A molecular investigation into the genus Bergenia

Scott Galloway RBGE - SRUC 27th Jun - 19th Aug 2022

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Introduction

I am 4th year Horticulture with Plantsmanship student at the Royal Botanic Garden Edinburgh and SRUC. In the last 5 years, I have completed the MacRobert Trust Horticultural Traineeship, worked with the National Trust for Scotland (NTS) as a gardener for two years and contributed to the RHS Find a Plant online database as a Plant Profile Writer.

While working at NTS Greenbank Garden I managed the Plant Heritage National Collection of *Bergenia* and began to cultivate a keen interest in the genus. Working closely with the collection and the garden's plant records, I noticed several unusual species which appeared to be morphologically distinct and which also carried unconfirmed names in Plants of the World Online (POWO) and SaxBase. It was through further investigation into these taxa that I discovered there was a need for greater taxonomic scrutiny of the genus.

Since leaving that role in 2021, I have been building my own collection of *Bergenia* as part of a personal research interest in the genus. This has allowed me to make more detailed observations of the plants and better understand their distinctive features. I have been greatly supported by my university who have given me the opportunity to undertake a growing trial of the cultivar *Bergenia* 'Oeschberg' in various peat free substrates and to produce a mini monograph on the unconfirmed species *Bergenia afghanica*.

It was this latter monographic study into *B. afghanica* that sparked a deeper interest in taxonomy and a pursuit of further research. However, there are several strands of interest within the genus that I hope to explore more in the future - these are explained in the main body of this report where I discuss my archival research at the Wisley library.

As of today, I hold the largest collection of *Bergenia* in the UK, including all but three of the species - *B. hissarica*, *B. scopulosa* and *B. ugamica*. These have never entered cultivation in the UK and field work to Eastern Uzbekistan and South West China would need to be organised for further research of these taxa.

I hope that with the opportunity of further molecular research and more closely detailed morphological observations that I will be able to produce a complete *Bergenia* monograph for reference in the near future.

Itinerary

Research	Department	Staff
DNA Extractions	Molecular Laboratory	Kalman Konyves
Taxonomic Research	Botany Team	John David, Dawn Edwards, Kalman Konyves and Richard Dee
Archival Research (trade catalogues, monographs and revision)	Wisley and Lindley Library	Library staff
Living collection identification, voucher sampling and propagation	Gardens Team	Matthew Pottage, John Ricketts and the diploma students
Herbarium specimen collecting	Herbarium Team	Toni Martin, Yvette Harvey, Lydia Walles and Clare Booth-Downs

Locations Visited

The studentship was based at RHS Garden Wisley for 8 weeks. I worked primarily with my supervisor Kálmán Könyves in the molecular laboratory on DNA extractions of *Bergenia* but I was also able to utilise other resources, including the Herbarium, Wisley Library and the garden's living collection of 66 taxa.



View of the Glasshouse from the top of the Rock Garden

Aims and Objectives

<u>Aim</u>

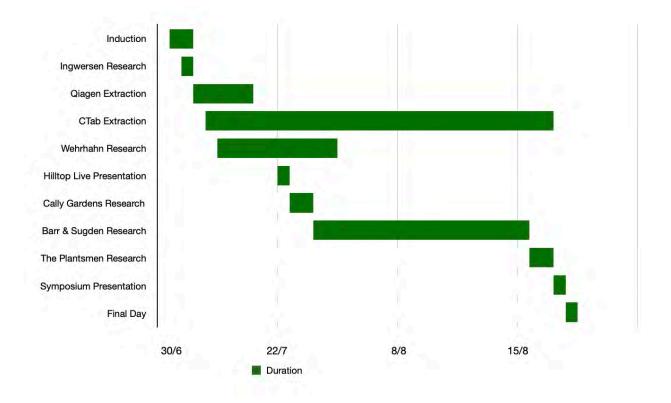
The aim of this project was to extract DNA from 27 *Bergenia* samples so I could produce the first phylogeny of *Bergenia* and allow me to better understand the relationship of the species within the genus.

Although the primary focus of the research was molecular work, I was able to utilise the many other resources available at RHS Garden Wisley during my time there, including the Library, Herbarium and Living Collection. Therefore my work broadened in scale to support wider research into *Bergenia*.

Objectives

- Choose the most appropriate protocol to extract DNA for Bergenia
- Extract DNA from 27 leaf material samples
- Send samples to Arbor Biosciences, USA for next generation sequencing
- Collect, press and mount Bergenia taxa from the garden for the Herbarium
- Utilise on-site resources, such as the Living Collection and Wisley Library to research Thomas Smith hybrids and *Bergenia afghanica*

Timeline



The above bar chart shows a timeline of the key tasks that I undertook during my placement. The longest tasks included the CTab extraction of the *Bergenia* samples and the archival research into H.R. Wehrhahn's *Die Gartenstauden* and the Barr and Sugden nursery catalogues. I have also included two presentations that I delivered during my placement, as part of the Hilltop Live series and Science Symposium, where I was able to share some of my favourite *Bergenia* with members and visitors and discuss the importance of my research with colleagues from across RHS Wisley.

Work Programme

At the beginning of the project I was introduced to the molecular laboratory and went through all the safety procedures with my supervisor. I practiced my pipette skills by repeating practice extractions, using the Qiagen DNeasy Plant Kit, on *Bergenia* samples from the garden. The early results of these extractions using Qubit showed a very low yield of DNA from the samples and Kalman decided to change the protocol to try to improve this.



Dried leaf material samples

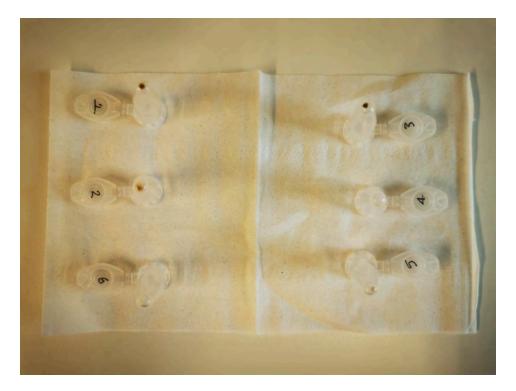
The difficulty in breaking down the cell structures of the samples required a different extraction method. Kalman suggested we use a CTAB buffer instead. At the beginning of each week I prepared a buffer for the week ahead, combining 25ml of CTAB buffer with 50ul of B-mercaptoethanol and 1g of PVP, to break down the tissues and remove contaminants.

The CTAB extraction process was carried out over two days and involved measuring 20mg of leaf material from each sample and grinding it down with 2 beads and a small amount of sand in the TissueLyser at 24hz for 2 mins. The tubes were removed and turned and the

process was repeated. 750ul of the CTAB buffer was then added and the samples vortexed to break up the powder at the base of the tubes.

The samples were then incubated at 65C for 30 mins in the Thermomixer. Following incubation, 3ul of RNase was added and the samples were incubated for a further 10 mins. The samples were then centrifuged at 13200 rpm for 3 mins to separate the debris. The aqueous phase was transferred to 1.5ml tubes and an equal volume of chloroform: iso-amyl alcohol was added. The samples were incubated for 30 minutes in the rotary mixer at 10 rpm.

Following incubation, the samples were centrifuged for 15 mins to separate the aqueous phase, the debris and the chloroform. Carefully using a pipette I extracted the upper aqueous phase to clean 1.5ml tubes and added a volume of ice cold isopropanol at 2/3 of the volume of the aqueous phase. These were mixed and stored in the freezer overnight.



Inverting samples on Kim wipe to dry the pellet

The next morning I removed the samples and centrifuged them at 13200 rpm for 15 minutes to pellet. The following steps involved pipetting off the liquid and adding 750ul of ice cold 70% ethanol and leaving it to sit for 5 mins to release the pellet. The samples were then centrifuged for 3 mins at 13200 rpm and the ethanol pipetted off. This was

repeated. The tubes, now containing the pellet, were inverted on a Kim wipe for about 30 mins until completely dry, after which 100ul of EB buffer was added and the samples stored in the fridge.

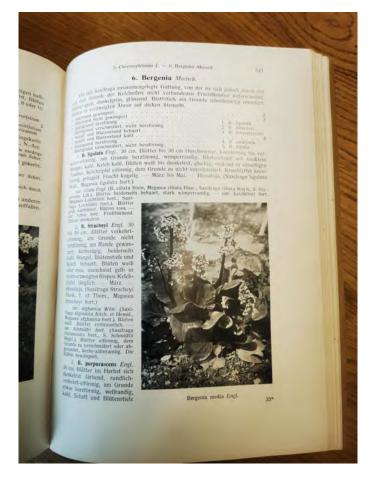
Having extracted my DNA, I had to measure it and used the Qubit Fluorometer to quickly quantitate the amount of DNA in each sample. The process was simple and gave me accurate results that determined whether I needed to repeat my extractions. I required at least 20ng/ml of DNA per sample before sending them off sequencing. Although the CTAB extraction improved the DNA yield compared to the Qiagen DNeasy Plant Kit, I had to repeat several extractions to get the required minimum yield.



Qubit Fluorometer measuring sample DNA of B. cyanea at 30.4ng/ml

Library Research

Having access to the Wisley Library archive allowed me to advance my research into the unconfirmed species *Bergenia afghanica*. I was specifically interested in historical descriptions of the plant, from botanists, plant hunters and nursery owners alike, and dates of its commercial availability here in the UK.



Wehrhahn's description of B. stracheyi

In Peter Yeo's *Supplementary list of cultivars of Bergenia* from 1970 I found a description of *B. afghanica*, which Yeo writes is "Probably intended for *B. stracheyi*". He references H.R. Wehrhahn's *Die Gartenstauden* from 1930, which I was able to request from the Lindley Library, where he describes the species as a variety under *Bergenia stracheyi*:

"var. *afghanica* Whn. (*Saxifraga afghanica* Aitch. et. Hemsl., *Megasea afghanica* hort.) Flowers white. Leaves reddish brown." Yeo's scrutiny of *Bergenia afghanica* is based solely on Wehrhahn's description and brings him to the same conclusion that the species is likely to be a variety of *Bergenia stracheyi*. Interestingly, Wehrhahn's description is one of the last until Yeo's 1966 taxonomic revision. This is intriguing as there were several descriptions of the plant between 1882 and 1931 and then a gap in the literature until *Bergenia afghanica* appears for sale from Will Ingwersen's nursery in the early 1960s.

Between the years 1901 and 1928, *Bergenia afghanica* is described in many popular garden books of the time. Unfortunately between 1880 and 1930 *Bergenia* was known as *Saxifraga*, *Saxifraga* (Section V., Giant Flowered and Large Leaved Saxifragas), *Saxifraga* (Group VI., Megasea) and *Megasea*. The discovery of new species, such as *Saxifraga milesii*, also added to the confusion of the nomenclature of the genus (this has now been confirmed as *Bergenia stracheyi*).



Bergenia afghanica, the only known UK specimen at Greenbank Garden

Bergenia afghanica is described as a species [Saxifraga afghanica] by John Weathers in 1901. It appears again in 1910 in Lewis B. Meredith's Rock Garden: How to Make and Maintain Them within a description of S. stracheyi (Group VI Megasea) and in 1916, A. Clutton-Brock writes that there is an Afghanica variety of S. stracheyi "a beautiful plant not often seen".

A description of *Megasea* var. *afghanica* appears in 1905 in *Flora and Sylva*, although no species is provided here, this is a change from the name *Megasea afghanica*, in Leichtlin's 1882 *Pflanzen Liste* from Baden-Baden. This later changes to *Bergenia afghanica* in 1928 in *Plantes nouvelles ou critiques des serres du Museum*. However, Hort. is applied at this time, confirming that in France the name was recognised as being misapplied and accepted as an invalid name. The name reverts back to a variety again two years later in *Die Gartenstauden*, where Wehrhahn suggests *Bergenia afghanica* is likely to be *Bergenia stracheyi* var. *afghanica*.

What is important about these descriptions is that they make a distinction between *Saxifraga afghanica* - later *Megasea* var. *afghanica*, *Bergenia afghanica*, then *Bergenia stracheyi* var. *afghanica* - and *Saxifraga afghanica*, the Kabschia saxifrage discovered by Aitch and Hemsl. in 1880. The latter is an accepted name in Plants of the World Online (POWO).

What adds further interest is that Max Leichtlin of the Baden-Baden Botanic Garden introduced *Saxifraga stracheyi* var. *alba* (now *Bergenia stracheyi* Alba group) as Saxifraga afghanistanica from Afghanistan c1880. Yet, comparing both *Bergenia afghanica* and *Bergenia stracheyi* Alba Group today there are very clear morphological differences between these plants that would suggest Leichtlin introduced a different variety to *B. stracheyi* Alba Group.

The descriptions between 1901 and 1928 highlight that both *Bergenia stracheyi* Alba Group and *Bergenia afghanica* share similar white flowers. A conclusion could be drawn that in 1886 Leichtlin introduced a variety of *Bergenia stracheyi* under the name *Saxifraga afghanistanica* that would later be known as *Megasea afghanica*, *Bergenia afghanica*, *Bergenia stracheyi* var. *afghanica* and *Bergenia stracheyi* 'Afghanica', or Leichtlin introduced *Bergenia stracheyi* var. *alba* but incorrectly named it *Saxifraga afghanistanica* rather than *Saxifraga stracheyi* var. *alba*, as it would have been known at the time.

This scrutiny of the unconfirmed species *Bergenia afghanica* allows for several conclusions to be drawn. Hence the need for DNA analysis and the creation of the first *Bergenia* phylogeny to see where both *B. stracheyi* Alba Group and *B. afghanica* sit within the tree. I hope that alongside morphometric data I will be able to resolve some of the taxonomic complexities surrounding this plant.

Trade Catalogues

While working with the National Collection of *Bergenia* at National Trust for Scotland Greenbank Garden, I discovered a cultivar called *Bergenia* 'Ingwersen's Minima'. This is an interesting plant as it does not appear in any online searches or the RHS Find a Plant database and I quickly recognised that it may be quite rare in cultivation.

In my search for more information, I asked the library staff if they hold any of Will Ingwersen's trade catalogues and was offered a selection from 1961-96. It was here that I made two quite big discoveries: the first being two listings of *Bergenia* 'Minima' from 1963-65 and five listings of *Bergenia afghanica* from 1963-68.



Ingwersen Nursery Catalogues, 1961-1969

The plant named *Bergenia* 'Ingwersen's Minima' at Greenbank Garden is most likely to be the one listed as 'Minima' in Ingwersen's catalogue. Aileen Stocks in the Hardy Plant Society publication *Saxifragaceae, Some Herbaceous Members of the Saxifragaceae Family* describes it as a "poor cultivar, not garden worthy; a compact plant, a shy bloomer with small, lilac pink flowers and no winter colour" (Stocks, 1995) and suggests that it is most likely a synonym of *Bergenia cordifolia minor*.

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Bergenia list from Ingwersen Nursery Catalogue, 1963/64

The five listings of *Bergenia afghanica* are of particular importance as they are the first mention of the plant since Wehrhahn's description in 1930. It should be noted that Ingwersen advertises the plant as a species rather than a variety of *B. stracheyi*, as Wehrhahn suggested in *Die Gartenstauden*.

Out of curiosity, I also asked the library staff about Michael Wickenden's catalogues from Cally Gardens and was given access to a selection from the years 1992 - 2014. It was in the 1995-96 catalogue that I made an exciting discovery. Wickenden lists *Bergenia ciliata* 'Smaller Form' for this year only but it is his description that provides greater insight into *Bergenia afghanica*:

"Bergenia ciliata 'Smaller Form'. - This arrived labelled B. Stracheyi afghanica but the large rounded leaves bristly on both surfaces suggest B. Ciliata, smaller pale pink flowers and leaves than the one offered last year, some yellow and red autumn colour. 15""

This description is the first indication that *Bergenia afghanica* may not be a variety or cultivar of *Bergenia stracheyi* as previously suggested, instead, it could be a form of *Bergenia ciliata*. The only plant growing in the UK as far as I am aware is within the collection at Greenbank Garden and Wickenden's description certainly matches the morphological features of this plant compared to *Bergenia stracheyi*. I hope that with the results of the sequencing data from my DNA extractions I will be able to observe whether *Bergenia afghanica* sits closer to *B. ciliata* or *B. stracheyi* or somewhere separately on the tree.



Cally Gardens Nursery Catalogues

Thomas Smith

Many of the earliest *Bergenia* hybrids were bred by Thomas Smith of Daisy Hill Nurseries, Newry, Ireland in the 1880s and 1890s and introduced into cultivation by Barr and Sons. The Wisley Library has a full collection of Barr and Sons catalogues from 1861 to 1960, from its establishment as Barr and Sugden Co., Barr and Sons Co. and finally Wallace and Barr.

I was very lucky to be able to go through every catalogue in the collection and see first hand many of Smith's introductions. These include: *Bergenia* x *smithii* 'Brilliant', 'Campana', 'Cascade', 'Corrugata', 'Distinction' and 'Progress'. Unfortunately, none of these are available commercially today and several have been lost in cultivation since the early 1920s.



Bergenia 'Profusion', photo credit Brendan Sayers

However, through the efforts of Brendan Sayers and Gary Mentanko at Glasnevin Botanic Garden in Dublin many of these hybrid cultivars are being trialled to try to match their descriptions to plants sourced from across Ireland and the UK. In partnership with the Irish Garden Plant Society (IGPS) the trial hopes to identify those of true Irish heritage and preserve their genetic material in the garden's living collection and herbarium.

One Smith cultivar of particular interest that I did not find in the catalogue was *Bergenia* 'Profusion'. I did, however, to my great surprise, find it listed in the living collection at Wisley! Working closely with gardener John Ricketts we were able to find an unnamed clump which I matched with a description by Aileen Stocks:

"Similar to B. cordifolia with pale pink flowers. C. 1880" (Stocks, 1995)

I later exchanged photographs with Brendan Sayers who was able to confirm it is likely to be the same plant as trialled at Glasnevin. Rather excitedly, I spoke with Matthew Pottage about the importance of this particular cultivar and the need to propagate and preserve it in the garden.

The Jekyll Mystery

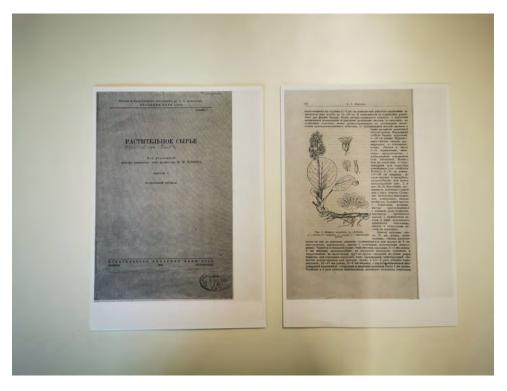
While researching Smith introductions in the Barr and Sons catalogues, I happened across one particularly intriguing plant from 1922. *Bergenia cordifolia* rubra munstead variety appears only once, and without reference to a source, but of course, the name is in reference to Gertrude Jekyll's home Munstead Wood. It is well known that she used *Bergenia cordifolia* in her designs, planting it en masse to create a bold, evergreen foliage effect, however, there has been no reference that I can recall to a *Bergenia* variety from Munstead. It may or may not have been selected by Jekyll and made available commercially but it is interesting that it appears only once in the catalogue list. Further investigation into Jekyll's writing and a visit to Munstead Wood to try to find the plant in the garden is necessary to uncover more information about this rather special introduction.

Challenges

Before beginning my placement I discussed with my supervisor the problems we may encounter extracting DNA from *Bergenia*. Kalman suggested that *Bergenia* might not give up its DNA easily and we may need to adjust our protocol. This was evidenced in the very low yield result from the Qiagen extractions carried out during my first week.

The requirement for a minimum of 20ng/ml of DNA meant we had to adjust the protocol. Kalman suggested using a CTAB extraction instead which resulted in a higher yield across the samples but some samples still did not meet the minimum required amount. I repeated extractions of those samples and instead of adding 100ul of EB buffer at the end of the protocol I added 50ul. Repeating this twice, I mixed the samples together to increase the overall yield of DNA to meet the minimum 20ng/ml.

One significant challenge was the week I had to take off to isolate at home due to my housemate contracting Covid-19. During this time, my supervisor sent me material to work on from home, such as Antonina Borissova's research into *Bergenia*, which I translated from Russian into English. I focused on *B. hissarica*, which has not entered cultivation in the UK or Europe, but is of particular interest for future research and field work.



Antonina Borissova's study into Bergenia species

Conclusion

The summer studentship gave me first hand experience of what it is like working as a molecular taxonomist and showed me the importance of taxonomic research in improving our understanding of genetic diversity in wild plant species. During my time at Wisley, I was able to advance my research into *Bergenia* by carrying out DNA extractions and begin the first steps of producing a phylogeny of the genus.

By the end of my placement, I had selected 16 samples to send to Arbor Biosciences, USA for next generation sequencing. When the sequencing data is returned to the RHS in October, I will be able to analyse it and produce a phylogenetic tree. However, this work will be carried out at RBGE under the supervision of Dr Greg Kenicer and supported by Dr Kálmán Könyves remotely while I complete my degree.

Although my work at Wisley was always going to be limited by time constraints due to the length of the placement, I seized the opportunity to build connections with staff across the garden who supported my research in ways that I couldn't have imagined. Some of the personal highlights include: finding the earliest Thomas Smith hybrid *B*. 'Profusion' from 1880, which I had believed to be lost in cultivation in the UK and only grown at Glasnevin, Ireland; discovering a Munstead variety of *B. cordifolia* in the Barr and Sugden catalogue of 1922, which may have been selected by Gertrude Jekyll herself; and uncovering several references to *B. afghanica* in Will Ingwersen and Michael Wickenden's nursery catalogues.

Working closely with the herbarium team, I also learned how we can preserve wild and cultivated genetic material for future research. I was shown the process the herbarium staff follow from taking cuttings to colour charting, pressing, drying and mounting specimens.

Overall, the placement far exceeded my expectations. I was able to develop my skills in the laboratory, as well as my understanding of modern day taxonomy. It confirmed that this is the field I want to work in after graduating and I am now looking at pursuing PhD level research.

Further Research

Following DNA extraction at RHS Wisley, my samples have been sent to Arbor Biosciences in the USA for next generation sequencing. Once I receive my sequencing data (6-8 weeks) I will be able to generate whole plastomes and produce a phylogenetic tree. In September, I will be returning to Edinburgh for my final year of the BSc (Hons) Horticulture with Plantsmanship degree at RBGE and I will use this data as part of my Honours Project to analyse the first phylogeny of *Bergenia* and better understand the relationships of the species within the genus.

I am keen to undertake further research as part of a PhD into the effects of climate change on wild populations across the Himalayan biome. Examining in particular the effect of increasing temperatures in the Khasi Hills region on the Shillong Plateau in Meghalaya state of India. I am currently in discussion with potential funding opportunities and supervisors across institutions, such as the RHS, RBGE and University of Edinburgh.

My research has been an on-going passion project for almost three years now and in that time I have built the largest collection of *Bergenia* in the UK. This has allowed me to make close observations of their growing habits and distinctive morphological features and now undertake molecular research. The collection is currently stored at a nursery site in Edinburgh but will need to be moved to a semi-permanent location for the duration of a PhD. I am in discussion with potential partners to make this happen to support further research into the genus.

Budget Breakdown

It was agreed between myself and John David that although I would not receive a stipend, the RHS would pay my rent during my placement. However, due to a delay in receiving the funds I had to use my RHS bursary to cover the cost of rent and essentials, such as food and travel. I would like to extend my thanks to the bursaries committee for their generous financial support of my taxonomic research during my placement as it would not have been possible to undertake this project without it.

See appendix A for breakdown.

Acknowledgements

First of all, I would like to thank Dr. John David for accepting me as the taxonomy student for the summer placement in 2022 at RHS Garden Wisley. My supervisor Kálmán Könyves greatly supported and mentored me throughout the placement and I am incredibly grateful to have had the opportunity to learn so much from him and the wider botany team.

I would also like to extend my thanks to everyone I worked with during my placement at Wisley. From the library staff who helped me with archival research, to the gardens team who helped me source *Bergenia* across the living collection. In particular, thanks to John Ricketts who helped me find the very rare *Bergenia* 'Profusion'. Special mention must go to the Herbarium team who were a fantastic support, inspiration and motivation.

Finally, I would like to thank the RHS bursaries committee for awarding me a bursary of £650 from the RHS Coke Trust Bursary Fund, as well as Dr Greg Kenicer and the William Steel Trust who awarded me a bursary of £650. Without their generous financial support I would not have been able to undertake this studentship. My research has greatly benefitted from this opportunity which gave me access to the incredible wealth of resources available at RHS Wisley.

References

Stocks, A., 1995. *Saxifragaceae Some Herbaceous Members of the Saxifragaceae Family*. 1st ed. Worcestershire: The Hardy Plant Society, p.22, 26.

Appendix A

Budget Breakdown

I generously received an RHS bursary of £650 and a William Steel Trust bursary of £650 from RBGE to support my research at Wisley. I used both of these to pay my rent and support me with the cost of essentials, such as food and travel. The breakdown is below:

Expenses	Weekly Cost	Total Cost	
Rent	120	960	
Groceries	50	400	
Canteen Lunch	3.25	130	
Bus travel	8	64	
Purchases from the Wisley Shop	N/A	100	
Other food expenses	15	120	
Total cost:		£1774	

When I had used both bursary funds I used my personal savings to support me through to the end of the placement.