Dear Flora Foundation,

Thank you for the valuable financial support for my research project. The report below details progress made towards each aim outlined in my initial application. In summary, the number of tree's sampled in 2022 and site visitations are less than originally anticipated following a redesign of the sampling strategy for Aims 1 and 3 due to exciting and novel findings of a pilot study (outlined below). While we have completed Aim 2 of the original proposal, I request that current funds from the approved term (1/12/2021) be rolled over for 2023, in addition to the release of the second term instalment of $7,495 to cover costs associated with the planned works to complete Aims 1 and 2.

<u>Project background:</u>

The object of this study was to undertake one of the first investigations of how population translocations and ongoing climate change may reshape resource provisioning, using the forest tree species *Eucalyptus viminalis* (manna gum) as a model. Specifically, we aimed to investigate how genetics and the environment influences plant survival and resource provisioning of manna, a critically important food resource. Manna is a sugary carbohydrate produced by *E. viminalis* that is a core food resource for honeyeaters (*Melithreptus* genus), insects (ants, beetles, butterflies, moths), gliders/possums (including the endangered Leadbeater's possum) and the endangered Forty-spotted Pardalote (*Pardalotus quadragintus*) [1-4]. It is anticipated that the results from this research will provide key information to inform the targeted seed sourcing of forest tree species, such as *E. viminalis*, to establish climate-resilient and biodiverse ecosystems.

<u>Research progress:</u>

A pilot study revealed that manna quality and production varied seasonally, suggesting that sampling should be conducted during late spring/summer to maximise likelihood of successfully extracting manna. Interestingly, this coincides with the peak breeding season of the Forty-spotted pardalote (endemic to Tasmania). While this novel finding provides an optimal window to study the genetic and environmental drivers shaping manna variation among populations, it has resulted in the unforeseen requirement to rescheduling some field work associated with the below aims.

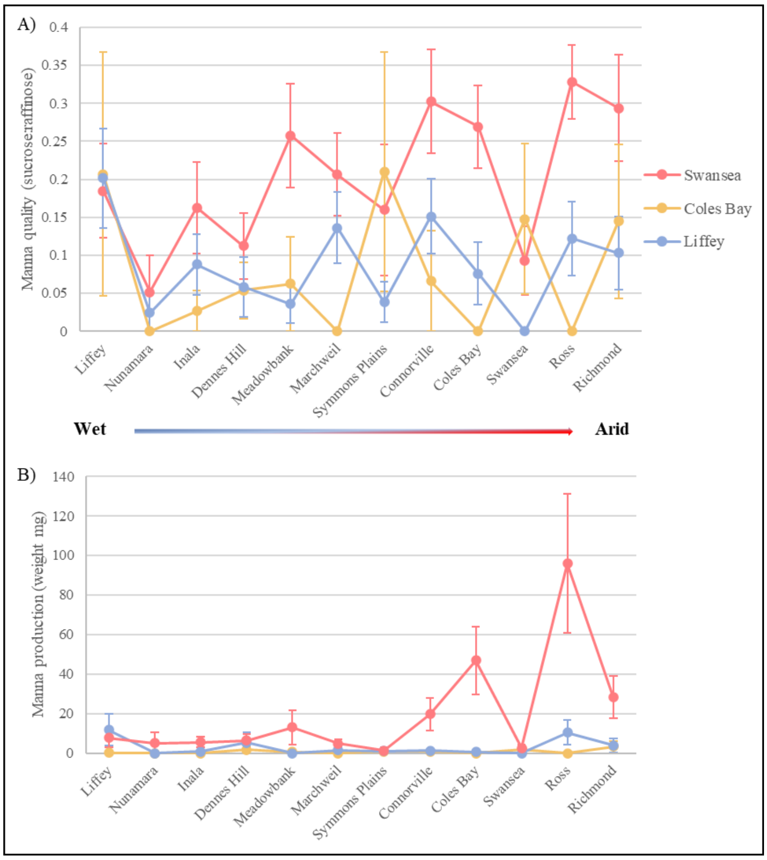
Aim 1: *Investigate differences in population performance (e.g., growth, survival, vigour) and variation in manna quality and quantity as underpinned by genetic and environmental factors.*

Three common garden sites were sampled to examine the extent of genetic by environment effects on manna production and quality. These sites, (Swansea, Liffey and Coles Bay) were targeted as they maintained the highest survival and growth rates and represented the extremes of the aridity gradients for these trials. The Swansea and Liffey sites fall at the driest and wettest ends of the aridity gradient, respectively, and Coles Bay falls between these two sites. While an additional three sites were planned to be sampled (Meadowbank, Marchwiel, Nunamara), growth rates have been slow, and the trials were deemed unsuitable for manna assessment. However, subsequent growth performance at these sites (based on assessment end of 2022) indicates they will be suitable for manna sampling in late spring/early summer 2023/24.

Two early findings have emerged from the initial sampling of 300 individuals across the three trial sites:

- At the site level, for those trees that produced manna the quality, as measured by the ratio between sucrose: raffinose (the two most common sugars found in manna), tended to be on average highest at the Coles Bay (0.679 ppm) site, compared to the Liffey and Swansea sites (0.319 and 0.385 ppm, respectively) which tended to be more consistent. Manna production, as measured by the total weight of manna produced per branch collected, also varied among the sites, with trees at the Swansea site producing on average higher quantities of manna, (20.12 mg) than Liffey (11.02 mg) and Coles Bay (7.03 mg).
- At the provenance level, there was evidence of variation in both manna quality and production between the provenances for all trees that were sampled (Figure 1).

Figure 1: Mean manna quality (A) and manna production (B) and standard error for each provenance across the three sites, as ordered by annual heat moisture index, from wet to arid.



Variation in manna quality was high between all provenances, and within provenances across the three sites. This suggests there is both environmental and genetic variation in manna quality occurring. Interestingly, trees from wetter provenances show greater overlap of error bars, indicative that variation is most likely underpinned by genetics rather than environment. Variation in manna production was more conserved and showed a general trend towards higher quantities of manna being produced by trees from more arid provenances.

Irrespective of site, trees from the Richmond provenance showed the best performance when considering both quality (0.189 ppm) and quantity of manna produced (13.2mg). With the Liffey provenance producing on average the highest quality manna at 0.196ppm, and the Ross provenance on average greatest quantity at 42.59 mg.

Given the large variation in manna quality at the provenance level, sampling manna from additional sites will enable us to further disentangle the extent of this variation. This will also involve re-sampling of initial sites (Swansea, Liffey and Coles Bay) to remove potential annual variation. Performance traits (height, survival, vigour) will also be considered in final analysis of GxE variation in manna production and quality.

Aim 2: *Effect of water-stress and provenance variation on manna production.*

The objective of this aim was to investigate if manna production varies among populations when grown under water-stress conditions. A controlled dry-down experiment was conducted on 72 *E. viminalis* saplings (approx. 2yrs old) representing 31 families from six populations (three wet and three arid) that spanned and aridity gradient across the species natural range within Tasmania. Saplings were randomly allocated into three treatments: hydrated, dehydrated and control, where control was included to consider the effect of repeated incisions on manna production. Plants in the dehydration treatment underwent a 5-day dry-down, followed by 10-day recovery, and final 10-day dry-down, with manna sampled from all trees across these three time points. Manna quality and production was then associated with the provenance homesite aridity as measured using the annual heat moisture (AHM) index (Table 1).

Table 1. Provenance/homesite details (Lat, Long, AHM) and associated climate classification of plants used in dry-down experiment.

Provenance/homesite	Lat	Long	AHM	Climate
Swansea	-42.15	148.07	40.46	Arid
Connorville	-41.84	147.16	35.88	Arid
Dennes Hill	-43.09	147.35	28.09	Arid
Inala	-43.39	147.25	23.24	Wet
Nunamara	-41.38	147.29	20.59	Wet
Liffey	-41.68	146.80	18.81	Wet

Homesite climate appeared to cause greatest variation in manna production and total concentration. Where those plants from drier climates overall were more likely to produce manna (6.36% more likely) and in higher concentration (155.22 ppm vs 59.93ppm) regardless of treatment. Within treatments only those plants from wetter climates showed significant variance in total quantity of manna produced due to water availability. With dehydrated plants producing on average 10.9 mg less manna than those that were hydrated.

These results suggest that *E. viminalis* plants from a drier homesite climate show an overall greater capacity to produce manna regardless of water availability.

Aim 3: *Develop spatially explicit model to explore how future climates may affect manna production.*

A total of 120 individuals representing 12 natural populations spanning the *E. viminalis* distribution in Tasmania have been sampled to capture the geographic and climatic variation in manna production and quality (Table 2). The individuals selected from these populations have previously been utilised in a genome-wide study of adaptive variation in *E. viminalis* [5] and include the wild mothers of the progeny in the common garden field trials in Aim 1. It is anticipated that the collected manna will be quantified for its quality and production using the same established method utilised in Aim 1 and modelled as a function of homesite climate to build a preliminary model of the geographic extent of manna quality across the species natural distribution in Tasmania. This model will then be validated by sampling new populations across regions within the model's parameter space and across regions where the model is being extrapolated.

Table 2. Sites used for natural population sampling and corresponding climate (Mean Annual Temperature, Temperature Maximum at Warmest Period, Mean Annual Precipitation and Annual Heat Moisture) and spatial variables (Longitude and Latitude) used to inform climate model.

Site/Population	Lat	Long	MAT	TMXWP	MAP	AHM
Black River	-40.85	145.324	12.91	21.62	950.95	24.09
Brushy Lagoon	-41.40	146.701	10.71	22.22	1037.01	19.97
Evercreech	-41.43	147.981	10.95	21.50	908.07	23.07
Connorville	-41.84	147.161	11.42	24.51	597.10	35.88
Ross	-42.01	147.552	11.28	23.81	501.03	42.48
Coles Bay	-42.08	148.233	13.25	22.23	632.98	36.73
Swansea	-42.15	148.074	13.03	22.71	569.17	40.46
Stonor	-42.44	147.415	10.21	21.71	589.54	34.29
Meadowbank	-42.64	146.823	10.73	23.03	728.98	28.44
Richmond	-42.74	147.407	12.61	23.32	485.06	46.61
Marchwiel	-42.78	147.868	12.65	21.47	664.05	34.11
Saltwater River	-43.03	147.724	12.41	20.62	716.56	31.28

Research Funding

Due to the unexpected, but exciting, discovery of seasonal variation some planned actions for this period have not been conducted. Instead, natural population sampling was conducted (2022), to allow time to complete common garden trial sampling in 2023, where natural population samples will be analysed in early 2023 (approx. $1,440). These actions remain within the budget proposed for this project and an updated summary of expenses for 2022 is detailed below.

	% Expended	Details
Lab research costs	40% of 2022 funds	Manna analysis @ $12 per sample by Central Science Laboratory (UTAS)
Field research costs	45% of 2022 funds	Car hire @ $0.60 (per km), Accommodation and overnight consumables $135 (per person per night)
Equipment	5% of 2022 funds	General lab/ field consumables (e.g., razors, vials, secateurs, buckets, pots, soil)

Thank you for your continued support!

Kind regards,

Erin Bok

References

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4. Steinbauer, M.J., *A note on manna feeding by ants (Hymenoptera: Formicidae).* Journal of Natural History, 1996. **30**(8): p. 1185-1192.
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