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What is the Best Germination Method for *Senecio macrocarpus*?

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Year 10

Questioning and predicting:

We have decided to investagate the question, "What is the best germination method for *Senecio macrocarpus?"* because we wanted to help effectively grow a plant species that is native to the Coorong, where we stayed at our school's campus there, for three weeks last year. We also know that this species is vulnerable in South Australia *(Senecio macrocarpus (Compositae))* and endangered in Victoria, *(Senecio macrocarpus (Belcher)) (Victorian Flora and Fauna Act 1988).* This plant is also listed as vulnerable in the Environmental Protection and Conservation Act 1999, so we wanted to find out more about how to grow them, so they don't become extinct in the future.

We are both very interested in plants and the environment and have been working with our science teacher, Dr Nobbs, to hand pollinate, collect seed heads, separate and plant the seeds of a critically endangered Coorong plant *Spyridium fontis-woodii.* Due to our interest and commitment working with the *Spyridium,* Dr Nobbs gave us the seeds of *Senecio macrocarpus* for us to take home and experiment ourselves. We decided we wanted to find out what the most effective way of growing them was, so that this plant could survive in the future and so we based our experiment around this idea.

Objectives:
- To grow *Senecio macrocarpus*
- To find the best medium for germination

Hypothesis:
The seeds grown in the sterile mix of sand and potting mix would germinate first, after around 10 days.

Our hypothesis was based on research that suggested a sterile mix would be the best for germinating the seeds. We believe this because the sand reflects the Coorong environment, but also includes potting mix to provide nutrients. Our research also led us to believe that sterilising the mix would help increase the germination rate of the seeds. *(Ferriss, 1984)*. We expected that they would begin germinating between 8-18 days after planting. *(Government of South Australia, 2020).* (We thought they would germinate slightly slower, and begin after around 10 days, as the conditions are not monitored as they would be at the Botanic Gardens, where we found this range.)

Planning and conducting:

Plan:
- To do 4 variations changing the variable of medium, growing the seeds in sterile soil, sterile sand, sterile mix of sand and soil (1:1 weight ratio) and a non- sterile mix of sand and soil (1:1 weight ratio)
- Check for signs of germination, which we expected mostly between the range of 8-18 days after planting.

Steps:

1. Research – We looked into where the *Senecio* was found and the characteristics of the plant in the wild. We also researched the germination methods that would be most compatible with this species. However, due to the fact that this plant is rare there was little information available about the growing of this plant.

Seed Information

Name derivation:	Lanin '*senex*' an old man, refers to the white pappus and Greek '*macros*' large, '*carpos*' fruit
Distribution:	Occuring in a variety of habitats including grasslands, sedgelands, shrublands and woodlands. Often in depressions that are waterlogged in winter, on sandy loam to heavy clay soils.
Status:	Vulnerable in South Australia
Plant description:	Perennial herb 20-70cm high. Leaves alternate, linear, 10cm long, 2-5mm wide, grey-green, covered with fine hairs. Inflorescence a cyme with 2-10 capitula 18mm x 20mm. Up to 150 yellow florets, surrounded by linear, pointed bracts. Flowering September - October.
Fruit type:	Achenes 4.5-5 rarely 6 mm long, brown, rostrate, covered in very short, greyish hairs; pappus deciduous, dimorphic.
Seed type:	No information available
Embryo type:	No information available
Seed collecting:	No information available
Seed cleaning:	No information available
Seed viability:	No information available
Seed germination:	No information available

2. Sterilising the mediums (soil and sand) – After speaking with Joan Gibbs, (an ecologist at the University of South Australia, who specialises in native seed germination and restoration on the Coorong) we found that the seeds would germinate better after sterilising the medium. She advised us to sterilise them by heating the medium in the oven for 24 hours. We then found from John Weber (a soil scientist) that we could heat them in a microwave in order to sterilise the sand and soil as well. We went with this method, because it was quicker and easier. We put the sand and soil in plastic zip-lock bags with some water to allow the water to evaporate and condense within the bag, helping with the sterilisation process. We then let it cool to room temperature before using it.

Figure 1: The mediums after being heated in the microwave

Figure 2: The sterilised potting mix

3. Combining the mediums – As well as having sterilised potting mix and sand as two separate mediums, we also decided to combine the sterilised potting mix and sand and non-sterilised potting mix and sand to form two separate mixtures, thus, creating the four mediums within this experiment.

Figure 3: Weighing the sterilise sand and potting mix.

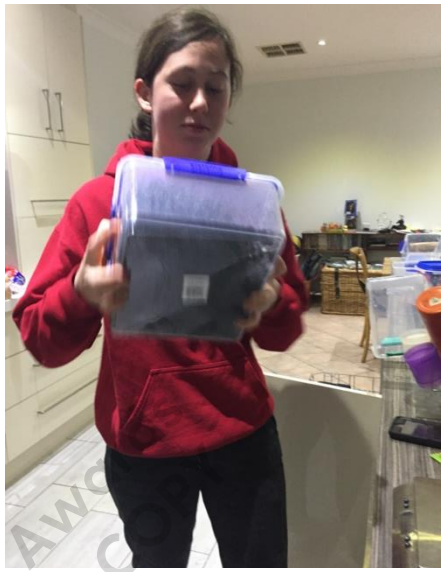


Figure 4: Inika mixing the sterile mixture

Figure 5: Weighing the non-sterile sand and potting mix

Figure 6: Trishna mixing the non-sterile mixture

4. Planting (4 seeds per pot, 6 pots per soil type) – We purchased small, compostable pots, so if the plants grew large enough, we could plant the entire pot. We then put 6 of these into a plastic container for each of the soil types (sterilised potting mix, sterilised sand, a mixture of non-sterile potting mix and sand, a mixture of sterilised potting mix and sand). With a pen dipped in isopropyl alcohol to ensure the sterile environment remains sterilised, we dug four holes around 10-15mm deep in each pot and placed a single seed in each hole. We covered these seeds with a thin layer of their respective medium and then watered them all.

Figure 7: Digging holes in the sterile sand

Figure 8: Seed in hole

5. Monitoring – We checked the seeds frequently to look for any signs of germination. We also had them outside and inside at different times and temperatures within the waiting period. Between the 8-18 day period, we left them outside to receive the winter conditions as we believed that the seeds would grow best at this time and temperature, as this was suggested on the Botanic Gardens' 'Seeds of South Australia' database.

Figure 9: Sterilised sand, day after planting.

Figure 10: Non-sterile mix, day after planting

Figure 11: Sterile mix, day after planting.

Figure 12: Sterile potting mix, day after planting.

6. Watering – We watered the seeds based on whether the medium was damp. When the pots were indoors, they were watered simply using tap water. Every second day of when it was outdoors during the 8-18 day period was watered with water collected from the rain using the lid of the box. However, after a heavy downpour of rain, the seeds were not watered for 9 days. This was because the medium was still damp.

Figure 13: Watering the sterile mixture

7. Doing viability test – After the seeds had not germinated after 20 days, we put some of the remaining seeds onto paper towel to perform a viability test. This would allow the seeds to germinate regardless of the medium, as they are growing from the moist paper towel. We scattered the seeds along with their seed heads along the diagonal of the paper towel and then rolled it, allowing them to be covered. We then watered this roll and left it in a plastic bag to retain this moisture.

Figure 14: The seeds and seed heads on paper towel as the viability test

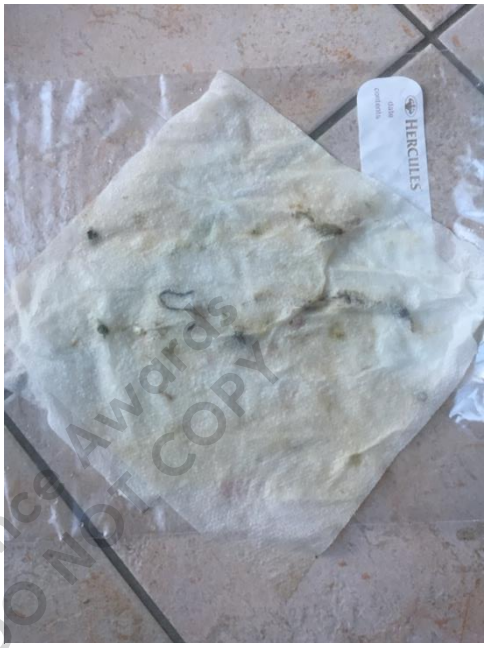
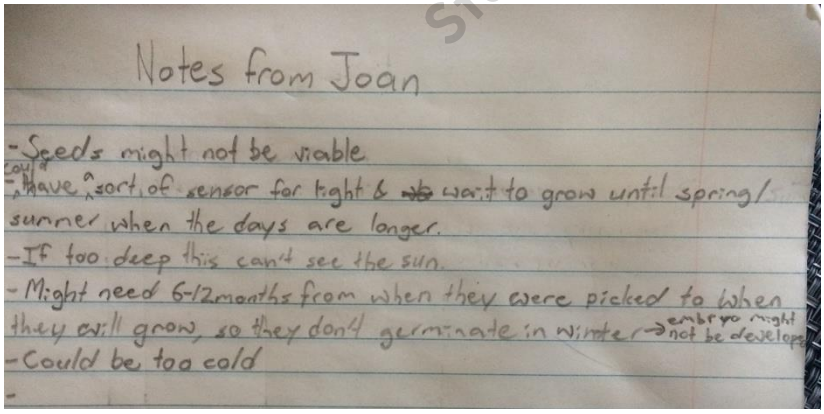


Figure 15: The unrolled and watered paper towel after 12 days.

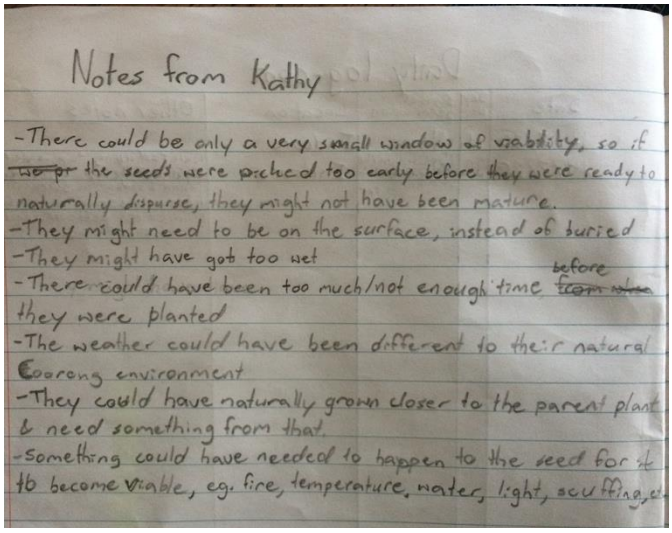
8. Talked to experts– After the seeds had not grown for 24 days, we decided to speak to experts about why this might be the case. We talked to Kathy Penny, a plant expert who used to work at the Botanic Gardens, and Joan Gibbs, an ecologist from UniSA, and found out some of the reasons why they thought these might not have grown.



Notes from Joan

- Seeds might not be viable
- Have a sort of sensor for light & ~~to~~ want to grow until spring/summer when the days are longer.
- If too deep this can't see the sun.
- Might need 6-12 months from when they were picked to when they will grow, so they don't germinate in winter → embryo might not be developed
- Could be too cold

Figure 16: Notes from Joan, Coorong plant expert.



Notes from Kathy

- There could be only a very small window of viability, so if ~~the~~ the seeds were picked too early before they were ready to naturally dispurse, they might not have been mature.
- They might need to be on the surface, instead of buried
- They might have got too wet
- There could have been too much/not enough time before ~~from when~~ they were planted
- The weather could have been different to their natural Coorong environment
- They could have naturally grown closer to the parent plant & need something from that.
- Something could have needed to happen to the seed for it to become viable, eg. fire, temperature, water, light, scuffing, etc

Figure 17: Notes from Kathy, plant expert.

Variables:
- Independent: (what we change) medium (soil and sand)
- Dependent: (what we measure) germination rate
- Constant: (what stays the same) number of seeds planted in each soil type and the conditions these receive (e.g. rain, heat, light)

We believe that this experiment was a fair test because we only changed one variable and the conditions received by each of the mediums was, to the best of our knowledge, the same. Even though some factors changed, such as the temperature and amount of water, we tried to keep these conditions the same between the mediums. However, there is still an error margin, for example some plants could have received more or less water than others.

Equipment and materials:

- Seeds of *Senecio macrocarpus* – We were given a paper bag of seeds that were collected at our school's Coorong campus on the 7/1/20. We put 24 seeds in each variation of soil medium. We also used these seeds and their seed heads in the viability test. The remained were planted in the leftover sterile mix, as we expected this to grow best.
The seeds are tiny with the base that is the fruit containing the seeds measuring around 3 or 4mm. There appears to be pappus attached to the end of the fruit.

Figure 18: Close-up of a Senecio macrocarpus *seed.*

- Pots
- Potting mix
- Sand
- Plastic bags (to use when sterilising sand and soil as well as over the viability test)
- Paper towel
- Pen/ pencil
- Isopropyl alcohol
- Notebook

<u>Risks:</u>
- Microwaving – Using a microwave can be dangerous because we could get burnt when taking hot substances out of the microwave. We controlled this by taking the mediums carefully out using tongs.
- Using potting mix and isopropyl alcohol – These could have been dangerous if we accidentally ingested them from having either potting mix or isopropyl alcohol still on our hands. We controlled this by washing our hands after using these and not touching our faces when using either substance.
- Losing the seeds – The seeds were feather light, so we had to be careful not to breathe too deeply when working with them or our breath could blow the seeds away. We controlled this by breathing further enough away from the seeds that our breath was not too heavy.

- Poking ourselves (sharps risk) – We had to poke the seeds with a pin to check their viability, which could be dangerous if we accidentally poked ourselves with the pin. We controlled this by putting the seeds on a surface that was away from our hand, (such as a plate or the table,) when we poked it.

Processing and analysing data and information:

We collected data in our field book using this table to record signs of germination as well as the date, day number, location and other important information such as if they were watered.

Date	Day No.	Signs of germination	Location	Other Notes
13/6/20	0	None	Indoors	Planted them, left them stacked away from window, lids on.
14/6/20	1	None	Indoors	
15/6/20	2	None	Indoors	
16/6/20	3	None	Outdoors	Moved them outside to get natural weather, lids on, watered.
17/6/20	4	None	Outdoors	
18/6/20	5	None	Outdoors	Lids slightly off to allow air flow.
19/6/20	6	None	Outdoors	
20/6/20	7	None	Outdoors	Watered using tap water.
21/6/20	8	None	Outdoors	
22/6/20	9	None	Outdoors	Watered using rain collected in lid.
23/6/20	10	None	Outdoors	
24/6/20	11	None	Outdoors	Watered using rain collected in lid.
25/6/20	12	None	Outdoors	
26/6/20	13	None	Outdoors	
27/6/20	14	None	Outdoors	Watered using rain collected in lid.
28/6/20	15	None	Outdoors	
29/6/20	16	None	Outdoors	
30/6/20	17	None	Outdoors	

1/7/20	18	None	Outdoors	Watered using rain collected in lid, lids put on.
2/7/20	19	None	Outdoors	Put remaining seeds on paper towel for germination test, put this in a plastic bag outside.
3/7/20	20	None	Outdoors	
4/7/20	21	None	Outdoors	Watered using rain collected in lid.
5/7/20	22	None	Indoors	Brought inside in case it was too cold and/or wet for them to germinate but next to window for light.
6/7/20	23	None	Indoors	
7/7/20	24	None	Indoors	Got moved around a bit as brought them with us to ask Joan.
8/7/20	25	None	Indoors	Lids off, still wet from the last time they were watered.
9/7/20	26	None	Indoors	
10/7/20	27	None	Indoors	
11/7/20	28	None	Indoors	
12/7/20	29	None	Indoors	
13/7/20	30	None	Indoors	
14/7/20	31	None within experiment pots, sprout in the germination test	Indoors	Watered using tap water, opened germination test and found one sprout and also one found in our box where we planted the remaining seeds

				not used in the experiment.

Since there was still no data as to what grew, after 24 days we decided to investigate why it might not have grown. After talking with Kathy Penny and Joan Gibbs we have come to the below list of reasons as to why these seeds might not have grown.

It is likely that the seeds were not viable, some of the reasons for this include:
- Picked too early before they were ready to naturally disperse
- Need something to happen to the seed for it to become viable, such as fire, a certain temperature, water, light, time of year, scuffing, etc.
- Not pollinated

Other reason for why they might not grow:
- They got too wet (although the pots allowed drainage, the containers we kept them in did not)
- Might need to be on the surface instead of buried (if they are too deep, they can't see the sun)
- Might have been not enough or too much time between when they were picked and when they were planted
- The weather might be too different to that of the Coorong
- May have naturally grown closer to the parent plant and need to receive something from that
- Might have a sort of 'sensor' that waits until there are long enough days in spring or summer
- Might need 6-12 months from when they were picked to when they will grow, to allow the embryo to develop, this is so the plants don't germinate in the middle of winter and die
- It is too cold

On the 14/7/20, the 12th day after, we found a tiny sprout in our box where we put the remaining seeds that were not used for the experiment. This box's medium was the sterile mixture of sand and potting mix.

Figure 19: The tiny sprout which grew in in separate box of the sterilised mixture.

Due to this recent discovery we checked the viability test, which we had left rolled since we planted them and were planning to open them after 18 days. We also found one germinated seed in this test.

Figure 20: The tiny sprout found in the viability test.

Figure 21: Prodding a seed with a needle

This led us to believe that, at least some of the seeds were viable. In order to test this, we prodded the fruit to feel whether there was a seed within the fruit, as there would be if the embryo was fully developed. This method was suggested by Joan. We took a sample of 10 seeds from the viability test and we concluded 5 as viable, so we can therefore assume that approximately 50% of the seeds in the viability test were viable. However, through measuring which seeds germinated the viability test could conclude that less than 1.5% of the seeds were viable. This shows that even though many of the seeds are viable, they still may not have grown. Perhaps they grow better at a different time of year or will take closer to or more than the 18 days, since we are not growing them in a controlled facility.

After one of the plants had grown, we decided to talk to Joan again and have found a few new reasons as to why they may not have grown as well as being able to prove against some of our previous ideas.

Our new list of reasons why the seeds might not have grown is as follows:

- Some of the flower heads we were given could have been picked too early before they were ready to naturally disperse, as they don't all mature at the same time – We were given our seeds in a paper bag, many of them were individual, but there were also a lot of seed heads, since these were all picked at the same time to the best of our knowledge, some could have been more mature than others.
- Not all the seeds on every flower head will be pollinated – The plant produces heaps of seeds within the seed head, but not all of these will get pollinated hence not being all viable. This could be why we found only around 50% to have a developed embryo. After talking with Joan, we found that many native plants have viability rates this low.
- We over watered them, so the seeds could have drowned – The extra box of remaining seeds and the viability test were planted later than the main four variations within the experiment. This means that they weren't outside for as long where we watered them using the rainwater quite often as it rained frequently. This could have meant that we watered the main seeds too much, too frequently, consequently drowning the seeds. Since the extra box wasn't outside for as long, it could have avoided the excess watering. We also only watered the viability test once and left it in a plastic bag so it could retain this moisture, this could have been not enough water to allow the seeds to drown.
- Seeds might be buried too deep, so they can't see the sun – The seeds in the box were probably slightly closer to the surface. This is because instead of digging individual holes for each seed, we removed some of the medium (the sterile sand and potting mix mixture,) sprinkled the seeds over the remaining medium and then replaced the medium we removed

by sprinkling it over the seeds that were previously on the surface. This could mean that they were closer to the surface than the seeds we buried in holes. The viability test seeds were not buried, instead rolled in paper towel, but would still be close to the surface of the paper towel roll.
- Might have a sort of 'sensor' that waits until there are long enough days in spring or summer – The seeds could have potentially been waiting for longer warmer days before they germinate. This period may have just started, and been amplified by being brought inside, so if this is the case, it could be expected that the seeds in the main part of the experiment may germinate in the near future.
Many of our other ideas we had after talking with Joan and Kathy were refuted by the germination of a seed in both the extra box and the germination test. These ideas include:

- Seeds need something to happen to the seed for it to become viable, such as fire, a certain temperature, water, light, time of year, scuffing, etc. – We did nothing different to the seeds that did germinate, in comparison to the ones that did, proving against this idea. If it is a temperature, light or time of year, based trigger and this has just started, we can expect more seeds to germinate in the near future.
- The weather might be too different to that of the Coorong – We have not taken the seeds to the Coorong, but instead inside, so they would be receiving warmer more predictable weather, but this is probably even more different to the Coorong than the weather outside.
- May have naturally grown closer to the parent plant and need to receive something from that - None of our seeds are near a parent plant, so the germination of two seeds proves that this could not be the case.
- Might need 6-12 months from when they were picked to when they will grow, to allow the embryo to develop, this is so the plants don't germinate in the middle of winter and die – All of our seeds had over 6 months from when they were picked to when they were planted, but both the extra box and the viability test had closer to 7 months. This could have made a difference, however, the planted seeds would have continued to develop under the soil, so they would have had the same amount of time, since all the seeds were picked on the same day. Some of the embryos could have been further developed than others, resulting in them germinating sooner. If this is the case, we would expect more germinating seeds soon, as more embryos mature.
- It is too cold – The temperature has been, to the best of our knowledge, the same between all the seeds, so if it were too cold none would have grown. The main variations were kept outside in the cold for longer than the viability test and extra box, which could have impacted the seed viability. Due to the seeds being moved inside, the temperature could have increased enough for the seeds to germinate, from this, we could expect more germination in the future.
- We killed the seeds by using tap water instead of rainwater – When speaking with Joan she told us that tap water could be toxic to some plants as it is sterilised to stop living things being able to live in it. However, this cannot be the case with *Senecio macrocarpus,* because we watered all the seeds with the same water. In fact, the seeds in the viability test and extra box could have received a higher percentage of their total water as tap water, because they were outside for less of their time and we watered the seeds with tap water when they were inside. After conducting some research on this point, we found that although tap water may slow the growth of most plants, there is only very few that are killed by it. (*Urban Garden Guide, n.d.)*

Within both the categories of reasons there is a lot of grey area, where the points could be proven the other way, especially if more germination were to occur in the near future.

From these points we believe it is most likely that the seeds were overwatered, and we drowned them. We think this because that is the biggest difference between the viability test and extra box in comparison to the other variations, since they were planted later. This could be proven incorrect if we find germination in any of the four variations within the near future. To give the seeds the best chance at survival, we have decided based on this information, to not water our seeds as frequently and only if they are entirely dry. Hopefully this way, when the seeds dry out, they will still be able to grow.
From our experiment although we managed to get two seeds to germinate, we can still conclude that natives are very hard to grow, and potentially difficult to keep alive after germination, which is why it is so important to look after our natural ecosystems. We also know that there are so many factors that impact plants, resulting in many reasons why most of the seeds did not grow.

Native seeds are also more difficult to grow than seeds for agriculture as their seeds are designed to produce variation and so don't have as high viability rate as those specially bred by humans. There is inadequate knowledge on how best to grow native plants, which is one of their biggest threats to survival, but we hope we have begun to shed some light in this area, through conducting this experiment.

Evaluation:

There were many improvements that could have been made and were fairly simple to outline as the seeds had not grown.

- Firstly, it would have been helpful to have tested the viability of the seeds before planting them. This will ensure that we are planting viable seeds and increase our chances of seeds growing, which in the long term, will ultimately save the plant species from extinction.
- Another thing we could have done was to attempt to replicate the plant's natural habitat. The little information on the websites we looked at whilst researching mentioned the different habitats in which the plant grows. If we separately researched these habitats and replicated them as best as we could, it could prove to have much higher results. However, this replication does not guarantee the plant's growth.
- During the research stage of our experiment, we also could have actively sought the plants in the wild and take notes as to what conditions they grow under, specific characteristics to its habitat, the method with which they were picked, etc. However, this may be slightly difficult as there is a minimal number of these plants growing. These plants would have to be strategically found, whether it is through an environmental company of sorts.
- Instead of growing the seeds in separate pots, it would have been much simpler to grow them in a natural environment, more specifically, in exclusion zones. This will minimize the amount of human impact and interference with the plant's growth, essentially, minimizing the number of errors that could be made.

- As there was insufficient information as to the weather conditions in which the plant grows in, growing it at a different time of the year could also make a difference. Although the Botanic Gardens website *(https://spapps.environment.sa.gov.au/SeedsOfSA/speciesinformation.html?rid=4054)* talks about growing the seeds in winter conditions, these would be closely monitored and not necessarily the same as the natural environment. We could find more information from analysing the plant growing in the wild as mentioned previously.

The outcome of this experiment has outlined many mistakes that could have occurred. Amidst these mistakes are elements that we don't fully understand and thus, questions arise regarding how best to grow the *Senecio*. These could be investigated by us or others in the future.

- Does the temperature or other weather conditions affect the germination of a plant (*Senecio macrocarpus)* more than the soil medium? Should the seeds have been planted in different conditions? Was more light needed? Was it too cold?
- When is the best time of year to pick or grow *Senecio macrocarpus*?
- How could more awareness be raised about the *Senecio macrocarpus* to keep it alive in its natural environment and how could this help scientists to better understand the plant?

Even though the plants didn't grow, outlining the possible errors that could have occurred and the improvements that could have been made is extremely helpful to know how to grow this *Senecio* in the future. As mentioned previously, there was minimal information on *Senecio macrocarpus*, thus, it was highly unlikely that we would receive optimal results without the information that is needed. Making mistakes when attempting to grow an endangered plant species is definitely a risk, however, these mistakes can outline what can be done differently next time. This adds to the knowledge already acquired about the plant species.

Growing the *Senecio macrocarpus* was an experience that not only extended our knowledge about plants, taught us and made us more aware of the real world, the world in which endangered plants need more attention. Joan Gibbs stated that there are three main problems when growing an endangered plant species: loss of habitat, collection of seeds that are not viable and low viability rates if they are, and *insufficient knowledge*. It is this insufficient knowledge that makes the most errors, thus, stressing the importance of analysing these endangered plants more carefully. *Senecio macrocarpus* is just one of these endangered plants that need extra attention and the only way to save these plants is to keep trying and learning from what was done. Even though only minimal *Senecio* grew for us, and none as part of the main experiment, we still acquired knowledge of the errors that should be avoided and improvements that can be made for next time.

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Kathy Penny – For sharing her plant knowledge with us, helping to give us more ideas as to why our seeds might not be growing.