

Persoonia longifolia

Seed Dormancy and Germination



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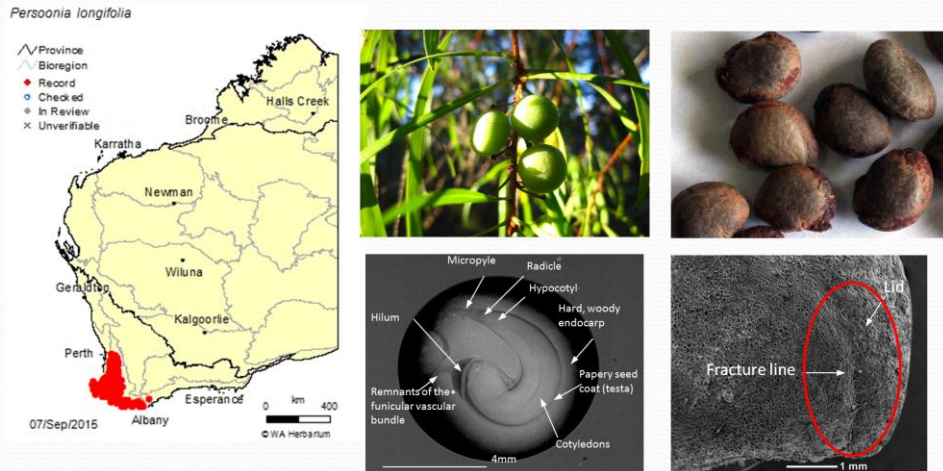


Worsley Alumina



Thank you for coming along to listen to my presentation. Today I want to talk about my research into a **very common and difficult** to germinate species from Western Australia, *Persoonia longifolia* which was the basis of my PhD study over the past 6 years.

Introducing *Persoonia longifolia*



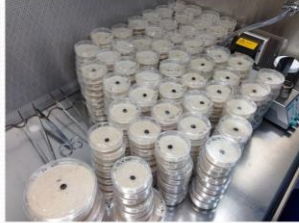
Species with hard woody endocarps are found worldwide. The *Persoonia* genus is part of the **Proteaceae** family, and is endemic to Australia. It includes **ninety eight different** species which vary from shrubs to small trees. Some are **threatened** and others like *Persoonia longifolia* are very common.

Persoonia longifolia is a **resprouter** species that occurs in the **south west** of WA **densities of around 250-400** plants per hectare. It is a species that is important for mining companies to return to restored areas of mine sites, it is of interest to **gardeners** and it's foliage is used as a filler in **floral arrangements**. The reproductive unit is a **fleshy drupe** with a hard, indehiscent endocarp surrounding the seed which emerges through a predetermined fracture line (**CLICK**) on the endocarp.

Aims and Objectives

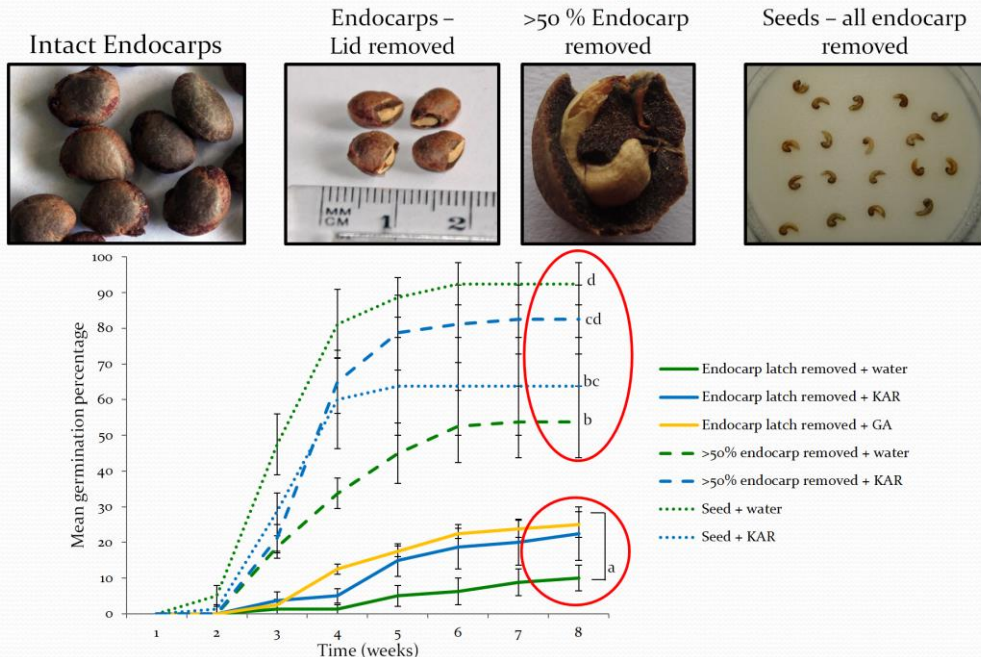
Specific Aims:

- Determine the natural sequence of environmental conditions that result in germination
- Determine the role of the endocarp in regulation of dormancy
- Investigate the impacts of field burial on endocarp opening
- Examine optimal germination requirements after dormancy was broken



Germination and dormancy in *Persoonia longifolia* had been studied for many, many years before I came along, but with only **limited success**. The broad objective of my studies was to determine the **dormancy breaking and germination** requirements of *P. longifolia* endocarps under natural conditions. With the ultimate objective of **replicating** these conditions in the laboratory and nursery in order to develop **reliable propagation** of this species.

Role of the Endocarp



Studies on other *Persoonia* species had found that **removal of the endocarp** improves germination. And we found that this was also the case for *Persoonia longifolia*. We found that **intact endocarps (CLICK)** did not germinate at all. Interestingly when we removed **just the lid** from the endocarp (**CLICK**), germination improved **but not to the same extent** as removal of most or all of the endocarp (**CLICK**). Indicating that the **endocarp plays some role** in the germination of these species.

Burial Trials

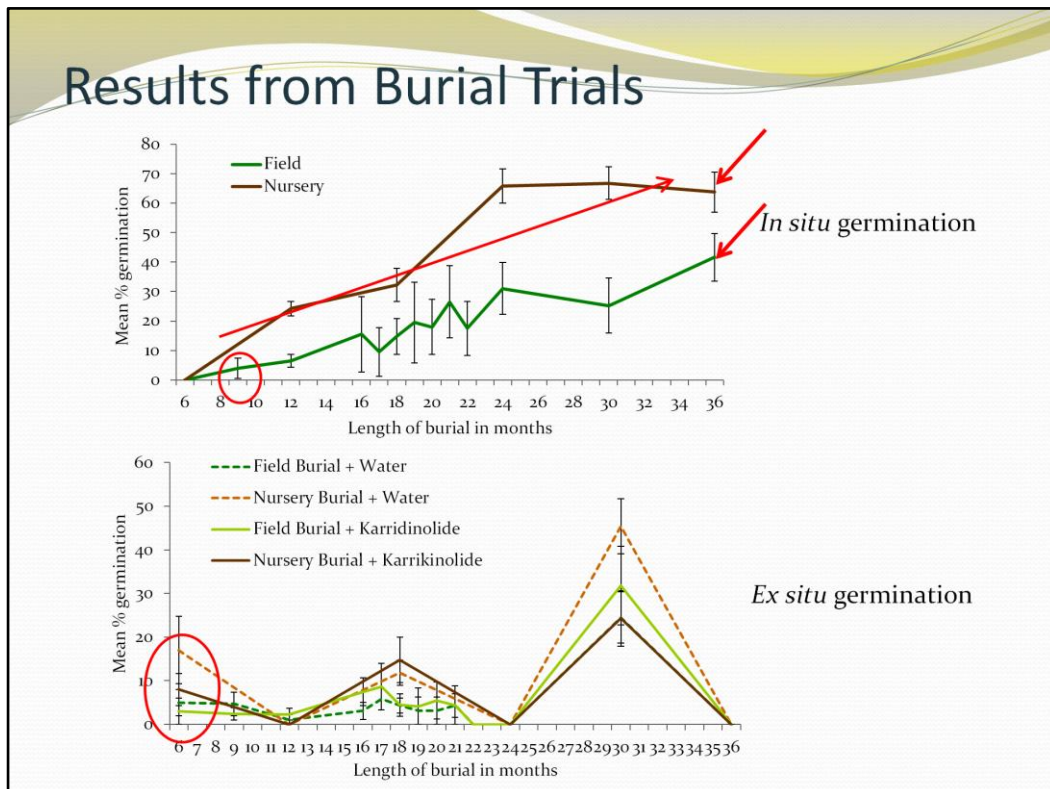
- Does burial increase germination?
- Can this be replicated in a nursery environment?
- What are the seasonal changes driving dormancy loss?



This all lead to the implementation of several **burial trials**. Previous burial trials with *Persoonia longifolia* undertaken at **Kings Park** had shown that germination of around 50% could be achieved after **18 months** burial, indicating that environmental conditions were playing a role in breaking seed dormancy. However these **environmental conditions** had not been identified so my trial was aiming to determine what these environmental conditions were.

Endocarps were buried in **the jarrah forest** and in a **nursery environment**. Retrievals **were six monthly** in the first year and then **monthly** from 16 months through to 24 months to try and pin down exactly when endocarps and seeds lost their dormancy. **Data loggers** were placed out at the same time to measure **soil temperature** and **moisture content**.

Once seeds were retrieved, **in situ germinants** were recorded, empty seeds were removed and the remaining seeds placed into germination cabinets for **three months** to observe ex-situ germination.



This trial provided quite a **break through** in terms of identifying what was happening while the endocarps were in the ground. The first point that should be made is that **no endocarps** stored at the constant temperature and RH germinated either *in situ* or *ex situ* throughout the entire trial – **as expected**.

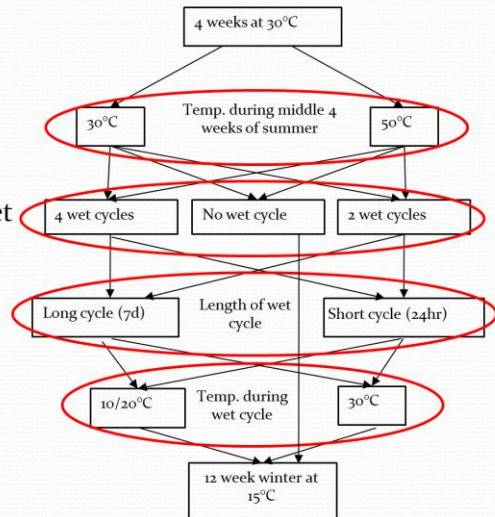
First **ex-situ germination** occurred at six months (**CLICK**). This was a full 12 months earlier than had been observed in previous burial trials undertaken which led me to think **something had happened** in this trial that may have already begun to break dormancy. I decided to do an additional retrieval from field buried endocarps at **9 months** to see what was happening. At that point I also had a small number of seeds that had germinated *in situ* as well **CLICK**.

So from here you can see ***in situ* germination** (**CLICK**) continued to rise over the three years of the trial. Interestingly there was a significantly greater germination observed in nursery buried endocarps compared with field buried endocarps **CLICK**. This was intriguing as they had both received the **same rainfall**.

Close inspection of the **soil temperature and moisture** data revealed that there was in fact quite a difference in the **environmental conditions** experienced in each situation, and in particular during the summer months, most notably any time there was a summer thunderstorm. The nursery soils stayed **wetter longer** and the soil temperatures **dropped to below 10 degrees** for at least 24 hours following summer rainfall events. When I investigated this further I found that previous burial trials had no summer rainfall and also minimal germination compared with my burial trial.

Wet/Dry Trial

- Constant 30°C vs alternating 30/50°C temperatures
- Two vs four wet cycles
- Short vs long wet cycles
- 10/20°C during wet vs 30°C during wet cycle



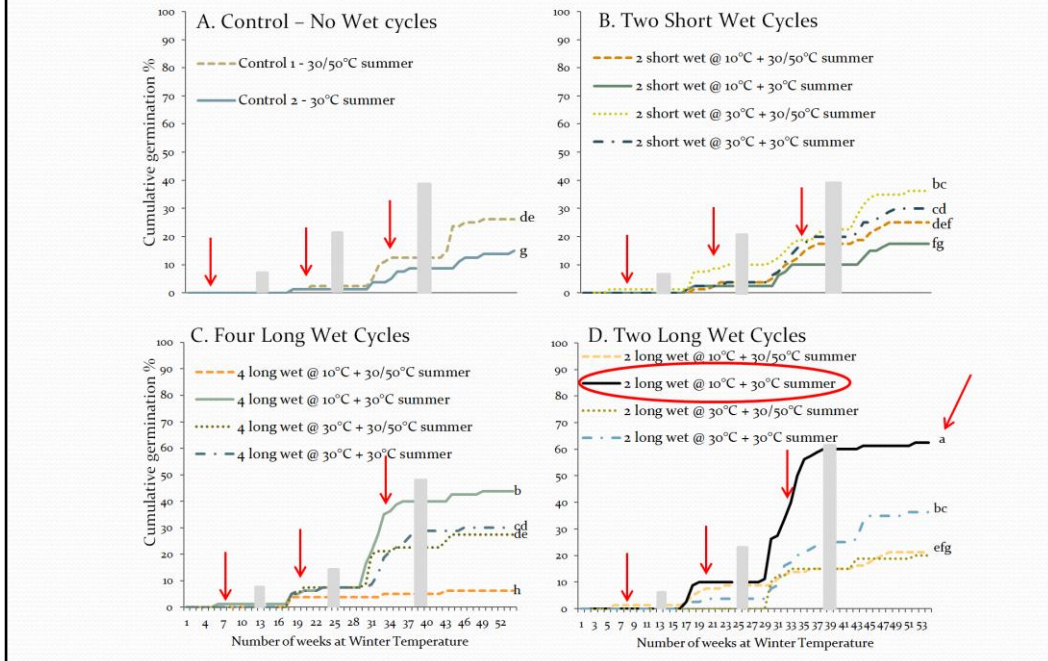
This all then led to some **laboratory germination** trials where I was aiming to **replicate the environmental conditions** experienced in the burial trials and try to narrow down exactly which **conditions** were resulting in the dormancy loss.

These trials were designed in the vein of Baskin and Baskin's move along trials. So endocarps were cycled through a summer period and then put through a winter period over the course of two years. The first trial I ran had four main treatments over a **12 week summer period**. These were:

- **(CLICK)** either **constant 30°C** all summer or a 30°C summer with the incorporation of a four week heat burst at the **extreme summer temperature** of 50°C, simulating the hottest soil temperatures that fruits would naturally be exposed during the summer months;
- **(CLICK)** **two or four** wet cycles simulating summer rainfall events;
- **(CLICK)** summer wet cycles were **short or long**; and
- **(CLICK)** endocarps were subject to a **temperature drop** to 10/20°C for 24 hours during the wet cycle or alternatively were **kept at 30°C** during the wet cycle simulating the change in soil temperature that occurred in the nursery soils during the burial trial.

All **combinations** of these summer treatments were included.

Results – Wet/dry trial One

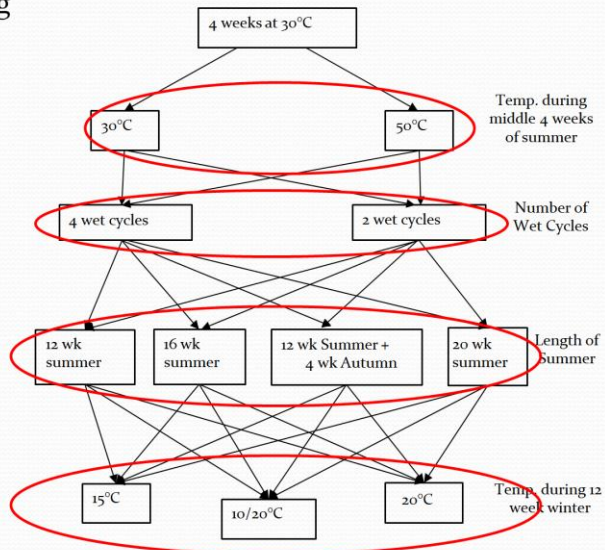


So these graphs show the **results** of that trial. I know there is a lot of information here so I have split the results over **four graphs** to make it easier to decipher. Each graph relates to the **four main treatments (CLICK)** – no wet cycles, two short wet cycles, two long wet cycles and four long wet cycles. The first thing to note here is that all treatments with **4 short wet cycles** resulted in **no germination** at all – so results are not presented. Each of the **grey bars** on the graph indicate a **summer cycle**.

Germination in the **first winter** was very low in all treatments **CLICK**. Germination after the **second winter CLICK** (i.e. 12 months after commencement of the trial), was still low but had begun to occur in most treatments. The **majority of germination** occurred during the **third winter phase CLICK**. At the **end** of the experiment greatest germination was observed in the treatment with **2 long wet** cycles with a temperature **drop to 10** degrees during that wet cycle and a constant **summer of 30** degrees **CLICK**. And this was significantly greater than in all other treatments. So it would seem that the **wet cycles** during the summer months definitely play a role in dormancy break.

Wet/Dry Trial Two

- Constant 30°C vs alternating 30/50°C temperatures
- Two vs four wet cycles
- Variations on the length of the summer period
- Variations on winter temperatures

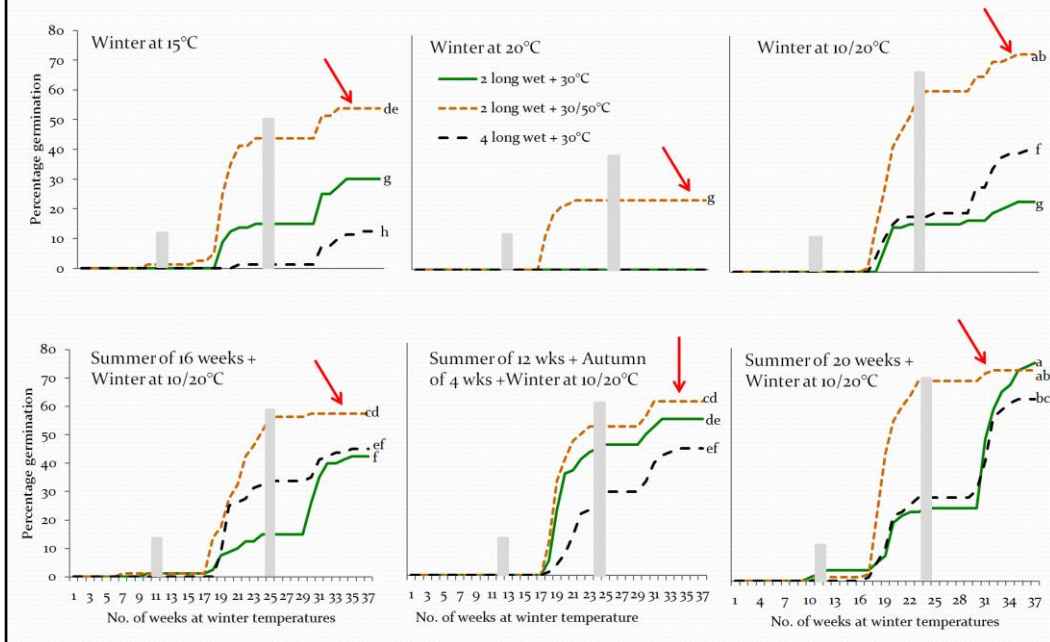


So whilst the results from the first trial were encouraging I was keen to tease out the dormancy drivers a bit further. Based on the results from the first wet/dry trial and some other previous trials that had been undertaken I established a second wet/dry trial which included:

- **Constant** 30°C vs 50°C again.
- **Two or four** wet cycles (**CLICK**).
- Variations on the **length of the summer** period (**CLICK**)
- Variations on **winter temperatures**. (**CLICK**)

This experiment was run for 3 summer and 3 winter cycles.

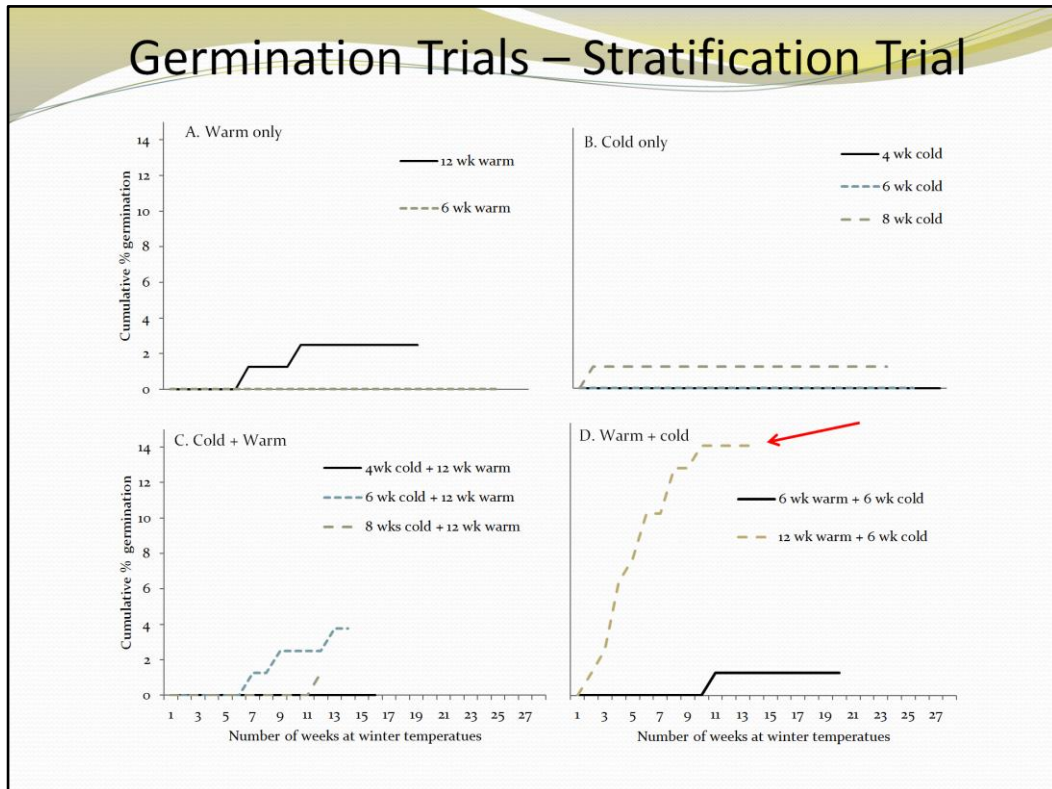
Results – Wet/dry trial two



So these graphs are presented in a **similar manner** to the previous wet/dry trial results with each of the main treatments on a different graph. So this graph is **winter at 15 degrees (CLICK)**, this one **winter at 20 degrees (CLICK)**, this one with **winter at 10/20 degrees (CLICK)**. The bottom three graphs (**CLICK**) all were winter at 10/20° but they had **varying lengths of summer**. Some real patterns began to emerge after this experiment and the most obvious one was that in all of the different main treatments those seeds exposed to **2 long wet cycles CLICK** plus a heat burst of **50 degrees** in summer germinated the best.

So these wet dry trials started to provide a real insight into some of the possible dormancy breaking mechanisms and it would seem that the conditions the seeds and endocarps experienced over the **summer months** appear to be critical for breaking seed dormancy. This

Germination Trials – Stratification Trial



This made me wonder if it was some form of **stratification** that was resulting in dormancy break so decide to run a more specific stratification germination trial which included **constant wet temperatures**, cold stratification only, warm stratification only and combinations of these.

What we found was that germination was still very low (<5%) after one cycle in all treatments except one (**CLICK**) and that was the treatment where the endocarps were exposed to warm stratification followed by cold stratification.

Results summary

- Wet cycles are necessary for germination. Two wet cycles (53%) were better than four wet cycles (42%)
- Long wet cycles (35%) are significantly better than short (27%) or no wet cycles (21%)
- Best germination occurred at the winter temperature of 10/20°C (58% compared with 32% at 15°C and 27% at 20°C)
- Length of summer was important – the longer the summer the greater the germination (70% for 20 weeks compared with 38% for 12 weeks).
- It appears that warm stratification followed by cold stratification breaks down the hard woody endocarp and allows the physiologically dormant embryo to develop the push power to emerge from within the endocarp.

Some further analysis was undertaken to tease out what was happening in these trials

Firstly, significantly more endocarps germinated after being exposed to summer **wet cycles** compared with those endocarps that experienced **no wet cycles** and **two wet cycles** appear better than **four**.

Secondly (**CLICK**), I found that **long wet cycles** during the summer months resulted in **significantly greater** germination than short wet cycles or no wet cycles (**CLICK**). So summer wet cycles obviously play a part in breaking dormancy.

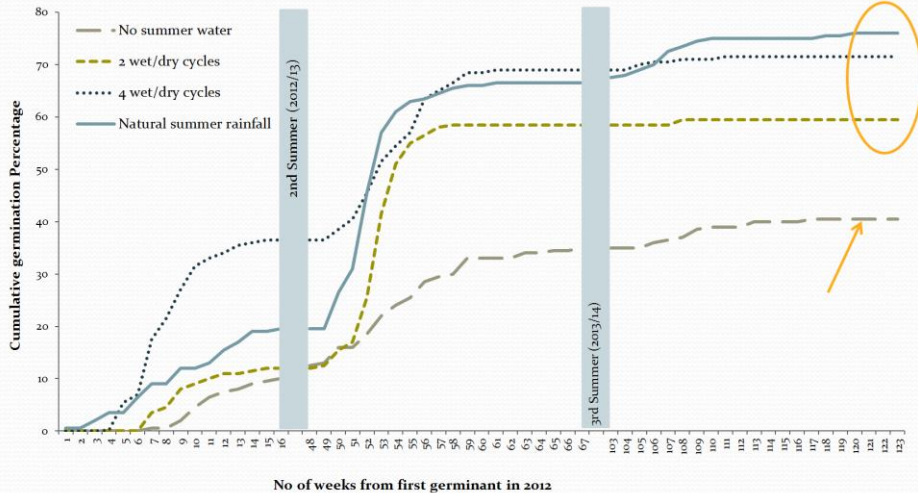
Germination occurred best at the **winter temperature of 10/20°C** (**CLICK**). This is the temperature that best equates to the temperatures that the endocarps experience in their natural environment.

The **length of time** that the endocarps are exposed to summer temperatures also appears to play a critical role in reducing seed dormancy **CLICK**. The **longer the summer period** the greater the germination that occurred, with the best germination occurring in the treatments with a 20 week summer. Once again reflecting the natural environmental conditions experienced by these endocarps.

Finally it appears that two principal processes are responsible for controlling germination: (1) mechanical dormancy imposed by the hard woody endocarp restricting embryo growth and (2) physiological dormancy residing within the embryo,

which is overcome by cycles of warm and cold stratification.

Nursery trial



So these results are all well and good but what do they mean if you a someone trying to **germinate these seeds**. In order to develop a way of germinating large numbers of these seeds I **implemented a nursery trial** manipulated the summer conditions and in particular the summer rainfall events in order to get the seeds germinating.

What we found was that germination in those treatments that received some form of summer rainfall (**CLICK**) was significantly greater than that which was observed in the treatment that received no summer water at all (**CLICK**). Once again showing that **summer rainfall** is important for breaking dormancy in *Persoonia longifolia*.

Propagating *Persoonia longifolia*

- Collect fruits late July/ early August
- Remove the fleshy mesocarp as soon as possible
- Bury endocarps 2 cm deep in clean washed sand in tubs
- Cover with shade cloth
- Water tubs 4 times over summer with 25mm rainfall equivalent
- Expose tubs to natural winter temperatures and rainfall
- Check weekly for germination
- Germination will commence Late July/early August



So what does this mean for propagating snottygobblers:

Collect seeds late July/early August when the mesocarp has turned a **yellowish colour** and the fruits readily drop to the ground.

Remove the **tough exocarp** as soon as possible either with a coffee depulping machine (**CLICK**) as shown here or by hand until they look like this (**CLICK**). Then agitated them in a pectinase solution or **blast the flesh** off with a high pressure cleaner to remove the flesh

Once you have the **clean endocarps** you then need to **bury them** – they will not germinate if scattered on the surface (**CLICK**). I found tubs that had a bit of depth to them and **clean washed sand** in a nursery worked well. I found that the sand held the **summer water** longer than normal forest soil or potting mix.

The endocarps should be **buried approximately 2 cm** below the surface and **shade cloth** put over the tubs. You then need to ensure that the tubs get water **four times** over the summer months (either naturally or by hand watering) with the equivalent of 25 mm of water and on each occasion between watering events, sand plus endocarps should be allowed to **completely dry out**. This allows the hard endocarp to crack and deteriorate through heating and cooling and wetting and drying cycles.

Then come April, **expose tubs** to natural rainfall and temperatures during **winter months** (**CLICK**). The seed and endocarp need a period of cold weather to give the

seed the push power to push through the deteriorated endocarp. This breaks the physiological dormancy within the seed itself.

Check weekly for germinants throughout winter (germination is likely to occur approximately 5-8 weeks after the soil is continuously wet – so around July/August) and **prick out into pots** as required.

Rehabilitation

- Seedling emergence in different areas
- Seedling survival
- The use of guarding treatments



It is all well and good to understand the **mechanisms** breaking seed dormancy but this information needs to be transferred into a more **practical** method of including either seeds, fruits or seedlings back onto restored areas of the mine site. I undertook a number of studies that examined the use and performance of *P. longifolia* in **post mining rehabilitation** and in particular to evaluate the utility of both seeds and seedlings in restoration through investigations into:

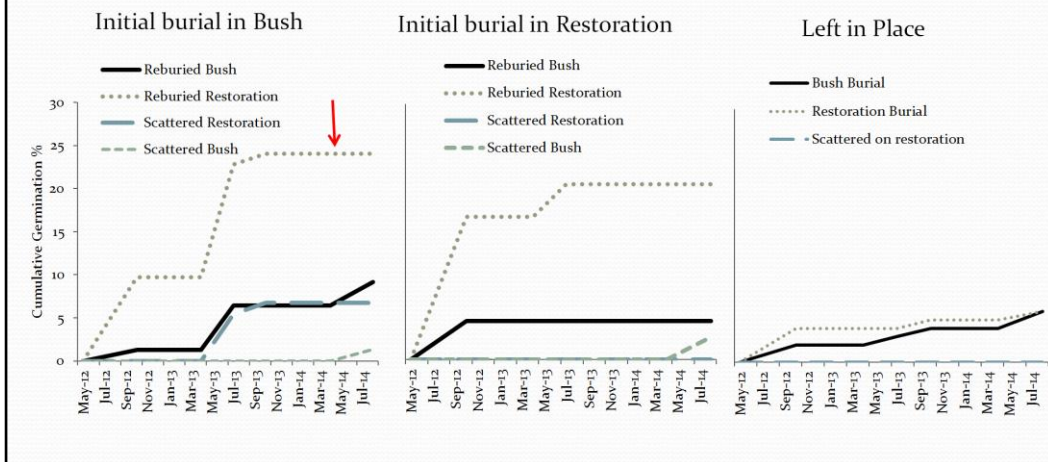
- seedling **emergence** on restored areas;
- seedling **survival** following planting onto different restored areas; and
- the use of different **tree guards** to enhance **survival and growth** of seedlings once planted out.

In the past the mining companies had been including *Persoonia longifolia* **fruits** into their **seed mixes** that were **scattered** directly onto the **rehabilitated** areas of the mine, however this was not resulting in successful germination. Quantification of this revealed that of **480 fruits buried** and scattered on the rehab only **2 germinated in a period of 3 years** – less than 1%. So it soon became obvious that the reason *Persoonia longifolia* seedlings are not observed on the rehab is because they are **not germinating!**

Rehabilitation – Use of fruits

Fruits were either:

- Buried or scattered (sowing treatment)
- Initially placed in natural bush or restored areas
- Re-used in natural bush, restored areas or left in place



So then I looked at the possibility of using a **synthetic seed bank** – can we bury the fruits, **cue** them for germination and then **retrieve** them and **re-use** them on restored areas to **promote** *in situ* germination? What we found here was that the best emergence **CLICK** of 24.0 % after 26 months was observed for fruits that had been initially **buried in the natural bushland site**, then **retrieved** and **reburied** in the **restored** areas. Those fruits that were scattered **on top** of the soil germinated very **poorly** compared to all the other **treatments** assessed.

Rehabilitation – Use of seeds



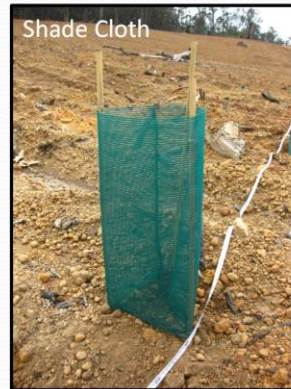
- 7% germination
- Of those that germinated only 6.4% survived

Of the **911 fruits** that were reburied in this trial a total of **64 germinated** over the two years of the - so around **7%** - with only **6.4 %** of these seedlings (so three seedlings) surviving until **the end of the trial** – so less than **1%** of the original seeds put out **germinated and survived** as seedlings to the completion of the trial. Most seedling deaths occurred as a result of **desiccation CLICK** or as a result of **heavy insect damage CLICK**. Only a few seedlings were observed to have been **grazed by herbivores**.

Rehabilitation – Use of seedlings

Aims:

- Impact of guarding on survival and growth
- Impact of age of seedlings at time of planting on survival and growth

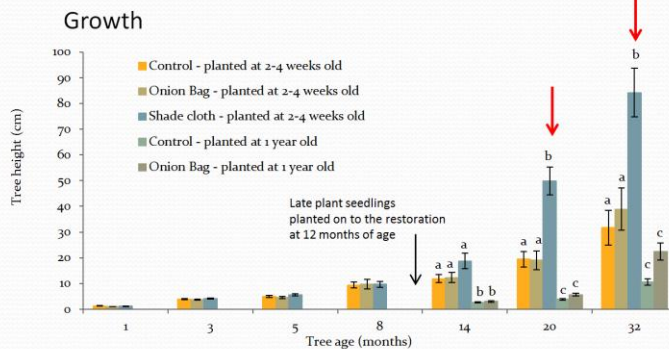


I also investigated the **impacts of using guards** to protect seedlings and to determine whether plants of different ages perform differently when planted into fresh restoration.

Seedlings were either planted **without** a tree guard (control plants), or either with an “**onion bag**” tree guard or a purpose built **shade cloth tree** guard and were either planted at **2 weeks** of age (so almost immediately after germination) or **1 year old**. There were **not enough** of the one year old seedlings to do all three different treatments with the guarding component of the trial so they were planted with either an **onion bag or no guard**. Shade cloth guards were considered to be the least likely to be used in large scale rehab because of the cost.

What we found in this trial was that when initially planted out seedlings survived better if they were older when planted – as you would expect. However after 20 months in the ground, survival was similar for seedlings planted at 2 weeks of age and those that were planted after a year in the nursery.

Rehabilitation Use of seedlings



- Initially older seedlings survived best.
- After 20 months in the ground, survival was similar for seedlings planted early and late.
- There was no difference in survival after 20 months between the different guarding treatments.
- However seedlings planted in shade cloth guards grew much better than trees in other treatments.

Guarding treatments did not affect survival but those trees planted in the shade cloth guards grew much taller (**CLICK**) than those that were planted in the onion bags or without a guard - as you can see in these pictures here. It is possible that the shade cloth guards provide a better **microclimate** than the onion bag or no guards. But also those seedlings in the shade cloth guards experienced **much less insect damage** than the other seedlings. So these results indicate that it is better to plant the seedlings out **earlier** rather than keeping them in the nursery over the summer months and the use of a shade cloth guard will **maximise** growth.

Conclusion



This work was kindly sponsored by the following organisations:



As you can see germination systems can be complex and often time and persistence is required in order to identify the conditions required to break dormancy and to piece together the jigsaw. In the case of *Persoonia longifolia* we now know **summer conditions and in particular thunderstorms**, are critical for breaking seed dormancy and have **developed a method of germinating** this species in the nursery environment which has allowed the **production of a large number** of seedlings for use in restoration. The technologies that have been developed here may also prove to be useful for breaking seed dormancy in **other species of *Persoonia*** or other species with **hard woody endocarps** and this is the direction that future work will take.

Finally I must thank those organisations that sponsored this work (**CLICK**) and thank you for listening to my presentation.