

DO GREVILLEA SEEDS HAVE A 'HARD' SEED COAT?

Background Reading - books

Baskin, CC, & Baskin, JM, 1998, *Seeds: ecology, biogeography and evolution of dormancy and germination*, Academic Press, San Diego.

Bewley, J D, Black, M, 1994, *Seeds: Physiology of Development and Germination*, Plenum Press, New York.

Background Reading – Journal articles

- Burrows, G E, Virgona, JM, & Heady, RD, 2009, 'Effect of boiling water, seed coat structure and provenance on the germination of *Acacia melanoxylon* seeds', *Australian Journal of Botany* **57**, 139-147.
- Briggs CL, Morris EC, & Ashford AE, 2005, 'Investigations into seed dormancy in *Grevillea linearifolia*, *G. buxifolia* and *G. sericea*; anatomy and histochemistry of the seed coat', *Annals of Botany* **96**, 965 – 980.
- Edwards, W, & Whelan, R, 1995, 'The size, distribution and germination requirements of the soil-stored seed-bank of *Grevillea barklyana* (Proteaceae)', *Australian Journal of Ecology* **20**, 548-555.
- Haigh, AM & Barlow, EWR, 1987, 'Water relations of tomato seed germination', *Australian Journal of Plant Physiology* **14**, 485-492.
- Morris, EC, 2000, 'Germination response of seven east Australian *Grevillea* species (Proteaceae) to smoke, heat exposure and scarification', *Australian Journal of Botany* **48**, 179 – 189.
- Morris, EC, Tieu, AL, & Dixon, K, 2000, 'Seed coat dormancy in two species of *Grevillea* (Proteaceae)', *Annals of Botany* **86**, 771-775.
- Morrison, DA, Auld, TD, Rish, S, Porter, C. & McClay, K, 1992, 'Patterns of testa-imposed seed dormancy in native Australian legumes' *Annals of Botany* **70**, 157 – 167.
- Morrison, DA, McClay, K, Porter, C, & Rish, S, 1998, 'The role of the lens in controlling heat-induced breakdown of testa-imposed dormancy in native Australian legumes', *Annals of Botany* **82**, 75 – 40.

At the end of this practical you should be able to:

- **answer a scientific question by designing, executing and analysing the results of an experiment.**

DO GREVILLEA SEEDS HAVE A 'HARD' SEED COAT?

3.1 INTRODUCTION

Seeds of many Australian native plants germinate in large numbers only after a bushfire, resulting in a post-fire flush of germination. Some germination of seeds can occur between

fires, but the subsequent survival of the seedlings is usually poor, because of the presence of adult plants that shade the seedlings and take water and nutrients from them. However, the passage of a fire removes these adverse effects of adult plants, and provides high light levels and an 'ashbed' of nutrients to assist in establishment of new seedlings.

Seeds of some plant species, eg *Banksia*, survive the long inter-fire period by being kept on the plant in woody fruits: the heat of the fire opens the fruit, and the seeds are then released post-fire, and germinate readily when water becomes available.

Seeds of many other species are released from the parent plant after each flowering and fruiting season, and lie dormant in the soil, in what is termed a 'soil-stored seed bank'. This is the case for many species of wattles (*Acacia*) and *Grevillea*.

How do the seeds present in the soil seed bank 'know' that a fire has passed? Intervals between fires can be several decades, and so seeds in the soil seed bank need some mechanism for detecting that a fire has passed, and that it is an appropriate time to germinate.

For some species, the mechanism by which the passage of a fire is detected is well-known: wattle (*Acacia*) and bush pea species have what is called a '**hard**' seed coat. This means that when the seed is released from the parent plant, the seed coat is **hard and impermeable to water**. Thus the seed cannot take up water from the soil, and germination cannot occur - the seed is said to be **dormant**. What **breaks the hard seed coat** is the flux of **heat** that passes down through the soil after a fire. The heat shock breaks seed dormancy by physically disrupting the hard seed coat, allowing water to enter the seed (Morrison *et al.* 1992, 1998). Thus, when it next rains, seeds of wattles and peas can take up water and complete germination. This pattern of water uptake in wattle seeds is illustrated in Fig. 3.1 below.

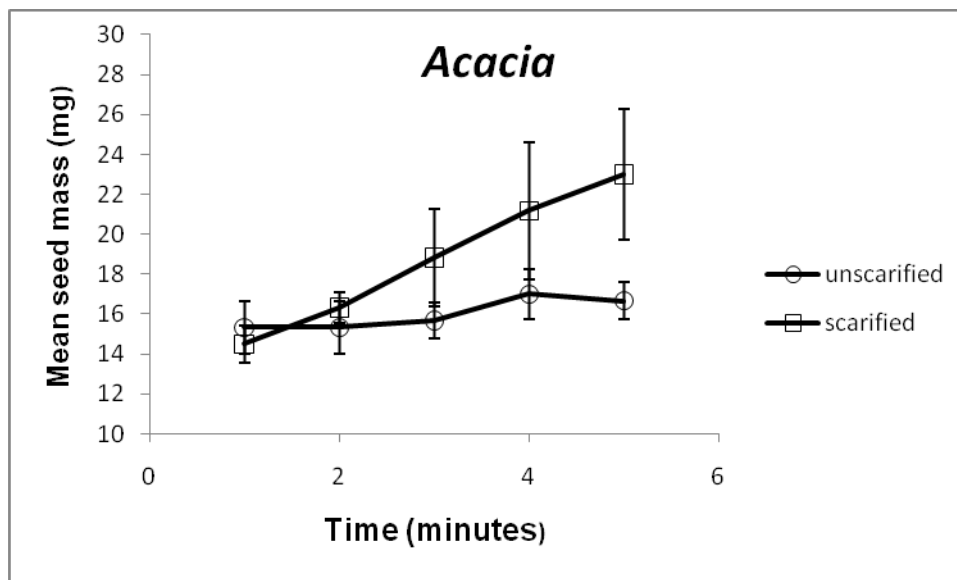


Fig. 3.1: Mean water uptake (measured as seed mass) of scarified and unscarified (control) seeds of *Acacia decurrens*. Data shown are means \pm Standard Errors of the means.

For species with a 'hard' seed coat, the effects of heat in breaking dormancy can be **mimicked in the laboratory** by putting the seeds in an oven for a short period, immersing

them briefly in boiling water, or by physically disrupting the hard seed coat eg by **scarifying** the seed coat with sandpaper or a scalpel.

Seeds of species other than wattles and peas germinate principally in the post-fire period, but the mechanism by which these seeds detect the passage of a fire is only starting to become known (Morris 2000). Thus seeds of *Grevillea*, the ‘spider-flower’ plant that belongs to the Family Proteaceae (to which also belong plants like the waratah, the banksias and hakeas) germinate in the immediate post-fire period.

Two workers from The University of Wollongong published a paper in 1995, in which they showed that seeds of a *Grevillea* species they were investigating, *G. barkylana*, showed a positive germination response to scarification and to heat (Edwards and Whelan 1995). If seeds of *G. barkylana* were given a heat shock in an oven (to simulate passage of a fire), or the seed coat was cut with a scalpel, germination occurred. Germination in response to heat shock, or to scarification of the seed coat, are classic responses observed in the wattles and peas, that have the ‘hard’ seed coat.

On the basis of their results, Edwards and Whelan (1995) argued that **dormancy in seeds of *G. barkylana* was imposed by the seed-coat.**

This led on to the question of whether the seed-coat dormancy seen in *Grevillea* was of the same kind already known for wattles and peas ie was the seed coat ‘hard’, preventing water uptake and subsequent germination. Edwards and Whelan (1995) argued that the seed coat was hard (but without being able to investigate whether this prevented uptake of water; other types of hardseededness that do not involve water impermeability are possible).

If the hard seed coat of *Grevillea* is of the same type as found in wattles (*Acacia*) and peas, then the seed-coat of *Grevillea* should be impermeable to water.

So the question we are asking today is: ‘Does *Grevillea* have a ‘hard’ seed coat?’

This question can be answered fairly simply by investigating the water uptake characteristics, or ‘imbibition’ of *Grevillea* seeds.

Q. 1. If *Grevillea* seeds show the same water uptake characteristics as wattles ie only scarified seeds take up substantial amounts of water (see Fig. 3.1), what would you conclude about the seed coat of *Grevillea*?? ‘Hard’, or ‘not hard’?

Q. 2. If *Grevillea* seeds *do not* show the same water uptake characteristics as wattles ie scarification is not required for substantial water uptake to occur, what would you conclude about the seed coat of *Grevillea*?? ‘Hard’, or ‘not hard’?

So the *aim* of today's investigation is to determine whether the seed coat of *Grevillea* requires breaking eg. by scarification, in order for water uptake to occur.

Students will work in groups and design an experiment that answers the question 'Does *Grevillea* have a 'hard' seed coat?'

Before you execute your experiment, you have to spend time thinking about the experimental design, what data you will obtain, and how you might analyse your data to answer your question.

Read the Section in Box 3.1 on Experimental Design BEFORE you proceed with your own experiment.

BOX. 3.1 EXPERIMENTAL DESIGN

In order to answer the question that you ask in this Practical, you will have to **design an experiment**. In order for you to be able to use the information you obtain from the experiment to answer your question, you need to give careful attention to the **correct elements of experimental design and data analysis**.

Experiments are conducted to answer questions (or 'test models') about how we think the world works. Thus, the logic underlying an experiment goes something like this:

I. model/idea of your system - this is how you think the system might be working; eg seeds of *Grevillea* have a 'hard' seed coat and so are impermeable to water.

II. making predictions from your model - if the system is working in the way you think it is, then you can make **predictions** about what will happen in new situations. This is a very important property of scientific investigations - seeing whether we can successfully predict what our system will do in new circumstances.

III. testing the predictions - this is the fun bit - running an experiment to see whether our predictions work. Will our predictions be borne out (and so we think our model is accurate), or will they not be borne out (back to the drawing board!).

In our case, we want to see whether *Grevillea* seeds show the same water uptake characteristics as *Acacia* showed in Fig. 3.1.

This means we will want to compare the water uptake of *Grevillea* seeds in two groups (see Fig. 3.1). The two groups will be

1. **scarified** seeds, and
2. **unscarified**, or **control** seeds.

If *Grevillea* seeds do have a hard seed coat, like *Acacia*, the results for *Grevillea* should show the same pattern as for *Acacia* in Fig. 3.1.

A problem that always occurs in testing predictions in Biology is that we are working with living organisms, that show a lot of **individual variation** in all sorts of characteristics. Individual organisms like seeds will differ from each other in characteristics like weight, thickness of the seed coat, their history since leaving the parent plant etc. Thus **individual seeds will differ from each other** in, say, their **water uptake characteristics**. The water uptake of any two seeds will differ from each other anyway, and from that of other seeds. So merely to show that the water uptake of one scarified seed is different from the water uptake of another, unscarified seed, is not much of an advance.

The usual approach to this problem is to **apply treatments to groups of organisms** (called **replicates**), and measure the **average or mean** response of the group to a treatment. Thus we would scarify a group of seeds, and have a second group of seeds as the control. Water uptake within any one of these groups will differ to a certain extent from seed to seed - this is the **background variability** in water uptake, due to small differences between individual seeds. So if the mean water uptake tells us what is the average response of seeds to the treatment, the **scatter of readings** around the mean (measured as the **variance** or **standard deviation** of the readings, or the **standard error of the mean**) tells us how much individual seeds differed from each other, within that treatment. This measure of background variability is sometimes referred to as the background **sampling error** by statisticians. It is not 'error' in the sense that it is wrong - it is called **sampling error** to distinguish it from **measurement error**.

Thus at the end of the experiment, you can **compare the mean difference between the groups** in your experiment, and compare this to the **amount of background variability** present in the experiment. If **differences between group means is within the range of background variability**, then you would usually conclude that there was **not much difference** between your different groups - they were all sampled essentially from the same population. If **differences between group means exceeds the range of background variability**, you begin to think that there might be **real, treatment differences** between the groups.

Examination of Fig. 3.1 shows this idea. At the end of the experiment, the difference in mean seed mass between scarified seeds of *Acacia*, and unscarified seeds, was about 25 mg. Background variability, as measured by the Standard Error of the means, was about 5 mg or less. So in this case, you would conclude that scarification has led to a significant increase in the water uptake of *Acacia*.

What we have just described is the thinking behind **inferential statistics** such as the *t*-test and Analysis of Variance. We use these statistical tests to sort out the background variability of the individual organisms that happen to be in all experiments, from any **treatment effect** or 'signal'.

So for experiments in Biology, we usually use statistical tests that proceed as tests of 'no (significant) difference between group means'. This statement of 'no difference between groups' is called the **null hypothesis**.

This means that conducting your experiment, you will need to have

- **two or more groups of seeds to compare (control and experimental groups)**
- **replication of subjects (seeds) within each group.**

Other elements of good experimental design come from the **requirements of the statistical tests** we may use to compare our groups. Tests such as the t -test or Analysis of Variance rely on **four assumptions** that must be met in order for you to obtain a valid answer from the test (if the assumptions of the test are not met, the test does not work as designed, and you can get strange answers from using them). The assumptions are:

1. data are obtained from a **random sample** of the population;
2. the data are **independent** of each other;
3. the variances of the groups in the experiment are similar (**homogeneity of variances**);
4. the data are **normally distributed**.

Of these four assumptions, **assumptions one and two must be met by correct experimental design**. Assumptions three and four are properties of the data, once collected, and we will think about them then.

So to ensure correct experimental design, we must incorporate the following elements into the experiment.

Randomness: this is required in experiments so that any effects we detect can be clearly inferred to be the result of the treatment, and not some artefact of the way we have run the experiment. Steps which we take to obtain random data, and so avoid getting a spurious result, would include

- **random allocation of seeds into treatments.** If for example in our seed experiment, we put all the big, healthy-looking seeds into one treatment, and all the small, unhealthy-looking seeds into another treatment, any differences between the treatments could simply reflect this initial non-random allocation of seeds into treatments. So **subjects must be randomly allocated into treatments** to avoid **bias** in the estimation of treatment effects. Can be done by tossing a coin to assign seeds into treatments.

Independence: as for randomness, independence of the data is required so that any effects that are detected can be unambiguously inferred to the result of the treatment. Many of the steps taken to ensure randomness will also ensure independence, but there are some additional procedures involved for independence of data from experiments:

- **independent application of the treatment to replicates:** as an individual seed will be a replicate, any treatment you impose on an individual seed must be independent of the same treatment imposed on other seeds. Since individual seeds are scarified individually, the treatment is applied independently to each seed. A further step to ensure water uptake is independent in each seed is to make individual containers out of foil to wet each seed in. Thus individual seeds get individual exposure to water. Seeds will need to be weighed individually also to ensure independence of the data.

To conclude, the components you must consider for your experimental design are:

- **what treatment(s) to include, and what are the appropriate controls?**
- **replication**
- **randomisation**

- independence of readings
- how you might graph and analyse your results.

Finally, you will need to consider what conclusion you will draw if the water uptake of scarified and control *Grevillea* seeds is

- the *same* (within the bounds of sampling variation)?
- *different* from each other?

3.2 DESIGNING YOUR EXPERIMENT

For your experiment, the first of the steps listed in Box. 3.1 has been taken already.

I. model/idea being tested - seeds of *Grevillea* have a 'hard' seed coat ie the seed coat is impermeable to water.

Now you have to take over.

II. making predictions

What predictions can you make about the water uptake of *Grevillea* seeds if they have a 'hard' seed coat?

Write your prediction here, for

- scarified seeds?

- unscarified or control seeds?

III. testing the prediction

This is where you design your experiment. What are you going to do to make sure you have included all the elements of good experimental design outlined in Box. 3.1?

Groups of seeds in the experiment:

- **experimental treatment**

- **control**

Seeds of which plant species to use?

Requirements for good experimental design

- **replication**

- **randomisation**

- **independence of data**

Recording data

- **what measurements you will take, and when?**

Graphing and analysis

- **how will you compare results at the end?**

When you have decided on an experimental design, write it out below. Show it to a Demonstrator before you commence.

3.3 METHOD

Work in groups of 4 students. Each group will be responsible for their own experiment.

We have made available for you the following materials:

- **seeds:** *Grevillea* species (*Grevillea* hybrid) and an *Acacia* species (*Acacia decurrens*). Supplied by Southern Biological Laboratory Suppliers, Melbourne, Australia. <http://www.southernbiological.com/About/AboutUsFrameSet.htm>..
- **foil and tissues:** to make small containers to expose the seeds to water
- **balances:** for following water uptake in the seeds
- **sandpaper and razor blades:** for scarifying seeds
- **tissues** to surface dry seeds before weighing

To scarify seeds

For *Grevillea* seeds, use a razor blade to make a single cut through the seed coat mid-way along the edge of the seed, to expose the white embryo underneath.

For *Acacia* seeds, cutting with a razor is **dangerous**. Instead, put a seed on a small square of sandpaper, and with a second small square of sandpaper on top of the seed, grind away the seed coat until the inside of the seed is exposed (seed coat is black; embryonic tissue is white, so stop once white tissue is visible. Check frequently to make sure you don't overdo it!).

To allow seeds to imbibe water

Place filter paper or tissue paper in the bottom of a foil container (1 for each seed), and add sufficient water to allow the seed to take up water (depth of 1 mm). Place seed on filter paper.

To follow water uptake

Water uptake can be easily followed as the change in mass of the seeds*. Once the seeds start to imbibe water, a standard procedure must be adopted to get reproducible values (variable amounts of free water will adhere to the outside of the seed coat as you remove it from the water, adding unwanted 'noise' to mass of individual seeds). For seeds once imbibition has commenced, remove the seed from the water, gently sponge dry with a tissue, let it stand for a further number of minutes (adopt a standard time in the range 1 - 3 minutes), and then weigh.

*The *Grevillea* hybrid seeds have a thin 'wing' which can break off during handling, affecting seed mass. It is often better to trim it off with a blade to avoid this problem.

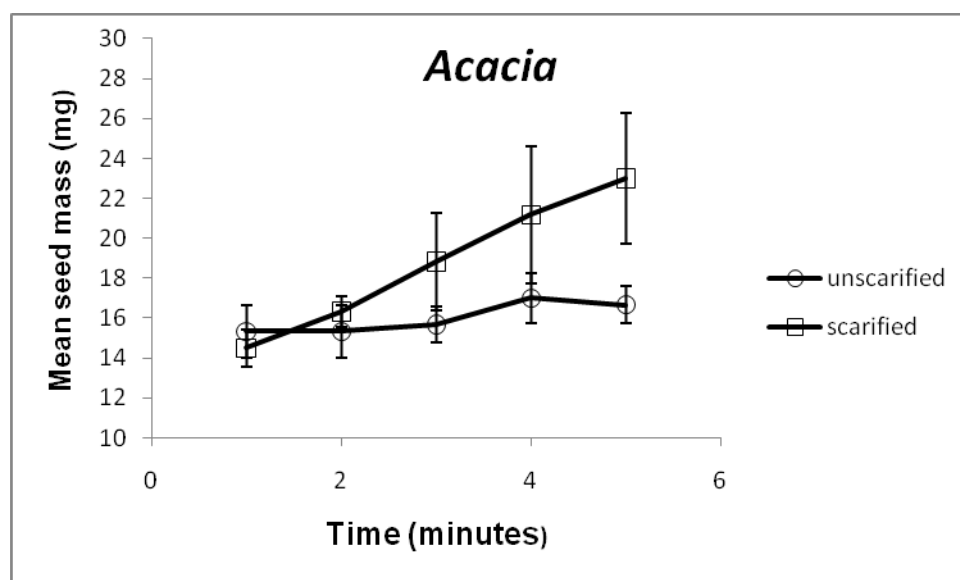
Record your results in Tables you draw up in the space below.

Sample data for *Acacia*

<i>Acacia decurrens</i>	replicate	0 min	15 min	30 min	45 min	60 min	
Unscarified control weight in g	1	0.015	0.015	0.015	0.015	0.017	
	2	0.02	0.02	0.02	0.023	0.021	
	3	0.015	0.015	0.015	0.016	0.015	
	1 seed per container	4	0.016	0.016	0.015	0.017	0.016
		5	0.016	0.016	0.015	0.016	0.016
		6	0.01	0.01	0.014	0.015	0.015
mean (mg)		15.3	15.3	15.7	17.0	16.7	
variance		10.27	10.27	4.67	9.20	5.07	
SE		1.31	1.31	0.88	1.24	0.92	

<i>Acacia decurrens</i>	replicate	0 min	15 min	30 min	45 min	60 min	
scarified weight in g	1	0.016	0.016	0.018	0.019	0.019	
	2	0.015	0.015	0.016	0.017	0.021	
	3	0.01	0.02	0.031	0.038	0.039	
	1 seed per container	4	0.015	0.015	0.016	0.02	0.023
		5	0.015	0.016	0.016	0.017	0.018
		6	0.016	0.016	0.016	0.016	0.018
mean (mg)		14.5	16.3	18.8	21.2	23.0	
variance		5.10	3.47	36.17	70.17	65.20	
SE		0.92	0.76	2.46	3.42	3.30	

Sample graph of means for *Acacia*



Note that unscarified controls show a small increase in mass (some water adhering to seed coat exterior); but only scarified seeds show substantial uptake of water.

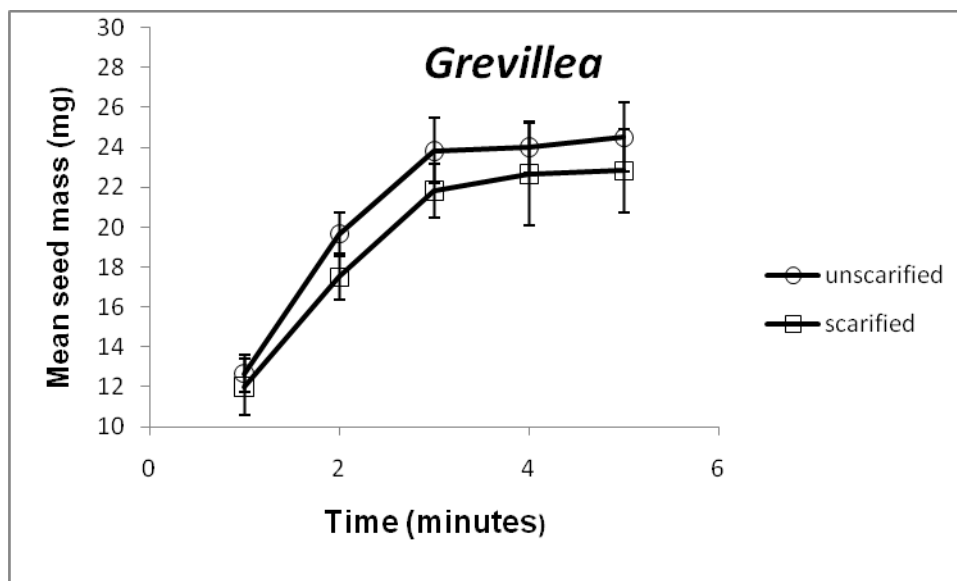
More space for results

Sample data for *Grevillea*

<i>Grevillea</i>		Time (min)				
		0	15	30	45	60
Unscarified control weight in g	rep 1	0.011	0.016	0.019	0.02	0.018
	rep 2	0.015	0.019	0.023	0.022	0.023
	rep 3	0.016	0.024	0.027	0.025	0.028
	rep 4	0.012	0.021	0.03	0.029	0.03
	rep 5	0.012	0.019	0.022	0.025	0.023
	rep 6	0.01	0.019	0.022	0.023	0.025
	mean (mg)		12.7	19.7	23.8	24.0
variance		5.47	7.07	15.77	9.60	17.90
SE		0.95	1.09	1.62	1.26	1.73

<i>Grevillea</i>		Time (min)				
		0	15	30	45	60
scarified weight in g	rep 1	0.006	0.014	0.02	0.02	0.02
	rep 2	0.014	0.019	0.024	0.026	0.025
	rep 3	0.016	0.022	0.027	0.033	0.03
	rep 4	0.013	0.016	0.018	0.015	0.016
	rep 5	0.012	0.016	0.022	0.023	0.026
	rep 6	0.011	0.018	0.02	0.019	0.02
	mean (mg)		12.0	17.5	21.8	22.7
variance		11.60	7.90	10.57	39.47	25.77
SE		1.39	1.15	1.33	2.56	2.07

Sample graph of means for *Grevillea*



Note that both unscarified and scarified seeds show substantial uptake of water. The seed coat of *Grevillea* is permeable to water; it does not have a 'hard' seed coat.

3.4 PRELIMINARY DATA ANALYSIS AND GRAPHING

To determining what conclusion to draw from you experiment,

- calculate the **MEAN seed mass** for each treatment (calculator, or see Appendix 3 for how to use Excel for the calculations)
- calculate the **scatter of readings** around each mean as the **Variance** or **Standard Deviation** of the readings.
- Once you have the variance or standard deviation of the individual readings, an estimate of the ‘sampling error’ associated with your mean can be calculated as the Standard Error (S.E.) of the Mean.

$$S.E. = \sqrt{\frac{s^2}{n}} = \frac{s}{\sqrt{n}}$$

where s^2 = variance of readings, s = standard deviation of readings, n = sample size.

- **graph** the **MEAN SEED MASS** (as a measure of water uptake) *vs.* time for each treatment (see Fig. 3.1 as an example). (Do not plot mass of individual seeds *vs.* time)

Add the **Standard Error of the Mean** as an error bar to the means, to indicate the ‘sampling error’ associated with each mean.

Graphs can be drawn in

- Excel (Appendix 3)
- by hand.

Final comments

The correct interpretation of this experiment hinges on the students observing that unscarified *Grevillea* seeds do not show the same pattern of water uptake as unscarified *Acacia* seeds. Some students observe this in their results, but still cannot draw the appropriate conclusion (*Grevillea* does not have a ‘hard’ seed coat).

The difference in water uptake should be apparent after about 60 minutes, and certainly by 90 minutes. An initial and final weighing would suffice to detect the pattern; weighing at intermediate times simply allow the time-course of water uptake to be graphed.