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Dormancy and germination in two Australian native species (*Acacia aneura* and *Rhodanthe floribunda*)

Paul Theophile Epee Misse^{1*}

¹Lincoln University New Zealand, Department of Soil and Physical Sciences, And The Ministry of Agriculture and Rural Development, Cameroon. E-mail: paul.epeemisse@lincolnuni.ac.nz

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Abstract

In the laboratory of plant physiology of the University of Queensland (Gatton Campus), a seed germination experiment was undertaken on seeds of two Australian native plant species – *Rhodanthe floribundata* and *Acacia aneura*. Most *Acacia*, including *A. aneura* exhibit a physical dormancy due to the waxy coat covering the seed. Comparably, just a few species of *Rhodanthe* are studied as to their dormancy. However, they are also known to present different forms of dormancy. To understand and describe these dormancy mechanisms, a seed germination experiment was conducted on *Acacia aneura* and *Rhodanthe floribunda*. This experiment will either add to the existing knowledge regarding these species' dormancy or corroborate them. It is expected that both species display some form(s) of dormancy.

Keywords: Germination, *Rhodanthe floribundata*, *Acacia aneura*, Physical dormancy

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1. Introduction

Germination constitutes an early stage of development for most naturally growing plants. A wide array of plant species, however, exhibit germination blocks termed dormancy. Five types of dormancy are identified: physiological, morphological, morpho-physiological, physical and combinational dormancy (Baskin and Baskin, 2004). To determine what type of dormancy a particular seed species exhibit, a specific set of experiments are performed. In the laboratory of plant physiology of the University of Queensland (Gatton Campus), a seed germination experiment was undertaken on seeds of two Australian native plant species – *Rhodanthe floribundata* and *Acacia aneura*.

Dormancy across the *Acacia* genera and ways to overcome it are well documented through a number of studies. Some of these include *Acacia caven* (Escobar *et al.*, 2010), *Acacia tortilis*, *Acacia oerfota* (Abari *et al.*, 2012), *Acacia aroma*, *A. cavenand* and *A. Furcatispina* (Funes and Venier, 2006). Most *Acacia*, including *A. aneura* exhibit a physical dormancy due to the waxy coat covering the seed (Auld, 1986; and Al-Mudaris *et al.*, 1999). Comparably, just a few species of *Rhodanthe* are studied as to their dormancy (Hoyle *et al.*, 2008). However, they are also known to present different forms of dormancy (Bunker, 1994). To understand and describe these dormancy mechanisms, a seed germination experiment was conducted on *Acacia aneura* and *Rhodanthe floribunda*. This experiment will either add to the existing knowledge regarding these species' dormancy or corroborate them. It is expected that both species display some form(s) of dormancy.

* Corresponding author: Paul Theophile Epee Misse, Lincoln University New Zealand, Department of Soil and Physical Sciences, And The Ministry of Agriculture and Rural Development, Cameroon. E-mail: paul.epeemisse@lincolnuni.ac.nz

2. Materials and Methods

Rhodanthe floribunda seeds used for this experiment were provided by the Center for Native Floriculture of the University of Queensland, while those of *Acacia aneura* were collected from the Nindethana seed service.

The seed germination trial was conducted in the plant physiology laboratory of the University of Queensland, Gatton campus. Seeds were first sterilized in 2% sodium hypochlorite for 2 to 3 minutes, rinsed in sterile distilled water and placed on 2 sheets of moistened filter paper in a Petri dish. The petri dishes were then carefully sealed with parafilm and incubated at 25 °C.

The trial was a completely randomized design of six treatments with eight replicates: intact seeds moistened with water (the control); scarified moistened seeds; intact seeds soaked in GA₃ at 100 mg L⁻¹ for 24 h; and two light levels (light and dark). For the dark treatments, dishes were covered with an aluminum foil. *Acacia* seeds were scarified by making incisions on the seed coat at the opposite side of the embryo using N°10 scalpel blade. Seeds of *Rhodanthe* were too small to be scarified and therefore, this treatment was not considered for this species. The dishes were then incubated at 25°C.

A day eight following incubation, the petri dishes were inspected for signs of germination or contamination. A seed was considered to have germinated when the radicle emerged from the seed coat. Contaminated seeds with fungi or bacteria were discarded. For each treatment, the number of contaminated and germinated seeds was recorded. With dark treatments, the aluminum foil was carefully and quickly peeled off to inspect for contamination, removal of contaminated seeds and germination count and then immediately reinstate. Petri dishes were all resealed and returned to the incubation room (25 °C). The week after, all Petri dishes were removed from their covers, checked for contamination and germination count.

The recorded data were statistically analyzed using the statistical software package R. The effects of the various treatments were assessed by the ANOVA followed by the Least Significant Difference test for means comparison at the probability level of $p < 0.05$.

3. Results

3.1. *Acacia dormancy*

3.1.1. *Effect of light on dormancy*

Acacia aneura displayed a positive response to dark eight days following imbibition. The germination rate of intact and GA₃ treated seeds in the dark was twice as much as in the light (Table 1). Scarified seeds incubated in the dark recorded a higher germination rate (80%) compare to light incubated seeds (57.5%). Fifteen days later, there was no significant difference for both light levels except for the GA₃ treatment (57.5% in dark and 35% in light).

Table 1: Effect of scarification, GA₃ and light/dark on percentage germination at 8 days and 15 days from imbibition on *Acacia aneura*

Periods of Observation from Imbibitions	Germination (%)					
	Intact (Control)		Scarified		GA ₃	
	Dark	Light	Dark	Light	Dark	Light
8 days from imbibition	37.5bc	17.5c	80a	57.5ab	40bc	17.5c
15 days from imbibition	80ab	65ab	92.5a	87.5a	57.5bc	35c

Note: Treatment means within the same row followed by the same letter(s) are not significantly different at $p < 0.05$.

3.1.2. *Effect of Scarification on Dormancy*

Scarified *A. aneura* seeds germinated faster and more than others. At the first seven days of the trial, their germination rate in both light levels was at least twice higher than the control and the GA₃ treatment (Table 1). Seven days afterward, scarified seeds still recorded the highest germination rates (92.5%), particularly in the dark.

3.1.3. Effect of GA₃ on Dormancy

The GA₃ treatment had a negative effect on *A. aneura* germination especially at the second period of the experiment. The germination rate of this treatment was about half the rate of intact seeds (control) independent of the light level.

4. Rhodanthe dormancy

4.1. Effect of light on dormancy

R. floribunda seeds responded positively to light. Eight days and 15 days from incubation, the number of intact seeds (control) germinated in the light was nearly six times as much as in the dark (Table 2). Irrespective of the treatment and the period, germinated seeds in the light were at about twice or higher as much as in the dark. Furthermore, the highest germination rate (100%) was recorded on seeds incubated in the light.

Periods of Observation from Imbibitions	Germination (%)			
	Intact (Control)		GA ₃	
	Dark	Light	Dark	Light
8 days from Imbibition	5c	27.5b	32.5b	80a
15 days from Imbibition	12.5c	52.5b	57.5b	100a

Note: Treatment means within the same row followed by the same letter(s) are not significantly different at $p < 0.05$

4.2. Effect of GA₃ on dormancy

R. floribunda seeds reacted positively to GA₃ treatment. At the first period of the trial, the germination rate under this treatment was threefold that of the control for light incubated seeds, and up to sixfold for dark incubated seeds. At the second period of observation, the rate of GA₃ treated seeds in light was almost twice as much as in the control.

4.3. Seed contamination

Seed contamination was monitored during the whole trial. Eight days from incubation micro-organisms began infesting seeds in petri dishes. At the second period, contamination kept progressing mostly on *Acacia* (Table 3). The *Rhodanthe* contamination remained quite stable over both periods with a little increase of the

Species	Periods of Observation from Imbibitions	Germination (%)					
		Intact (Control)		Scarified		GA ₃	
		Dark	Light	Dark	Light	Dark	Light
<i>Acacia aneura</i>	8 days	2.5a	2.5a	0a	5a	2.5a	12.5a
	15 days	7.5a	7.5a	2.5a	2.5a	2.5a	17.5a
<i>Rhodanthe floribunda</i>	8 days	7.5a	2.5a			5a	0a
	15 days	12.5a	2.5ab			5ab	0b

Note: Treatment means within the same row followed by the same letter(s) are not significantly different at $p < 0.05$.

contamination rate of dark incubated intact seeds. However, these are just tendencies since no major significant difference was noticed over both periods and across treatments except between the *Rhodanthe* intact dark incubated seeds and those treated with GA₃ in the light, 12.5% and 0% respectively.

5. Discussion

5.1 *Acacia aneura* dormancy

Scarified seeds of *A. aneura* germinated faster than the intact seeds. This result is consistent with a number of studies on *Acacia* dating back in the 1980's (Auld, 1986). Pound *et al.* (2014) report that mechanical scarification enhances *Acacia* germination, with a higher percentage being achieved between 10 to 15 days. *Acacia* seeds are generally recovered with a seed coat. To germinate, seeds need water and the seed coat recovering the *Acacia* seed is a barrier to water absorption. Scarification provides openings through which water and oxygen are absorbed triggering imbibition, the first germination stage. It is also likely that some other compounds inhibiting germination are released through these openings (Pound *et al.*, 2014). However, not all *Acacia* exhibit physical dormancy due to seed coat for Schelin (2004) found that *Acacia macrostachya* lacked physical dormancy and could germinate properly under favorable conditions despite its seed coat.

The darkness promoted *Acacia* germination. Although it is established that seeds with physical dormancy do not exhibit any sensitivity to light (Baskin and Baskin, 2004), our observations contradict this assumption, particularly during the first week following incubation.

The germination percentage of *Acacia* seeds was lower when treated with GA₃. GA₃ appears to be an inhibitor of *Acacia* germination. Although the control and the GA₃ treatment were quite similar a week after incubation, a significant difference was noticeable a week later, with GA₃ treated seeds displaying a lower germination rate. This indicates that GA₃ slows down or blocks *Acacia* germination.

5.2 *Rhodanthe floribunda* dormancy

Rhodanthe floribunda seeds responded to light as well as GA₃, an indication that dormancy mechanisms may be controlled by both factors. This corroborates other findings on Asteracea to which the *Rhodanthe* genera belongs to. For example, Merritt (2006) noted that Gibberellic acid (or GA₃) and light stimulated the germination of some Australian Asteracea seeds. Similarly, Bunker (1994) reported the germination stimulating effects of GA₃ on *Rhodanthe moschata* and *Rhodanthe polygalifolia* as well as that of light on *Rhodanthe humboldtiana* and *Rhodanthe stricta*. In some dormant seeds, germination is triggered by light which activates phytochromes. Phytochromes seem to control the synthesis of Gibberellins (Bewley, 2013). Gibberellins concentrations usually increase during germination to support active cell enlargement by controlling the transcription of genes encoding hydrolytic enzymes. Upon activation of these genes by Gibberellins, enzymes are released into the endosperm to decompose proteins and starch into nutrients assimilated by the developing embryo (Hopkins and Hüner, 2009). Similar biochemical processes might have occurred in GA₃ treated *Rhodanthe* seeds incubated in light, suggesting that the concentration levels of Gibberellins were lower to spark germination.

5.3. Seed contamination

The number of contaminated seeds tended to increase over time. *Rhodanthe* seeds were less subject to infestation than *Acacia*. The highest contamination occurred on *Acacia* GA₃ treated seeds followed by the *Rhodanthe* intact seeds (control). This suggests that the sterilization with 2% sodium hypochlorite was not strong enough to destroy completely the microbes off the seeds or that the growth media were not completely sterile. Therefore, sterillising seeds with 4% sodium hypochlorite with the adjonction of pesticides application could prevent contamination. For instance, Bunker (1994) added fungicide on the petri dishes at the start of his experiment although other aseptic measures might have been taken to keep the medium sterile.

6. Conclusion

This experiment revealed that *Rhodanthe floribunda* and *Acacia aneura* present different forms of dormancy. The former exhibited a physiological dormancy while the later displayed a physical dormancy. Mechanical scarification proved effective to break the physical dormancy of *A. aneura*. However, this method can be time-consuming and risky for the embryo, particularly if done manually on a large number of seeds. Consequently, further investigation on chemical scarification could provide an alternative. To break *R. floribunda* dormancy, seeds should be soaked in a solution of GA₃ at the concentration of 100 mg L⁻¹ for 24 h and incubated in the light. Finally, to limit seed microbial contamination strict aseptic measures should be combined with the use of environmentally friendly pesticides.

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