2009; Emmertz, 2010; Revell et al., 2014; Williams et al., 2014; Newstrom-Lloyd, 2015; Nickless et al., 2017; Bong et al., 2018; Clearwater et al., 2018; Noe et al., 2019;). However, these studies are restricted to analysing just nectar volume and the major nectar sugars, or focus on honey. I am unaware of other New Zealand nectar studies that delve deeper into the nectar's chemical composition beyond stating the major three sugars — sucrose (S), fructose (F), and glucose (G) — and dihydroxyacetone (DHA) in the case of *Leptospermum scoparium*.

Examining variation in floral nectar production presents challenges due to its intricate physiology, complex phylogenetic structure, and considerable environmental variability (see Chapters 1 & 3). Percival (1961) and Baker (1982) compiled large comprehensive datasets (n ~ 1600 spp., including some NZ species or relatives), categorising nectar types by their sugar ratios. They revealed that nectar types vary broadly and are linked to pollinators and phylogeny. Notably, the late 20th-century technical advancements, such as HPLC, LC-MS (Barceló & Petrovic, 2007) and phylogeny analysis (e.g. Ornelas et al., 2007; Schmidt-Lebuhn et al., 2007), contributed significantly to our possibilities to identify chemical compounds beyond sugars and to analyse their phylogenetic constraints. For instance, a study of 31 North American species (Palmer-Young et al., 2018) identified 102 non-carbohydrate nectar compounds, including chlorogenic acids, ALK, amino acids (AA), and phenolics (PHE). The latter three are categories of nectar compounds that I have also investigated in this study.

Nectar composition is crucial in attracting nectar consumers, given the diverse nutritional requirements of different animal groups and species. Birds, butterflies, moths, ants, and long-tongued bees typically favour sucrose-rich nectars. However, there are important exceptions: some nectarivorous birds and ants lack the sucrose-cleaving enzyme invertase, rendering them incapable of assimilating sucrose. Consequently, these species favour sucrose-free nectars (Martínez del Rio, 1990; Heil et al., 2005). Similarly, flies and short-tongued bees (the only type of native bee present in NZ; Heine, 1938) prefer nectars rich in hexoses such as glucose and fructose (Blüthgen & Fiedler, 2004; Nepi & Stpiczynska, 2008; González-Teuber & Heil, 2009; Nepi et al., 2009).

The composition of nectar plays a dual role: it can influence attraction of mutualistic partners, such as pollinators and defenders (e.g. ants and parasitoids) (González-Teuber & Heil, 2009), while simultaneously repelling or discouraging nectar robbers (Adler, 2000) and nectar-infecting microorganisms (Schmitt et al., 2021), for example through the presence of dark, bitter-tasting nectar as found in the South African *Aloe vryheidensis* (Johnson et al., 2006). Essential attractive compounds include mono- and disaccharides, AA, PHE and volatile components like benzyl acetone. Conversely, repellent effects are attributed to secondary compounds such as gelsemine and iridoid glycosides (monoterpenoids, ALK). Notably, gelsemine has repelling effects on legitimate pollinators and potential threats (Heil, 2011). Nectar proteins, specifically nectarins, primarily safeguard the nectar, and possibly the nectary, from microbial infections (Goss, 2009); however, they are not the focus of this study.

While nectar sugars typically exhibit concentrations approximately 100 - 1000 times higher than AA, the presence of AA significantly influences nectar attractiveness. Birds and bats, having alternative nitrogen sources, can derive nitrogen from sources other than nectar, whereas many adult insects rely exclusively on liquid diets. Consequently, insect-pollinated flowers are expected to contain higher concentrations of AA in their nectar than those pollinated by vertebrates. Indeed, elevated AA concentrations have been documented in flowers adapted to butterflies (Baker, 1982), flies (Potter & Bertin, 1988), and bees (Petanidou et al., 2006). Similarly, ants prefer nectars rich in AA, and ants and several other insect pollinators demonstrate distinct preferences for specific essential AA (Blüthgen & Fiedler, 2004; Carter et al., 2006; González-Teuber & Heil, 2009). Overall, interspecific

variation in nectar composition is therefore very likely to be influenced by variation in the suite of animals and microbes associated with each species of plant.

Despite New Zealand's modest tally of native angiosperms — numbering approximately 2080 species (McGlone et al., 2010), inclusive of 215 trees (those that reach ≥ 6 m at maturity, McGlone et al., 2010) — the flora exhibits a remarkable endemism rate of approximately 85%, with an impressive 11% of these species constituting endemic genera (McGlone et al., 2001). Such figures underscore New Zealand as a unique biodiversity hotspot (Ogden, 1995).

The country's phenological cycle predominantly features spring and summer (September - January) as the primary flowering seasons. Nevertheless, a notable fraction of species exhibit winter blooming — a testament to the diverse temporal niches occupied by these plants (Heine, 1938; Castro & Robertson, 1997; Richardson et al., 2023).

Most of New Zealand's plants display floral colouration in less conspicuous hues—whitish, yellow, or green, comprising about 82% of the diversity, indicating insect-mediated pollination. Conversely, a smaller portion exhibits the ornithophilous syndrome with large, vividly coloured flowers (yellow, pink, red), catering to the visual preferences and body size of bird pollinators (Godley, 1979).

The absence of social bees and a paucity of butterfly fauna almost certainly limited the proliferation of blue flowers. However, the dominance of white, yellow or green flowers is typical for evergreen moist forests (McGlone & Richardson, 2023). The prevalence of small, dull, simple flowers within New Zealand's flora is unlikely to be solely attributed to the lack of specialised pollinators, despite the possible influence of this factor on the scarcity of large, complex blossoms (Newstrom & Robertson, 2005; McGlone & Richardson, 2023). For instance, regions such as South West China and New Caledonia, despite having a broader and more specialised range of pollinators, including long-tongued social bees, exhibit a comparable proportion of small, white, yellow, or yellow-green flowers that appeal to generalist pollinators (Chen & Li, 2008; Schlessman et al., 2014). Similarly, in North America's tree flora, small-flowered trees constitute about 60% of species, closely aligning with New Zealand's 53% (McGlone & Richardson, 2023). Thus, unremarkable, unspecialised flowers in New Zealand's flora align with global patterns and, while associated with gender

dimorphism, do not stand out as a unique determinant of New Zealand floral diversity (Richardson et al., 2023).

New Zealand has a limited but ecologically crucial group of vertebrate pollinators — seven bird species, one bat species, and several lizard species — contrasted by a more varied insect pollinator guild comprising butterflies and moths (Lepidoptera), solitary bees (Hymenoptera), thrips, beetles, flies, and introduced species such as the honey bee (*Apis mellifera,* Hymenoptera) (Heine, 1938; Norton, 1984).

Many insects have a trichromatic vision system sensitive to UV, blue, and green light. Advanced visual adaptations in Hymenoptera and Lepidoptera, through acquiring additional photoreceptor types, have broadened their colour perception spectrum, facilitating the detection of a broader range of flower pigments (Chen et al., 2016; see Chapter 1).
Conversely, most birds have tetrachromatic vision, with photoreceptors sensitive to UV, blue, green and red light (Bowmaker, 1980; Goldsmith, 2006). UV signatures or patterns, more frequently observed on yellow, red, and blue flowers and seldom on white ones, are produced by specialised epidermal structures rather than pigments that generate other colours (Kugler, 1963). These signatures are not visible to humans (Backhaus, 1998; Goldsmith, 2006) and New Zealand's endemic honeyeaters (passerine Meliphagidae: tūī *Prosthemadera novaeseelandiae*, bellbird *Anthornis melanura*), with both lacking UV-sensitive photoreceptors (Ödeen & Håstad, 2010). In contrast, native kākāriki/parakeets (*Cyanoramphus* spp.) and other Passeriformes (hihi/stitchbird, *Notiomystis cincta*; tauhou/silvereye, *Zosterops lateralis*; tīeke/saddleback *Philesturnus* spp.) are capable of perceiving UV-signatures, either on other birds' plumage or on floral surfaces (Hunt et al., 2009, Burd, 2014). The emergence of colour vision in the animal kingdom predates and thus has driven the evolution of chromatic diversity in angiosperm flowers (Chittka & Menzel, 1992). As UV patterns on flowers cannot be detected using standard RGB photography, they were not included in this study, as their detection would have exceeded the timeframe of an already broad research scope.

Evidence indicating multiple independent origins of pollinator syndromes supports this view, reflecting a deep evolutionary alignment between floral colouration and pollinator colour vision (Cronk & Ojeda, 2008). Despite the overlap in spectral sensitivity among animals, applying the term ‘colour’ is not straightforward as animals differ not only in photoreceptor

types but also regarding colour perception. Colour perception depends on the photoreceptor's absorbance of the maximum reflected wavelength (λ_{max}) of an object (Wang et al., 2022). Determining a λ_{max} for specific colours, such as 'pink' or 'white', proves challenging because they reflect a broad spectrum of wavelengths compared to straightforward colours, such as purple, blue, green, yellow and red. Red is associated with the longest visible reflectance wavelengths (for birds and lepidopterans above 600 nm; Cronk & Ojeda, 2008; Wang et al., 2022). A single λ_{max} might not fully capture potential variation in floral spectral properties for specific colours. Hence, the need for more objective measures of colour, using a defined colour space, was additionally considered in my analysis.

Objectives and Hypotheses

I aimed to quantify the following:

i) The interspecific variation in nectar traits (volume, concentration, and composition, including carbohydrates and non-carbohydrates) for common New Zealand trees and

ii) The correlations among nectar and floral traits (size, weight, shape and colour).

Furthermore, I tested whether nectar and floral trait variation is driven by the species'

iii) Pollinator syndrome and

iv) Phylogeny.

I tested the following hypotheses:

i) Nectar traits vary among New Zealand tree species;

ii) Floral traits correlate with nectar traits;

iii) Species sharing the same pollinator syndrome are expected to exhibit particular nectar traits: Ornithophilous species, characterised by heavy, large, brightly-coloured flowers, are expected to show higher nectar volumes with low concentrations. Entomophilous species featuring small, inconspicuously coloured flowers are expected to show small nectar volumes with high concentrations. Generalists, species visited by both insects and birds, are expected to exhibit nectar traits that are intermediate between bird- and insect-pollinated species based on the assumption that these flowers cater to the diverse needs of a wide range of potential pollinators and

iv) Variation in nectar composition and floral traits is phylogenetically conserved.

4.2 Materials and Methods

4.2.1 Quantification of Nectar Volume, Concentration and Floral Traits

To quantify interspecific variation in nectar traits for common New Zealand woody species (excludes herbaceous species such as New Zealand flax, harakeke, *Phormium tenax*), I sampled 48 native (incl. the variety *Metrosideros excelsa aurea,* MET EX A) and three exotic species (*Paraserianthes lophanta* and *Cordyline stricta*, originating from Australia, and *Robinia pseudoacacia* from North America), totalling 428 individuals, including 46 tree (incl. MET EX A), two liane and three shrub species (Table 1).

The exotic species have become naturalised in New Zealand since 1870 (de Lange, 2024). I sampled 15 common core species (15 - 32 trees, 5 - 10 sites; Table 1, Figure 1) and a further 38 less commonly found species for comparison (2 - 9 trees, 1 - 3 sites) to facilitate a comprehensive analysis within a broader phylogenetic framework.

Species were from 15 orders, 21 families, and 31 genera: *Sophora* (6 spp.), *Metrosideros* (4 spp. + 1 variety), *Pittosporum* (5 spp.), *Cordyline*, *Lophomyrtus*, and *Melicytus* (3 spp. each), and *Griselinia*, *Kunzea*, and *Pseudopanax* (2 spp. each), and one species per genus of the remaining genera, as detailed in Table 1. Species comprised 44 core eudicots (27 rosids and 17 asterids) and six basal species (one magnolid, four monocots, and one basal eudicot).

Species were selected based on their wide distribution, abundance and basal area in National Vegetation Survey vegetation plot data at a national scale (core species), and on their previously reported nectar production (Martin, 1961; GBIF.org, 2018; Landcare Research, 2018a - d).

Table 1: Sampled species (including abbreviations and n of nectar samples), with '*' indicating exotic species. Species are trees except for species with '~' = liane or '#' = shrub.

CORE SPECIES		*n*
Aristotelia serrata	ARI SER	15
Cordyline australis	COR AUS	21
Fuchsia excorticata	FUC EXC	29
Geniostoma rupestre #	GEN RUP	19
Griselinia littoralis	GRI LIT	21
Kunzea ericoides agg.	KUN ERI	18
Leptospermum scoparium	LEP SCO	24
Melicytus ramiflorus	MEL RAM	20
Metrosideros excelsa	MET EXC	23
Myoporum laetum	MYO LAE	22
Pittosporum crassifolium	PIT CRA	23
Pittosporum eugenioides	PIT EUG	23
Pittosporum tenuifolium	PIT TEN	23
Sophora microphylla	SOP MIC	24
Vitex lucens	VIT LUC	18

SPECIES FOR COMPARISON					
*Paraserianthes lophantha **	PAR LOP	1	*Metrosideros fulgens ~*	MET FUL	1
Carmichaela australis #	CAR AUS	1	*Metrosideros umbellata*	MET UMB	1
Carpodetus serratus	CAR SER	7	*Myrsine australis*	MYR AUS	3
Clianthus maximus #	CLI MAX	1	*Pittosporum colensoi*	PIT COL	1
Cordyline banksii	COR BAN	1	*Pittosporum ralphii*	PIT RAL	5
*Cordyline stricta **	COR STR	2	*Pseudopanax arboreus*	PSE ARB	2
Corokia cotoneaster	COR COT	1	*Pseudopanax crassifolius*	PSE CRA	1
Corynocarpus laevigatus	COR LAE	5	*Pseudowintera colorata*	PSE COL	1
Elaeocarpus dentatus	ELA DEN	3	*Rhabdothamnus solandri*	RHA SOL	1
Griselinia lucida	GRI LUC	3	*Rhopalostylus sapida*	RHO SAP	5
Knightia excelsa	KNI EXC	9	*Robinia pseudoacacia **	ROB PSE	1
Lophomyrtus bullata	LOP BUL	4	*Sophora chathamica*	SOP CHA	5
Lophomyrtus obcordata	LOP OBC	1	*Sophora godleyi*	SOP GOD	2
Melicope ternata	MEL TER	9	*Sophora longicarinata*	SOP LON	1
Melicytus lanceolatus	MEL LAN	1	*Sophora prostata*	SOP PRO	1
Melicytus novae-zealandiae	MEL NOV	1	*Sophora tetraptera*	SOP TET	1
Metrosideros colensoi ~	MET COL	2	*Veronica diosmifolia*	VER DIO	1
Metrosideros excelsa aurea	MET EX A	9	*Weinmannia racemosa*	WEI RAC	3

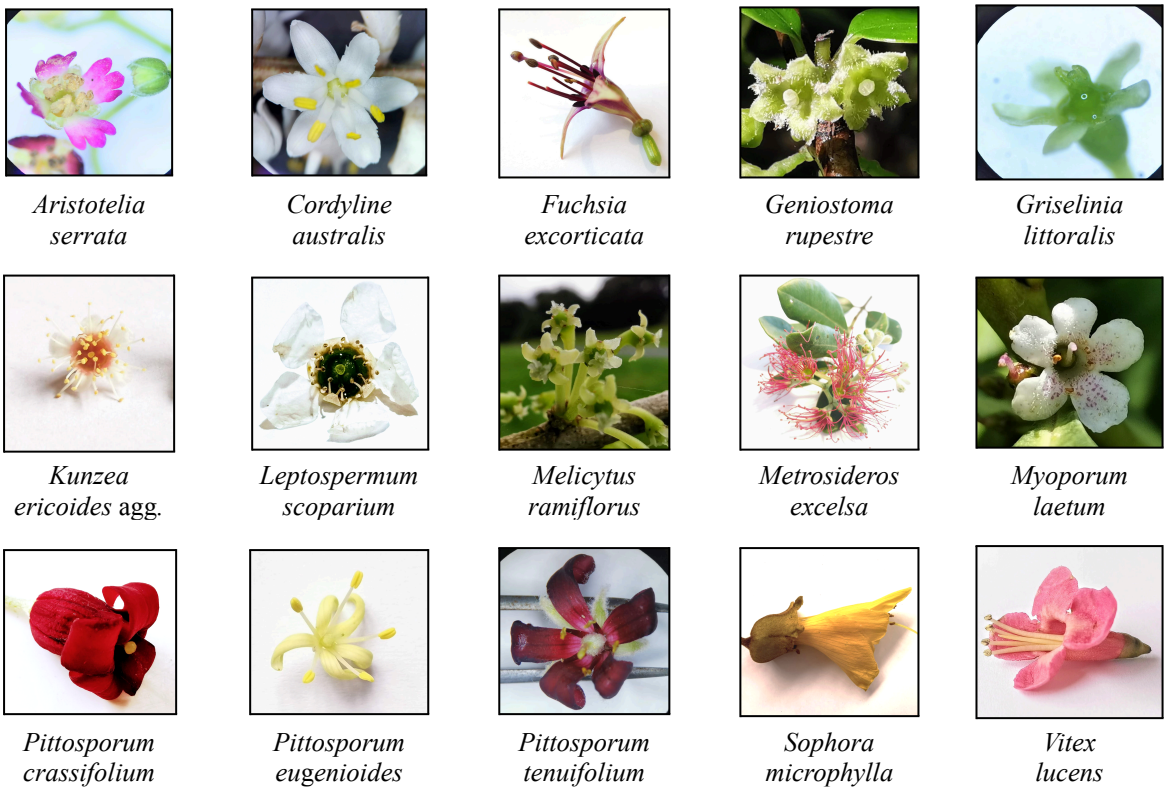

Figure 1: Flower appearance of the sampled 15 core species (own photographs).

Flowers were collected across ten out of 15 NZ regions between 35 - 45° S and 170 - 177° E across the main two islands of New Zealand: Northland, Auckland, Taranaki, Hawke's Bay, and Wellington in the North Island, and Nelson-Tasman, Marlborough, West Coast, Canterbury, and Otago in the South Island (Figure 2). The chosen sites are located across all six NZ climate regions (Kidson, 2000).

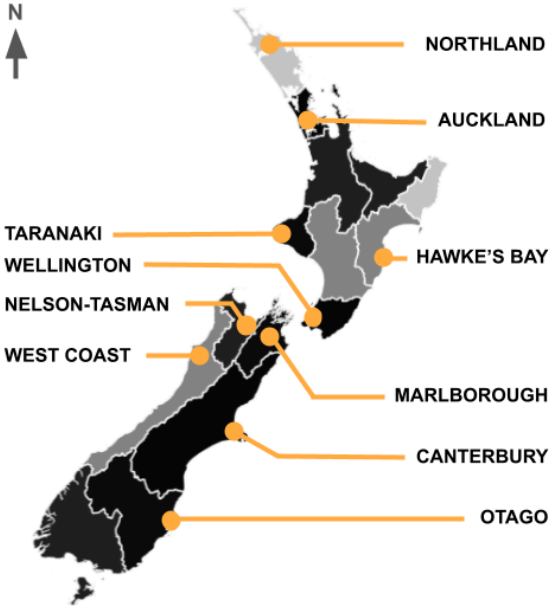

Figure 2: Sampled New Zealand regions (●; grey-shaded areas represent New Zealand council districts).

Sampling was conducted during the species' peak anthesis periods, particularly between September and January of two consecutive years, 2019/20 and 2020/21. To collect nectar, I covered flowering shoots with transparent synthetic organza bags, with transparent rain shields when necessary, to exclude nectar feeders, and allowed the nectar to accumulate for 24 hours. The following day, I cut the shoots from the plants and sealed them in plastic bags with damp paper tissue for their short transport to the respective field laboratory.

Immediately after collecting all bagged inflorescences from the various sub-sites in the morning, I began processing the flowers. For this, I randomly removed 10-40 suitable flowers per tree, totalling 10 - 703 flowers per species and extracted their nectar using micropipettes. I worked on a hydrophobic plastic sheet to collect potential runoff. Nectar was pooled into a pre-weighed 1.5 mL vial (Eppendorf, Germany) assigned to the respective tree. Pure nectar was collected from *Fuchsia*, *Vitex*, *Metrosideros*, *Sophora*, *Clianthus*, *Rhapolostylis* and *Robinia*. Flowers of the remaining genera were rinsed with 5 - 20 µL of distilled water (after Morrant et al., 2009) to remove smaller nectar volumes efficiently.

Subsequently, I weighed nectar-filled vials and the sampled fresh flowers using a three decimal place balance (MS 120, Mettler, Greifensee, Switzerland, accuracy ± 1 mg). Nectar mass was calculated by comparing the weights of empty and filled vials. For samples of rinsed flowers, I subtracted the added water volumes from the total vial weight to determine the nectar amount. Nectar concentrations were measured in °Brix using a digital refractometer (Atago PAL-1 3810, Tokyo, Japan, accuracy ± 0.1° Brix) using 20-100 µL of nectar or nectar solution (of rinsed flowers). Concentration values were used to calculate specific gravity and convert nectar mass to volume units. Any remaining nectar was frozen for consecutive High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) analysis.

Flower size was measured with digital callipers and defined for each flower shape: corolla diameter was measured for dish- or bowl-shaped flowers, and corolla length for funnel-, flag-, gullet- or bell-shaped flowers (see Figure 4 in Chapter 1). The flower size of brush-like flowers was calculated by summing the height of a nectar capsule with the mean length of 20 measured filaments per inflorescence.

Flower colours were determined using software-calculated colour values of digital flower images, which were aligned with photometrically detected λ_{max} provided by recent literature (Zhang et al., 2022; Wang et al., 2022). I used common colour names based on the human visual system for ease of readability. Given the constraints of the study's timeframe, my study uses digital RGB and wavelength estimation as its primary methodological tools, which excluded any UV signatures.

To quantitatively analyse flower colours (excl. UV-spectrum; Table 2), I first extracted HEX colour codes from digital photographs (iNaturalist, 2018-2020) of the flower's main display structure – mainly their petals, in some cases fused sepals or stamens from brush-like flowers using the ColorZilla online application (Tillman & Dyer, 2004 - 2024), which calculates a HEX value by averaging the RGB (red, green, blue) values within a manually selected region of an image (main floral display structure). HEX values were converted into CIE L*a*b* (or CIELAB, with 'CIE' = abbreviation for Commission Internationale de l'Éclairage – International Commission on Illumination; CIE, 1986; Figure 3) colour space values (after Lee et al., 1994) using the Hextoral online tool (Hextoral.com, 2023).
The L* component represents perceptual lightness, ranging from 0% (black) to 100% (white), while a* denotes the red-to-green spectrum, and b* captures the blue-to-yellow spectrum.

CIELAB can be represented in cylindrical coordinates, wherein the chromaticity components are substituted with chroma (saturation) and hue correlates. Hue is "the degree to which a stimulus can be described as similar to or different from stimuli described as red, orange, yellow, green, blue, violet" per CIECAM02 model (Fairchild, 2013).

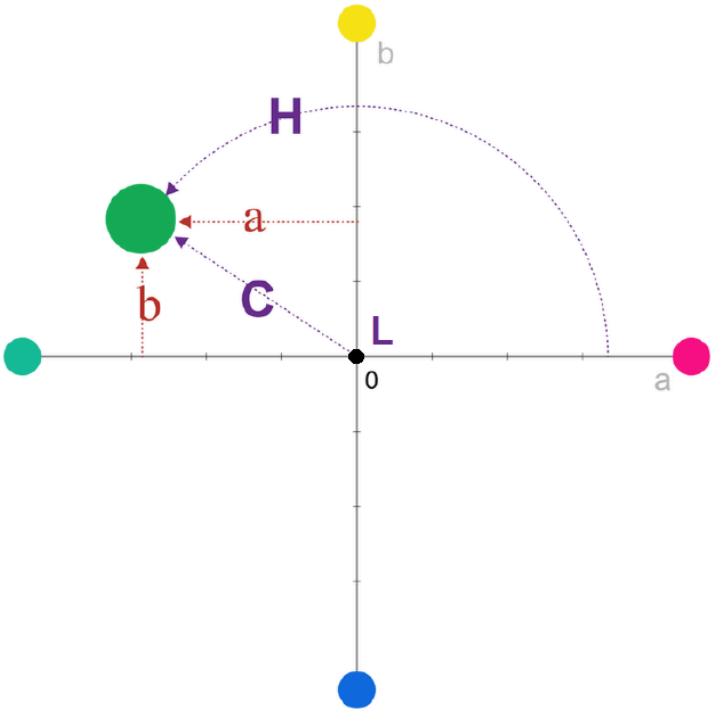

Figure 3: HLC colour atlas based on CIELAB, with 'H' = hue, 'L' = perceptual lightness, 'C' = chroma, 'a' = red-to-green spectrum, 'b' = blue-to-yellow spectrum (Der freie Farbe e.V., 2023).

Chroma and hue were calculated using *chroma* = $\sqrt{(a^{*2} + b^{*2})}$ and *hue* = atan2(b*, a*) and chroma was further transformed from rectangular to polar coordinates to facilitate my analyses. Lastly, I used the online converter 405nm.com (2024) to approximate the maximum wavelength of reflectance for each HEX colour and aligned them with published photometric values (Zhang et al., 2022; Wang et al., 2022). I placed 'pink' and 'white' to the shorter wavelength reflectance spectrum as this was also the closest match for their approximate wavelengths based on digitally averaged flower colour. The term 'purple' was used as an umbrella category to group and simplify flower colouration encompassing shades of purple, violet, and lilac.

Table 2: Analysis parameters for the appearance of flower colour (1 image per species), with species grouped by their CIELAB values. Parameters include assigned 'Colour' names, hexadecimal codes (HEX), approximate wavelengths based on CIELAB value conversions, the CIELAB variables hue, L*, a*, b*, and chroma, and species exhibiting corresponding flower colours. The colour name 'purple' is representative for shades of purple, lilac, and violet.

COLOUR NAME	HEX	WAVE-LENGTH (nm)	HUE	L	a* (CIE)	b*	CHROMA	*n*	SPECIES
purple	#8B008B	380	2.15	33	60	-39	71.56	5	*Fuchsia excelsa* *Melicytus lanceolatus* *Pittosporum colensoi* *Pittosporum ralphii* *Pittosporum tenuifolium*
dark red	#A8113C	740	1.25	37	58	19	61.03	2	*Knightia excelsa* *Pittosporum crassifolium*
crimson red	#DC143C	635	1.11	48	71	35	79.16	3	*Metrosideros excelsa* *Metrosideros fulgens* *Metrosideros umbellata*
pink	#E000e0	400	2.15	53	85	-55	101.24	4	*Aristotelia serrata* *Clianthus maximus* *Rhopalostylis sapida* *Vitex lucens*
orange	#DB621F	625	0.66	56	44	57	72.01	1	*Rhabdothamnus solandri*
green	#32CD32	519	-0.80	73	-62	60	86.28	7	*Geniostoma rupestre* *Griselinia* spp. *Melicope ternata* *Melicytus ramiflorus* *Pseudopanax* spp.
light purple	#CAC8F1	409	2.72	82	9	-20	21.93	2	*Carmichaelis australis* *Veronica diosmifolia*
pale yellow	#E1F75A	575	-0.31	93	-22	70	73.38	3	*Paraserianthes lophanta* *Pittosporum eugenioides* *Pseudowintera colorata*
yellow	#FFF159	584	-0.11	94	-8	72	72.44	7	*Corokia cotoneaster* *Sophora* spp.
cream	#FDF5E6	583	0.12	97	1	8	8.06	1	*Melicytus novae-zealandiae*
white	#FFFEFC	480	0.00	100	0	1	1.00	15	*Carpodetus serratus* *Cordyline* spp. *Corynocarpus laevigatus* *Elaeocarpus dentatus* *Kunzea ericoides* *Leptospermum scoparium* *Lophomyrtus* spp. *Metrosideros colensoi* *Myoporum laetum* *Myrsine australis* *Robinia pseudoacacia* *Weinmannia racemosa*

4.2.2 Quantification of Nectar Components

I used HPLC to separate carbohydrate compounds within 420 nectar samples from 50 woody species (+ one variety). For further identification of unknown components of interest ('COI'), Liquid Chromatography-Mass Spectrometry (LC-MS) was employed, analysing 240 samples from 39 species (+ one variety) based on the nectar I had collected for the HPLC analysis. I restricted the LC-MS data analysis to *i*) confirming the presence of HPLC-identified carbohydrates and *ii*) identifying HPLC-detected COI, with a focus on specific alkaloids (ALK), amino acids (AA), phenolics (PHE), and vitamers (VIT).

Quantification of Carbohydrates

Nectar solutions (50 - 200 µL) were transferred to 0.3 mL clear PP short screw micro-vials (9 mm thread, 11.6*32 mm with inserts from interlab.co.nz, NZ) and placed in a chilled (10 ± 5°C) Alliance Waters WAT034282 autosampler. Operating at a high limit pressure of 4000 PSI, an operating pressure of ~ 250 PSI, and a flow rate of 0.4 mL/min, the Alliance system used the Shodex KS-801 sugar column (8.0 x 300 mm), the Shodex KS-G HPLC guard column (7 µm, 6 x 50 mm), and the PDA 410 (Waters). Columns were maintained at 65°C, and the internal detector (Refractive Index, RI) at 40°C. The mobile phase and wet prime solvent used was HPLC grade water (type 1), prepared from a Barnstead E-Pure Water System (18.2 MΩ cm) and purified in situ. Following each 35-minute sample run, the Alliance system needle was washed with 5% MeOH (methanol 100%, HPLC grade) and reloaded with an injection volume of 10 µL. To monitor and flush the system, I periodically injected samples of a standard solution – containing sucrose (S), fructose (F), glucose (G) and DHA – and purified water using Empower 3 software (Waters). The software automatically computed peak areas and retention times (RT), totalling 40 recurring components.

To identify components, I prepared standard solutions of 20 common sugar types (90 - 829 g/mol), processed them under the same HPLC conditions and matched their mean retention times with detected components. To achieve this, I prepared stock solutions for sucrose (S), fructose (F), glucose (G), maltose (grade 1, purity ≥ 97%), alpha-lactose (monohydrate, beta-anomer content 2.6%), D-mannose, myoinositol, D-galactose (pur. ≥ 97%), xylose (pur. ≥ 99.0%), L-rhamnose monohydrate, D(-)-ribose, stachyose hydrate (purity ≥ 98%) (purchased from SIGMA-ALDRICH CO., USA); cellobiose, L(+)-arabinose, L(-)-sorbose, sorbitol (purchased from BDH Chemicals Ltd Poole England); D(+)-mannitol (Riedel-de

Haen AG, Germany); D(+)-melezitose (SIGMA Chemie GmbH, Germany); raffinose and verbascose (pur. > 95%, purchased from Megazyme, USA).

For fructose and glucose, 2500 mg of each sugar was dissolved in a 50-mL volumetric flask with 50 mL of type 1 water, resulting in a 50 mg/mL stock solution. This solution was then diluted in a 1:1 ratio to obtain three additional solutions with 25, 12.5, and 6.25 mg/mL concentrations. The same method was applied to 300 mg of sucrose to generate solutions with 6, 3, 1.5, and 0.75 mg/mL concentrations. For all other sugar types, 400 mg of each was dissolved in an 8-mL volumetric flask with 4 mL of type 1 water to obtain stock solutions of 100 mg/mL. These stock solutions were further diluted to 50, 25, and 5 mg/mL concentrations per sugar type. Components were considered elementary to a species' nectar composition if present in at least 50% of the species samples.

The sugars S, G, and F represented the dominant peaks in most samples. Early eluting components (2 - 17.1 min retention time, 'RT') were classified as (larger) oligosaccharides (*n* = 15, > 500 mol/g), including hexasaccharides (*n* = 12, RT = 2 - 15.1 min), the pentasaccharide verbascose (RT = 15.9 min), and the trisaccharides raffinose and melezitose (RT = 16.3 and 17.1 min, respectively). Components such as mannitol, sorbose, sorbitol, galactose, mannose, and xylose were likely overshadowed by larger S, G, and F peaks, preventing confirmation of their presence or quantification of their amounts.
Raffinose-Family-Oligosaccharides "RFO" (~ 504-828 mol/g; C_{18} - C_{30}) eluted between 15.8 - 17.0 min, disaccharides (~ 342 mol/g; $C_{12}H_{22}O_{11}$) between 18.6 - 19.2 min, and monosaccharides (~ 90 - 180 mol/g; C_3 - C_5) between 22.5-33 min. The two polyols D(+)-mannitol and sorbitol (sugar alcohols, ~ 182 mol/g; $C_6H_{14}O_6$) peaked at 22.6 and 23.3 min, respectively.
Of the 40 detected components, 26 did not match any tested sugar standards (Table S1, Supplementary Material). Unknown components were consecutively numbered [in order of HPLC retention time (RT) and therefore with decreasing molecular weight] and termed 'components of interest'(COI). To accurately quantify each identified component, I calibrated detected peak area amounts through linear regression analysis, plotting the concentration of the analytes (*x*) against their peak areas (*y*) (Table 3). COI were calibrated by comparing their peak area or height to a known compound with similar properties for which the concentration was known. The ratio of the COI's peak area or height to that of a known compound is termed

the 'response factor', allowing me to estimate concentrations of unknown compounds (common method after González & Herrador, 2007). Subsequently, I used the calibrated component amounts to determine their concentrations. Following a 3.3% discrepancy between HPLC and refractometer nectar concentration values, I used the HPLC values in all analyses.

Table 3: Regression equations of tested sugar standards for component calibration, with 'RT' = High Performance Liquid Chromatography retention time.

CLASSIFICATION		SUGAR TYPE	REGRESSION EQUATION	AREA per mg/ml	R^2	*n*	CALIBRATION RANGE (mg/mL)	RT (min)
Oligosaccharides	Pentasaccharides	Verbascose	143369*x + 835403	157000	0.999	4	25-200	15.77 ± 0.02
	Tetrasaccharides	Stachyose	158633*x + 190032	166344	0.999	4	12.5-100	16.25 ± 0.02
	Trisaccharides	Melizitose	1.77E+06*x + -50032	1723700	0.999	4	5-100	17.01 ± 0.04
	Disaccharides	Sucrose	165704*x + 20192	171204	0.999	9	1.7-6.1	17.47 ± 0.01
		Cellobiose	170120*x + -18283	205533	0.999	5	2.5-100	18.61 ± 0.04
		Maltose	151768*x + 947012	165974	(1)	2	2.5-100	18.98 ± 0.03
		Lactose	167675*x + 139569	230300	0.999	3	2.5-100	19.12 ± 0.05
Monosaccharides	Hexoses	Glucose	167097*x + 16799	168336	0.999	8	6-48	20.92 ± 0.01
		Fructose	167385*x + 5838	168477	0.999	8	6-48	22.46 ± 0.01
		Rhamnose	125967*x + 2E+06	144982	0.997	4	5-250	22.90 ± 0.05
		Sorbose	na	na	na	2	2.5-100	23.34 ± 0.62
		Galactose	160157*x + 480397	176303	0.999	4	12.5-100	23.65 ± 0.02
		Mannose	166689*x + -15115	157194	0.987	4	5-100	23.75 ± 0.04
	Hexanols	Sorbitol	na	160785	na	2	2.5-12.5	23.31 ± 0.01
		Mannitol	175023*x + 30093	177081	0.999	4	5-100	22.54 ± 0.05
		Inositol	203447*x + -70179	193622	(1)	2	5-12.5	24.74 ± 0.01
	Pentoses	Xylose	142360*x + 952222	177725	0.996	4	2.5-500	24.05 ± 0.13
		Arabinose	163962*x + 174007	173046	0.999	5	5-100	25.50 ± 0.04
		Ribose	119978*x + -123033	129767	0.999	5	2-100	28.11 ± 0.07
	Trioses	DHA	110966*x + 15011	122683	0.996	7	0.06-3.6	31.03 ± 0.02

Quantification of Nectar Sugar Types

I adapted the traditional nectar sugar classification system by Baker & Baker (1990), which uses the ratio of sucrose to hexoses (fructose and glucose), which I termed Baker's ratio. My approach used the author's threshold values of the Baker's ratio to divide species into two categories: those with a predominance of sucrose (Baker's ratio > 0.5) and those with a predominance of hexoses (Baker's ratio < 0.499).

Considering Baker & Baker's (1990) identification of a correlation between a plant's Baker's ratio and its pollination syndrome, I investigated the applicability of this correlation to New Zealand species. I tested Baker & Baker's associations of plant species pollinated by insects generally possessing hexose-rich nectar, whereas those pollinated by birds are more likely to have sucrose-rich nectar. Species' pollination syndromes were extracted from Kelly et al., (2010). Additionally, I examined the validity of Percival's (1961) observations of tubular (or 'closed') flowers typically producing sucrose-rich nectars, with 'open' flowers tending to produce hexose-rich nectars within the context of New Zealand species.

Quantification of Non-Carbohydrates

To quantify non-carbohydrates and identify the remaining COI, I analysed the LC-MS dataset (including positive and negative ion mode sub-datasets based on Hydrophilic Interaction Liquid Chromatography 'HILIC' and using a C18 column for separating small molecules; for *n* of samples see Table 4), which I had collaboratively processed externally (van Klink & Joyce, Plant and Food, Lincoln) based on an MS setup after McDougal et al. (2018). The resulting data was compared with the HPLC observations and analysed for the presence of carbohydrates and the amounts of ALK, AA, PHE, and VIT, which had been detected in nectar in previous studies (Agar et al., 2020; Lingwan et al., 2021) or been recommended to me, e.g. ALK in *Sophora* plant tissue (McDougal et al., 2018).

Besides searching for 22 sugar types to confirm their presence I had identified by HPLC, I focused on finding eight ALK (Table 5), all AA, eleven PHE (Table 5) and all VIT. I achieved targeted compound identification through *i*) comparison of exact mass and retention time with available standards, and *ii*) in the absence of standards, tentative identification was conducted by comparing the theoretical exact mass of the molecular ion with the measured accurate mass. This comparison was further verified through searches in metabolite databases such as PubChem and ChemSpider (following Pereira-Caro et al., 2021). LC-MS data analysis narrowed down the identity of 21 COI and detected 14 more components not identified via HPLC. However, five HPLC COI remained unidentified (COI 17 - 19, 23, 26) and were excluded from further statistical analysis. COI 1 - 12 were grouped as hexasaccharides and thus classified as carbohydrates (Table S1, Supplementary Material).

Table 4: Number (*n*) of separately analysed nectar samples per species using LC-MS, with 'M' referring to a single master mix sample created by combining 15 nectar samples.

SPECIES	*n*	SPECIES	*n*	SPECIES	*n*	SPECIES	*n*
ARI SER	M	KUN ERI	M	MET EX A	6	PSE COL	1
COR AUS	M	LEP SCO	M	MET FUL	1	PSE CRA	2
COR BAN	1	LOB OBC	1	MET UMB	1	RHA SOL	1
COR LAE	4	LOP BUL	3	MYO LAE	M	RHO SAP	2
COR STR	2	MEL LAN	1	PIT COL	1	SOP CHA	4
ELA DEN	3	MEL NOV	1	PIT CRA	M	SOP GOD	2
FUC EXC	M	MEL RAM	4	PIT EUG	7	SOP LON	1
GEN RUP	5	MEL TER	8	PIT RAL	4	SOP MIC	M
GRI LIT	2	MET COL	2	PIT TEN	9	SOP TET	1
KNI EXC	6	MET EXC	M	PSE ARB	3	VIT LUC	M
						WEI RAC	1

Table 5: Selected ALK and PHE used for searching within LC-MS results (component data from the National Center for Biotechnology Information, 2021; LOTUS IDs from Rutz et al., 2022).

		COMPONENT	ALTERNATIVE COMPONENTS	CLASSIFICATION		FORMULA	MW	PSA	CID (PubCHEM)	LOTUS ID (Presence in plants)
Alkaloids	1	5,6-Dehydrolupanine	Sophocarpine	Lysine alkaloids	Quinolizidine alkaloids	C15H22N2O	246	24	115269	LTS0179391
	2	Lupanine				C15H24N2O	248	24	91471	LTS0039153
	3	Matrine				C15H24N2O	248	24	91466	LTS0113091
	4	Lupinine				C10H19NO	169	23	91461	LTS0232754
	5	N-Methyl cytisine	3-(3-Hydroxyphenyl)-2-propenoic acid			C12H16N2O	164	58	670971	LTS0230668
	6	Anagyrine	Monolupine		Pyridine alkaloids	C15H20N2O	244	25	5351589	LTS0180035
	7	Cytisine				C11H14N2O	190	34	22407	LTS0139944
	8	Ammodendrine			Piperidine alkaloid	C12H20N2O	208	32	442625	LTS0084410
Phenols	1	Luteolin	Carlinoside	Flavonoids	Flavonol	C26H28O15	581	249	44566720	LTS0088939
	2	Quercetin	Sophoretin			C15H10O7	302	131	5280343	LTS0004651
	3	Rutin				C27H30O16	611	269	5280805	LTS0042292
	4	Aromadendrin	Eriodictyol, Swertinin		Flavanonol	C15H12O6	288	89	5491517	LTS0153299
	5	Apigenin/Galangin	Galangin		Flavones	C15H10O5	270	91	5280443	LTS0210648
	6	Vitexin	Afzelin			C21H20O10	432	181	5280441	LTS0199581
	7	Kaempherol				C15H10O6	286	111	5280863	LTS0155822
	8	Spinosin	Flavoayamenin			C28H32O15	609	249	155692	LTS0097829
	9	3-O-Feruloylquinic acid		Phenolic acids	Hydroxycinnamic acids	C17H20O9	368	154	9799386	LTS0062037
	10	Coumaric acid				C9H8O3	164	58	637541	LTS0022256
	11	Gentisic acid			Hydroxybenzoic acids	C7H6O4	154	78	3469	LTS0170793
	12	Cinnamic Acid			Cinnamic acids	C9H8O2	148	37	444539	LTS0128130

4.2.6 Analysis

Construction of the Phylogenetic Framework

The phylogenetic framework was constructed using the 'phylo.maker' function in the V.PhyloMaker R package (Qian & Jin, 2016), drawing on a previously published supertree (Zanne et al., 2014) and adhering to the Angiosperm Phylogeny Group APG IV classification for flowering plants (APG IV, 2016). The scientific names of all species analysed were verified and standardised according to the World of Flora Online (2024), with varieties and subspecies of the same species being excluded from the analysis. I applied the 'phylosig' function from the phytools R package to examine the phylogenetic signal in species distribution, Pagel's lambda (λ). λ varies between 0 and 1. A λ value of 0 indicates phylogenetic independence in trait evolution, whereas a λ of 1 suggests that trait evolution is consistent with Brownian motion. Any λ significantly greater than zero is considered to exhibit a phylogenetic signal, indicating a degree of similarity to Brownian motion (Arène et al., 2017).

Statistics

I used a suite of statistical tests to assess relationships between nectar traits—such as volume, concentration, solubles, specific sugars, the ‘Baker’s ratio’ and grouped non-carbohydrate components—and predictor variables including floral size, weight, shape, colour, pollinator syndrome, and phylogeny. These included Spearman's correlation test, linear regression, Generalised Additive Models (GAM), One-Way ANOVA, the Kruskal-Wallis test (KW), and the 'phylo.maker' and 'phylosig' function from the V.PhyloMaker and phytools R package, respectively. ANOVA and KW tests were followed by respective post-hoc tests after conducting normality checks with the Shapiro-Wilk test. Based on the outcomes, post-hoc analyses were carried out using the Tukey's Honest Significant Difference (HSD) or the Dunn's (incl. Bonferroni correction) test. Value standardisation on a scale of 0 - 100, and natural log transformation were used in some analyses to aid interpretation. My analyses were supported by R packages including 'dunn.test', 'ade4', 'adephylo', 'lm4', 'lmerTest', 'phytools', 'phylobase', ‘V.PhyloMaker’ and 'picante', utilising R version 4.3.1 in RStudio (R Team, 2020).

4.3 Results

4.3.1 Interspecific Variation in Nectar and Floral Traits

Flowers ranged between 2 - 67 mm in size, 3 - 879 mg in fresh weight, 1 - 49 µL of nectar volume and 0.01 - 54% nectar concentrations (Tables 6 and 7), equivalent to 0.0001 - 19 mg of solubles, with *Sophora* species responsible for all maximum values. Solubles comprised 62 components based on combined HPLC and LC-MS analysis data. LC-MS analysis confirmed the presence of hexasaccharides generally and further large components beyond 834 g/mol. However, this method is not suitable to identify molecules of this size. Hence, for simplicity, the 12 components detected by HPLC (COI 1 - 12, Table S3) that eluted before verbascose were collectively categorised as hexasaccharides (C_{36}).

The carbohydrate fraction constituted 72 - 100% of solubles comprising mono-, di-, tri-, penta- and hexasaccharides (Tables 6 - 9), resulting in 25 distinct sugar types (including 12 unidentified large components, 'COI', Table S1 - 3). The non-carbohydrate fraction constituted 0.03 - 8.1% of solubles comprising specific alkaloids (ALK) of interest, amino acids (AA), phenolics (PHE) and vitamers (VIT) (see 4.3.1 *Section* 'Variation in Non-Carbohydrates'), resulting in 37 distinct non-sugars.

Table 6: Mean ± SD and range of flower and nectar measurements (with relative compound concentrations given in %). Nectar composition was derived from HPLC for carbohydrates and LC-MS for non-carbohydrates, with *n* referring to the number of flowers, nectar samples or detected compounds.

TRAIT	*n*	MEAN ± SD	RANGE	
Flower Size	8,446 flowers	13 ± 13	2 - 67	mm
Flower Fresh Weight	10,290 flowers	156 ± 234	3 - 879	mg
Nectar Concentration	420 samples of 8,164 flowers	13 ± 10	0.01 - 54	%
Nectar Volume	420 samples of 8,164 flowers	10 ± 12	1 - 49	µl
Nectar Solubles	420 samples of 8,164 flowers	2 ± 4	0.0001 - 19	mg
Carbohydrate Compounds	25 compounds	11 ± 3	5 - 19	n
Carbohydrate Fraction	Sum of 25 compounds	98 ± 5	72 - 100	%
Hexasaccharides	Sum of 12 compounds	7 ± 11	0.3 - 60	%
Pentasaccharide	Sum of 1 compound	1.2 ± 2.6	0.01 - 14	%
Trisaccharide	Sum of 2 compounds	1.3 ± 1.4	0.01 - 5	%
Disaccharide	Sum of 3 compounds	24 ± 23	0.4 - 81	%
Hexoses	Sum of 4 compounds	64 ± 25	6 - 97	%
Pentoses	Sum of 2 compounds	0.3 ± 0.3	0.02 - 1.5	%
Triose	Sum of 1 compound	0.04 ± 0.11	0.0002 - 0.32	%
ALK	Sum of 6 compounds	2 ± 2.3	0.00003 - 0.13	%
AA	Sum of 16 compounds	0.5 ± 1.1	0.01 - 4.7	%
PHE	Sum of 11 compounds	0.4 ± 0.9	0.003 - 3.7	%
VIT	Sum of 4 compounds	0.05 ± 0.05	0.004 - 0.26	%

Table 7: Summarising table of species trait means (and their standard deviation, SD) and number (*n*) of samples per trait, with carbohydrate data derived from HPLC; DBH = diameter at breast height.

SPECIES	TREES		FLOWERS			NECTAR					
	DBH		Weight		Size	*n*		Volume	Concentration	Carbohydrate Compounds	Total Volume
	n	cm ± SD	*n*	mg ± SD	*n*	mm ± SD	Flowers	Samples	µL ± SD	°BRIX ± SD	*n* ± SD	µL
ARI SER	15	27 ± 21	384	6 ± 1	282	5 ± 1	285	15	2 ± 1	3 ± 3	11 ± 2	570
CAR AUS	1	3 ± NA	30	4 ± NA	29	4 ± 0	30	1	1 ± NA	15 ± NA	11 ± NA	30
CAR SER	7	34 ± 12	190	18 ± 4	138	7 ± 1	190	7	2 ± 1	14 ± 4	8 ± 2	380
CLI MAX	1	1 ± NA	10	169 ± NA	10	67 ± 1	10	1	70 ± NA	18 ± NA	14 ± NA	700
COR AUS	21	12 ± 8	490	19 ± 6	457	7 ± 2	395	21	3 ± 1	6 ± 3	13 ± 1	1304
COR BAN	1	10 ± NA	20	20 ± NA	20	11 ± 1	20	1	3 ± NA	7 ± NA	12 ± NA	60
COR COT	1	1 ± NA	20	11 ± NA	20	8 ± 0	20	1	1 ± NA	7 ± NA	15 ± NA	20
COR LAE	5	95 ± 33	220	18 ± 3	101	5 ± 1	100	5	2 ± 2	7 ± 4	18 ± 1	200
COR STR	2	5 ± 0	40	40 ± 1	40	10 ± 1	40	2	1 ± 0	24 ± 1	13 ± 2	40
ELA DEN	3	50 ± 17	60	29 ± 12	49	12 ± 2	60	3	5 ± 0.4	10 ± 1	12 ± 6	300
FUC EXC	29	42 ± 30	703	253 ± 92	486	17 ± 5	767	29	23 ± 15	16 ± 10	4 ± 1	17641
GEN RUP	19	5 ± 7	494	11 ± 5	348	6 ± 1	426	19	1 ± 0	13 ± 8	16 ± 2	426
GRI LIT	21	45 ± 62	555	3 ± 1	399	4 ± 1	375	21	2 ± 0	2 ± 1	8 ± 2	750
GRI LUC	3	20 ± 0	80	6 ± 2	57	4 ± 1	60	3	2 ± 0	3 ± 3	9 ± 3	120
KNI EXC	9	39 ± 22	185	216 ± 45	178	40 ± 4	165	9	11 ± 5	16 ± 12	14 ± 2	1815
KUN ERI	21	15 ± 11	480	11 ± 3	430	7 ± 1	420	18	2 ± 1	9 ± 7	14 ± 1	840
KUN SPP	1	15 ± NA	20	9 ± NA	20	7 ± 1	20	1	1 ± NA	11 ± NA	11 ± NA	20
LEP SCO	23	17 ± 15	539	41 ± 16	529	14 ± 2	450	24	3 ± 1	6 ± 7	11 ± 2	1350
LOP BUL	1	15 ± NA	20	45 ± NA	18	12 ± 1	20	1	3 ± NA	3 ± NA	11 ± NA	60
LOP OBC	1	5 ± NA	20	16 ± NA	17	7 ± 1	20	1	2 ± NA	2 ± NA	12 ± NA	40
LOP SPP	2	4 ± 2	30	35 ± 15	16	14 ± 2	30	2	3 ± 0	3 ± NA	9 ± 3	90
MEL LAN	1	20 ± NA	20	12 ± NA	7	5 ± 1	20	1	2 ± NA	1 ± NA	12 ± NA	40
MEL NOV	1	30 ± NA	20	10 ± NA	16	5 ± 1	20	1	2 ± NA	9 ± NA	11 ± NA	40
MEL RAM	19	19 ± 15	530	9 ± 2	402	6 ± 1	400	20	1 ± 1	1 ± 1	10 ± 2	400
MEL TER	9	13 ± 8	195	33 ± 4	155	8 ± 1	155	9	2 ± 0	13 ± 9	10 ± 1	310
MET COL	1	4 ± NA	13	325 ± 85	13	29 ± 4	13	2	17 ± 5	32 ± 18	11 ± 0	221
MET EXC	23	32 ± 23	697	226 ± 96	528	33 ± 4	360	23	23 ± 10	23 ± 12	12 ± 2	8280
MET EX A	9	61 ± 24	382	241 ± 49	219	26 ± 3	227	9	13 ± 6	24 ± 4	14 ± 1	2951
MET FUL	1	2 ± NA	20	24 ± NA	21	11 ± 1	20	1	2 ± NA	14 ± NA	6 ± NA	40
MET UMB	1	250 ± NA	40	155 ± NA	20	24 ± 2	40	1	13 ± NA	8 ± NA	12 ± NA	520
MYO LAE	22	41 ± 35	543	44 ± 13	522	14 ± 2	536	22	6 ± 1	9 ± 5	12 ± 1	3216
MYR AUS	2	5 ± 0	40	3 ± 2	36	3 ± 1	40	3	1 ± 0	3 ± 0	11 ± 2	40
PAR LOP	1	20 ± NA	40	34 ± NA	24	2 ± 0	25	1	3 ± NA	7 ± NA	14 ± NA	75
PIT COL	1	5 ± NA	40	150 ± NA	20	7 ± 0	20	1	3 ± NA	10 ± NA	15 ± NA	60
PIT CRA	32	29 ± 29	290	84 ± 33	434	9 ± 1	446	23	7 ± 4	13 ± 7	14 ± 2	3122
PIT EUG	23	40 ± 26	549	17 ± 8	428	11 ± 2	410	23	3 ± 1	8 ± 6	14 ± 2	1353
PIT RAL	5	16 ± 25	140	97 ± 25	55	9 ± 2	100	5	3 ± 1	13 ± 3	14 ± 1	300
PIT TEN	23	32 ± 26	450	65 ± 17	364	7 ± 2	348	23	4 ± 1	9 ± 7	15 ± 3	1392
PSE ARB	3	20 ± 10	60	15 ± 5	2	8 ± 1	60	2	5 ± 1	10 ± 7	19 ± 0	300

PSE COL	1	10 ± NA	20	42 ± NA	19	13 ± 1	20	1	1 ± NA	< 0.1	12 ± NA	26
PSE CRA	1	15 ± NA	17	29 ± NA	17	4 ± 0	17	1	2 ± NA	< 0.1	10 ± NA	34
RHA SOL	1	5 ± na	21	66 ± na	20	18 ± 3	21	1	26 ± NA	15 ± NA	14 ± NA	546
RHO SAP	5	30 ± NA	140	38 ± 9	114	8 ± 2	110	5	7 ± 4	14 ± 8	13 ± 1	770
ROB PSE	1	30 ± NA	20	117 ± NA	13	20 ± 1	20	1	82 ± NA	41 ± NA	13 ± NA	1640
SOP CHA	5	39 ± 36	170	675 ± 205	119	40 ± 5	126	5	26 ± 19	25 ± 4	14 ± 0	3276
SOP GOD	2	9 ± 1	40	637 ± 199	60	42 ± 2	40	2	25 ± 28	28 ± 7	12 ± 1	1000
SOP LON	1	10 ± NA	20	688 ± NA	20	47 ± 1	30	1	49 ± NA	20 ± NA	12 ± NA	1470
SOP MIC	23	52 ± 45	642	730 ± 205	505	46 ± 6	488	24	37 ± 14	24 ± 12	7 ± 1	18056
SOP PRO	1	3 ± NA	NA	392	20	25 est.	20	1	4 ± NA	9 ± NA	12 ± NA	80
SOP TET	1	80 ± NA	40	879 ± NA	19	56 ± 2	20	1	35 ± NA	54 >	11 ± NA	700
VER DIO	1	1 ± NA	10	16 ± NA	20	8 ± 1	20	1	1 ± NA	4 ± NA	15 ± NA	20
VIT LUC	18	88 ± 85	431	371 ± 95	289	32 ± 4	311	18	26 ± 7	19 ± 8	11 ± 2	7993
WEI RAC	3	18 ± 3	60	3 ± 2	19	5 ± 1	60	3	1 ± 0	8 ± 6	12 ± 1	60
Mean ± SD	8	29 ± 38	10290	145 ± 215	8164	24 ± 18	8446	420	12 ± 17	13 ± 6	12 ± 2	1605
Maximum	1	250	10	879	2	67	10	1	82	54	19	20
Minimum	32	1	703	3	529	2	767	29	1	<0.1	4	18056

*Caption for **Table 8** on the next page:*

Summarising table of species nectar composition means (per cent by weight, with standard deviation for overall means, SD), with carbohydrate data derived from HPLC (number of samples see Table 7) and non-carbohydrate data derived from LC-MS (number of samples see Table 4). Abbreviations as follows: HEXA = hexasaccharides, TRI = Trisaccharides, DI = disaccharides (Oligosaccharides); HEXO = hexoses, PENTO = pentoses, TRIO = trioses (Monosaccharides); ALK = alkaloids, AA = amino acids, PHE = phenolics, VIT = vitamers. non-carbohydrates shown in parts per million (ppm) for better readability; all other component groups given in percent (%).

SPECIES	CARBOHYDRATES %						NON-CARBOHYDRATES ppm				
	OLIGOSACCHARIDES			MONOSACCHARIDES							
	HEXA	TRI	DI	HEXO	PENTO	TRI	ALK	AA	PHE	VIT	TOTAL
ARI SER	2.6	1.1	37	58			1	2052	482	104	2639
CAR AUS	0.4	0.3	46	52	0.1			---------- not tested ----------			
CAR SER	2.1	0.9	41	54				---------- not tested ----------			
CLI MAX	4.1	1.1	39	55	0.1			---------- not tested ----------			
COR AUS	1.0	0.2	29	68	0.3		2	3325	833	119	4279
COR BAN	0.4	0.3	42	57	0.0		1	3373	920	59	4353
COR COT	5.2	0.3	2	91	0.3			---------- not tested ----------			
COR LAE	3.0	0.2	1	95	0.3		1	2409	90	552	3052
COR STR	1.4	1.3	2	95	0.0	0.0020	3	458	205	601	1267
ELA DEN	4.1	2.4	41	48	0.2		6	323	34	260	623
FUC EXC	2.8	0.01	4	93			1	762	162	1156	2081
GEN RUP	2.3	1.8	46	49	0.3		1	1403	700	308	2412
GRI LIT	5.8	0.8	24	68	0.9		1	2109	1373	994	4477
GRI LUC	5.7	1.0	45	47	0.3			---------- not tested ----------			
KNI EXC	2.7	4.7	67	24	0.2	0.0090		---------- not tested ----------			
KUN ERI	2.1	0.1	1	97	0.3		1	1945	19798	575	22319
LEP SCO	3.7	0.2	1	94	0.3	0.3170	21	43598	36579	1097	81295
LOP BUL	39.1	1.2	12	32	0.9		6	14150	16328	855	31339
LOP OBC	30.1		5	22			3	894	762	267	1926
MEL LAN	6.8	1.2	3	88			1	2047	196	332	2576
MEL NOV	2.8	0.3	1	96	0.2		6	46900	2427	1130	50463
MEL RAM	19.0	2.1	4	72	0.3		2	8794	9693	850	19339
MEL TER	3.8	0.2	4	90	1.4			146	49	160	355
MET COL	1.3	0.3	39	59	0.1		1	293	357	393	1044
MET EX A	6.1	0.3	2	89	0.1			---------- not tested ----------			
MET EXC	4.2	0.2	1	92	0.3		1	216	249	93	559
MET FUL	2.0	0.1	3	94			1	1326	5046	74	6447
MET UMB	2.9	0.1	0.4	96	0.9			---------- not tested ----------			
MYO LAE	1.2	0.1	9	89	0.1	0.0070		---------- not tested ----------			
MYR AUS	36.6		6	41	0.3		4	613	1145	1110	2872
PAR LOP	10.7	0.7	11	75			1	235	185	148	569
PIT CRA	3.0	1.4	40	54	0.1		2	842	379	182	1405
PIT EUG	1.3	2.0	44	52	0.1	0.0020	24	234	123	153	534
PIT RAL	5.5	1.4	64	28	0.1	0.0002	3	908	729	223	1863
PIT TEN	3.1	1.4	56	38	0.2			262	426	365	1053
PIT COL	6.3	0.7	1	89	1.5	0.0070	3	994	3513	637	5147
PSE ARB	2.8	1.8	24	71	0.3		6	33974	5366	707	40053
PSE COL	27.8	3.5	2	57			235	6288	33629	2645	42797
PSE CRA	59.7	4.9	2	21				1807	35	110	1952
RHA SOL	2.4	0.03	1	96	0.1			---------- not tested ----------			
RHO SAP	7.1	4.4	81	6	0.9		974	933	135	226	2268
ROB PSE	5.4	1.6	72	19	0.2		1193	6322	252	137	7904
SOP CHA	1.1	4.4	38	55	0.2		36	297	128	162	623
SOP GOD	2.1	4.1	31	60	0.4		643	2255	262	186	3346
SOP LON	1.1	3.0	49	44	0.2			---------- not tested ----------			
SOP MIC	2.0	0.9	44	52			341	13063	90	149	13643
SOP PRO	1.1	3.0	49	44	0.2			---------- not tested ----------			
SOP TET	0.3	0.9	40	57			1	146	4875	252	5274
VER DIO	2.2	0.3	1	96	0.5		1	761	112	35	909
VIT LUC	2.0	0.6	14	82	0.2	0.0100	1193	46900	36579	2645	87317
WEI RAC	2.2	1.5	38	56	0.2			---------- not tested ----------			
Mean	**7**	**1.3**	**24**	**64**	**0.3**	**0.04**	**131**	**6552**	**5016**	**520**	12219
SD	**11**	**1**	**23**	**25**	**0.3**	**0.11**	**328**	**12953**	**10015**	**606**	23902
Minimum	**0.3**	**0.01**	**0.4**	**6**	**0.02**	**0.0002**	**1.0**	**146**	**34**	**35**	216
Maximum	**60**	**5**	**81**	**97**	**1.5**	**0.317**	**1193**	**46900**	**36579**	**2645**	87317

Variation in Nectar Carbohydrate Composition

Identified sugars (Tables 8, S1 & 2) ranged between 90 - 828 g/mol in molecular weight, comprising a single triose, two pentoses, four hexoses, three disaccharides, two trisaccharides, and one pentasaccharide. In particular, I identified DHA (C_3), ribose, arabinose (C_5), inositol, fructose, rhamnose, glucose (C_6), lactose, maltose, sucrose (C_{12}), melezitose, raffinose (C_{18}), and verbascose (C_{30}).

As expected, sucrose, glucose, and fructose (Table 9) were found in all species. Other sugars were present in 6-96% of species. Melezitose was the most common of the other sugars, found in 96% of species, followed by COI 11 at 94% and arabinose at 75% (Table S2). Verbascose (65%), COI 7 (62%), COI 9 (60%), and raffinose (63%) were also prevalent. Less frequently detected were COI 6 and COI 12 (46% each), ribose (37%), lactose (35%), inositol (22%), DHA (16%), maltose (12%), and rhamnose (6%). The remaining early-eluting COIs were present in 2 - 30% of species tested.

Hexoses were the most dominant components with mean additive concentrations of 6 - 97%, followed by disaccharides at 0.4 - 81% and hexasaccharides at 0.3 - 60%. The triose DHA was detected in seven other species besides *Leptospermum scoparium* (LEP SCO). Those seven species had 30- to 1600-fold lower concentrations compared to LEP SCO, with *Vitex lucens* (VIT LUC), *Knightia excelsa* (KNI EXC), *Pittosporum tenuifolium* (PIT TEN) and *Myosporum laetum* (MYO LAE) nectar containing approximately 0.01%, *Cordyline stricta* (COR STR) and *Pittosporum crassifolium* (PIT CRA) 0.002%, and *Pittosporum eugenioides* (PIT EUG) 0.002% DHA. I observed a relatively balanced division between species with hexose-rich (54% of 48 native species) and sucrose-rich (44%) nectar (Table 9).

Table 9: Species sorted by increasing relative sucrose (S) concentrations, with 'Hex-rich' = hexose-rich (Baker's ratio 0 - 0.499), 'S-rich' = sucrose-rich (Baker's ratio > 0.5), 'Entomo.' = entomophilous syndrome, 'Ornitho." = ornithophilous syndrome, 'Pol. Syndrome' = Pollinator syndrome (after Kelly et al., 2010).

SPECIES	S% OF SFG	FG% OF SFG	BAKER'S	SIMPLIFIED SUGAR TYPE	POL. SYN-DROME	CLADE	SUBCLADE	ORDER	FAMILY
Corokia cotoneaster	0.3	99.7	0.003	Hex-rich	Entomo	Asterids	Campanulids	Asterales	Argophyllaceae
Corynocarpus laevigatus	0.6	99.4	0.006	Hex-rich	Entomo	Rosids	Fabids	Cucurbitales	Corynocarpaceae
Fuchsia excelsa	0.6	99.4	0.006	Hex-rich	Ornitho	Rosids	Malvids	Myrtales	Onagraceae
Griselinia littoralis	0.6	99.4	0.006	Hex-rich	Entomo	Asterids	Campanulids	Apiales	Griseliniaceae
Kunzea ericoides	0.7	99.3	0.007	Hex-rich	Entomo	Rosids	Malvids	Myrtales	Myrtaceae
Leptospermum scoparium	0.7	99.3	0.007	Hex-rich	Entomo	Rosids	Malvids	Myrtales	Myrtaceae
Lophomyrtus bullata	0.8	99.2	0.008	Hex-rich	Entomo	Rosids	Malvids	Myrtales	Myrtaceae
Lophomyrtus obcordata	1.1	98.9	0.011	Hex-rich	Entomo	Rosids	Malvids	Myrtales	Myrtaceae
Melicope ternata	1.1	98.9	0.011	Hex-rich	Entomo	Rosids	Malvids	Sapindales	Rutaceae
Melicytus lanceolatus	1.6	98.4	0.017	Hex-rich	Entomo	Rosids	Fabids	Malphigiales	Violaceae
Melicytus novae-zealandiae	3.1	96.9	0.032	Hex-rich	Entomo	Rosids	Fabids	Malphigiales	Violaceae
Melicytus ramiflorus	3.4	96.6	0.036	Hex-rich	Entomo	Rosids	Fabids	Malphigiales	Violaceae
Metrosideros excelsa	3.6	96.4	0.038	Hex-rich	Ornitho	Rosids	Malvids	Myrtales	Myrtaceae
Metrosideros fulgens	4.1	95.9	0.043	Hex-rich	Ornitho	Rosids	Malvids	Myrtales	Myrtaceae
Metrosideros umbellata	4.4	95.6	0.046	Hex-rich	Ornitho	Rosids	Malvids	Myrtales	Myrtaceae
Myoporum laetum	4.5	95.5	0.047	Hex-rich	Entomo	Asterids	Lamiids	Lamiales	Scrophulariaceae
Myrsine australis	6.8	93.2	0.073	Hex-rich	Entomo	Asterids	Asterids	Ericales	Primulaceae
Pittosporum colensoi	8.9	91.1	0.097	Hex-rich	Entomo	Asterids	Campanulids	Apiales	Pittosporaceae
Pseudopanax arboreus	12.8	87.2	0.147	Hex-rich	Generalist	Asterids	Campanulids	Apiales	Araliaceae
Pseudopanax crassifolium	14.4	85.6	0.168	Hex-rich	Generalist	Asterids	Campanulids	Apiales	Araliaceae
Pseudowintera colorata	19.7	80.3	0.245	Hex-rich	Entomo	Magnoliids	Magnoliids	Cannelales	Winteraceae
Rhabdothamnus solandri	25.2	74.8	0.337	Hex-rich	Ornitho	Asterids	Lamiids	Lamiales	Gesneriaceae
Veronica diosmifolia	25.8	74.2	0.347	Hex-rich	Entomo	Asterids	Lamiids	Lamiales	Plantagina
Vitex lucens	27.5	72.5	0.379	Hex-rich	Ornitho	Asterids	Lamiids	Lamiales	Lamiaceae
Carmichaelis australis	29.6	70.4	0.420	Hex-rich	Generalist	Rosids	Fabids	Fabales	Fabaceae
Carpodetus serratus	32.9	67.1	0.491	Hex-rich	Entomo	Asterids	Campanulids	Asterales	Rousseaceae
Clianthus maximus	39.0	61.0	0.639	S-rich	Ornitho	Rosids	Fabids	Fabales	Fabaceae
Cordyline banksii	39.6	60.4	0.655	S-rich	Entomo	Lilioids	Lilioids	Asparagales	Asparagaceae
Elaeocarpus dentatus	40.3	59.7	0.676	S-rich	Entomo	Rosids	Fabids	Oxalidales	Elaeocarpaceae
Geniostoma rupestre	40.7	59.3	0.686	S-rich	Generalist	Asterids	Lamiids	Gentiales	Loganiaceae
Griselinia lucida	40.9	59.1	0.692	S-rich	Entomo	Asterids	Campanulids	Apiales	Griseliniaceae
Knightia excelsa	41.3	58.7	0.704	S-rich	Ornitho	Basal	Basal	Proteales	Proteaceae
Pittosporum crassifolium	42.4	57.6	0.737	S-rich	Entomo	Asterids	Campanulids	Apiales	Pittosporaceae
Pittosporum eugenioides	42.8	57.2	0.748	S-rich	Entomo	Asterids	Campanulids	Apiales	Pittosporaceae
Pittosporum ralphii	43.5	56.5	0.769	S-rich	Entomo	Asterids	Campanulids	Apiales	Pittosporaceae
Pittosporum tenuifolium	45.4	54.6	0.831	S-rich	Entomo	Asterids	Campanulids	Apiales	Pittosporaceae
Rhopalostylis sapida	45.6	54.4	0.837	S-rich	Entomo	Commelinids	Commelinids	Arecales	Arecaceae
Sophora chathamica	46.0	54.0	0.852	S-rich	Ornitho	Rosids	Fabids	Fabales	Fabaceae
Sophora longicarinata	46.8	53.2	0.879	S-rich	Ornitho	Rosids	Fabids	Fabales	Fabaceae
Sophora microphylla	48.8	51.2	0.952	S-rich	Ornitho	Rosids	Fabids	Fabales	Fabaceae
Sophora prostata	48.8	51.2	0.952	S-rich	Ornitho	Rosids	Fabids	Fabales	Fabaceae
Sophora tetraptera	52.5	47.5	1.105	S-rich	Ornitho	Rosids	Fabids	Fabales	Fabaceae
Weinmannia racemosa	52.5	47.5	1.105	S-rich	Generalist	Rosids	Fabids	Oxalidales	Cunoniaceae
Aristotelia serrata	59.8	40.2	1.490	S-rich	Entomo	Rosids	Fabids	Oxalidales	Elaeocarpaceae
Cordyline australis	70.0	30.0	2.329	S-rich	Entomo	Lilioids	Lilioids	Asparagales	Asparagaceae
Metrosideros colensoi	73.9	26.1	2.829	S-rich	Generalist	Rosids	Malvids	Myrtales	Myrtaceae
Sophora godleyi	91.3	8.7	10.455	S-rich	Ornitho	Rosids	Fabids	Fabales	Fabaceae

Variation in Non-Carbohydrates

I quantified non-carbohydrates using LC-MS, based on the mean abundances of components relative to dry weight, with mean abundances of ALK ranging between 0 - 1193 ppm, AA between 146 - 47,000 ppm, PHE between 34 - 37,000 ppm (Figure 4, Table 10) and VIT ranging between 35 - 2,645 ppm among tested species (n = 40). These compounds comprised between 0.04 - 8.13% of total solubles among species, excluding unspecified non-carbohydrates (0 - 1.3%).

In particular (Figure 4), I detected the ALK (in decreasing order of their relative abundance within their group): anagyrine, cytisine, sparteine, n-methyl cytisine, ammodendrine, and lupinine, with the three most abundant ALK ranging from 0.026 - 2%.
Additionally, I detected the AA: valine, proline, arginine, glutamic acid, glutamine, GABA, isoleucine and leucine, histidine, phenylalanine, asparagine, lysine, serine, tyrosine, methionine, and aspartic acid, with the three most abundant AA ranging from 0.2 - 0.4%.
Moreover, I found the PHE: 3-O-feruloyl quinic acid, gentisic acid, apigenin, kaempferol, rutin, quercetin, vitexin, coumaric acid, luteolin, aromadendrin, and cinnamic acid, with the five most abundant PHE ranging from 32 - 193 ppm.
Lastly, I detected the four VIT: niacin, ascorbic acid, pantothenic acid, and riboflavin, of which the first two showed the highest abundances, with 110 and 75 ppm.

Among species, the highest overall relative abundances of ALK were detected in *Sophora godleyi* at 0.12%, of AA in *Melicytus ramiflorus* at 4.7%, of PHE in *Lophopmyrtus bullata* at 3.7%, and of VIT in *Rhabdothamnus solandri* with 0.3% (Table 10).

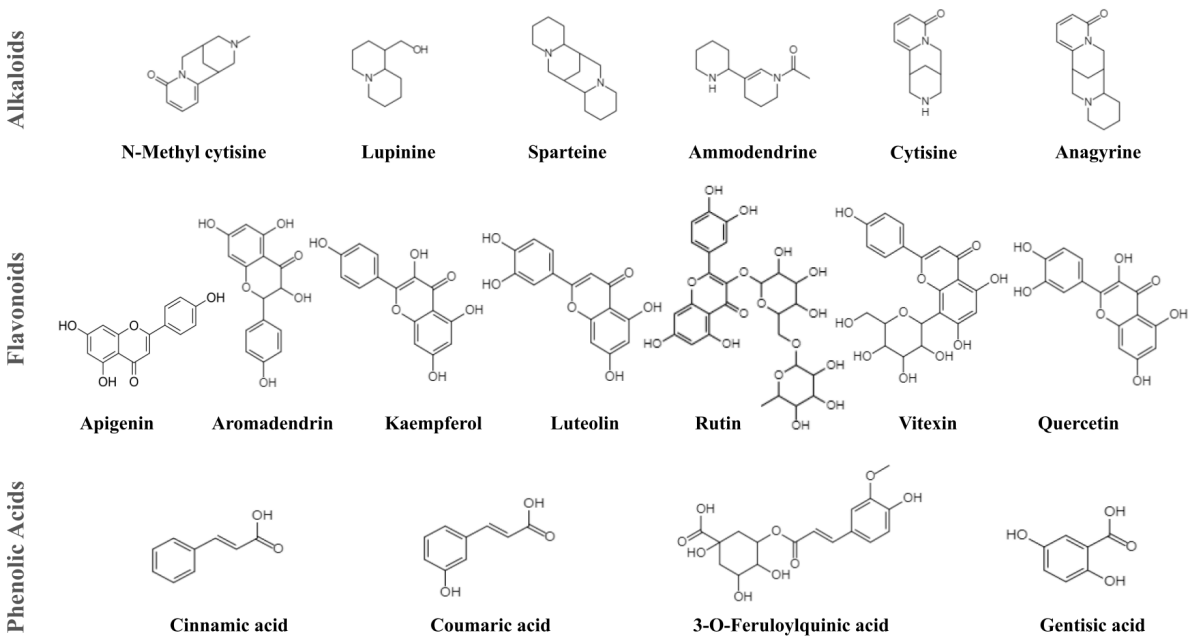

Figure 4: Structures of alkaloids (top row) and phenolics (flavonoids and phenolic acids) identified in the nectar of my tested New Zealand tree species (n = 40) (structures from Rutz et al., 2022).

Table 10: List of species ranked by the highest quarter (10 of 40 LC-MS analysed species) of relative abundances (% dry weight) of total alkaloids (ALK), amino acids (AA), phenolics (PHE), and vitamers (VIT), sorted in decreasing order.

SPECIES	ALK %	SPECIES	AA %	SPECIES	PHE %	SPECIES	VIT %
SOP GOD	0.1193	MEL RAM	4.69	LOP BUL	3.66	RHA SOL	0.26
SOP CHA	0.0974	LOP BUL	4.36	RHA SOL	3.36	FUC EXC	0.12
SOP MIC	0.0643	PSE CRA	3.40	KUN ERI	1.98	MEL RAM	0.11
SOP TET	0.0341	LOP OBC	1.42	LOP OBC	1.63	PIT COL	0.11
RHA SOL	0.0235	SOP TET	1.31	LEP SCO	1.14	LOP BUL	0.11
SOP LON	0.0036	MEL TER	0.88	MEL TER	0.97	GRI LIT	0.10
PIT RAL	0.0024	SOP GOD	0.63	PSE CRA	0.54	KNI EXC	0.09
LOP BUL	0.0021	RHA SOL	0.63	MYO LAE	0.50	LOP OBC	0.09
MEL RAM	0.0006	COR BAN	0.34	VIT LUC	0.49	MEL TER	0.08
PSE CRA	0.0006	COR AUS	0.33	PSE COL	0.35	PSE CRA	0.07
				Ranges of the Remaining 30 species:			
0.00003 -	0.0006	0.01 -	0.24	0.003 -	0.24	0.004 -	0.06

4.3.2 Correlations

Spearman correlation matrices (Figure 5, Table S4 - 5), supported by subsequent generalised additive model (GAM) (Figure 6, Table S6), non-parametric ANOVA, and phylogenetic signal analyses (Figure 10 shows all significant traits displayed on a phylogenetic tree, Table 16 with statistical parameters), revealed a complex network of associations among plant traits, pollination syndromes, and phylogeny.

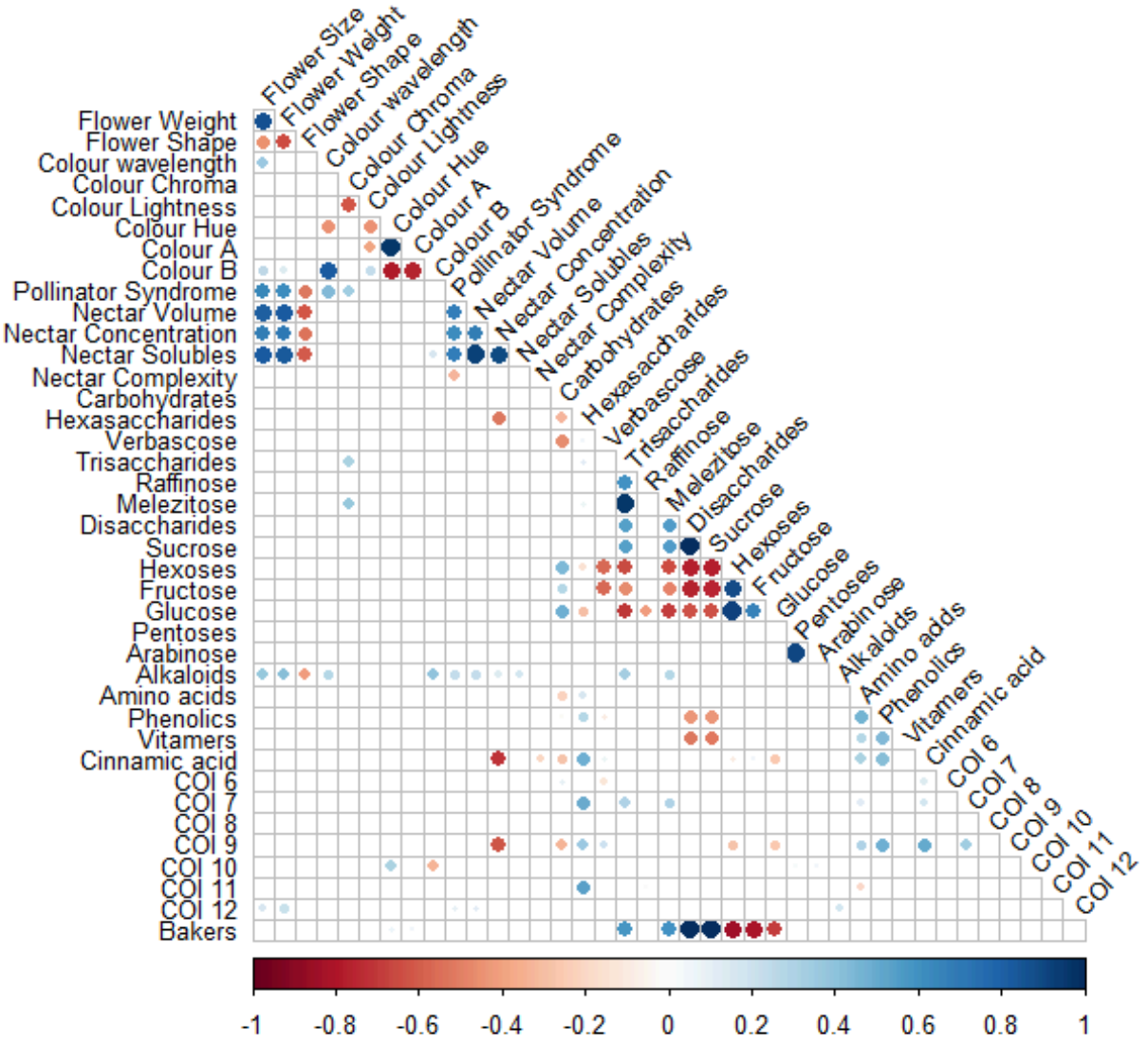

Figure 5: Spearman's correlation matrix of nectar traits (variables with at least 20 finite values) and predictor variables of 39 species. Nectar data is based on LC-MS (alkaloids, amino acids, phenolics, vitamers) and HPLC (other nectar variables) analysis, with 'Pollinator Syndrome' values set as 0 ~ entomophilous, 50 ~ generalist, 100 ~ ornithophilous (based on Kelly et al., 2010); 'Flower Shape' values defined by accessibility and set as 2 ~ flag-, 3 ~ gullet-, 4 ~ bell- or funnel-, 5 ~ brush-, 6 ~ dish-shaped flowers, with no tube-shaped flowers in my dataset (~ 1); 'Nectar Complexity' = number of HPLC-detected components.

Correlations among Nectar Traits

The analysis revealed strong correlations among nectar traits (Table 11), with absolute values of Spearman's correlation coefficients ($|r_s|$) ranging from 0.2 to 0.7. Nectar volume was positively correlated with nectar concentration, total solubles, and ALK content. Hexose-rich nectar lacked larger sugars. Conversely, sucrose-rich nectars contained an abundance of di- and trisaccharides, especially melezitose, but were deficient in PHE and VIT. Hexasaccharides (notably COI 9), AA, PHE, VIT, and cinnamic acid tend to co-occur and were more commonly found in nectars with low concentration.

Table 11: Extract (full tables S4-5 in Supplementary Material) of significant correlations among nectar variables based on Spearman's correlation coefficients $|r_s| > 0.2$ for the variable matrix shown in Figure 5.

		NECTAR CON-CENTRATION	HEXA-SACCHAR-IDES	COI 7	COI 9	DI-SACCHAR-IDES	SUCROSE	HEX-OSES	ALK	AA	PHE	VIT	CINNA-MIC ACID
Nectar Volume	*P*	<0.001							0.001				
	r□	0.684							0.233				
Nectar Concentration	*P*		0.007		0.017								0.029
	r□		-0.528		-0.625								-0.712
Carbohydrates	*P*		<0.001		<0.001			0.012		0.021			<0.001
	r□		-0.329		-0.332			0.441		-0.211			-0.280
Hexasaccharides	*P*			<0.001	<0.001						0.016		0.000
	r□			0.495	0.352						0.276		0.488
Verbascose	*P*							0.019					
	r□							-0.568					
Trisaccharides	*P*			0.012		0.003	0.005	<0.001	0.005				
	r□			0.305		0.547	0.546	-0.651	0.324				
Sucrose	*P*							<0.001			0.018	0.001	
	r□							-0.772			-0.433	-0.519	
Hexoses	*P*				0.014								
	r□				-0.276								
AA	*P*				<0.001						0.004	0.017	0.002
	r□				0.294						0.468	0.277	0.314
PHE	*P*				0.001							<0.001	0.027
	r□				0.481							0.439	0.427
Cinnamic acid	*P*				<0.001								
	r□				0.506								

Correlations between Nectar and Floral Traits

Based on trends detected using Spearman's correlation (Table 12), nectar volume, concentration, total solubles, and alkaloids were higher in larger, heavier, less accessible (such as gullet/flag-shaped), ornithophilous flowers, with ALK also higher in flowers with higher wavelengths and colour b*-values (based on CIELAB). Additionally, nectar complexity (defined as the number of detected HPLC compounds) was higher in entomophilous species. Trisaccharides, especially melezitose, were more concentrated in flowers with high chroma values. COI 10 was higher in flowers with high colour hue and b* value. Lastly, COI 12 was significantly higher in large, heavy and ornithophilous flowers.

Table 12: Extract (full tables S4-5 in Supplementary Material) of significant correlations between nectar variables and predictors (floral traits) based on Spearman correlation coefficients $|r_s| > 0.2$ for matrix shown in Figure 5. Flower shape is based on scaling floral accessibility from 1-5, with 1 ~ highly accessible (e.g. dish-shaped) and 5 ~ hardly accessible (see Chapter 1, 1.5.1 *Flower Shape*).

		NECTAR VOLUME	NECTAR CONCENT-RATION	NECTAR SOLUBLES	NECTAR COMPLEXICITY	TRI-SACCHA-RIDES	MELE-ZITOSE	ALK	COI 10
Flower Size	*P*	<0.001	<0.001	<0.001				<0.001	
	r□	0.818	0.689	0.829				0.376	
Flower Weight	*P*	<0.001	<0.001	<0.001				<0.001	
	r□	0.826	0.719	0.834				0.417	
Flower Shape	*P*	<0.001	<0.001	<0.001				0.003	
	r□	-0.618	-0.539	-0.603				-0.412	
Colour Wavelength	*P*							0.035	
	r□							0.273	
Colour Chroma	*P*					0.019	0.016		
	r□					0.315	0.372		
Colour Hue	*P*								0.020
	r□								0.316
Colour b*	*P*			0.033				0.012	0.044
	r□			0.153				0.392	-0.335
Pollinator Syndrome	*P*	<0.001	<0.001	<0.001	0.029			<0.001	
	r□	0.675	0.628	0.677	-0.328			0.231	

GAM analysis (Figure 6, Table S6) further confirmed these results and clearly showed that larger flowers were heavier ($P < 0.001$; $R^2 = 0.76$) and tended to produce higher nectar volumes ($P < 0.001$; $R^2 = 0.63$) with higher soluble concentrations ($P < 0.001$; $R^2 = 0.5$), in particular, based on higher levels of total carbohydrates ($P < 0.05$; $R^2 = 0.14$) and ALK ($P < 0.01$; $R^2 = 0.2$) associated with more concentrated nectars ($P < 0.001$; $R^2 = 0.32$ - 0.39) in comparison to smaller, lighter flowers. Lastly, less concentrated nectar contained slightly lower total concentrations of PHE ($P < 0.01$; $R^2 = 0.26$), when removing one outlier.

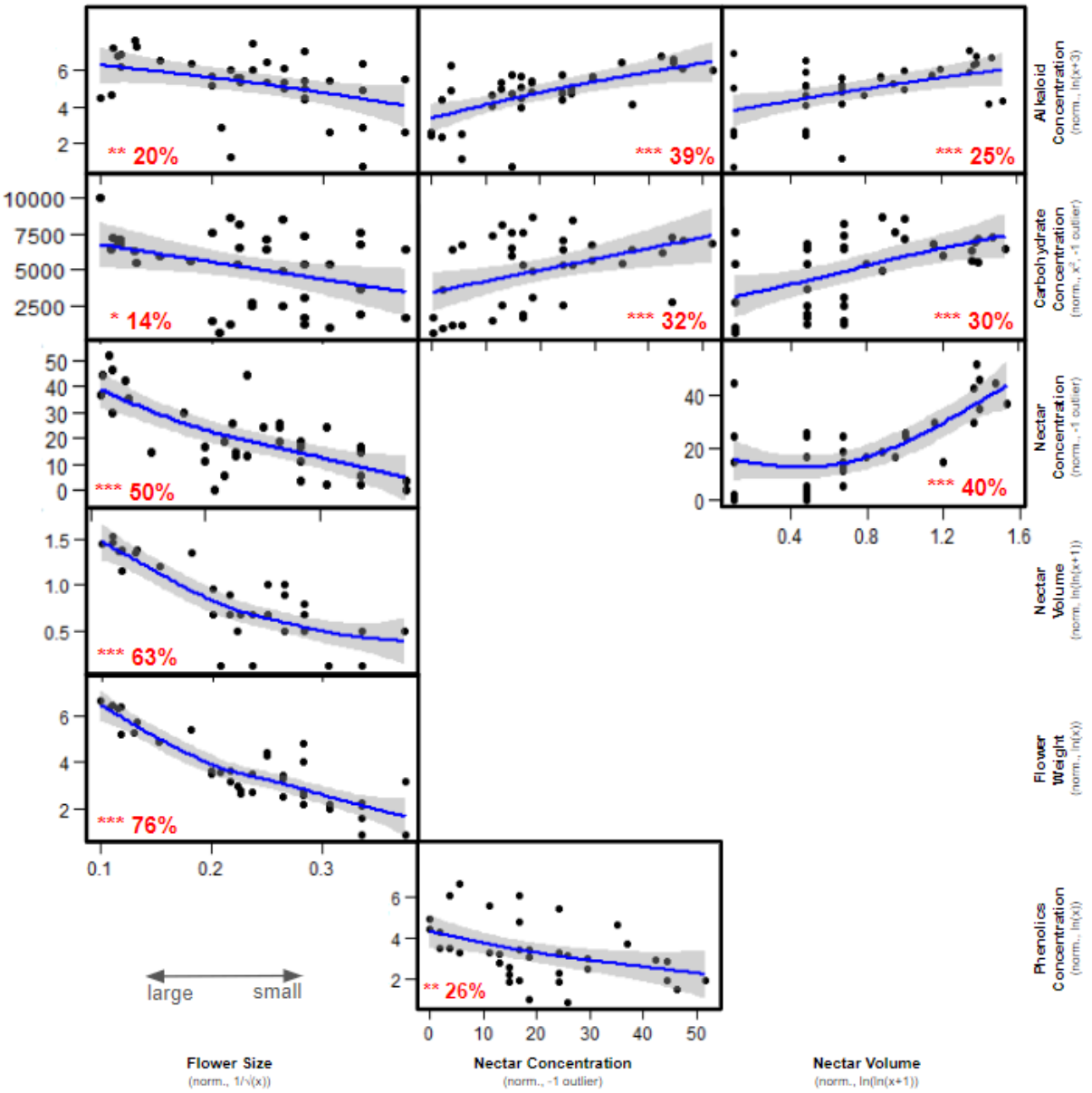

Figure 6: Scatterplots with fitted Generalised Additive Models (GAM) and confidence intervals illustrating significant correlations between floral traits and nectar composition (% deviance explained, with significance levels indicated by * to *** for *P*-values ranging from < 0.05 to < 0.001, see Table S5). The term 'norm.' refers to normalised values scaled from 0 - 100. Traits were transformed for normality either using the natural log or x^2. Transformations were applied to both *x* and *y* variables.

Flower Shape

Nectar traits varied significantly among my five tested flower shapes (after Faegri & van der Pijl, 2013; Figure 7, Table 13). Nectar volume, solubles, concentration, and nectar complexity varied significantly among flower shapes. This variation also extended to specific components, including hexasaccharides, ALK, PHE, and COI 9. On average, nectar volumes were highest in the flag- and gullet-shaped flowers; nectar solubles, concentrations, and ALK contents in gullet-shaped flowers; PHE in flag; the number of nectar components and hexasaccharides in dish-shaped, and lastly, COI 9 in brush-shaped flowers. Moreover, certain components were exclusively associated with specific flower shapes: ribose was only detected in dish-, brush-, and bell/funnel-shaped flowers. DHA was absent in gullet-shaped flowers. COI 2 and 5 appeared solely in bell/funnel- and dish-shaped flowers, COI 1, 3, 8, and 10 only in dish-shaped flowers, and COI 4 in flag-, bell/funnel-, and dish-shaped flowers. Furthermore, I could confirm Percival's (1961) association between tubular flowers (including flag-, gullet-, bell-, and funnel-shaped) having sucrose-rich nectars and the propensity for 'open' flowers (dish- and brush-shaped) to have hexose-rich nectars.

Table 13: Kruskal-Wallis tests for differences in nectar traits between flower shape categories, with bold post-hoc Dunn's test *P*-values (with Bonferroni correction) denoting statistical significance.

NECTAR TRAIT	*n*	χ^2	*P*	POST-HOC DUNN'S TEST *P*-VALUES FOR PAIRS									
				Flag vs. gullet	Flag vs. bell	Flag vs. brush	Flag vs. dish	Gullet vs. bell	Gullet vs. brush	Gullet vs. dish	Bell vs. brush	Bell vs. dish	Brush vs. dish
Nectar Volume	50	16.3	<0.01	0.3	**0.059**	0.1	**0.008**	**0.003**	**0.01**	0	0.3	**0.1**	0.06
Nectar Solubles	50	18.2	<0.01	0.3	**0.04**	0.08	**0.003**	**0.004**	**0.01**	**0.0000**	0.4	0.08	**0.04**
Nectar Concentration	50	17.3	<0.01	0.5	**0.02**	**0.04**	**0.001**	**0.01**	**0.02**	**0.0001**	0.4	0.1	0.1
Nectar Complexity (number of components)	50	14.9	<0.01	0.5	0.2	0.3	**0.03**	0.1	0.3	**0.007**	**0.02**	0.1	**0.0003**
Hexasaccharides	50	10.3	<0.05	0.1	0.2	0.2	0.2	**0.004**	**0.004**	**0.004**	0.5	0.4	0.4
COI 9	50	10.7	<0.05	0.4	**0.02**	**0.003**	**0.02**	**0.05**	**0.009**	0.1	0.1	0.4	**0.04**
ALK	40	10.7	<0.05	**0.04**	0.2	0.3	0.3	**0.04**	**0.01**	**0.001**	0.3	0.1	0.3
PHE	40	9	0.05	**0.05**	0.1	0.2	0.3	0.2	0.1	**0.003**	0.3	**0.03**	0.1
Tubular versus open flowers:													
Sucrose	50	10.7	0.03										
Hexoses	50	9.4	0.05										

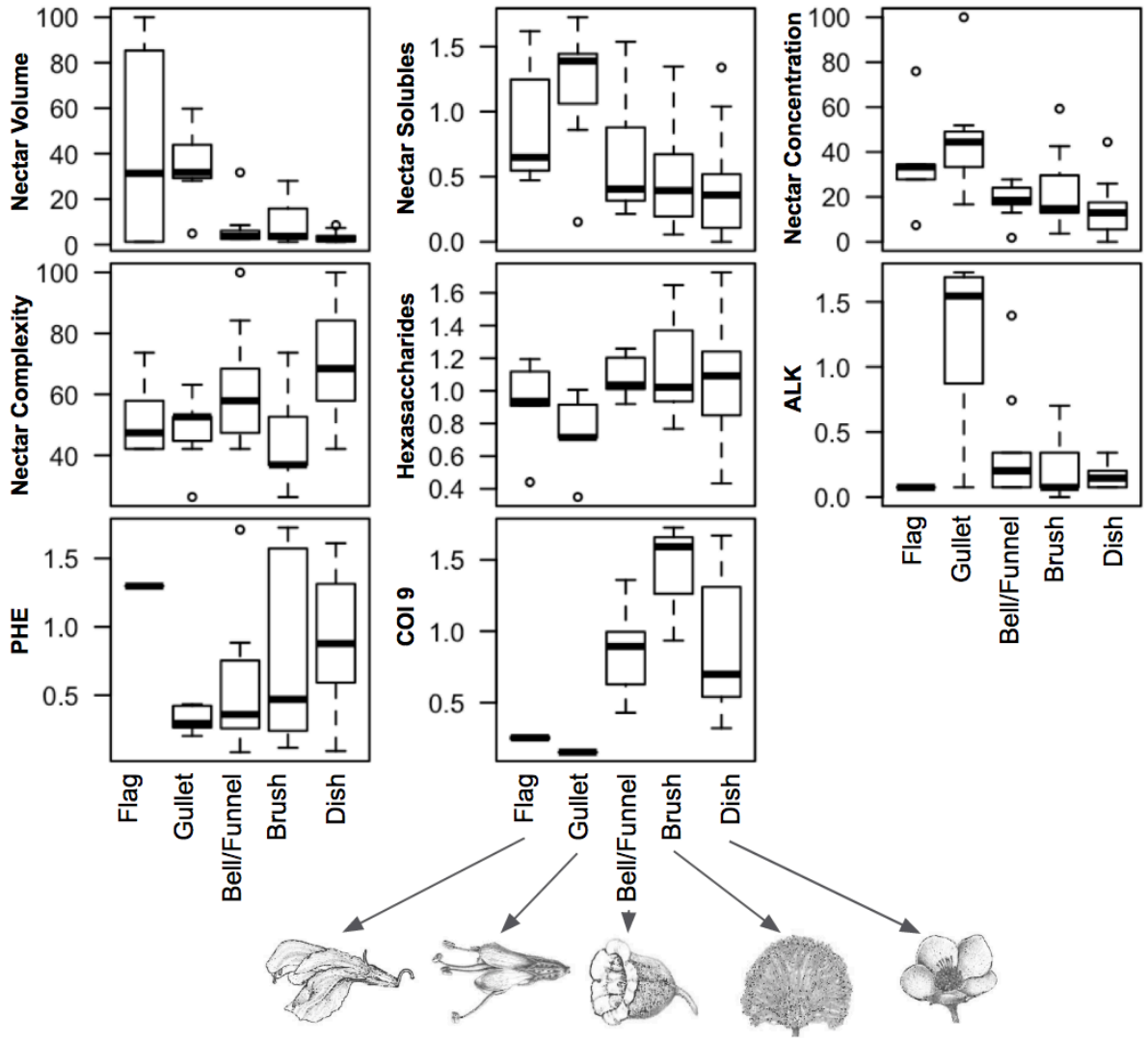

Figure 7: Boxplots displaying nectar traits that vary significantly with flower shape, ranging from hardly accessible (flag) to highly accessible (dish). All trait values were standardised on a scale of 0 - 100. Most traits, except for nectar volume, complexity, and concentration, have been transformed using the natural log function to facilitate interpretation.

Flower Colour

I observed significant differences in nectar traits by flower colour (Figure 8, Table 14) based on human vision colour grouping (Backhaus, 1998), following Spearman correlation and Kruskal-Wallis tests using calculated colour categories. Nectar volume was notably higher in yellow, orange, and red flowers; nectar concentration levels were elevated in red/pink and yellow flowers; ALK in orange, yellow and yellow-green flowers; Melezitose in yellow and yellow-green flowers; hexasaccharides in yellow-green, green, purple and white flowers; and VIT in orange, green, purple and white flowers. Furthermore, I observed a distinct pattern of component absence across flower colours: Orange flowers lacked COI 2 - 5, 7, 9, 12, DHA, and the PHE cinnamic acid. Yellow flowers were missing DHA, cinnamic acid, and COI 2, 3, and 5. These COI were also absent in red/pink flowers, while purple flowers exclusively lacked COI 3. In contrast, green flowers were only deficient in DHA. COI 8 was uniquely present in red/pink and white flowers and COI 10 was found only in green and red/pink flowers.

Table 14: Tests for significant nectar trait variation across flower colours. Level comparisons indicate the results of Dunn's pairwise multiple comparison tests (with Bonferroni correction).

NECTAR TRAIT	HIGHER LEVELS	LOWER LEVELS	COVARIANCE		
			Kruskal-Wallis Test		Dunn's Test
			P-value	χ^2	*P*-value range
Volume	yellow, orange, red	> green, white, purple	0.003	20.1	0.05 to 0.0001
Concentration	red/pink, yellow	> all but orange	0.008	17.5	0.05-0.0005
Alkaloids	orange yellow yellow green	> red/pink, white > green, purple > red/pink	0.003	20	0.04-0.000
Hexasaccharides	green red/pink, purple, white, yellow green	> yellow, red/pink > yellow	<0.05	13.5	0.05-0.0004
Vitamers	orange, green purple, white	> red/pink, white, yellow > red/pink	<0.05	13.8	0.05-0.002
Melezitose	green orange yellow	> orange, white > yellow, yellow green > all but green	<0.05	13.5	0.05-0.004

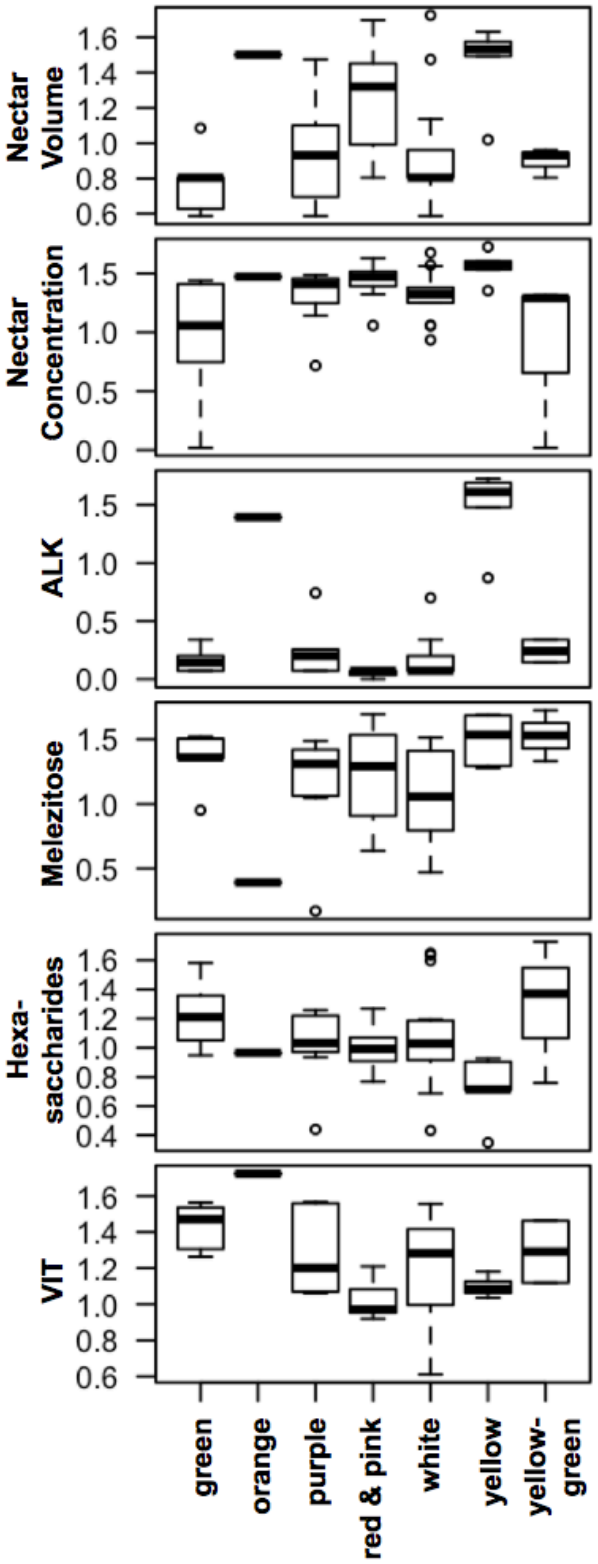

Figure 8: Boxplots of significantly varying nectar traits by flower colour. Trait values were scaled (0 - 100) and transformed using the natural log to aid interpretation.

Pollinator Syndrome

ANOVA analysis (Table 15) demonstrated that the pollinator syndrome of a species (Figure 9) significantly influences nectar volume, concentration, solubles, complexity, hexasaccharides and ALK content, with ornithophilous species displaying elevated levels compared to others, except for nectar complexity and hexasaccharides.

Entomophilous species showed a higher nectar complexity, mainly based on a higher diversity and concentration of hexasaccharides. Additional findings partially corroborate the observation by Baker & Baker (1990) regarding ornithophilous species being more likely to have sucrose-rich nectar and entomophilous species to have hexose-rich nectar. Specifically, among the ornithophilous species, 57% (8 out of 14) showed sucrose-rich nectar, while among the entomophilous species examined, 63% (17 out of 27) exhibited hexose-rich nectar (Table 9). Nectar in generalist species was equally likely to be sucrose- or hexose-rich. However, the mean Baker's ratio (n = 50) did not show significant differences across pollinator syndromes.

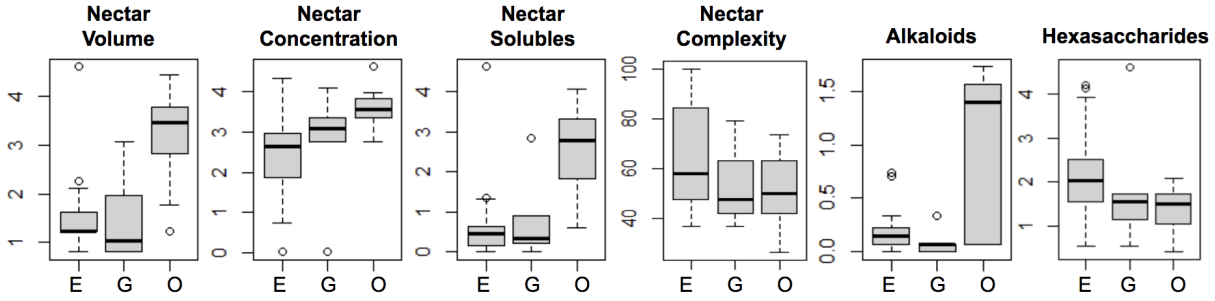

Figure 9: Significant differences between nectar and floral traits (scaled data, with all traits transformed using the natural log function except for nectar complexity) and pollinator syndromes. The nectar of the entomophilous sister taxa *Pittosporum eugenioides* and *P. tenuifolium* each contain 100% of the total HPLC compounds detected. Pollinator syndromes are denoted as 'E' for entomophilous, 'G' for generalists, and 'O' for ornithophilous species. Species' pollinator syndromes were defined according to Kelly et al. (2010).

Table 15: Effects and post-hoc test parameters for nectar trait variation across pollinator syndromes, using the Kruskal-Wallis test ('KW') or ANOVA ('A'), with 'E' = entomophilous, 'G' = generalists, 'O' = ornithophilous species, Tukey's HSD = Tukey's Honest Significant Difference, and bold *P*-values denoting statistical significance, Dunn's tests with Bonferroni correction.

NECTAR TRAIT	*n*	χ2 or F	*P*-VALUE	TEST	POST-HOC TEST *P*-VALUES FOR PAIRS			TEST
					E vs. O	E vs. G	O vs. G	
Nectar Volume	50	21.1	**<0.001**	KW	**<0.001**	0.3	**<0.001**	Dunn's Test
Nectar Concentration	50	17.9	**<0.001**	KW	**<0.001**	0.1	**0.05**	Dunn's Test
Nectar Solubles	50	21.6	**<0.001**	KW	**<0.001**	0.5	**0.002**	Dunn's Test
Nectar Complexity	50	3.4	**0.04**	A	**0.05**	0.3	0.9	Tukey's HSD
ALK	40	7.8	**0.02**	KW	**0.03**	0.1	**0.004**	Dunn's Test
Hexasaccharides	50	8.5	**0.01**	KW	**0.002**	0.08	0.29	Dunn's Test
Baker's Ratio	50	1.0	0.62	KW	-	-	-	-

Phylogenetic Signals in Nectar and Floral Traits

Based on the species examined, I detected strong phylogenetic signals (Table 16) in nectar volume and solubles, flower weight and size, pollination syndrome and the component ribose. Furthermore, I found strong signals in glucose, sucrose, and flower colour parameters. Weaker signals were detected in raffinose, melezitose, and ALK, and a significant but very weak one was detected in nectar concentration. Most tested floral traits were more similar in phylogenetically closely than in distantly related species. Given the limited number of species representing each phylogenetic group in my study, these results should be considered indicative trends and interpreted cautiously. Relative floral trait sizes are displayed along a phylogenetic tree in Figure 10 to compare interspecific trait variation directly.

Table 16: Log-likelihood (logL) values for different lambda (λ) parameters within linear regression (LR) models. Specifically, LR(λ=0) denotes a standard linear regression with no regularisation applied, serving as a baseline for comparison. Higher LogL(λ) values indicate a better fit of the model to the observed data. Variables are sorted by Pagel's lambda and *P*-values, which indicate the statistical significance of the Likelihood-ratio (LR) tests.

TRAIT	λ	logL(λ)	LR(λ=0)	*P*	DETECTED IN *n* SPECIES	*n* SPECIES TESTED
Pollinator Syndrome	1.00	-237	46.9	7.4E-12	all	50
Flower Weight	1.00	-209	45.9	1.3E-11	all	50
Flower Shape	1.00	-68	33.0	9.1E-09	all	50
Flower Size	1.00	-211	32.7	1.1E-08	all	50
Nectar Solubles	1.00	-203	20.4	6.2E-06	all	50
Nectar Volume	0.99	-210	27.3	1.7E-07	all	50
Ribose	0.97	-195	27.9	1.3E-07	18	50
Baker's Ratio	0.95	-193	26.9	2.2E-07	all	50
Flower Colour Hue (CIE)	0.90	-237	8.5	0.0036	all	50
Flower Colour a* (CIE)	0.86	-236	8.4	0.0037	all	50
Glucose	0.72	-226	7.1	0.0076	all	50
Flower Colour Chroma (CIE)	0.71	-249	3.9	0.0481	all	50
Flower Colour b* (CIE)	0.69	-243	5.6	0.0181	all	50
Sucrose	0.65	-237	10.2	0.0014	all	50
Raffinose	0.56	-223	5	0.0253	31	50
ALK	0.51	-173	12.5	0.0004	35	40
Melezitose	0.46	-227	4.5	0.0338	48	50
Nectar Concentration	0.34	-216	6.4	0.0115	all	50

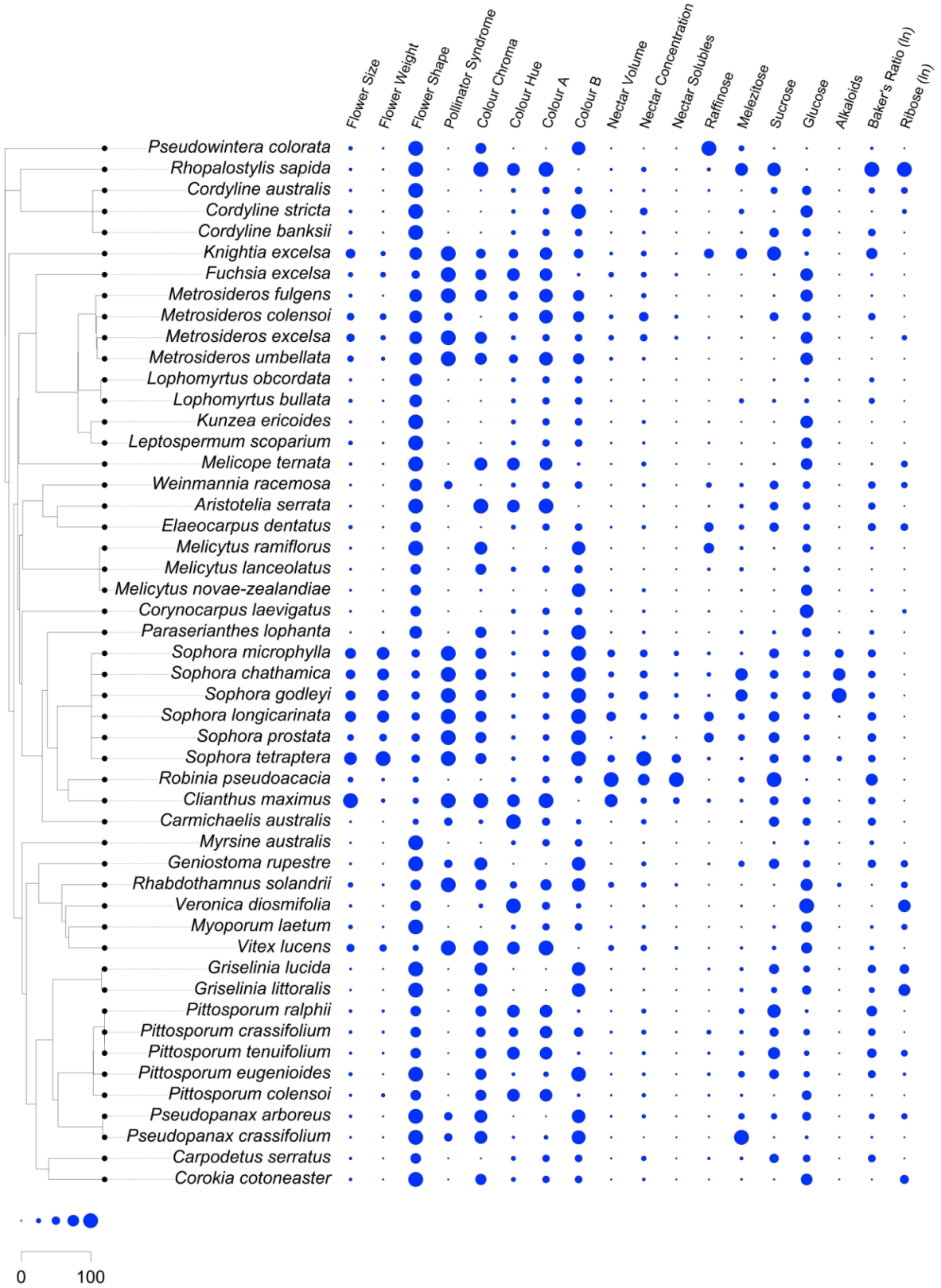

Figure 10: Phylogenetic tree (derived from a supertree using V.PhyloMaker in R) of the species analysed by HPLC ($n = 50$) and LC-MS (only alkaloids, $n = 40$), with dot size representing the average trait value on a standardised scale (0-100). Only traits exhibiting significant phylogenetic signals are displayed, with all traits $\lambda > 0.5$, except for nectar concentration at $\lambda = 0.3$). For 'Pollinator syndrome', entomophilous is scored as 0, generalists are scored as 50, and ornithophilous is scored as 100. For 'Flower Shape', flag is scored as 0, gullet as 33, bell/funnel as 67, brush as 83, and dish as 100, based on their accessibility. 'Baker's ratio (ln)' and 'Ribose (ln)' values were transformed using the natural log function to aid interpretation.

Nectar volume differed significantly (KW, χ^2 = 23.4, DF = 14, P = 0.05) between orders, with tested Fabales higher in volumes than Apiales, Asparagales, Asterales, Ericales, Gentiales, Malphigiales and Oxalidales (Dunn's test, $P < 0.05$).

Nectar concentrations did not differ across any phylogenetic level above species, indicated by the weak phylogenetic signal.

Nectar solubles differed significantly on the order level, with tested Fabales nectar containing more solubles than Cannelales, Ericales, Malpighiales and Oxalidales, as well as Lamiales containing more than Malpighiales (KW, χ^2 = 23.1, DF = 14, P = 0.05).

Sucrose (λ = 0.65, P = 0.01), ranging from 0.24 - 72%, differed significantly on the subclade level (KW, χ^2 = 15.2, DF = 8, P = 0.05), based on lower concentrations in Malvids than in Fabids, Campanulids, Commelinids and Proteaceae (Dunn's-test, $P \leqq 0.01$). I found sucrose-dominated (with S = 38 - 72% of additive amounts of S, G, F and O) nectar in 20 species across seven subclades, with Fabids and Campanulids comprising 75% of sucrose-dominant species.

Glucose (λ = 0.72, $P < 0.01$), ranging from ~ 2 - 58%, differed high significantly on the order level (ANOVA, F = 3.7, DF = 14, $P < 0.001$), with higher concentrations in Lamiales than in Apiales, Fabales, Cannelales and Arecales, which comprise 46% of species (Tukey HSD test, $P < 0.05$). I found glucose-dominated nectar (with G = 41 - 58% of additive amounts of S, G, F and O) in 15 species across seven orders, comprised of 73% Myrtales and Lamiales.

Baker's ratio (λ = 0.95, P = 0.001), ranging from 0.003 - 10.68, differed significantly on the subclade level (KW, χ^2 = 16.5, DF = 8, P = 0.036), with lower Baker's values in Malvids than in Campanulids, Fabids, Basal Eudicots and Commelinids, which comprise 60% of species (Dunn's test, P = 0.01 - 0.001).

Raffinose (λ = 0.56, $P < 0.05$), present in 31 spp. ranging from 0.002 - 1.95%, was significantly different on the subclade level (KW, χ^2 = 18.8, DF = 8, P = 0.01) and greater in Magnoliids, Fabids and basal eudicots than in Malvids. The magnoliid (*Pseudowintera*

colorata) was also richer in raffinose than Lamiids, Lilioids and Asterids (Dunn's test, $P < 0.05$).

Melezitose ($\lambda = 0.46$, $P < 0.05$), present in 48 spp. (missing in *Lophomyrtus obcordata* and *Myrsine australis*) ranging from 0.01 - 4.9%, differed high significantly on the subclade level (KW, $\chi^2 = 23.4$, DF = 8, $P < 0.01$,), based on higher concentrations in the Commelinid (*R. sapida*), Campanulids and basal eudicots than in Malvids and Asterids (Dunn's test, $P < 0.05$) and Fabids higher than Malvids ($P < 0.001$).

Ribose ($\lambda = 0.97$, $P < 0.001$) was detected in 18 spp. (10 asterids, 4 rosids, 3 monocots) with concentrations of 0.006 - 0.88%. The sugar differed significantly on the subclade level (KW, $\chi^2 = 16.4$, DF = 8, $P < 0.05$), with the Commelinid, Campunilids and Lamiids higher in concentrations than Fabids. The Commelinid sp. and Lamiids were higher in ribose than Malvids (Dunn's test, $P \leqq 0.01$).

ALK ($\lambda = 0.51$, $P < 0.001$), present in 35 of the 40 tested species with mean concentrations of 1 – 1193 ppm, differed significantly on the phylogenetic order level (KW, $\chi^2 = 20.5$, DF = 12, $P = 0.058$). I detected higher concentrations in the tested Fabales (*Sophora* spp.) in comparison with 70% of other orders, particularly Lamiales, Proteales, Gentiales, Myrtales, Apiales, Malphigiales, Oxalidales, and Asparagales (all < 25 ppm) (Dunn's test, $P < 0.05$).

Lastly, I could confirm Percival's and Baker & Baker's shared hypothesis on nectar sugar types being more conserved in phylogeny than pollination syndrome. Regarding phylogenetic constraints, all Lamiids ($n = 4$) and Malvids ($n = 9$), except for one species (MET COL), and six out of eleven Campanulids exhibited hexose-rich nectar. Conversely, most Fabids (ten out of 15) and five Campanulids produced sucrose-rich nectar.

4.4 Discussion

This study demonstrated that New Zealand 9nz0trees' floral and nectar characteristics strongly correlate, aligning with global observations on pollinator syndromes (Faegri & van der Pijl, 2013). NZ flowers of the ornithophilous syndrome display large, brightly colour-saturated flowers, which produce nectar of high volume, concentration, total solubles and alkaloids. Native entomophilous tree species in NZ typically feature less conspicuously coloured flowers and produce lower volumes of nectar, with an elevated nectar complexity (number of detected compounds using HPLC) and hexasaccharide content.

The predominance of sucrose and hexoses and their ratio, the Baker's ratio [sucrose/(fructose + glucose)], is strongly influenced by phylogeny and exhibits limited variation due to pollination syndrome, aligning with historical findings (Percival, 1961; Baker & Baker, 1990). Qualitative nectar sugar classification systems, such as Percival's division into up to ten sugar subtypes (1961) limit interspecific comparisons. Hence, a more quantitative framework seems necessary to improve analytical reproducibility for future studies. While the Baker's ratio is a useful albeit restricted metric for delineating nectar sugar composition, it fails to differentiate dominance among hexoses or incorporate other significant nectar components. Notably, in 16% (eight out of 50) of the species examined, including *Lophomyrtus* spp.*, Melicytus ramiflorus, Myrsine australis, Pseudowintera colorata, Rhopalostylis sapida, Pseudopanax crassifolium*, and the naturalised *Paraserianthes lophanta*, concentrations of non-SGF components ranged from 25-72%. The traditional SGF classification (Percival, 1961; Baker & Baker, 1990) can alternatively be represented by simplified S to G to F ratios, such as '1:1:1'. This approach could be expanded to incorporate a fourth category, 'O', to account for the sum of other detected components, thereby encompassing hexa- and trisaccharides and additional di- and monosaccharides beyond S, G, and F. Such an enhancement would allow for more precise interspecific comparisons, facilitating quantitative statistical analyses. By adopting this more refined 'SGFO' classification, the depiction of nectar sugar types in species with high 'O' fractions, such as *L. bullata* and *M. australis,* shift notably (here from a 1:2:1 SGF to a more descriptive 1:2:1:6 SGFO ratio). The 'O' value typically ranges between 0.1 and 1 for 84% of the New Zealand species studied, therefore its inclusion in the SGF classification is crucial for more accurately describing nectar sugar types.

To integrate the 'O' variable, the Baker's ratio [S/(G+F)] needs to be expanded. The primary issue with the Baker's ratio is that it attempts to represent sugar variation in a

multidimensional space using a single index, resulting in inevitable information loss when measurements are condensed onto a single scale. For example, a very uneven ratio between G and F will not be identifiable based on the single number, based on the formula summing the two monosaccharides. However, collapsing S, G, and F into a single number is still logical because G and F are thought to be derived from S by invertase, supporting the simple logic behind the S/(G+F) formula. To refine the Baker's ratio, 'O' could be introduced as an additive factor that adjusts the ratio either upwards or downwards depending on its value. This method acknowledges the impact of 'O' on the sugar balance while still factoring it into the overall evaluation. In this revised formula, γ is a scaling factor that determines the extent of 'O's contribution to the overall score. The value of γ should be calibrated based on experimental data or literature regarding the relative impact of the nectar components included in 'O'.

$$New\ Ratio\ = \frac{S}{G+F} + \gamma O$$

However, since 'O' comprises various compounds potentially derived from different metabolic pathways and transporters, an increase in 'O' might dilute SGF without directly influencing the S/(G+F) ratio. Therefore, I suggest that γO should not be included in the index but rather noted as a separate index:

$$New\ Ratio\ = \frac{S}{G+F} \quad \text{with } \gamma O,$$

e.g. for *Pittosporum crassifolium,* with a ratio of ~ ‘40 : 21 : 33 : 5.5’ (with sugars in %), this would mean :

$$New\ Ratio_{\ \text{PIT CRA}} = \frac{40}{21+33} \text{ with } \gamma 5.5$$
$$= 0.741 \text{ with } \gamma 5.5.$$

Nevertheless, I personally prefer to compare the species' nectar sugar types based on their individual concentrations. This can be simplified by dividing each sugar's concentration by 10 and then rounding each value to the nearest whole number. In this approach, 'O' does not require a γ factor. For example, consider the species *Pittosporum crassifolium* (PIT CRA), which would result in a ratio of ‘4 : 2 : 3 : 1’ for S : G : F : O. This method allows for quick visual comparison between closely related species. For instance, *Pittosporum eugenioides* shares the same ratio as *Pittosporum crassifolium*, at 4 : 2 : 3 : 1, whereas *Pittosporum tenuifolium* presents a distinct ratio of 6 : 2 : 2 : 1.

Furthermore, I found that the relative abundance of non-carbohydrates was up to 9.4%, with identified compounds making up 8.1% of this total. This is in line with the findings of Lüttge (1977), who reported values up to 10%. The non-carbohydrate compounds identified in this study included amino acids (AA), alkaloids (ALK), phenolics (PHE), and vitamers (VIT), which were found in different amounts across the species examined. The distribution of ALK was notably influenced by the plant's phylogeny and pollinator syndrome. However, none of the predictors I tested could account for the interspecific variations observed in AA, PHE, and VIT. This limitation could be attributed to the study's focus on the total concentrations of these compounds rather than a more granular analysis. A detailed examination, for instance, of specific AA and their relationship to the actual pollinators visiting the species (as opposed to the generalised pollinator syndrome analysed in this study), might provide insights into the observed variability of these compound classes across different species.

4.4.1 Floral Traits Signal Level of Nectar Quantity, Energy Supply & Toxicity

Floral visual traits, including colour, shape, and size, are reliable indicators of various nectar characteristics such as volume, concentration, abundance of total carbohydrates, hexasaccharides and ALK of NZ tree flowers. These visual cues are often termed 'honest' signals (Armbruster et al., 2005; Knauer & Schiestl, 2015), reflecting the nature and quantity of nectar rewards. For example, large, less accessible flowers typically offer abundant nectar, whereas small, easily accessible flowers provide minimal nectar volumes. This pattern tends to be consistent across plant communities, including ~ 100 Mediterranean species (Ortiz et al., 2021). In NZ, the distinct floral traits of *Clianthus maximus* and *Griselinia littoralis* illustrate the concept of 'honest' signalling in floral characteristics, which directly correlate with nectar properties. Specifically, the bird-pollinated *C. maximus* features large, conspicuous, bright pink, flag-shaped flowers measuring 70 mm long and weighing 169 mg, producing around 70 µL of nectar daily. In contrast, *G. littoralis*, an insect-pollinated species, presents with small, unobtrusive, dish-shaped flowers that are white or green, have a diameter of 4 mm, and weigh an average of 3 mg, and only produce about 2 µL of nectar daily. These adaptations likely reflect the specific demands of their pollinators, emphasising the evolutionary responses to the size variance between bird and insect visitors. While the concept of 'honesty' in signalling may be subjective, analysing nectar yield in relation to flower size or weight would present a

quantitative approach, providing objective insights into the nectar productivity of a species in future studies.

Flower colour, for instance, of the yellow and orange pigmented NZ flowers with high ALK levels could indicate toxicity. However, the presence of ALK does not seem to be limited to a certain flower colour within a global context, as white, purple and red flowering species can also contain toxic levels of ALK, e.g., *Aconitum* spp. (Ameri, 1998), *Atropa belladonna* (Ashtiania & Sefidkonb, 2011), *Conium maculatum* (Vetter, 2004), *Datura* spp. (Cinelli & Jones, 2021), and *Delphinium* spp. (Shamma et al., 1979) just to name a few. It is more likely that ALK levels are higher in Fabaceae species, with many of them showing yellow pigmentation. As ALK showed phylogenetic constraints, this might be a logical conclusion.

Furthermore, bird-pollinated species in New Zealand exhibit significantly higher relative alkaloid levels in their nectar, averaging 312 ± 435 ppm (n = 11, range 1 - 1193 ppm), compared to insect-pollinated species, which average 4 ± 6 ppm (n = 21, range 1 - 24 ppm). Similarly, species with less accessible flower shapes tend to have higher alkaloid levels than those with more accessible flowers, which I expected as flower shape is a major factor in determining pollination syndromes. However, since phylogenetic factors strongly influence the alkaloid abundance and flower shape, the observed correlations may reflect species' evolutionary histories rather than direct adaptations to pollinator types. Current research, including larger cohort studies, is limited in assessing whether nectar alkaloid levels directly correlate with pollination syndromes on a global scale. Existing studies primarily examine the interaction between nectar alkaloids and pollinator behaviour, focusing on the attraction or repulsion of different pollinator guilds based on nectar ALK content and toxicity (e.g., Lerch-Henning & Nicolson, 2013; Nicolson et al., 2015).

Although energy-intensive, the production of secondary metabolites such as alkaloids offers considerable benefits to the plant. Alkaloids are often a defence mechanism against nectar robbers based on their bitter taste or toxicity (Wink & Twardowski, 1992; Adler, 2000; Johnson et al., 2006; Stevenson et al., 2017). For instance, kōwhai nectar (*Sophora* spp.), known for its high alkaloid content (McDougal et al., 2018), has been linked to narcosis and even death in honey bees (Clinch et al., 1972). Particular bird species are tolerant to these toxic substances (Banko et al., 2002), such as the main pollinators of NZ kōwhai (McEwen & McEwan, 1978; Campbell, 2006; Anderson et al., 2011), including the tūī (*Prosthemadera novaeseelandiae*) and the bellbird (*Anthornis melanura*). Recent studies confirmed alkaloids

can have antibacterial properties, reducing pathogen loads in nectar consumers – ultimately benefiting their health (Schmitt et al., 2021).

Moreover, NZ ornithophilous species secrete more concentrated nectars (21 ± 11%, ranging from 8 - 54%, n = 14) than their entomophilous counterpart (7 ± 4%, ranging from 0.01-14%, n = 27, exclusive naturalised species) and generalistic species (13 ± 11, ranging from 0.01 - 32%, n = 6). These results conflict with results from elsewhere (Baker, 1975). However, concentration levels of ornithophilous NZ species align with mean concentrations of 21 - 25% found in ornithophilous, specifically hummingbird-pollinated, species from other habitats, such as Puerto Rico (Kodric-Brown et al., 1984), Jamaica (Percival, 1974) or Costa Rica (Baker, 1975). However, Costa Rica's entomophilous species secrete nectar of more than twice the concentration of ornithophilous species (48%). Also, species growing in California exhibit higher nectar concentrations in entomophilous species, with averages of 31% versus 21% found in ornithophilous flowers (Baker, 1975). Interestingly, Jamaican flowering species do not significantly differ in nectar concentrations among pollination syndromes, even with predominantly honey bee-pollinated species not exceeding 30% and fly-associated flowers showing even lesser maximum values (23%). As New Zealand's pollinating insect fauna is strongly dominated by dipterans, with only a few solitary hymenopterans (40 spp.) and butterflies (16 spp.) next to moths, beetles and thrips (Heine, 1938) – there may have been a different selection pressure on New Zealand's insect-pollinated plant species towards lower nectar concentrations, particularly knowing NZ bees mainly gather pollen (Thomson, 1881, 1927; Heine, 1938).

However, based on my analysis of nectar traits at the individual flower level, volume emerges as the primary driver of total solubles and sugar content, with concentration playing a lesser, albeit still significant, role. Large flowers produce more total solubles per flower, primarily due to their higher nectar volumes. In contrast, small flowers physically cannot contain or produce large amounts of sugar.

A critical variable missing from my ecological analysis is the number of individual flowers per inflorescence or tree, which was not investigated. For instance, large New Zealand flowers such as *Rhabdothamnus solandri* and *Fuchsia excorticata* often grow either solitarily or in small clusters, including racemes in *Sophora* spp. and panicles in *Vitex lucens*, typically featuring fewer than 16 flowers per inflorescence and relatively few inflorescences per tree (de Lange, 2024). Despite their limited flower number per tree, these large endemic species are significant nectar producers.

Conversely, New Zealand's small tree flowers frequently occur in large numbers, collectively providing an important nectar source for both small insects and large birds. For example, the small flowers of *Weinmannia racemosa* (wineberry) produce only 1 µl of nectar a day (Table 7). However, each inflorescence averages 92 flowers, and the trees bear many racemes (Nepia, 2020), flowering extensively over four months from November to February (Perrot & Armstrong, 2000). Another species, *Pseudopanax arboreus*, produces about 5 µl of nectar a day per flower (Table 7), which might be compensated by the species' high abundance and floral density (Castro & Robertson, 1997).

In addition to flower density, another unexplored variable is the species' phenology — specifically, the timely restricted availability of nectar to pollinators — which may also compensate for the lesser nectar amounts in smaller flowers. A prime example is the copious nectar production by the large flowers of *Sophora* spp., *Fuchsia excorticata*, and *Rhabdothamnus solandri*, which bloom for several weeks. In contrast, species with many small flowers, such as *Weinmannia racemosa* and *Aristotelia serrata*, flower over several months. Despite *Vitex lucens*' year-round blooming, it is an exception; its branches bear a small number of inflorescences, typically 4 - 15 flowers per panicle, even at peak times (de Lange, 2024).

A study on Mokoia Island (Perrot & Armstrong, 2000) concerning flower and fruit availability for the reintroduced hihi bird (*Notiomystis cincta*) noted that only a few species flower simultaneously, with flowering times of these species closely consecutive to others, suggesting reduced competition among nectar-producing species and enhancing pollination success. Specifically, during New Zealand's winter from July to August, the main nectar sources are *Pseudopanax arboreus* and *Fuchsia excorticata*. *P. arboreus* begins flowering a month earlier in high abundances and dense flower arrangements, while *Fuchsia* is rarer and bears only a few clusters of flowers per tree. The least amount of species in flower occurs from August to September on Mokoia, which aligns with the main flowering period of *Sophora* on the mainland (de Lange, 2024). Furthermore, *Pittosporum tenuifolium* is noted as the primary nectar source in spring on Mokoia, followed by *Metrosideros excelsa* and *Aristotelia serrata* in summer, with *M. excelsa* producing over 100,000 flowers at its peak.

4.4.2 Phylogeny Constrains Carbohydrate Composition and Alkaloid Concentration in Nectar

Regarding the interspecific variation in nectar composition, I could confirm Wykes' (1952) and Percivals's (1961) postulations that nectar sugar types and their ratios to each other are constrained by phylogeny. Their presence seemed to be distinctive for certain plant families. Based on my access to more advanced methods of analysis – including testing for phylogenetic signals – I could confirm certain dominant components associated mostly with a specific plant order or clade, such as Myrtales and Lamiales being rich in glucose, Fabids and Campanulids in sucrose, and Fabales in ALK.

The major nectar sugars, sucrose, fructose, and glucose, and their ratios were often similar among closely related genera or higher taxonomic groups. For example, Violaceae, Malpighiales, Lamiales, and most Malvids predominantly showed hexose-rich nectar. In contrast, monocots, Griseliniaceae, Proteales, Fabales, and Oxalidales were predominantly classified as sugar-balanced (equal amounts of sucrose, fructose, and glucose) or sucrose-rich. This pattern echoes the findings of Percival's extensive nectar study of approximately 460 species. In her research, the tested Fabales (including *Carmichaelia* spp. and *Clianthus* spp.), Proteales, and Griseliniaceae were predominantly sugar-balanced or sucrose-rich. Similarly, her Malvids (like the Myrtaceae species *Leptospermum scoparium, L. lamberthii,* and *Metrosideros diffusa*) and Malpighiales predominantly exhibited hexose-rich nectar. *Fuchsia fulgens* was identified as hexose-rich, aligning with my findings on *Fuchsia excorticata*. Her examined Lamiales showed variation, with most being sugar-balanced. However, *Veronica* spp. mostly displayed hexose-rich nectar, corroborating my observations regarding the hexose-rich species *Veronica diosmifolia* (Table 9). Percival's findings on Liliales, being either sugar-balanced or hexose-rich, align with results on my tested lilioid monocots, further validating the consistency of these nectar patterns across various studies.

Furthermore, confirming further observations by Percival, my findings also showed that nectar sugar types can vary within the genus. For instance, the NZ native monocots *Cordyline australis* and *C. banksii* produce sucrose-rich nectar. In contrast, their close Australian relative, *Cordyline stricta*, produces hexose-rich nectar. This variability extends to the *Pittosporum* genus, where *Pittosporum colensoi* has hexose-rich nectar, while *P. crassifolium*, *P. eugenoides*, and *P. tenuifolium* produce sucrose-rich nectar, showcasing significant differences in nectar composition, even among closely related species. The species' differences in eccrine nectar secretion may stem from minor mutations in their invertase gene,

a key enzyme in sugar transformation, according to the eccrine secretion model involving SWEET9 (Minami et al., 2021, Chapter 1).

ALK levels were universally detected across all species, with significantly higher concentrations observed in the *Sophora* species (Fabids), *Rhabdothamnus solandri*, *Pittosporum ralphii*, and *Lophomyrtus bullata*, underpinning the identified phylogenetic trend of elevated ALK levels in Fabids. In a study by McDougal et al. (2018), eight major ALK were identified in seeds and leaves of eight New Zealand *Sophora* species: ammodendrine, anagyrine, cytisine, 5,6-dehydrolupanine, lupanine ($C_{15}H_{24}N_2O$), matrine, N-methyl cytisine, and sparteine. Lupinine ($C_{10}H_{19}NO$) was not detected. Seed chemistry varied interspecifically only in anagyrine concentrations. My results on five *Sophora* species confirmed that most of these ALK are also in nectar, except for lupanine. In particular, *Sophora godleyi* nectar contained elevated levels of cytisine, lupinine and sparteine, whereas its sister taxon, *S. chathamica*, showed the highest relative abundances of N-methyl cytisine and ammodendrine. Additionally, I detected *S. microphylla* exhibiting tenfold higher anagyrine concentrations than *S. chathamica* and *S. godleyi* and a hundredfold more than *S. longicarinata* and *S. tetraptera*, confirming McDougal's observation on anagyrine dominating ALK components.

4.4.3 Floral Traits, Carbohydrate Concentrations, and Phylogeny Do Not Explain Interspecific Variation in Non-Carbohydrate Nectar Traits

None of the tested factors exhibited significant effects on non-carbohydrate components except ALK, which could be attributed to the general importance of these compounds in nectar, rather than specific effects in particular taxa. For example, AA offer universal advantages to all pollinators (Brodschneider & Crailsheim, 2010; Jervis & Boggs, 2005; Nicolson, 2011; Wright et al., 2018). At the same time, other components like PHE and VIT contribute to the plant's fitness and reproductive success by preserving nectar and inhibiting microbial growth. The presence of microorganisms in nectar can diminish the concentration of secondary metabolites and impact the consumption behaviour of pollinators, as noted by Vannette & Fukami (2016). However, the presence of a species-specific microfauna (e.g. Mittelbach et al., 2015) may be crucial to the nectar's unique composition and taste — an aspect that future studies could explore in more detail. Moreover, specific compounds in nectar do not always directly increase pollinator attraction. Instead, the interaction between

multiple compounds, which can be either synergistic or antagonistic, plays a crucial ecological role, as Roy et al. (2017) suggested. Further studies could conduct experiments to test whether pollinators exhibit feeding preferences among different types of openly accessible floral nectars presented on a feeding tray, which were manually extracted from flowers beforehand or enhanced with key components.

Amino Acids Promote Feeding Behaviour and Pollinator Health

Amino acids (AA), key nitrogen sources for mutualists, have been detected in all nectar samples examined to date (Blüthgen & Fiedler, 2004; Heil, 2011) – including my New Zealand samples. In the biological process of protein synthesis, only 20 AA, known as primary or proteinogenic, are coded (Hardy, 1985). Various studies have emphasised these AA as crucial for pollinator health, as cited above. Notably, ten AA are essential for honey bees and likely other pollinators, as they cannot synthesise them internally. These include methionine, arginine, tryptophan, lysine, isoleucine, phenylalanine, histidine, valine, leucine, and threonine (De Groot, 1953), all of which I detected in NZ nectar.

Furthermore, AA have also been identified in gymnosperms' pollination drops, sugary secretions chemically akin to nectar (Nepi et al., 2009). Evidence suggests that extinct Eurasian scorpionflies (Mecoptera) might have fed on these pollination drops, contributing to gymnosperm pollination (Ren et al., 2009). This implies that pollination involving sugary exudates containing AA may have predated and coincided with the early diversification of angiosperms in the Cretaceous period. This evolutionary parallel supports the hypothesis that angiosperm nectar evolved to mimic the chemical composition of gymnosperm pollination drops, as proposed by Nepi et al. (2009) and Ren et al. (2009).

Among my 16 AA identified, valine (50 - 2600 ppm), arginine (50 - 2200 ppm), and proline (7 - 1800 ppm) showed the highest abundances, with a total abundance range from 0.01 - 0.91%. *Melicytus ramiflorus*, bearing small green insect-pollinated flowers, showed the most elevated relative abundances of these particular AA, in addition to having the highest overall relative AA abundance. Not only do AA contribute to the taste (e.g. proline) favoured by insects (Alm et al., 1990; Bertazzini et al., 2010), but they also encourage increased feeding behaviours, which can extend the foraging time and thereby enhance pollen collection and deposition on stigmas (Hansen et al., 1998). For instance, bees utilise oxidative degradation of proline for a rapid energy boost during the initial phase of flight, while sugars supply energy for sustained flight activities (Carter et al., 2006). Proline accumulates in high concentrations

in the nectars of various angiosperms, including soybean *Glycine max*, ornamental tobacco *Nicotiana alata*, field pumpkin *Cucurbita pepo*, and rapeseed *Brassica napus* (Carter et al., 2006; Nepi et al., 2012). Furthermore, proline is linked to plant cellular responses to water stress, acting as a compatible solute, and the process of nectar secretion likely imposes significant osmotic stress on the involved floral tissues (Yoshiba et al., 1997). An increase in nectar proline levels can also indicate stress factors such as salinity and frost, which enhance its biosynthesis (Carter et al., 2006). My exclusive sampling in coastal regions raises questions about the potential influence of salinity, particularly from ocean spray, on coastal plants. In New Zealand's South Island, temperatures can still drop below zero during the early flowering season in spring – September (Macara, 2020; Meyer, 2021), when I sampled many of my species, so frost could play a role. Additionally, considering that I also sampled other plants during the warmest and driest period (November - January), the impact of drought cannot be dismissed either. In this study, I did not investigate these effects on AA concentration. However, they certainly present intriguing prospects for future research.

Phenolics and Vitamers Inhibit Nectar Contamination and Foster Plant Health

3-O-feruloyl quinic acid and the flavonoids apigenin/galangin, kaempferol, quercetin, and rutin were the most abundant PHE identified. Quercetin, apigenin and kaempferol possess antimicrobial properties (Arima et al., 2002; Palmer-Young et al., 2022; Emiliano & Almeida-Amaral, 2023), and 3-O-feruloyl quinic acid is valued for its antifungal effects (Kyselka et al., 2018). Rutin has antimicrobial, antifungal, anthelmintic, and larvicidal effects (Dubey et al., 2013). Given the prevalence of *Candida* and other yeasts in floral nectars (Misra et al., 2012), rutin's presence in nectar suggests a strategic defence by the plant. Moreover, rutin can bolster the antibacterial effects of other flavonoids like quercetin and kaempferol, hinting at potential synergistic interactions (Arima et al., 2002; Sharma et al., 2013; Sarian et al., 2017).

In particular, 3-O-feruloyl quinic acid ($C_{17}H_{20}O_9$), a hydroxy-cinnamic acid, has been identified in nectar, fruits, and coffee beans but mainly in pollen (Gómez-Caravaca et al., 2006; Gao et al., 2020). In my nectar samples, I detected the hydroxy-cinnamic acid and its precursors, benzoic and cinnamic acid (Gallardo et al., 2006).

Quercetin ($C_{15}H_{10}O_7$) is frequently found in both nectar and pollen. It is preferred by honeybees in preference tests and has been shown to upregulate detoxification genes (Mao et al., 2013; Liao et al., 2017; Palmer-Young et al., 2018).

Apigenin ($C_{15}H_{10}O_5$) has been found in Campanulids (Asteraceae, Apiaceae), Lamiids (Gentianaceae, Lamiaceae, Plantaginaceae) and Fabids (Fabaceae) (Sen et al., 2016). I found the highest apigenin concentrations in the Malvids *Lophomyrtus bullata* and *L. obcordata*, the Lamiales *Myoporum laetum* and *Vitex lucens*, and the Fabids *Sophora godleyi*, *Melicytus ramiflorus* and *M. lanceolatus* – which match the plant taxa studied by Sen et al. (2016).

Kaempferol ($C_{15}H_{10}O_6$) is found in numerous fruits and vegetables. It has been sourced from *Justicia spicigera, Pteridium aquilinum, Acacia nilotica, Rosa rugosa, Moringa oleifera, Tilia americana* var. *mexicana, Lycium barbarum, Diospyros kaki,* and *Camellia sinensis*, among others (Euler & Alam, 1982; Imperato, 1995; Xiao et al., 2006; Singh et al., 2008; Sultana & Anwar, 2008; Aguirre-Hernández, 2010; Dong et al., 2011; Liu et al., 2012; Barreto-Silva et al., 2013; Mercader-Ros et al., 2013). Within this study, the highest abundances of kaempferol were found in members of Malvids (*Leptospermum scoparium*, *Lophomyrtus bullata*, *Kunzea ericoides* agg., *Melicope ternata*, and *Fuchsia excorticata*) and the Apiales (*Pseudopanax crassifolius*, *Pittosporum tenuifolium*, *P. eugenoides*, and *P. crassifolium*).

Rutin ($C_{27}H_{30}O_{16}$) is found in over 70 plant species. Buckwheat (*Fagopyrum esculentum*, Polygonaceae) is a major natural source of rutin (Kim et al., 2005). Other significant sources include *Ruta graveolens* (Rutaceae), *Sophora japonica* (Fabaceae), and *Eucalyptus* spp. (Myrtaceae) (Chua, 2013). In this study, the highest rutin concentrations were detected in Ruta- and Myrtaceae species (*Metrosideros colensoi*, *M. fulgens*, *M. umbellata*, *Lophomyrtus bullata*, *L. obcordata*, *K. ericoides,* and *Melicope ternata*). Interestingly, my *Sophora* species exhibited one of the lowest rutin concentrations.

VIT play a role in the redox cycle and are well-known as antioxidants in nectar and its consumers (Nicolson & Thornburg, 2007). For example, riboflavin helps plants resist diseases. In experiments by Dong & Beer (2000), riboflavin boosted the defence systems of *Arabidopsis thaliana* and tobacco against various pathogens without harming the plants. Riboflavin activates defence-related genes in plants through a specific signalling pathway that does not rely on the typical defence molecule, salicylic acid. Among the four detected VIT, ascorbic acid, niacin, pantothenic acid and riboflavin comprised 0.004 - 0.26% relative abundance. Ascorbic acid was most abundant in *Pseudowintera colorata*, niacin in *Lophomyrtus obcordata,* pantothenic acid in *Fuchsia excorticata* and riboflavin in *Kunzea ericoides* agg. Overall, *Lophomyrtus obcordata* nectar contained the highest VIT abundance.

However, VIT showed neither statistically significant relations to pollinator syndromes nor species' phylogeny.

Conclusion

In conclusion, I have confirmed that nectar traits vary among New Zealand tree species and that floral traits can predict specific nectar characteristics, including nectar volume, and levels of total and specific solubles. Additionally, I found that pollinator syndromes correlate with these nectar traits, though the patterns may differ from those seen in other regions. My analysis also indicates that phylogeny predicts certain nectar traits, particularly carbohydrate composition and alkaloid concentration, as well as floral traits such as weight, size, and shape.

Entomophilous tree species in New Zealand (NZ) can be associated with small, lightweight, dish-, brush-, bell-, or funnel-shaped flowers of white, green, pale yellow, or purple colour. These flowers produce small volumes of low-concentrated nectar containing a notable number of various carbohydrates, many of which are exclusively present in insect-pollinated species. Conversely, NZ ornithophilous tree species tend to feature large, heavy, gullet-, flag-, or funnel-shaped flowers in bright yellow, orange, pink, or red, secreting copious, more concentrated nectar with high total soluble content, as well as elevated levels of alkaloids. The ornithophilous syndrome is typical for the (tested) members of Fabales and Myrtales.

NZ Fabales produce higher nectar volumes containing higher amounts of solubles, particularly alkaloids, than species of other clades, and Myrtales are particularly rich in glucose. The three major nectar sugars – sucrose, glucose, and fructose – showed no significant association with flower size or colour but were linked to phylogenetic relatedness.

My research indicates that phylogeny predominantly determines the species' nectar sugar type, while pollination syndromes have little contribution. I included many sugar types beyond sucrose, glucose, and fructose. I showed that only a few of these sugars are influenced by phylogenetic or floral trait-related factors, prompting inquiry into other potential mechanisms driving their diversity across species.

I chose to use phylogenetic signal testing rather than phylogenetically independent contrasts (PIC) because my aim was solely to assess whether nectar traits exhibit a phylogenetic pattern. By quantifying the strength of phylogenetic signals (e.g., using Pagel's λ), I could determine whether closely related species share similar trait values beyond random expectation. In contrast, PIC assumes the presence of such a signal and corrects for it when analysing trait correlations, which can be unnecessary or misleading if no signal is present. Thus, phylogenetic signal testing provides a more appropriate and informative first step for evaluating evolutionary influences in my study. Future studies could build on my phylogenetic signal-based findings and apply PIC to further disentangle evolutionary effects on nectar traits across related species.

In addition to my broad analytical approach — which already combined phylogenetic analyses, regression models, boxplots, and GAMs on 50 species and 62 compounds — future studies could apply further multivariate analyses, such as principal component analysis (PCA), canonical correspondence analysis (CCA), or redundancy analysis (RDA), to explore the underlying drivers of interspecific nectar trait variation in even greater detail.

Future studies should also consider using UV-imaging methods to explore whether variations in nectar traits among the studied species can be further explained by incorporating this additional colour parameter.

Recent advancements in nectar research have significantly enhanced our understanding of both primary and secondary nectar constituents. With the reliance on subjective interpretations of results decreasing, there is now an opportunity to establish a more precise sugar classification system based on quantitative data, including non-SGF sugar types. My proposed SGFO classification system would allow for improved detailed categorisation of nectars and statistical testing. It would facilitate a better analysis of their phylogenetic connections and uncover new insights into their ecological significance.

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Supplementary Material

Table S1: HPLC peak alignment against the best matching component, with 'RT' = retention time, 'COI' = component of interest (not matching any sugar standards), 'MW' = molecular weight in mol/g, 'PSA' = polar surface area.

HPLC Detection				Component Identification					
COI Nr.	RT (min)	Peak Name	MW	Verification Method	Best Match	Chemical Formula	PSA	Group	Class
26	34.50	Unknown 8	-	-	-	-	-	?	
-	33.00	DHA	90	HPLC + LC-MS	**DHA**	C3 H6 O3	58	Carbohydrate	
25	32.00	Unknown 7.5	100	LC-MS	**GABA**	C4 H9 N O2	63	Amino acid (AA)	
24	31.50	Unknown 7	105	LC-MS	**Serine**	C3 H7 N O3	84		
23	30.00	Unknown 6.5	-	-	-	-	-	?	
22	29.50	Unknown 6.25	132	LC-MS	**Asparagine**	C4 H8 N2 O3	106	Asparagine	Amino acid
			133	LC-MS	**Aspartic acid**	C4 H7 N O4	108		
21	29.00	Unknown 6	146	LC-MS	**Glutamine**	C5 H10 N2 O3	106	Glutamine	
			147	LC-MS	**Glutamic-acid**	C5 H9 N O4	101		
20	28.11	Negative peak	148	LC-MS	**Cinnamic acid**	C9 H8 O2	78	other	
-	28.00	Ribose	150	HPLC + LC-MS	**Ribose**	C5 H10 O5	90	Carbohydrate	
19	27.00	Unknown 5.75	-	-	-	-	-		
18	26.50	Unknown 5.5	-	-	-	-	-	?	
17	26.00	Unknown 5.4	-	-	-	-	-		
-	25.52	Arabinose	150	HPLC + LC-MS	**Arabinose**	C5 H10 O5	90	Carbohydrate	
-	-	-	154	LC-MS	**Gentisic acid**	C7 H6 O4	78	other	
-	-	-	155	LC-MS	**Histidine**	C6 H9 N3 O2	96	Amino acid	
-	-	-	164	LC-MS	**M-Coumarin acid**	C9 H8 O3	24	Alkaloid (ALK)	
-	-	-	169	LC-MS	**Lupinine**	C10 H19 N O	24		
-	-	-	174	LC-MS	**Arginine**	C6 H14 N4 O2	128	Amino acid	
-	24.75	Inositol	180	HPLC + LC-MS	**Inositol**	C6 H12 O6	121	Carbohydrate	
-	24.00	Fructose	180	HPLC + LC-MS	**Fructose**	C6 H12 O6	110		
-	22.90	Rhamnose	180	HPLC + LC-MS	**Rhamnose**	C6 H12 O6	90		
-	22.40	Glucose	180	HPLC + LC-MS	**Glucose**	C6 H12 O6	110		
-	-	-	181	LC-MS	**Tyrosine**	C9 H11 N O3	84	Amino acid	
16	21.50	Unknown 5.25	190	LC-MS	**Cytisine**	C11 H14 N2 O	32	Cytisine	ALK
			204	LC-MS	**N-Methyl cytisine**	C12 H16 N2 O	85		
			208	LC-MS	**Ammodendrine**	C12 H20 N2 O	32		
15	20.10	Unknown 5	244	LC-MS	**Anagyrine**	C15 H20 N2 O	24	Lupanine	
			246	LC-MS	**5,6-Dehydrolupanine**	C15 H22 N2 O	24		
			248	LC-MS	**Lupanine/Matrine**	C15 H24 N2 O	24		
14	19.70	Unknown 4.75	270	LC-MS	**Apigenin/Galangin**	C15 H10 O5	87	Apigenin	BIO
			286	LC-MS	**Luteolin/ Kaempferol**	C15 H10 O6	107		
			288	LC-MS	**Schwertinin/ Aroma-**	C15 H12 O6	107		
			302	LC-MS	**Quercetin**	C15 H10 O7	127		
-	19.20	Lactose	342	HPLC + LC-MS	**Lactose**	C12 H22 O11	190	Carbohydrate	
-	18.90	Maltose	342	HPLC + LC-MS	**Maltose**	C12 H22 O11	190		
-	18.70	Sucrose	342	HPLC + LC-MS	**Sucrose**	C12 H22 O11	190		
13	18.20	Unknown 4.5	368	LC-MS	**3-O-Feruloylquinic acid**	C17 H20 O9	154	Vitexin	BIO
			432	LC-MS	**Vitexin/Afzelin**	C21 H20 O10	177		
-	17.01	Melezitose	504	HPLC + LC-MS	**Melezitose**	C18 H32 O16	269	Carbohydrate	
-	16.36	Raffinose	504	LC-MS	**Raffinose**	C18 H32 O16	269		
-	15.76	Verbascose	828	HPLC + LC-MS	**Verbascose**	C30 H52 O26	427		
12	15.10	Unknown 4	> 829	-	Hexasaccharides?	C36 +		Carbohydrate	
11	14.00	Unknown 3		-					
10	13.70	Unknown 2.5		-					
9	13.30	Unknown 2		-					
8	12.50	Unknown 1.5		-					
7	12.00	Unknown 1		-					
6	11.00	Unknown 11 MIN		-					
5	10.00	Unknown 10 min		-					
4	9.00	Unknown 9 MIN		-					
3	7.00	Unknown 7 MIN		-					
2	5.00	Unknown 5 MIN		-					
1	2.00	Unknown 2 MIN		-					

Table S2: Relative mean carbohydrate abundances (%) based on HPLC detection, for *n* of species-specific nectar samples see Table 1; concentration levels displayed as heatmap from white to orange (high).

	COI 1-12	VERBASCOSE	RAFFINOSE	MELIZITOSE	SUCROSE	MALTOSE	LACTOSE	GLUCOSE	RHAMNOSE	FRUCTOSE	INOSITOL	ARABINOSE	RIBOSE	DHA	SUM
ARI SER	3	0.2	0.1	1	37			25		32					98.3
CAR AUS	0.4	0.1		0.3	46			28		24		0.1			99.2
CAR SER	2		0.2	1	41		0.1	26		28					97.9
CLI MAX	4		0.4	1	39			26		29		0.1			99.1
COR AUS	1	0.3	0.1	0.04	29	0.2	0.1	33		35		0.2	0.04		99.1
COR BAN	0.4			0.3	42			29		27		0.0			99.9
COR COT	5			0.3	2		0.1	44		47		0.2	0.1		98.0
COR LAE	3	0.01	0.1	0.1	1	0.1	0.1	52		43		0.3	0.01		99.5
COR STR	1	0.1		1	2		0.1	46		48			0.02	0.002	99.9
ELA DEN	4	3	1	1	41			22		27		0.2	0.1		99.5
FUC EXC	3			0.01	4			48		45					99.9
GEN RUP	2	0.1	0.1	2	46			25		24		0.2	0.1		99.1
GRI LIT	6	0.1		1	24			33		35	0.02	0.6	0.3		99.7
GRI LUC	6	0.1	0.2	1	45	0.1		23		25	0.1	0.1	0.1		99.6
KNI EXC	3	0.3	1	4	67			11	0.001	12		0.2		0.01	99.2
KUN ERI	2	0.04	0.1	0.04	1			48		49		0.3			100.0
LEP SCO	4		0.1	0.1	1		0.1	41		53	0.2	0.3		0.32	99.4
LOP BUL	39	3		1	12			13		18		0.9			88.4
LOP OBC	30	14			5			13		9					71.9
MEL LAN	7		0.3	1	3			20		68					98.9
MEL NOV	3			0.3	1		0.1	42		54		0.2			99.7
MEL RAM	19	0.2	1	1	3		0.2	31		41		0.3			96.7
MEL TER	4	0.1	0.002	0.2	4		0.03	44		46		1.4	0.04		99.4
MET COL	1			0.3	39			30		30		0.1			99.4
MET EX A	6	2	0.2	0.2	1		0.3	43		46	0.01	0.1	0.02		99.5
MET EXC	4	2	0.1	0.1	1		0.2	45		47		0.3	0.02		99.5
MET FUL	2			0.1	3			47		48					99.9
MET UMB	3			0.1	0		0.2	47		48		0.9			100.0
MYO LAE	1	0.1	0.02	0.1	9			41		49		0.1	0.03	0.01	99.6
MYR AUS	37	6			6			15	9	17		0.3			89.1
PAR LOP	11	0.3		1	11			33		25	18.1				98.6
PIT CRA	3	1	0.5	1	40			21		33		0.1		0.002	99.5
PIT EUG	1	0.2	0.3	2	43	0.2		20		32		0.1	0.01	0.0002	99.4
PIT RAL	6	0.3		1	64			9		19		0.1			99.1
PIT TEN	3	0.2	0.2	1	56		0.02	16		21	0.6	0.2	0.04	0.01	99.2
PITCOL	6		0.2	1	1		0.3	35		54		1.5			98.7
PSE ARB	3		0.1	2	24			31		40		0.3	0.03		99.7
PSE COL	28		2	2	2			2		55					90.6
PSE CRA	60	2		5	2			8		13					89.5
RHA SOL	2			0.03	1		0.2	46		50		0.1	0.04		99.8
RHO SAP	7		0.3	4	64		16	2	0.1	4	0.04		1		99.1
ROB PSE	5	0.2		2	72			4		15		0.2			98.1
SOP CHA	1	0.2	0.4	4	37		1	24		30	0.1	0.2			98.6
SOP GOD	2	0.1	0.2	4	30		1.6	28		32	0.2	0.4			98.2
SOP LON	1	1	1	2	49			19		25	0.4	0.2			98.0
SOP MIC	2	0.4	0.3	1	44			22		31					99.4
SOP PRO	1	1	1	2	49			19		25	0.4	0.2			98.0
SOP TET	0.3	0.3	0.3	1	40			25		32					98.5
VER DIO	2			0.3	1	0.2		58		38		0.2	0.4		99.4
VIT LUC	2		0.1	1	14	0.03		41		41		0.2		0.01	98.4
WEI RAC	2	1	1	1	38			26		30		0.2	0.04		98.9
Mean	7	1	0.4	1	24	0.1	1	29	3	34	2	0.3	0.1	0.04	97.8
SD	12	3	0.5	1	22	0.1	4	14	5	14	5	0.3	0.2	0.1	4.6
n	51	33	32	49	51	6	18	51	3	51	11	38	19	8	
In % spp. present	100	65	63	96	100	12	35	100	6	100	22	75	37	16	
Minimum	0.3	0.01	0.002	0.01	0	0.03	0.02	2	0.001	4	0.01	0.04	0.01	0.0002	71.9
Maximum	60	14	2.0	5	72	0.2	16	58	9	68	18	1.5	0.9	0.3	100.0

Table S3: HPLC-based mean data on large (> 830 mol/g) components of interest (grouped as possible hexasaccharides), concentration levels displayed as heatmap from white to orange (high).

	COI 1	COI 2	COI 3	COI 4	COI 5	COI 6	COI 7	COI 8	COI 9	COI 10	COI 11	COI 12	SUM
ARI SER							0.10	0.3	0.60	1.4		0.20	2.60
CAR AUS									0.12		0.32		0.44
CAR SER							0.30		1.40			0.40	2.10
CLI MAX											3.50	0.58	4.08
COR AUS		0.060	0.020		0.04		0.11		0.48		0.27	0.03	1.01
COR BAN						0.06			0.31		0.06		0.43
COR COT		0.080					0.12		4.90		0.09		5.19
COR LAE				0.03		0.08	0.02		0.24		2.52	0.12	3.01
COR STR		0.021		0.01			0.02				1.04	0.29	1.37
ELA DEN							0.28		0.60		3.18		4.06
FUC EXC											2.79		2.79
GEN RUP		0.060		0.25		0.17	0.11		0.55		1.03	0.13	2.30
GRI LIT		0.007		0.04	0.03	0.03	0.13		4.66		0.85	0.01	5.76
GRI LUC			0.070			0.02	0.31		0.22		5.08		5.70
KNI EXC						0.02	0.03		0.001		1.45	1.23	2.73
KUN ERI			0.040		0.09	0.04		0.3	0.60		1.00		2.07
LEP SCO		0.006	0.098		0.07		0.01		0.88		2.60	0.06	3.72
LOP BUL									34.22		4.87		39.09
LOP OBC						11.14	2.23		16.71				30.08
MEL LAN							2.09				3.92	0.81	6.82
MEL NOV				0.10		0.08					2.65		2.83
MEL RAM					0.10	0.01	0.10				18.10	0.70	19.01
MEL TER				0.04	0.04	0.04	0.19		0.41	3.0	0.06		3.78
MET COL											1.25	0.05	1.29
MET EX A						0.02	0.03		0.02		3.08	2.93	6.08
MET EXC							0.01				1.31	2.85	4.17
MET FUL											2.02		2.02
MET UMB											2.94		2.94
MYO LAE	0.05	0.005	0.003		0.05		0.23		0.26		0.54	0.08	1.17
MYR AUS				0.67		11.30	5.55		19.10				36.62
PAR LOP											10.66		10.66
PIT CRA				0.01		0.05	0.09				2.11	0.70	2.96
PIT EUG		0.005	0.012	0.01	0.02	0.02	0.07		0.88		0.21	0.04	1.27
PIT RAL					0.02		0.11		0.38		4.56	0.47	5.54
PIT TEN		0.034		0.01	0.01	0.13	0.15		1.70		0.67	0.44	3.13
PITCOL				0.04		0.23			5.82		0.21		6.30
PSE ARB				0.10		0.02	0.03		0.16		2.50		2.80
PSE COL				1.99			2.59		20.43		2.80		27.81
PSE CRA							28.91		25.17		5.58		59.66
RHA SOL						0.03					2.41		2.44
RHO SAP							0.64				6.44		7.07
ROB PSE						0.09	0.31				4.97		5.37
SOP CHA											1.10		1.10
SOP GOD							0.03				0.09	2.03	2.15
SOP LON						0.02					1.07		1.09
SOP MIC											0.40	1.60	2.00
SOP PRO						0.02					1.07		1.09
SOP TET									0.06		0.02	0.23	0.31
VER DIO				0.36			0.64		1.11		0.10		2.21
VIT LUC				0.01		0.01	0.02		0.11		1.60	0.27	2.02
WEI RAC									1.26		0.95		2.21
Mean	0.05	0.03	0.04	0.2	0.05	1	1	0.3	5	2.2	2	0.7	7.0
SD	NA	0.03	0.04	0.5	0.03	3	5	0	9	1.1	3	0.9	11.5
n	1	9	6	15	10	23	32	2	31	2	47	24	51
In % spp present	2	18	12	29	20	45	63	4	61	4	92	47	100
Minimum	0.05	0.005	0.003	0.01	0.01	0.01	0.01	0.3	0.001	1.4	0.02	0.01	0.31
Maximum	0.05	0.08	0.1	2	0.1	11.3	29	0.3	34	3	18	2.9	59.7

Table S4: Spearman correlation *P*-values for the analysis of correlations among nectar traits and between nectar and floral traits. Nectar data is based on LC-MS (alkaloids, amino acids, phenolics, vitamer variants) and HPLC (other nectar variables) analysis, with 'Pollinator syndrome' values set as 0 ~ entomophilous, 50 ~ generalist, 100 ~ ornithophilous (based on Kelly et al., 2010); 'Flower Shape' values defined by accessibility and set as 2 ~ flag-, 3 ~ gullet-, 4 ~ bell- or funnel-, 5 ~ brush-, 6 ~ dish-shaped flowers, with no tube-shaped flowers in my dataset (~ 1).

	Flower Size	Flower Weight	Flower Shape	Colour wavelength	Colour Chroma	Colour Lightness	Colour Hue	Colour A	Colour B	Pollinator Syndrome	Nectar Volume	Nectar Concentration	Nectar Solubles	Nectar Complexicity	Carbohydrates	Hexasaccharides	Verbascose	Trisaccharides	Raffinose	Melezitose	Disaccharides	Sucrose	Hexoses	Fructose	Glucose	Pentoses	Arabinose	Alkaloids	Amino acids	Phenolics	Vitamer Variants	Cinnamic acid	COI 6	COI 7	COI 8	COI 9	COI 10	COI 11
Flower Weight	0.000																																				
Flower Shape	0.000	0.000																																			
Colour wavelength	0.012	0.087	0.625																																		
Colour Chroma	0.202	0.115	0.283	0.892																																	
Colour Lightness	0.582	0.501	0.209	0.562	0.000																																
Colour Hue	0.977	0.973	0.110	0.019	0.066	0.000																															
Colour A	0.478	0.672	0.092	0.284	0.338	0.000	0.000																														
Colour B	0.033	0.032	0.979	0.000	0.900	0.004	0.000	0.000																													
Pollinator Syndrome	0.000	0.000	0.000	0.024	0.017	0.379	0.837	0.423	0.053																												
Nectar Volume	0.000	0.000	0.000	0.089	0.064	0.987	0.726	0.353	0.077	0.000																											
Nectar Concentration	0.000	0.000	0.001	0.106	0.511	0.585	0.533	0.270	0.119	0.000	0.000																										
Nectar Solubles	0.000	0.000	0.000	0.099	0.161	0.455	0.827	0.796	0.033	0.000	0.000	0.000																									
Nectar Complexicity	0.156	0.094	0.061	0.351	0.952	0.874	0.986	0.637	0.491	0.029	0.056	0.359	0.089																								
Carbohydrates	0.406	0.442	0.655	0.689	0.153	0.140	0.297	0.339	0.999	0.255	0.311	0.093	0.404	0.052																							
Hexasaccharides	0.108	0.150	0.138	0.921	0.909	0.645	0.167	0.115	0.568	0.279	0.091	0.007	0.132	0.055	0.000																						
Verbascose	0.613	0.564	0.948	0.743	0.092	0.222	0.430	0.639	0.700	0.509	0.607	0.262	0.630	0.095	0.000	0.002																					
Trisaccharides	0.165	0.165	0.910	0.079	0.019	0.774	0.472	0.376	0.067	0.237	0.542	0.784	0.618	0.874	0.916	0.047	0.644																				
Raffinose	0.223	0.470	0.892	0.134	0.470	0.689	0.179	0.139	0.138	0.817	0.539	0.613	0.667	0.908	0.732	0.511	0.793	0.000																			
Melezitose	0.250	0.184	0.935	0.144	0.016	0.638	0.727	0.623	0.121	0.203	0.632	0.622	0.677	0.889	0.999	0.042	0.663	0.000	0.115																		
Disaccharides	0.115	0.109	0.499	0.590	0.216	0.440	0.119	0.126	0.643	0.557	0.262	0.074	0.188	0.406	0.222	0.065	0.443	0.003	0.216	0.004																	
Sucrose	0.091	0.089	0.426	0.487	0.260	0.476	0.153	0.161	0.763	0.498	0.239	0.068	0.164	0.377	0.215	0.056	0.452	0.005	0.200	0.007	0.000																
Hexoses	0.620	0.541	0.822	0.679	0.339	0.889	0.673	0.736	0.935	0.855	0.987	0.906	0.784	0.497	0.012	0.009	0.019	0.000	0.097	0.000	0.000	0.000															
Fructose	0.483	0.459	0.904	0.910	0.699	0.817	0.430	0.371	0.665	0.666	0.716	0.616	0.616	0.793	0.035	0.070	0.012	0.002	0.754	0.000	0.000	0.000	0.000														
Glucose	0.845	0.709	0.778	0.517	0.164	0.610	0.973	0.761	0.764	0.425	0.690	0.455	0.994	0.328	0.015	0.003	0.088	0.000	0.004	0.000	0.000	0.000	0.000	0.000													
Pentoses	0.238	0.313	0.293	0.058	0.279	0.262	0.072	0.206	0.075	0.269	0.209	0.353	0.170	0.260	0.663	0.951	0.508	0.618	0.300	0.837	0.455	0.330	0.449	0.679	0.327												
Arabinose	0.418	0.493	0.601	0.151	0.581	0.394	0.162	0.358	0.201	0.470	0.278	0.375	0.234	0.328	0.774	0.884	0.605	0.205	0.296	0.283	0.065	0.065	0.090	0.181	0.080	0.000											
Alkaloids	0.000	0.000	0.003	0.035	0.271	0.367	0.455	0.701	0.012	0.000	0.001	0.001	0.001	0.479	0.632	0.351	0.607	0.005	0.652	0.002	0.165	0.155	0.421	0.418	0.514	0.543	0.698										
Amino acids	0.447	0.578	0.273	0.993	0.972	0.346	0.116	0.091	0.335	0.459	0.367	0.174	0.757	0.290	0.021	0.000	0.081	0.348	0.659	0.360	0.111	0.104	0.130	0.148	0.199	0.489	0.336	0.853									
Phenolics	0.406	0.200	0.525	0.806	0.136	0.329	0.764	0.869	0.722	0.636	0.721	0.195	0.440	0.420	0.015	0.016	0.045	0.134	0.209	0.208	0.020	0.018	0.746	0.911	0.623	0.309	0.190	0.660	0.004								
Vitamer Variants	0.174	0.184	0.959	0.676	0.566	0.399	0.958	0.739	0.673	0.911	0.932	0.245	0.475	0.279	0.226	0.063	0.395	0.187	0.583	0.189	0.001	0.001	0.100	0.152	0.118	0.171	0.118	0.552	0.017	0.000							
Cinnamic acid	0.218	0.268	0.282	0.890	0.432	0.267	0.213	0.232	0.758	0.318	0.185	0.029	0.265	0.038	0.000	0.000	0.000	0.470	0.866	0.444	0.098	0.093	0.008	0.030	0.009	0.373	0.478	0.483	0.002	0.027	0.156						
COI 6	0.484	0.535	0.871	0.676	0.171	0.364	0.702	0.831	0.656	0.417	0.489	0.309	0.559	0.207	0.000	0.056	0.000	0.314	0.515	0.358	0.414	0.409	0.101	0.069	0.256	0.464	0.511	0.703	0.447	0.155	0.407	0.000					
COI 7	0.304	0.481	0.277	0.552	0.377	0.922	0.328	0.294	0.321	0.917	0.397	0.141	0.470	0.225	0.181	0.000	0.329	0.012	0.730	0.002	0.257	0.244	0.065	0.139	0.061	0.347	0.364	0.644	0.008	0.842	0.586	0.000	0.803				
COI 8	0.468	0.503	0.277	0.615	0.214	0.390	0.810	0.960	0.554	0.396	0.475	0.674	0.574	0.719	0.653	0.661	0.733	0.359	0.622	0.383	0.332	0.325	0.205	0.360	0.161	0.945	0.956	0.684	0.739	0.070	0.866	0.759	0.866	0.819			
COI 9	0.200	0.230	0.226	0.722	0.482	0.286	0.201	0.165	0.751	0.210	0.123	0.017	0.187	0.071	0.000	0.000	0.002	0.216	0.650	0.210	0.097	0.095	0.014	0.108	0.004	0.496	0.359	0.407	0.000	0.001	0.066	0.000	0.070	0.001	0.733		
COI 10	0.386	0.457	0.182	0.060	0.172	0.651	0.020	0.084	0.044	0.298	0.381	0.732	0.497	0.298	0.713	0.693	0.700	0.383	0.454	0.465	0.579	0.584	0.421	0.561	0.371	0.012	0.006	0.621	0.878	0.677	0.672	0.750	0.831	0.813	0.976	0.660	
COI 11	0.142	0.152	0.336	0.937	0.276	0.363	0.446	0.261	0.710	0.316	0.221	0.055	0.184	0.489	0.977	0.014	0.731	0.150	0.036	0.364	0.435	0.331	0.570	0.883	0.367	0.826	0.832	0.284	0.000	0.685	0.121	0.526	0.462	0.254	0.652	0.391	0.352
COI 12	0.005	0.034	0.137	0.169	0.134	0.134	0.838	0.990	0.545	0.017	0.044	0.058	0.075	0.834	0.372	0.448	0.897	0.421	0.640	0.451	0.647	0.613	0.936	0.852	0.961	0.403	0.556	0.005	0.826	0.202	0.292	0.371	0.597	0.582	0.602	0.231	0.588	0.959

Table S5: Spearman correlation R^2-values for the analysis of correlations among nectar traits and between nectar and floral traits. Nectar data is based on LC-MS (alkaloids, amino acids, phenolics, vitamer variants) and HPLC (other nectar variables) analysis, with 'Pollinator syndrome' values set as 0 ~ entomophilous, 50 ~ generalist, 100 ~ ornithophilous (based on Kelly et al., 2010); 'Flower Shape' values defined by accessibility and set as 2 ~ flag-, 3 ~ gullet-, 4 ~ bell- or funnel-, 5 ~ brush-, 6 ~ dish-shaped flowers, with no tube-shaped flowers in my dataset (~ 1).

	Flower Size	Flower Weight	Flower Shape	Colour wavelength	Colour Chroma	Colour Lightness	Colour Hue	Colour A	Colour B	Pollinator Syndrome	Nectar Volume	Nectar Concentration	Nectar Solubles	Nectar Complexicity	Carbohydrates	Hexasaccharides	Verbascose	Trisaccharides	Raffinose	Melezitose	Disaccharides	Sucrose	Hexoses	Fructose	Glucose	Pentoses	Arabinose	Alkaloids	Amino acids	Phenolics	Vitamer Variants	Cinnamic acid	COI 6	COI 7	COI 8	COI 9	COI 10	COI 11
Flower Weight	**0.868**																																				
Flower Shape	**-0.459**	**-0.650**																																			
Colour wavelength	0.357	0.253	-0.140																																		
Colour Chroma	0.002	0.050	0.017	0.005																																	
Colour Lightness	0.028	-0.202	0.228	0.098	**-0.610**																																
Colour Hue	0.087	0.285	-0.249	**-0.445**	-0.075	**-0.445**																															
Colour A	0.120	0.276	-0.211	-0.364	-0.029	-0.384	**0.968**																														
Colour B	0.259	0.150	-0.078	**0.829**	0.094	0.239	**-0.786**	**-0.761**																													
Pollinator Syndrome	**0.643**	**0.629**	**-0.521**	**0.438**	0.342	-0.202	-0.053	-0.005	0.368																												
Nectar Volume	**0.818**	**0.826**	**-0.618**	0.285	0.166	-0.180	0.233	0.268	0.129	**0.675**																											
Nectar Concentration	**0.689**	**0.719**	**-0.539**	0.257	0.117	-0.096	0.182	0.182	0.198	**0.628**	**0.684**																										
Nectar Solubles	**0.829**	**0.834**	**-0.603**	0.277	0.140	-0.148	0.249	0.269	0.153	**0.677**	**0.921**	**0.891**																									
Nectar Complexicity	-0.193	-0.238	0.327	-0.223	0.079	0.001	-0.025	-0.084	-0.141	-0.328	-0.178	-0.046	-0.087																								
Carbohydrates	0.103	0.053	0.048	0.027	-0.036	-0.149	0.280	0.283	-0.156	0.123	0.239	0.173	0.260	0.132																							
Hexasaccharides	**-0.452**	-0.288	0.169	-0.297	0.136	-0.343	0.104	0.058	-0.298	-0.378	**-0.427**	**-0.528**	**-0.538**	-0.129	-0.329																						
Verbascose	0.059	0.063	-0.107	0.082	-0.087	0.184	-0.144	-0.193	0.154	0.064	0.034	0.010	-0.001	0.026	**-0.462**	0.051																					
Trisaccharides	0.027	0.039	0.015	0.260	0.315	-0.165	-0.316	-0.321	0.367	0.084	-0.030	0.067	-0.012	0.082	**-0.480**	0.113	0.285																				
Raffinose	0.174	0.106	-0.149	0.178	0.100	-0.092	-0.148	-0.171	0.189	0.073	0.118	0.087	0.123	0.244	-0.241	-0.059	0.267	**0.599**																			
Melezitose	0.042	0.066	0.026	0.243	0.372	-0.196	-0.283	-0.281	0.361	0.097	0.003	0.120	0.030	0.078	**-0.435**	0.073	0.236	**0.962**	**0.441**																		
Disaccharides	0.212	0.193	-0.142	0.045	0.145	-0.115	0.100	0.084	0.023	0.103	0.295	0.329	0.331	0.112	-0.065	-0.294	0.338	**0.547**	**0.395**	**0.558**																	
Sucrose	0.209	0.186	-0.146	0.058	0.139	-0.113	0.091	0.075	0.035	0.106	0.291	0.330	0.329	0.105	-0.070	-0.304	0.337	**0.546**	0.388	**0.557**	**0.999**																
Hexoses	-0.032	-0.095	0.029	-0.016	-0.105	0.067	0.021	0.037	-0.038	0.008	-0.073	0.033	0.012	0.110	**0.441**	-0.157	**-0.568**	**-0.651**	-0.330	**-0.643**	**-0.777**	**-0.772**															
Fructose	-0.057	-0.095	0.050	0.004	-0.078	-0.057	-0.047	-0.060	0.018	-0.124	-0.147	-0.115	-0.111	0.107	0.278	0.003	**-0.568**	**-0.468**	-0.102	**-0.484**	**-0.761**	**-0.757**	**0.889**													
Glucose	0.006	-0.048	0.007	-0.104	-0.098	0.149	0.075	0.090	-0.108	0.111	0.013	0.172	0.138	0.173	**0.482**	-0.290	**-0.431**	**-0.706**	**-0.418**	**-0.688**	**-0.638**	**-0.637**	**0.914**	**0.667**												
Pentoses	-0.138	-0.123	0.163	-0.240	0.152	-0.014	0.049	0.040	-0.198	-0.113	-0.064	-0.027	-0.001	**0.437**	-0.015	0.218	-0.105	-0.007	0.041	-0.004	-0.148	-0.158	0.190	0.070	0.288												
Arabinose	-0.086	-0.081	0.070	-0.147	0.061	0.017	-0.017	-0.053	-0.095	-0.028	-0.076	-0.064	-0.027	**0.440**	0.030	0.130	-0.034	-0.098	-0.001	-0.108	-0.251	-0.254	0.288	0.171	0.374	**0.907**											
Alkaloids	0.376	**0.417**	**-0.412**	0.273	-0.054	0.003	-0.126	-0.219	**0.392**	0.231	0.233	0.147	0.174	-0.139	**-0.445**	0.028	**0.462**	0.324	0.288	0.274	0.141	0.146	-0.311	-0.193	-0.379	-0.160	-0.041										
Amino acids	-0.253	-0.289	0.239	-0.090	-0.012	0.349	-0.309	-0.342	0.122	-0.225	-0.283	-0.385	**-0.396**	-0.027	-0.211	0.158	0.173	-0.111	-0.105	-0.158	-0.185	-0.184	-0.030	-0.063	-0.041	0.025	0.010	0.332									
Phenolics	-0.208	-0.266	**0.410**	-0.201	0.017	0.035	-0.056	-0.092	-0.150	-0.252	-0.306	**-0.512**	**-0.462**	0.153	-0.023	0.276	-0.096	-0.311	-0.298	-0.298	**-0.437**	**-0.433**	0.189	0.276	0.092	0.091	0.204	0.097	**0.468**								
Vitamer Variants	-0.246	-0.148	0.008	-0.151	0.089	-0.104	-0.170	-0.259	0.051	-0.114	-0.280	-0.186	-0.316	-0.092	-0.111	**0.515**	-0.088	-0.190	-0.264	-0.202	**-0.521**	**-0.519**	0.272	0.308	0.219	0.116	0.167	0.148	0.277	**0.439**							
Cinnamic acid	**-0.527**	**-0.559**	0.377	-0.178	-0.125	0.097	-0.247	-0.259	-0.082	**-0.451**	**-0.657**	**-0.712**	**-0.766**	-0.201	-0.280	**0.488**	0.080	0.056	-0.049	0.009	-0.313	-0.311	-0.103	0.058	-0.267	-0.164	-0.077	-0.049	0.314	**0.427**	0.245						
COI 6	-0.356	-0.285	0.022	-0.110	-0.014	-0.096	0.017	-0.067	-0.096	-0.233	-0.229	-0.125	-0.181	0.276	0.100	0.069	-0.136	-0.201	-0.083	-0.205	-0.013	-0.001	0.051	-0.005	0.118	0.224	0.285	-0.131	0.101	0.264	0.294	0.145					
COI 7	**-0.423**	-0.380	**0.406**	-0.222	0.196	-0.180	-0.031	-0.057	-0.174	**-0.471**	**-0.398**	**-0.448**	**-0.465**	0.325	-0.234	**0.495**	0.146	0.305	0.132	0.295	0.152	0.143	-0.369	-0.163	**-0.461**	-0.082	-0.192	-0.026	0.130	0.203	0.053	0.185	-0.017			
COI 8	-0.216	-0.314	0.249	-0.198	0.039	0.053	0.133	0.184	-0.249	-0.188	-0.208	-0.152	-0.205	0.059	0.088	-0.115	-0.003	-0.159	-0.053	-0.124	-0.150	-0.140	0.191	0.118	0.150	-0.061	-0.035	-0.201	0.082	0.190	-0.066	0.274	0.022	-0.079		
COI 9	**-0.460**	**-0.425**	**0.400**	**-0.491**	-0.165	0.214	-0.067	-0.122	-0.292	**-0.492**	**-0.516**	**-0.625**	**-0.626**	0.179	-0.332	0.352	0.194	0.002	-0.108	0.016	-0.063	-0.068	-0.276	-0.158	-0.264	0.102	0.151	-0.004	0.294	**0.481**	0.198	**0.506**	0.172	0.343	0.171	
COI 10	-0.173	-0.203	0.249	-0.316	0.330	-0.130	0.316	0.313	-0.335	-0.188	-0.208	-0.078	-0.147	0.133	-0.007	0.044	0.035	-0.116	-0.096	-0.055	-0.013	-0.013	0.096	0.065	0.097	0.035	0.045	-0.109	0.199	0.158	-0.024	0.037	0.022	0.187	**0.446**	0.127	
COI 11	-0.087	-0.020	-0.035	0.069	0.047	-0.228	-0.018	0.010	-0.032	-0.014	-0.067	-0.188	-0.153	-0.231	-0.074	**0.538**	-0.160	0.248	0.015	0.188	-0.212	-0.214	-0.004	0.056	-0.127	0.093	-0.004	-0.049	-0.191	-0.117	0.255	0.172	-0.285	0.116	-0.239	-0.191	-0.350
COI 12	0.167	0.215	-0.181	0.196	0.123	-0.268	0.054	0.043	0.104	0.104	0.120	0.248	0.187	0.360	0.179	-0.104	0.194	0.141	0.247	0.119	0.222	0.216	-0.023	0.006	-0.055	-0.146	-0.049	0.172	-0.116	-0.137	-0.242	-0.151	-0.140	0.152	-0.043	-0.344	-0.043	-0.100

Table S6: GAM-correlation coefficients for floral and nectar traits.

		Flower Size	Flower Weight	Nectar Concentration	Nectar Volume
Flower Size	Deviance explained %	-	75.5		62.5
	R-sq	-	0.748		0.615
	p	-	1.53E-12		3.49E-09
	t	-	-10.53		-7.75E+00
	GCV	-	0.65057		0.074981
Flower Weight	Deviance explained %	75.5	-	58	70.8
	R-sq	0.748	-	0.568	0.7
	p	1.53E-12	-	4.49E-08	3.60E-11
	t	-10.53	-	6.946	9.352
	GCV	0.65057	-	0.54459	0.77407
Nectar Concentration	Deviance explained %	49.8	58	-	40.1
	R-sq	0.484	0.568	-	0.384
	p	1.06E-06	4.49E-08	-	2.58E-05
	t	-5.896	6.946	-	4.843
	GCV	0.0031982	0.54459	-	0.11486
Nectar Volume	Deviance explained %	62.5	70.8	40.1	-
	R-sq	0.615	0.7	0.384	-
	p	3.49E-09	3.60E-11	2.58E-05	-
	t	-7.75E+00	9.352	4.843	-
	GCV	0.074981	0.77407	0.11486	-
Phenolics	Deviance explained %	-	-	32	-
	R-sq	-	-	0.305	-
	p	-	-	0.000234	-
	t	-	-	-4.099	-
	GCV	-	-	0.85288	-
Alkaloids	Deviance explained %	20	20	39	25
	R-sq	0.177	0.172	0.37	0.226
	p	0.00501	0.00556	3.79E-05	0.000272
	t	-2.99	2.95	4.715	4.048
	GCV	2.3338	2.138	1.8241	0.13065
Carbohydrates	Deviance explained %	14	-	26	30
	R-sq	0.11	-	0.24	0.28
	p	0.0234	-	0.00101	0.000348
	t	-2.367	-	3.578	3.951
	GCV	6.27E+06	-	286.47	0.13958

5. General Discussion

This chapter synthesises the findings presented in Chapters 2 - 4, evaluates their implications, acknowledges the experimental limitations, and outlines directions for future research. My investigations covered the response of a native bird-pollinated species to frequent nectar removal (Chapter 2), regional variation of floral and nectar traits across New Zealand's diverse climates, and the role of abiotic factors on these traits (Chapter 3). Additionally, I studied the variability of these traits among species, their interrelationships, and their preservation across phylogenies (Chapter 4).

A schematic diagram (Figure 1) is provided to clarify the intricate topic of nectar variation, summarising key findings and their connections. Initial summaries in Section 5.1 lead into a detailed comparison with existing literature and the interpretation, implications and limitations of my research in subsequent sections.

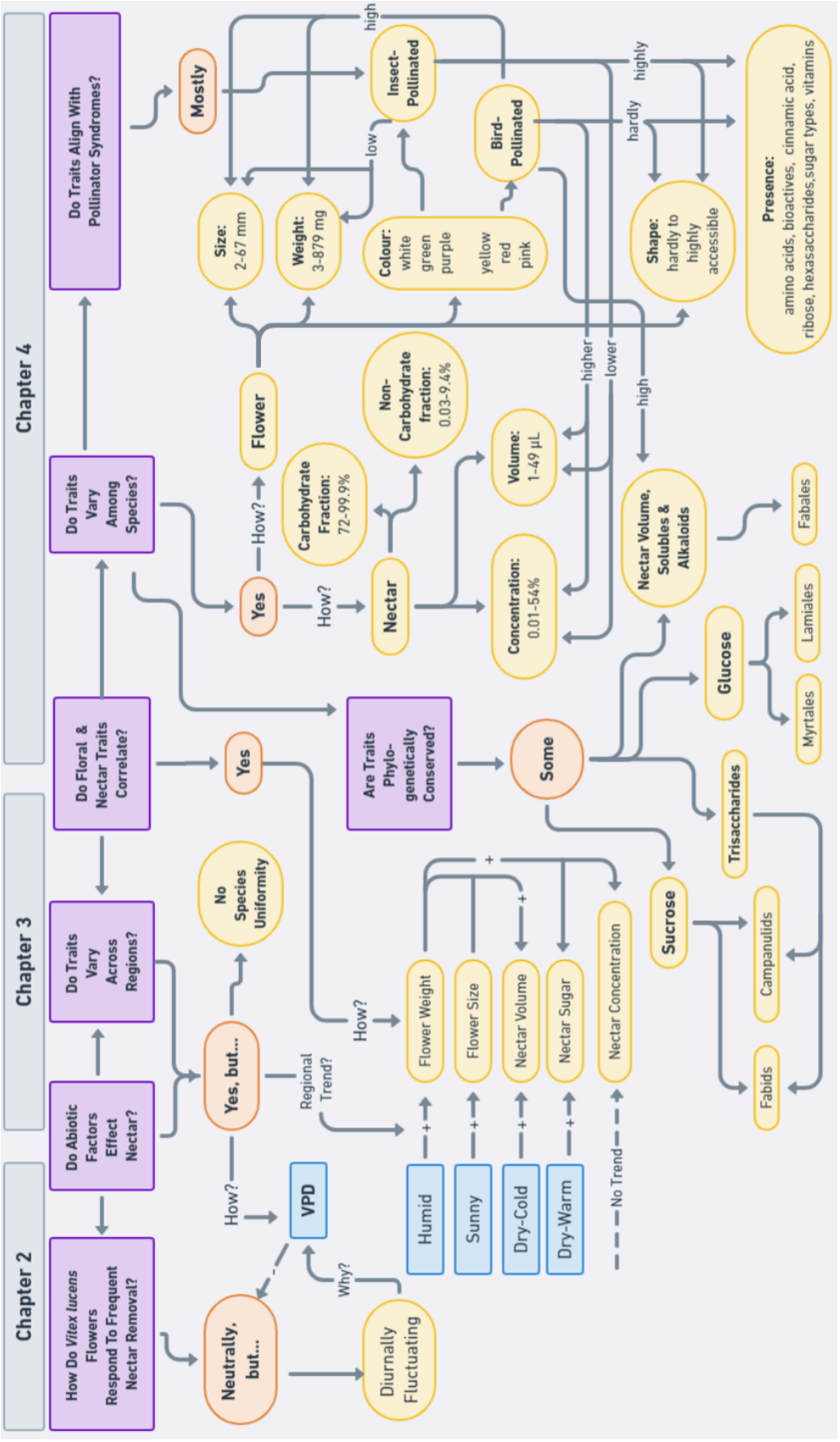


Figure 1: Schematic diagram of studied key questions (purple) and respective answers (orange) with details and their interrelations to findings from other chapters in yellow. Abiotic factors are in blue.

5.1 Key Findings

My study on *Vitex lucens* (Chapter 2) revealed a neutral response to frequent nectar removal, with similar nectar compositions in manipulated and unmanipulated flowers (Figure 2). During periods of peak vapour pressure deficit (VPD), increased VPD negatively impacted nectar replenishment, decreasing nectar volume and sugar content, in alignment with previous research (Fairhurst et al., 2021).

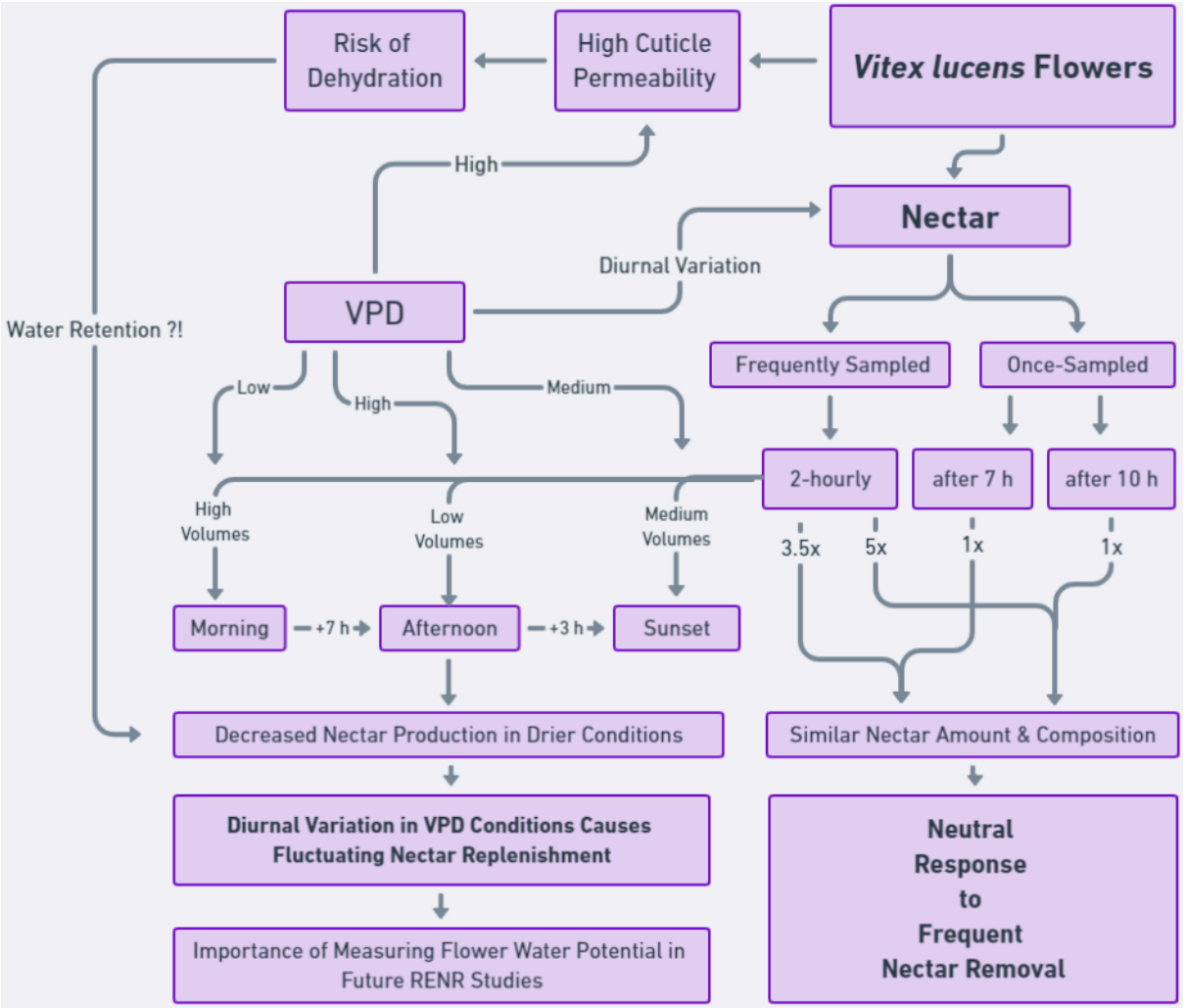


Figure 2: Schematic diagram of the Removal-Enhanced Nectar Replenishment (RENR) experiment on *Vitex lucens* flowers synthesising observations and conclusions, with VPD = vapour pressure deficit.

My study on regional plant trait (nectar and floral traits) variation (Chapter 3) found that climate was an influence on at least one and up to three traits in all species (Figure 3). The results indicate that climate is a major driver for floral and nectar trait variation, and floral trait-nectar trait correlations were often similar across species, with some exceptions.

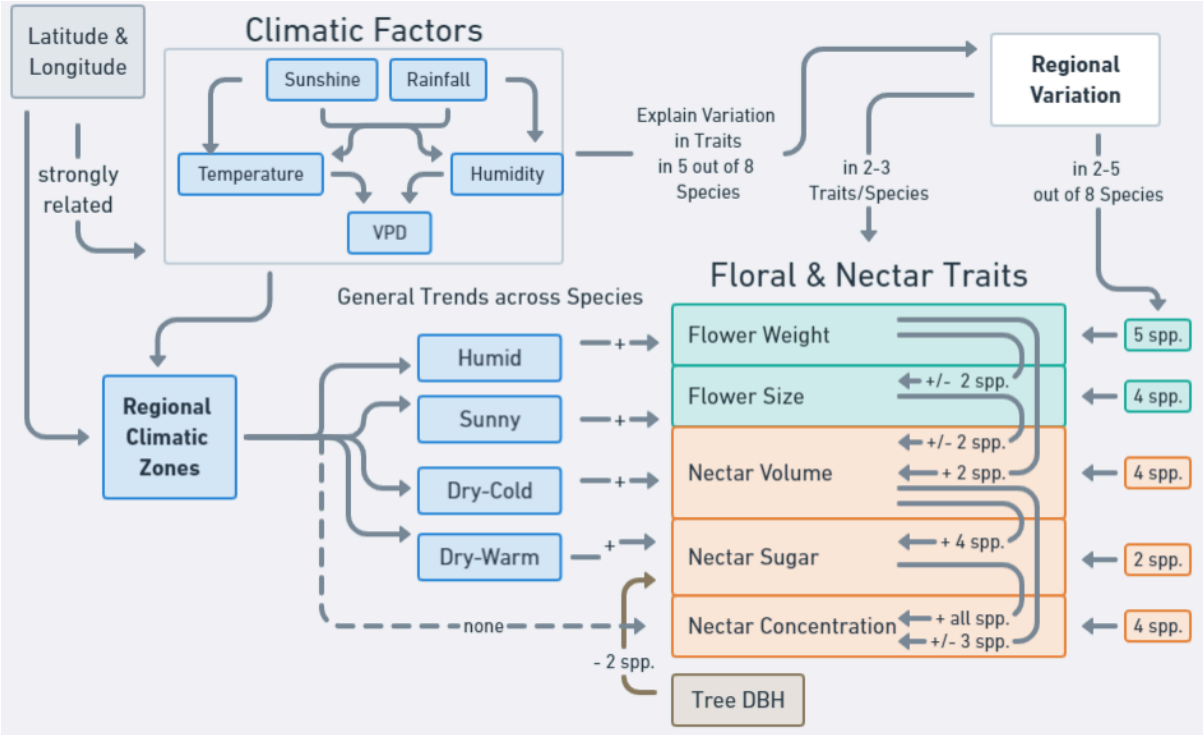


Figure 3: Schematic diagram of an inter-regional experiment analysing eight species. Observations are categorised by abiotic factors in blue, floral traits in green, and nectar traits in orange. The symbols on the arrows '+ x spp.', '- x spp.', and '+/- x spp.' indicate statistically significant positive, negative, or variable relationships between two traits across x number of species, respectively.

My study on interspecific nectar variation (Chapter 4) demonstrates that New Zealand's tree flowers produce nectar mainly composed of carbohydrates, with the remaining 3% comprised of alkaloids, amino acids, phenolics and vitamers (Figure 4). My findings demonstrated a strong phylogenetic signal in many nectar traits, as well as clear associations with pollinator syndromes. Given the limited phylogenetic range (50 species), and the tendency for multiple species sampling within some clades, it is difficult to separate phylogeny from pollinator-mediated selection.

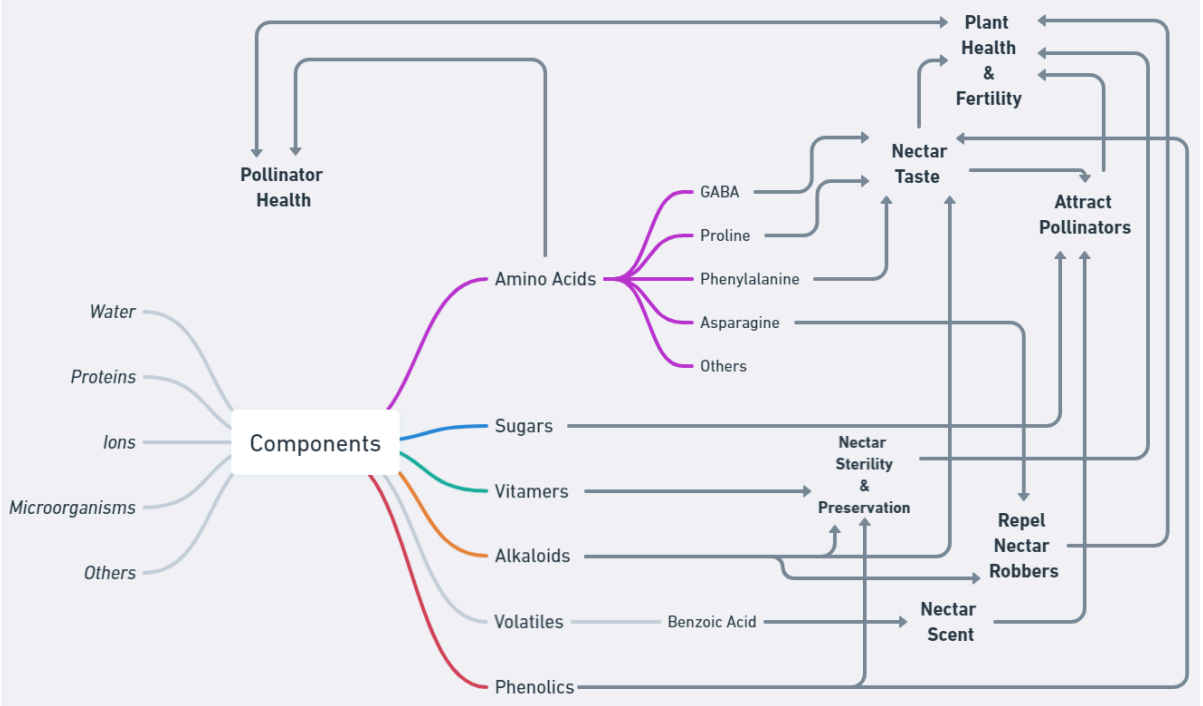


Figure 4: Simplified overview of nectar component categories. This figure divides nectar components into 11 groups (depicted in colour and light grey, with italicised compounds not studied in the thesis), with a detailed investigation of five groups (in colour) concerning their effects on nectar, plants, and pollinators.

5.2 Interpreting the Findings

In this section, I interpret my findings, examining how various factors such as floral visual traits, phylogeny, and environmental conditions influence nectar characteristics within and across species and regions.

In Chapter ('Ch.') 2, my findings on the model organism *Vitex lucens* indicate that New Zealand bird-pollinated flowers do not continuously secrete nectar when the nectar is frequently depleted throughout the day. Flowers reduce nectar production under high vapour pressure deficit (VPD) conditions. Relative humidity and air temperature— the factors determining VPD, also influence nectar variation across populations. This pattern was confirmed in Chapter 3, where I found decreasing nectar volumes linked to higher air temperatures in *Metrosideros excelsa*. Nectar volumes in two species (*Leptospermum scoparium, Pittosporum crassifolium*) were negatively influenced by annual sunshine hours, and the remaining species, which showed regional variation in nectar volumes, *Pittosporum eugenioides,* showed increasing nectar volumes related to higher annual rainfall amounts. All of these findings suggest that plant water status has a significant influence over nectar volume in many species.

Flowers require a steady water supply for bud expansion and nectar production, they can lose substantial water through transpiration, and they compete with the shoots for water during periods of high evaporative demand, all of which are possible explanations for reduced nectar volumes in hotter and sunnier conditions (Ch. 3). Moreover, flowers potentially have a higher cuticle permeability than leaves and may be more prone to dehydration, risking accelerated water loss and xylem cavitation (Zhang et al., 2017). This raises further questions about whether other species also exhibit distinct diurnal nectar replenishment responses, as seen in *V. lucens* in Chapter 2. Interspecific variation in refill responses could be driven by differences in flower cuticle permeability or the total number of flowers per plant, which could offset increased water loss. Potential variations in refill responses may also be attributed to differences in species' flower shape or colour (Ch. 4), nectary vascularisation or nectar secretion mechanisms.

The enclosed shape of bird-pollinated flowers (Chs. 3 and 4), such as the flag-shaped flowers of *V. lucens* (Ch. 2), could lead to decreased nectar secretion during periods of high VPD by trapping heat, potentially damaging reproductive organs, especially when exposed to direct sunlight (Patiño & Grace, 2002; Little et al., 2016). This situation might result in some

flowers ceasing nectar secretion altogether, which I observed in *Vitex lucens* in an entire 10-flower panicle during the hours of peak VPD. Species with highly accessible nectar sources, such as those with dish- and brush-shaped flowers (Chs. 3 and 4), may be more sensitive to high VPD conditions due to their open structure, potentially leading to a quicker and more prolonged reduction in nectar refill volumes. In upward-facing disc-, bowl-, or bell-shaped flowers, often linked to the entomophilous syndrome (Ch. 4), the reproductive organs can overheat under direct sunlight, exacerbated by additional light reflection from the petals (van der Kooi et al., 2019). Thus, species with these flower shapes, such as the dish-shaped New Zealand flowers of *Leptospermum scoparium, Cordyline australis* (Chs. 3 and 4), *Kunzea ericoides*, or *Myoporum laetum* (Ch. 4), which likely exhibit increased light reflectance on reproductive organs due to their white perianths, might be significantly affected by heat and its management. This suggests a higher likelihood of these species ceasing nectar secretion under high VPD conditions.

Additionally, the reproductive organs of the dark purple-coloured, bell-shaped *Pittosporum* species (Chs. 3 and 4) likely experience elevated perianth temperatures due to their dark colouration absorbing more light, which is then re-emitted as heat (Little et al., 2016; van der Kooi et al., 2019). An increased flower temperature on cooler days might offer a fitness advantage by offsetting potential decreases in nectar production during warmer parts of the day. This theory is supported by research showing that bees can associate specific colours with temperature, suggesting that colour may indirectly boost pollination success via the learned behaviours of pollinators (Dyer et al., 2006). Furthermore, insects can detect the temperature of flowers, which can be significantly warmer than the ambient air, attracting insects during foraging in cooler temperatures. These insects might prolong their visit within the warmer flower, potentially enhancing the plant's reproductive success. An extreme example is the arctic *Dryas integrifolia*, whose flowers are over 7°C warmer than the surrounding air on average, increasing the body temperature of visiting insects by up to 15°C (Kevan, 1975). Referring to other 'thermal rewards' for pollinators, the flowers of the Asian species sacred lotus (*Nelumbo nucifera*) exhibit thermogenic properties, heated through respiratory pathways (Watling et al., 2006). In that regard, the purple flowers of *Pittosporum crassifolium* (Chs. 3 and 4) and the pink flowers of *V. lucens* (Chs. 2 and 4) in New Zealand bloom during the cooler months—*Pittosporum* in September to early October and *Vitex* mainly from June to August (Landcare Research, 2018)—making them potentially attractive

to insects seeking warmth. Insects visit *V. lucens* despite the species primarily being categorised as bird-pollinated.

Moreover, flower colour (Ch. 4) adaptability to temperature changes can influence floral temperature, providing reproductive benefits (van der Kooi et al., 2019). This adaptability manifests in how anthocyanin levels vary with light intensity, with increased production under full light potentially responding to higher temperatures from more intense solar radiation (Ch. 3; Del Valle et al., 2018; van der Kooi et al., 2019). Intriguingly, in a trial RENR study on six *V. lucens* trees with most sunlit flowers facing in different cardinal directions, I observed possible light-induced differences in perianth colour saturation (from bright to pale pink), although this was not quantitatively measured.

Since flower orientation directly affects sunlight absorption, altering this orientation—through heliotropism—allows some species to regulate heat capture throughout the day. Heliotropism is observed in at least 17 genera across seven plant families, predominantly in Astera- and Ranunculaceae (van der Kooi et al., 2019). This phenomenon might influence species' variability in repeatable nectar removal (RENR) responses. New Zealand's flora is particularly rich in Asteraceae, with 287 species (91% endemic), including tree species like *Olearia* (Wagstaff & Breitwieser, 2002). However, the complex inflorescences of Asteraceae pose significant challenges for RENR studies, mainly due to the small size of individual flowers. Conducting RENR experiments on these inflorescences, especially when they remain on trees, is impractical following my experience handling approximately 10,000 individual flowers of various shapes and sizes.

The findings of Chapter 3 support the conclusions drawn from Chapter 2. Differences in air temperature and other abiotic factors along a geographical gradient foster divergence in nectar properties, e.g. increasing nectar volumes with decreasing air temperature and higher latitude. This has also been observed in various other habitats (Devoto et al., 2006; Chalcoff et al., 2008; Paiaro et al., 2012). For example, a study on *Monttea aphylla* (Plantaginaceae; Ferreiro et al., 2015), resembling New Zealand's Fabaceae *Carmichaelia australis* studied in Chapter 4*,* also found latitude significantly explaining population variation in nectar (and oils), with nectar volume increasing significantly with increasing latitude. Other investigations have documented intraspecific floral variations along geographical gradients, highlighting population divergence often driven by shifts in the types and abundances of pollinators (Pérez-Barrales et al., 2007; Chalcoff et al., 2008; Nattero et al., 2011; Paiaro et

al., 2012; Baranzelli et al., 2014). Variations in nectar composition may signal adaptation to local pollinator groups, distinguished by their dietary and ecological needs (Simpson & Neff, 1983; Fenster et al., 2004; Petanidou, 2005), which was beyond the scope of my study. However, I did reveal variations in nectar composition across species, which I could partly link with pollinator syndromes (Ch. 4). Therefore, the floral composition of a community, influenced by the abundance, diversity, and spatial arrangement of plants producing floral rewards, can shape the composition, abundance, and behaviour of pollinator guilds (Sargent & Ackerly, 2008; Lázaro et al., 2009).

While I observed regional variations in most plant characteristics in most species, total nectar sugar amounts hardly varied (Ch. 3). This finding was supported by the results of Chapter 4, indicating that variation in nectar composition is predominantly driven by phylogeny and flower morphology, which includes the flower's pollination syndrome. Broadly, New Zealand's nectar resources appear non-specialised, with a roughly equal distribution between species producing hexose-rich nectars (55%, predominantly among Lamids and Malvids) and those with sucrose-rich nectars (45%, mainly Fabids). Nonetheless, only a minority of tree species (13 out of 47) exhibited sucrose dominance (sucrose > 50%), such as the dark pink or purple flowering species *Rhopalostylis sapida, Knightia excelsa, Pittosporum ralphii,* and *P. tenuifolium*. However, the correlation between flower colour and sucrose content is not straightforward, suggesting that colour is not an indicator of nectar sugar composition, except possibly for the association of higher hexasaccharide levels with the purple-hued *Pittosporum* spp.

Conversely, particularly rich in hexoses was the nectar of the Malvids *Kunzea ericoides* agg., *Leptospermum scoparium*, *Metrosideros excelsa,* and *M. umbellata*; and the two Lamids *Rhabdothamnus solandri* and *Veronica diosmifolia*. My observed patterns (Ch. 4) confirmed previous observations by Percival (1961), who found hexose-rich nectar in the same or related species. The variation in the ratio of sucrose (S) to hexoses (fructose and glucose, 'F+G') among the *Metrosideros* and *Pittosporum* species I studied reinforces Percival's (1961) observation that nectar composition can vary within genera. The variability in sucrose dominance is likely driven by differences in invertase enzyme expression or activity (Minami et al., 2021), suggesting significant intrageneric variation without the need for new genetic developments. Similarly, variation in the ratio of fructose to glucose in nectar could be driven by relatively small differences in hexose transporter activity (Lin et al., 2014).

Additionally, species traditionally classified under Scrophulariaceae, now distributed among Orobanchaceae, Phrymaceae, Plantaginaceae, and others, also demonstrate diversity in nectar composition. Some genera, such as *Veronica*, produce hexose-dominated nectar, consistent with my observations in *Veronica diosmifolia* (Ch. 4). In contrast, genera like *Pedicularis, Mimulus*, and *Cymbalaria* feature sucrose-rich nectars (Percival, 1961; Freeman et al., 1985; Baker & Baker, 1990). The taxonomic reassignment of these species into different families corroborates my findings of sugar-type variability across families. My study (Ch. 4) also reaffirms Percival's (1961) observation of an association between tubular flowers and sucrose-rich nectars (indicated by high Baker's ratios, with Baker's ratio = [S/(F+G)]) and the tendency for open flowers to possess hexose-rich nectars (denoted by low Baker's ratios).

Interestingly, my analysis revealed only a limited number of nectar traits that varied according to pollination syndrome (Ch. 4). Confirming the high phylogenetic conservation of flower shape and the sugars sucrose and glucose, my findings align with the consensus that nectar sugar types are less influenced by pollinator preferences and more by phylogenetic constraints (Percival, 1961; Elisens & Freeman, 1988; Baker & Baker, 1990). This suggests that the composition of floral nectar sugars in many plant families follows a common pattern. Indeed, similar observations were made by Chalcoff et al. (2006), who reported that in South American species sampled across temperate forests, nectar sugar composition was independent of pollinators. Notably, the majority (17 out of 26 species) of the South American species had nectar with more than 50% sucrose content. In contrast, in my study, only four out of 50 species showed sucrose-dominated nectar, underscoring the significant influence of phylogeny and environment on nectar composition. It is noteworthy that separating the effects of pollination syndrome and phylogeny is challenging, not only because pollinator syndrome itself is influenced by phylogeny, but also because sampling biases further complicate the analysis.

Alternatively, my findings (Ch. 4) suggest that the presence of non-SFG sugars in nectar is partly influenced by pollinator type, as certain carbohydrates, including some larger sugars classified as hexasaccharides and the smaller pentose ribose, were notably absent in bird-pollinated species. The significance of hexasaccharides in insect diet and preference is less documented, likely because these sugars are less common in nectar and require more complex metabolic processes for digestion than simpler sugars. However, recent research (Fujii et al., 2023) has demonstrated that dipterans (flies) can detect and are attracted to

ribose, suggesting that nectar rich in ribose could prolong pollinator visits. This suggests that the presence of ribose in the nectar of insect-pollinated plants has conferred a selective advantage by appealing to specific pollinators, resulting in the conservation of this trait over time through enhanced pollination efficiency and success.

Unexpectedly, aside from alkaloids, none of the tested non-carbohydrates (amino acids, phenolics, and vitamers; Ch. 4) correlated with phylogenetics. Yet, I discovered that specific vitamers were particularly concentrated in flowers of colours typically associated with insect pollination—white, green, yellow-green and purple. Notably, the high vitamer concentration in the bird-pollinated *Rhabdothamnus solandri* (Gesneriaceae, Lamiales), the only orange-flowering species sampled, was the exception. Given the rarity of orange-flowering species in New Zealand (Ladley et al., 1997), this finding, while limited by the natural rarity of such species, offered valuable insights into nectar composition dynamics. Interestingly, this species also exhibited elevated alkaloid levels, similar to the high levels found in the tested *Sophora* spp. (Fabales). This supports the link between such compounds and ornithophily, which is attributed to their ability to repel nectar robbers with bitterness, dark colouration, and toxicity (Janzen, 1977; Stephenson, 1982; Hagler & Buchmann, 1993; Adler, 2000; Johnson et al., 2006; McDougal et al., 2015). Alkaloids, but also other secondary metabolites, can alter the nectar's appearance, taste, and digestibility, potentially attracting specific pollinators (Baker, 1978; Hagler & Buchmann, 1993; Mione & Anderson, 1996; Olesen et al., 1998; Weller et al., 1998; Adler, 2000; Gardener & Gillman, 2002). For instance, benzoic acid is known for its role in floral fragrance (Knudsen et al., 2006; Kong et al., 2013) and was present in many of my samples, although not quantified. My data on nectar components, beyond the common sugars sucrose, glucose and fructose, highlights the complex interplay of nectar composition with pollinator attraction.

Intriguingly, my findings indicate that certain nectar compounds are exclusively present in the nectar of insect-pollinated species, which is less concentrated than in other regions. This characteristic might stem from the long-standing isolation and environmental history of New Zealand's flora and fauna. Climatic cooling, increasing isolation, and tectonism have acted as significant environmental filters, leading to regional extinctions, reduced floral diversity, and major turnovers in the floristic composition of New Zealand (Lee et al., 2001). Lee et al. estimated that the New Zealand flora lost at least 15 families and 36 genera during the Miocene and Pliocene due to cooling conditions and the absence of significant refugia at

lower latitudes. This loss indicates that the current characteristics of New Zealand's flora are largely shaped by selective pressures from climate changes during the Late Miocene, Pliocene, and Pleistocene rather than events of greater geological antiquity.

New Zealand's prolonged geographic isolation, dating back about 70 million years, likely contributed to a slower coevolution rate and radiation than continental regions. This isolation has restricted the influx of new species, thus limiting opportunities for new evolutionary pressures and interactions, which explains New Zealand's high rate of endemism (Wallis & Trewick, 2009). In contrast, continental regions experience a constant introduction of new species and diverse habitats that promote rapid speciation and varied evolutionary pathways (Wright et al., 2006; Pérez‐Escobar et al., 2017; Harvey et al., 2020).

In New Zealand, the speciation of insects and trees might have been influenced by the absence of the intense competitive pressures typical of more biodiverse ecosystems like the tropics. With fewer species present (Thomson, 1881; Heine, 1938; see Chapter 1), insects and plants likely have experienced less selective pressure to diversify rapidly in response to competition.

5.3 Implications of the Research

This section discusses the implications of my findings on nectar and flower traits and how these insights can aid in forecasting food availability and foster conservation efforts for wildlife reliant on flowers and nectar.

My data (Ch. 4) imply that the plant's clade can be a useful proxy for the predominant nectar sugar type. This information allows for a broad classification of landscapes based on their hexose or sucrose nectar availability, given knowledge of plant community composition and the number of flowers a plant or tree produces throughout its flowering season. For calculating nectar volume at this scale, the average flower size within the plant's genus and the number of flowers per tree provide a basis to infer the total nectar volume. In general, such an approach does not necessitate an additional correction factor for regional climate differences between New Zealand's North and South Islands, as relative nectar volumes do not vary significantly between these locations, as shown in Chapter 3 (Wilcoxon rank sum test; $P = 0.456$; based on 164 nectar samples from 8 species, with 79 samples from the South Island and 85 from the North Island).

However, for the species *Pittosporum crassifolium*, *P. tenuifolium*, *Cordyline australis* and *Leptospermum scoparium* (out of the eight species I analysed in Ch. 3), regional variation in nectar traits would need to be considered when studying relationships between nectar-feeding pollinator guilds and plant communities. *P. crassifolium* and *C. australis* produce higher sugar amounts, and all four species produce higher nectar volumes in New Zealand's south, specifically in Otago–Dunedin (with *P. tenuifolium*'s nectar volume also significantly high in Auckland).

For studies focused on predicting food availability for flower-feeding birds, it is noteworthy that O'Donnell & Dilks (1994) recorded seven indigenous species primarily feeding on *Fuchsia excorticata* flowers (Chs. 3 and 4), constituting approximately 50% of these birds' flower-based diets. *Fuchsia* flowers are largest in terms of length in New Zealand's sunniest regions, Nelson-Tasman and Marlborough (Ch. 3), suggesting a potential for greater energy intake with less foraging effort for flower-feeding birds. Notably, *F. excorticata* exhibits a higher flower fresh mass in regions such as Hawke's Bay, Wellington, and Otago (Ch. 3). While I lack data on the flower's dry weight, this difference could be attributed to a higher water content within the flower tissue. This increased water content may benefit birds during the drier seasons, especially in the Hawke's Bay region, which has high VPD conditions and

low annual rainfall amounts (Kidson, 2020). Although *F. excorticata* does not vary in nectar volume across regions, it may show diurnal volume fluctuations based on the influence of VPD, as I demonstrated for *V. lucens* (Ch. 2). Both species have 'tube-shaped' flowers (*Vitex*: flag-shaped, Figure 5a; *Fuchsia*: funnel-shaped, Figures 5b-c) of relatable fresh mass (~ 200 - 400 mg) and size (2 - 3 cm of corolla lengths; Ch. 4). *Fuchsia*'s flower size and average nectar amounts could be sampled feasibly with flowers remaining on the tree, ideal characteristics for this species being used as another model organism for future RENR studies, and subsequent comparisons to my data of *Vitex* in Chapter 2. *Vitex lucens* has highly resilient flowers, which cope well with repetitive handling. Notably, only a single flower out of 120 fell off during my experiments, making this species a suitable model organism for future flower studies.

a **b** **c**

Figure 5: Tube-shaped flowers of the species: a) *V. lucens*, b) *F. excorticata* with pink pigmentation, and c) *F. excorticata* showing variations in pigmentation - intermediate (top, pinkish petal apices and nectar chamber) and green-purple (bottom).

Similarly, the flowers of *F. excorticata* are resiliently connected to their petioles with little likelihood of detachment due to repeated handling, as observed from personal experience. However, I encountered difficulties when inserting the micropipette tip through the narrow corolla passage (see Figure 6, arrow) to reach the nectar chamber, which required significant force. Although tissue damage did not play a role in nectar removal in single instances with flowers detached from trees (Chs. 3 and 4), it might impact the flower's ability to replenish nectar when repetitively handled while remaining on the tree. Therefore, I suggest that future studies considering *F. excorticata* as a model organism should select an appropriate tip size to minimise tissue damage.

Figure 6: Longitudinal section of a *Fuchsia excorticata* flower showing nectar in the nectar chamber, with the tip of a simulated micropipette tip inserted into it. The arrow indicates the narrow passage that could be damaged by the tip during repetitive sampling.

The gynodioecious *F. excorticata* (Delph & Lively, 1985, 1989), displays corollas in green-purple or pink. Although selecting pink *F. excorticata* flowers for a direct RENR comparison with *V. lucens*'s pink flowers may seem logical, the variation in *F. excorticata*'s colouration is not due to different variants but is instead a result of floral age, with colour change occurring after 4 - 5 days — even in bagged flowers — as shown by Delph & Lively (1985). Despite their vibrant colour, pink flowers are less frequented by birds as they contrast less with the orange bark background compared to the ostensibly more subtle green-purple flowers. Pink flowers also produce significantly less nectar than green-purple ones, with no observed differences between female and hermaphrodite flowers (Lim & Burns, 2024). My sub-analysis of 767 *Fuchsia* flowers sampled between 2019 - 2020 (green-purple: $n = 528$; intermediate: $n = 90$; pink: $n = 149$) confirmed this pattern. A Kruskal-Wallis test indicated significant differences in nectar volume based on corolla pigmentation ($\chi^2 = 15.22$, DF = 2, $P < 0.001$). Green-purple (25 ± 15 µL, range 2 - 60 µL) and intermediate (29 ± 24 µL, range 6 - 72 µL) flowers produced significantly more nectar than pink flowers (4 ± 5 µL, range 0.002 - 15 µL), with no significant variation between green-purple and intermediate flowers (Dunn's test incl. Bonferroni correction, green-purple/pink: $P = 0.0001$, intermediate/pink: $P = 0.001$, green-purple/intermediate: $P = 0.41$). Based on these observations, which predated Lim & Burns (2024), I chose to include only green-purple flowers in my analyses for Chapters 3 and 4.

5.4 Study Limitations

This section outlines the limitations encountered during the study, organised by chapter, to provide clarity and coherence regarding the challenges faced and their implications on the study's findings.

The initial plan for the experiment on frequent nectar removal (Ch. 2) included conducting five repetitions over two weeks during the winter of 2020 in New Zealand, with the assistance of at least a second handler. Due to pandemic-related restrictions, only one repetition could be completed within the intended season. Despite this limitation, using two controls for each experiment allowed for significant conclusions. A more granular approach of collecting nectar amounts per 2-hour refill interval and for each flower individually into separate vials, rather than pooling by inflorescence, could have provided a larger dataset for analysis and greater statistical power. However, given the time constraints in fieldwork, this level of detail was not feasible; handling 80 flowers per tree, even with pooling of the nectar of 20 flowers, already required 40 minutes for a single handler. Introducing additional handlers could lead to discrepancies in data acquisition, suggesting an alternative approach of sampling trees on separate consecutive days. However, this method also presents challenges as environmental conditions can fluctuate daily, potentially affecting the data. Conducting experiments in a controlled environment could mitigate these day-to-day variations. Nonetheless, larger woody species, such as mature trees of *F. excorticata* and *V. lucens*, are less suited for study under these idealised conditions due to their size.

My research in Chapter 3 focused solely on the influence of abiotic factors on regional variations in nectar and floral traits, employing a simplified approach based on 30-year climate norms. Due to the limited scope of factors tested, my analysis may not have fully captured the drivers behind the observed species-specific variations. My approach was predicated on the assumption that the observed variations were primarily driven by environmental factors rather than plant-specific variations such as genotypes. Ideally, future analyses would incorporate more detailed data on weather conditions during sampling and plant factors like water status and genotype. Additionally, it is important to consider biotic factors, such as the varying composition of pollinators, to fully understand the regional differences in floral phenotype and nectar traits. However, combining plant-pollinator interactions with nectar sampling introduces logistical and time constraints, particularly in areas with overlapping pollinator communities and within larger geographic scales.

Future studies on nectar variation should apply an integrative approach by gathering botanical and zoological data simultaneously. For example, monitoring flower visitors per species across regions and using the number and nature of visits per species as additional predictors in statistical models could help identify why regional nectar variation is species-specific. These models should incorporate measured abiotic factors, particularly VPD, rainfall, and solar radiation, drawing on insights from Chapters 2 and 3, as well as literature on local soil types. Considering the potential influence of a flower's water status on nectar production, integrating measurements of soil moisture, sap flow, and the flower's water status into these studies could yield valuable insights.

Following the classification by Baker & Baker (1990), which distinguishes plants based on their nectar sugar composition, my study found trends consistent with their findings: 63% of insect-pollinated species had hexose-rich nectar, and 57% of bird-pollinated species had sucrose-rich nectar. However, these findings, particularly the latter, were derived from a small sample size (14 ornithophilous species) and thus may not robustly support the binomial classification into bird- vs. insect-pollinated species proposed by Baker & Baker. Further analysis using continuous values of the Baker's ratio did not show significant differences across pollinator syndromes, suggesting a more nuanced relationship than previously indicated.

My study focused on sampling individual trees with accessible flowers, excluding flowers from high canopies and species that flower at unreachable heights. Specifically, notable nectar-producing species such as *Ixerba brexioides* and *Beilschmiedia tawa* (de Lange, 2024a, b) were omitted due to the elevation at which their first flowering branches are found. As a result, the flowers I collected were more likely to undergo significant diurnal sun exposure variations compared with flowers higher up, especially those from forest edges or open grasslands, indicating that these species were subject to edge effects.

Burgess et al. (2005) identified significant negative impacts on fruit or seed set for 50 plant species in their literature review, while 26 species showed no effect, and only nine, including the New Zealand mistletoe *Peraxilla tetrapetala* (Loranthaceae), exhibited positive responses. This particular species showed increased fruit set at forest edges, likely due to enhanced bird and insect visitation rates, with a notable preference among pollinators — especially native bees — for flowers in direct sunlight. This trend of improved reproductive success at edge habitats was similarly noted in the other eight positively affected species, suggesting that similar patterns might apply to nectar traits.

Consequently, further nectar studies should investigate whether: *i*) the species studied in this thesis display positive or negative edge effects concerning nectar traits, and *ii*) flowers in the canopy, which are less protected from sun, wind, and rain yet more affected by high water potentials, exhibit different nectar traits compared to those at lower heights.

Overall, these limitations underline the importance of cautious interpretation of the study's results and point towards potential areas for future research. This includes employing more comprehensive experimental designs and expanding sampling strategies to understand better the ecological impacts and evolutionary responses across different floral habitats.

5.5 Future Research Directions

Exploring the intricate world of flower morphology and environmental interactions unveils the potential of flower shape and colour as drivers for species-specific nectar refill responses. It is crucial to delve into how plants manage their water resources during high evaporative demand or soil water stress, which involves measuring the species' flower and shoot water potential concerning varied nectar vascularisation, especially under RENR conditions or in the face of regional climate and nectar variation.

To further explore how regional climate variations affect nectar production, one could assess the 24-hour nectar production rate (NPR) of plants under controlled conditions that simulate the climate of specific regions, depleting their nectar daily, minimising the effect of naturally foraging pollinators to simplify the model. Before conducting these experiments, it is crucial to determine whether the regional populations of the target species are genetically uniform, since nectar traits can vary with genotype (Leiss & Klinkhamer, 2005; Clearwater et al., 2018). If they are not, pot-raised plants of the respective regional genotypes should be utilized to test their NPR under the corresponding simulated regional climate conditions.

Controlled NPR-assessment or RENR experiments in a glasshouse using pot-raised, nectar-secreting plants would enable detailed analysis of nectar production in response to environmental factors like lighting, air quality (including VPD and CO_2 concentration), and soil moisture. Continuous monitoring of plant sap flow and flower corolla temperature during experiments could provide essential data on the influence of water relations on nectar supply.

Particularly, monitoring NPR or RENR under elevated CO_2 conditions, while monitoring flowers via thermal imagery for estimating floral stomatal conductance (Leinonen et al., 2006), could offer critical insights into future nectar availability amidst global change. This approach would facilitate scaling up individual plant nectar volumes to landscape levels, referencing studies such as Amthor (1999). The impact of increased CO_2 on plant growth and nectar secretion has been extensively documented in studies (Idso et al., 1987; Field et al., 1995; Cech et al., 2003; Asshoff et al., 2006; Leuzinger & Körner, 2007; Leuzinger & Bader, 2012; Bader et al., 2013), including its specific effects on floral nectar (Rusterholz & Erhardt, 1998; Erhardt et al., 2005; Hoover et al., 2012).

Hoover et al.'s study (2012) provides a useful model for exploring these dynamics in New Zealand species. They used a pumpkin system to demonstrate how nitrogen deposition, climate warming, and CO_2 enrichment collectively influence floral traits, including changes in

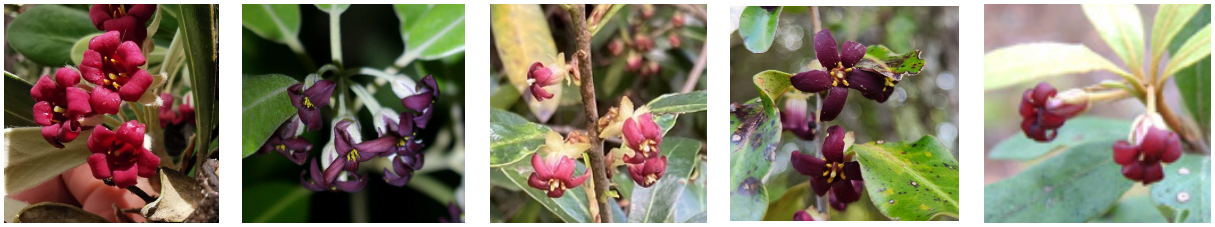
nectar chemistry, such as sugar and amino acid profiles. Their treatments significantly increased nectar attractiveness and reduced bumble bee worker longevity, with bees showing a marked increase in attraction to and consumption of nectar from treatments that decreased their survival by 22%. In a scenario where elevated CO_2 levels enhance nectar volumes but negatively alter its composition for pollinators, pollinator numbers could decline, thus reducing the plant's fertilisation success. Similarly, if elevated CO_2 levels decrease nectar volumes, offering lesser rewards, this could repel potential pollinators, reducing the plant's chances for successful fertilisation.

These outcomes pose significant risks for tree species that rely solely on sexual reproduction, as they may face an increased risk of extinction without reproductive alternatives. In contrast, species capable of self-pollination, such as *Vitex lucens* (Barrel et al., 1997), *Fuchsia excorticata*, and *Sophora microphylla* (Robertson et al., 2011) endemic to the New Zealand, may endure these adverse conditions slightly longer than those dependent exclusively on sexual reproduction. However, self-pollination in these species is associated with reduced seedling fitness. For instance, in the study by Robertson et al. (2011), fewer than 10% of selfed seedlings survived, and in three of the four cases studied, less than 1% survived; none flowered. Thus, the authors concluded that self-pollination is likely an ineffective reproductive strategy for these species, potentially leading to a decrease in their abundance due to the limited viability of self-pollinated offspring. These effects were confirmed by van Etten et al. (2015, 2018) in *Fuchsia excorticata* and *Sophora microphylla*.

To further explore whether and in which way flower morphology influences nectar replenishment responses across species, one could compare refill responses of species with tube-shaped versus open-shaped flowers. Further investigation could explore variations among different types of tube-shaped flowers — such as flag-, gullet-, funnel-, or bell-shaped — to ascertain whether specific morphological differences drive variations in nectar replenishment responses (Ch. 4).

To delve deeper, we could focus on a single type of tube-shaped flowers, specifically bell-shaped ones, to explore whether replenishment responses are consistent among closely related species, which may indicate phylogenetic constraints. During my fieldwork across New Zealand, I was intrigued by the genetic diversity among the many endemic *Pittosporum* species, which often exhibit high phenotypic similarity. Notably, the dark pink to purple-coloured bell-shaped flowers of *Pittosporum crassifolium* (Figure 7a), *P. ralphii*

(Figure 7b), *P. colensoi* (Figure 7c), and *P. tenuifolium* (Figure 7d) (Ch. 3 and 4) showed remarkable resemblance, making them ideal subjects for the proposed investigation. Adding *P. virgatum* (Figure 7e), another endemic species with similarly hued flowers, could enrich this group.



a **b** **c** **d** **e**

Figure 7: Closely resembling New Zealand *Pittosporum* species, with a) *P. crassifolium*, b) *P. ralphii* (iNaturalist, 2024a), c) *P. colensoi* (iNaturalist, 2024b), d) *P. tenuifolium* (iNaturalist, 2024c), and e) *P. virgatum*, naturally uncommon (New Zealand Plant Conservation Network, 2024).

Expanding the scope, this experiment could be replicated with light-coloured *Pittosporum* species (Figure 8) from diverse habitats to extend the phylogenetic framework, including the white to yellow flowering species *P. obcordatum* and *P. umbellatum* (New Zealand, figure 8a-b), *P. angustifolium* and *P. undulatum* (Australia, Figure 8c-d), *P. tobira* (North-East Asia, Figure 8e), and *P. ferrugineum* (South-East Asia and Australia, Figure 8f). Accompanying studies might include temperature measurements inside the corollas to determine if darker colours absorb more radiation than lighter ones, affecting the flower's temperature (van der Kooi et al., 2019) and, in turn, the species' RENR.

a **b** **c** **d** **e** **f**

Figure 8: White to yellow-flowering *Pittosporum* species, with a) *P. obcordatum* (iNaturalist, 2024d), b) *P. umbellatum,* c) *P. angustifolium* (iNaturalist, 2024e), d) *P. undulatum* (iNaturalist, 2024f), e) *P. tobira* (iNaturalist, 2024g), and f) *P. ferrugineum* (iNaturalist, 2024h).

However, as these analyses focus on petal colour effects on RENR *across* different species, they might not isolate the impact of petal pigmentation on RENR within a species. Thus, I propose a final experiment comparing RENR differences across colour variants within a

species, as reported for *P. tenuifolium*, where petals (of flowers on different plants) can have white, pink, dark purple or maroon pigmentation (Cooper, 1956).

The relationship between light exposure and nectar production presents an intriguing area for research. Investigating the subjective colour variations in *Vitex lucens*, potentially due to differing sunlight exposures (e.g. in Nozaki et al., 2015), and their effects on RENR could reveal the significant impact of colour and shape on nectar refill dynamics, for example, by conducting a shading/warming experiment using plastic shields. Nozaki et al. (2015) investigated the impact of various controlled temperatures on the intensity of flower colour and the content of the main anthocyanins in six pink flower genotypes of greenhouse chrysanthemum (*Chrysanthemum morifolium*). Their research aimed to elucidate the effects of high temperature on flower colouration. It was observed that poor colouration occurred at 30°C in most tested genotypes.

On the level of an individual plant, controlled experiments with constant environmental conditions could shed light on the eccrine mechanism of sugar secretion and whether species' varying responses to frequent nectar removal correlate with the plant's particular type of nectary vascularisation, as nectaries can be xylem- or phloem-supplied or not vascularised at all (Frei, 1955; Davis et al., 1986, 1988).

Fuchsia excorticata is a promising candidate for future research on flowers, particularly because it produces significantly larger flowers in New Zealand's sunniest regions (Ch. 3). These flowers develop into konini fruit, an essential part of the diet for several native bird species (McEwen & McEwan, 1978; Delph & Lively, 1985; Kelly et al., 2010). Investigating whether konini fruit size follows a similar regional pattern could yield fascinating insights. A positive correlation might suggest that birds in the Nelson-Tasman region benefit from a higher nutritional intake than those in other areas — a finding that could inform strategies for optimising bird conservation efforts.

Given that some New Zealand species, such as *V. lucens* and *F. excorticata*, flower over extended periods (de Lange, 2024c - d), examining how seasonal changes affect nectar variation presents a promising research direction. Insights from such studies could shed light on the combined effects of abiotic conditions and pollinator activity on nectar availability at

the community level. Incorporating a geographical perspective would further enrich our understanding of ecological and evolutionary dynamics across different populations.

Furthermore, exploring how lesser-studied abiotic factors, like sea salt spray, affect nectar production could enhance our knowledge of environmental impacts on nectar characteristics. This research might reveal the extent to which saltwater spray influences nectar production across coastal and inland populations.

While the effects of salt on nectar remain unclear, we have gained insights into the factors that influence sugar levels within nectar. Given the strong evidence from my data on the influence of phylogeny on the sugar composition of the nectar of New Zealand flowering trees, investigating whether South American species from Chalcoff's study show a similar trend could enrich our understanding of how phylogenetic factors constrain nectar composition.

Finally, although sugars are key in attracting pollinators, amino acids have also been shown to influence pollinator preferences (Ch. 4). My study, however, did not delve into the nuanced interactions between specific amino acids and pollinator syndromes, concentrating instead on overall amino acid levels. Investigating these interactions more closely, perhaps through feeding preference experiments, might shed light on the observed variations in amino acid concentrations among species, which were not directly associated with particular pollinator syndromes in my research. Similar experiments could be conducted for the phenolics and vitamers observed in Chapter 4.

My study highlights the complexity of nectar variation, yet it only partially explains observed differences. It underscores the need for future research to actively uncover and understand the myriad of factors influencing nectar variation across populations, species, and environments.

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