Breaking dormancy in seed germination

Abstract:

Introduction:

A dormant seed will fail to germinate under normally favourable conditions of moisture, temperature and oxygen supply. Temperate climate plants especially produce dormant seeds, but they are also found in tropical and subtropical species. Methods for breaking seed dormancy have been devised which override the physiological mechanisms involved in dormancy preservation. In this essay I intend to outline the most popular mechanisms of dormancy breaking and discuss the physiological mechanisms and ecological significance of stratification, photosensitivity and scarification.

Seeds are the agency of propagation for plants. Dormancy offers plants the opportunity to regulate this stage of their growth cycle. Through dormancy plants can pause and later benefit from both seasonal and fortuitous changes in the environment. Dormant seeds can remain viable for remarkably long periods of time. Lotus seeds, Nelumbo nucifera, dug from archeological sites over 1000 years old have been found to be viable (Weir et al., 1982). In certain species, the dormancy characteristics of seeds within the same harvest are different. The timing of germination would be spread out over months or years, increasing the likelihood of some seed survival. Other seeds display secondary dormancy. These seeds when shed will germinate readily if conditions are favourable. However if conditions are not favourable, secondary dormancy is induced. Studies of dormancy by plant physiologists have provided valuable knowledge on the mechanisms of seed dormancy initiation of germination. “ Dormancy mar be due to an immature embryo, impermeability of the seed coat to water or to gases, prevention of

embryo development due to mechanical causes, special requirements for temperature or light, or the presence of substances inhibiting germination” (Mayer & Poljakoff-Mayber, 1982). An understanding of germination initiation is of economic importance in agriculture, horticulture and forestry. Without this, important seed which is dormant would be reduced to germinating naturally in a very narrow range of micro-environmental conditions. To break dormancy, special treatments may be necessary before sowing or during imbibment of batches or seed banks.

The mechanisms for breaking dormancy correspond to the various dormancy inducing and maintaining mechanisms in action for each species. In a few species dormancy is related to the undeveloped state of the embryo at the dehiscence. A considerable period of time must pass during which the embryo will continue to develop until it reaches `maturity’. In other seeds there are no obvious changes anatomically or morphologically but still a period of after- ripening is required. The subtle differences which occur within the seed must be the consequence of chemical or physical changes within the seed or seed coat.

A hard seed coat is capable of causing dormancy in three was; it may be impermeable to water, impermeable to gases or it may mechanically restrain the embryo (Mayer & Poljakoff-Mayber, 1982). `Impaction’, vigorous shaking of the seeds can be effective in making the seeds permeable to water. Treatment of the seeds with microwave energy (2450 MHZ) Tran 1979; Mayer & Poljakoff-Mayber, 1982) has also been successful for hard coated seeds. Dry heat and boiling water treatments are commonly used for seeds of Acacia melanoxylon. Shaking with abrasive material causes mechanical

breakage of the seed coat and is a frequently used method as are scratching and pricking. Chemical treatment is usually with a solvent such as alcohol or an acid. Treatment with alcohol is suggested for members of the family Caesalpiniaceae (Mayer & Poljakoff-Mayber, 1982). Often combined types of dormancy restrain germination. Seeds of Rosa and Crataegus sp. have extremely hard and durable coats. Great mechanical pressure is needed to destroy the stony endocarps. Additionally, endogenously imposed dormancy mechanisms complicate the inducement of germination in Rosa species. Long imbibment periods without chilling will initiate secondary dormancy (Bradbeer, 1988).

In order to germinate many seeds require exposure to specific temperatures (high or low), at which germination is not normally favourable. In temperate regions chilling or ‘ stratification’ is an extremely important control factor in dormancy breaking. This involves exposure of imbibed seeds to temperatures normally between 10 and 100 C, for extended periods. The term stratification was coined from the procedure used in temperate forest nurseries. The seed material is placed in alternating layers of sand or soil. At the beginning of spring the seeds are dug up for sowing in seed beds (Bradbeer, 1988). Routinely though, standard chilling methods employ refrigerated incubation.

Treatments with high temperatures are not particularly successful even with tropical species. Although brief exposure to slightly elevated temperature has promoted subsequent germination at lower temperatures after exposure to light n Poa pratensis and Lepidium virginicum (Mayer & Poljakoff-Mayber,

1982). Tropical species more often respond to combinations of temperature and light.

Bradbeer recognizes specific groups of light related dormant seeds:

(1) Positively photoblastic seed in which germination was either induced or promoted by light

(2) Negatively photoblastic seed in which germination was either wholly or partially inhibited by light

(3) Apparently non-photoblastic seed in which no difference between dark and light germination has been reported.

For positively photoblastic seeds the level of illumination required may be low and germination relies more upon the quantity of light received (Villiers, 1972).)Only imbibed seeds will respond to light and there is usually a complex interaction between light and other external factors as well as the age of the geed (Mayer & Poljakoff-Mayber, 1982).

The control of dormancy in some species is dependent on the combined actions of inhibitory and promotive substances. Frequently many of these dormancies can be overcome by the use of chemical promoters. Commercial products such as Dalapon, Thiourea, Knop’s potassium nitrate, and hormones such as gibberellins and cytokines have been used. These substances can wholly or partly replace light or temperature in breaking of dormancy. More simply, natural substances enveloping the seed can produce an environment of high osmotic pressure. Seeds of plants from saline environments often are held dormant by this mechanism. Tomato seedlings

are encapsulated in a gelatinous substance which when removed allows germination of the seed (David Midmore, pers com). This gel may also contain a germination inhibitor such as caffeic and or ferulic acids (Mayer & Poijakoff-Mayber, 1982). Leaching can be used to attempt to remove any of these water-soluble inhibitors. Birch seeds need prolonged rinsing, 16 hours whereas Xanthium pennsylvanicum require only a small amount of water (Wareing & Foda 1975; Badbeer, 1988).

Stratification

The exact molecular mechanisms of breaking dormancy through chilling are shadowy. Frankland and Wareing (1966), working on hazel seeds, found that initially dorrmant seeds contained no detectable gibberellic acid (GA) substances.) however, after 12 weeks of chilling, the hazel seeds contained the biological equivalent of 0. 2 picomoles of GA3 (Bradbeer, 1988.). Through further work it was established that GA is synthesized in the embryonic axis and is translocated to the cotyledons not during stratification but in the first days after the temperature is increased (Ross and Bradbeer, 1971; Bradbeer, 1988). This would imply that chilling brings about changes which capacitate the biosynthesis of GA when the temperature is raised after stratification. Bradbeer (1988) continued by suggesting it was the presence of growth inhibitors which retarded synthesis of GA in the axis. Moreover that chilling influenced the levels of growth inhibiting substances. Studies by Mayer et al. (1982) upheld this. In the seed of Fraxinus americana, Juglans regis and Corylus avellana, it was reported the amount of growth inhibiting substance ABA,(abscisic acid), dropped during stratification. Walker-Simmons et al. (1989), whilst working on wheat embryos from dormant grain, showed that

ABA deficient mutants in maize, potato and tomato precociously germinate. It has been shown that gibberellins interact with ABA in the synthesis of hydrolytic enzymes in the aleurone layers of germinating cereal seeds (Chrispeels and Varner, 1966; T. A. Villier , 1972). It is generally accepted that dormancy is regulated by a balance between and sensitivity to the activities of growth-promoting and growth-inhibiting substances. During stratification this balance and sensitivity is changed considerably.

In temperate climates where dormant seeds are most abundant, many seeds are dispersed in autumn and covered by moist leaf litter or soil over the winter months. It is strategic to delay germination until spring when conditions are more promising for germination and seedling growth. Stratification mimics these conditions. For the seeds of tropical plants stratification is not as effective. An alteration between high temperatures and exposure to light, with low temperatures and high humidity is often needed to induce germination. Dormant seeds of Oldenlandia corymbosa will not germinate in the light at high temperatures without prior exposure to lower temperatures 25° C, at high relative humidity (Attims & Come, 1978; Mayer & Poljakoff-Mayber, 1982). In and regions such as the desert of Western Australia summer grasses remained dormant during winter rains and only germinated in the following rains of summer (Mott, 1972; Mayer & Poljakoff-Mayber, 1982).

Light in Dormancy Control

“ The effect of light on dormancy is dependent on the intensity and duration of irradiation, on the wavelength, on moisture content of the seed and on the time of the exposure to irradiation, including the whole of the previous

history of the seed during development on the parent plant and subsequently" Bradbeer, 1988. This statement hints at the enigmatic role of light as a mechanism for breaking dormancy in seeds. The effective wavelength of light for promotion of seed germination has been shown to be the red region of the spectrum, 660 nm. Far-red irradiation, 730 nm, inhibits the germination of light promoted seeds (Borthwick et al., 1952; T. A. Villiers, 1972). The regulatory pigment involved is Phytochrome, a chromoprotein in which the chromophore is a tetrapyrrole (Bradbeer, 1988). It has two photoconvertible forms PR and PFR. The pigment is converted from one energy form, PR, germination inhibitor, to the other, PFR, germination promoter, by exposure to red irradiation. Exposure to far red irradiation converts the pigment back to PR form. Villiers (1972) suggests that it is the higher energy of the active PFR form that drives the initiation reactions which promote germination. The lower energy levels of PR form are insufficient to bring about the cellular response required to initiate germination. Seeds may be light requiring when newly formed on the plant but over time and with air drying or chilling become less or no longer light sensitive. Unchilledseeds of Betula pubescens require day long illumination for dormancy release but chilled seeds will germinate in the darkness (Black & Wareing, 1959; Villiers, 1972). Moreover light sensitivity can be induced in negatively photoblastic seeds by maintaining seeds in unfavourable conditions. Light insensitive tomato seeds exposed to far-red irradiation for 18 hours from the beginning of imbibition then required red irradiation to promote germinate. It is suggested that prolonged irradiation causes far red absorbing pigment to be synthesised (Mancinelli et al., 1966; Villiers 19-72).

The conditions under which a particular batch of seeds has matured on the plant might be important in manipulating light sensitivity (Koller, 1962; Villiers 72).

The use of Phosphon D, an inhibitor of gibberelin synthesis, can reverse the effect of light on dormancy of Verbascum seeds (McDonough, 1965; T. A. Villiers, 1972). Such a finding suggests that gibberelin synthesis may. play a pivotal role in light-stimulated dormancy breaking of seed. The ecological value of light regulated germination is not totally clear.

Superficially, seeds requiring light for dormancy breaking are likely to be pioneering species. Understorey species are presumably less light sensitive and seeds would need to be covered or shaded to germinate. In studies on soil light penetration it was found that transmittance was strongly dependent on soil type and decreased with decreasing particle size and with -eases in darker components. It was also established that increasing soil depth lead progressive decline in the red/far red ratio (Benvenuti, 1995). Therefore light requiring seed buried deeply in soil will receive lower levels of red light. These seeds will remain dormant until the soil is disturbed. Furthermore the ratio of red/far red changes during the day. The lower angles Elevation of the sun at dusk and dawn increase the ratio. Light passage through leaf canopy also alters the spectral composition of the light reaching the seeds. Vazquez-Yanes et al., (1996) compared the effect of this phenomenon on two pioneering tree species. Passage of radiation through the rainforest opy in Veracruz, New Mexico produces a decrease in the red/far red ratio. e the major components of the annual seed deposition were seeds of Cecropia obtusifolia and of some species of ficus. Seeds of C. obtusifolia require light

to germinate but do not germinate in low red/far red conditions and become dormant forming a relatively persistent viable seed bank. Seeds of the ficus however do germinate in such conditions and do not have a dormant stage. They cannot however build up a seed bank on the forest floor and are therefore not as opportunistic as C. obtusifolia might be.

Seed Coat Damage

In many seeds the coating is the cause of dormancy. The physiological mechanisms involved in release from dormancy with these techniques are far more clear. Thick and water-proof of seed coats such as those of Leguminosae and Solanaceae interfere with water uptake. Leguminosae often control the entry of water into the seed by a small opening in the seed coat, the strophiolar cleft. Suberized cells line this cleft and if the cells are disturbed or the suberized layer is fractured or removed then water can enter the seed (Meyer & Poljakoff, 1982) Impaction loosens this plug of cells. Heating causes the plug to ‘ erupt’. When there is no strophiolar cleft, abrading the seed coat can make the seed permeable to water. Dormancy breaking by artificial methods of opening the seed coat mimic natural mechanisms; fungal and soil microbial attack, passage through the digestive tract of animals, trampling by animals, uncompleted predation by animals such as rodents, birds and insects, extreme changes in temperature such as fire. Although heating erupts the strophiolar plug, bush fires may have more than one dormancy breaking mechanism. In studies by Van Staden et al., (1995), it was suggested that plant derived smoke affected the membrane permeability or receptor sensitivity to G3 or ABA in dormant lettuce seeds. Chemical treatments with solvents and alcohols generally remove the waxy

coat and increase the permeability of seeds to water as well as gases. It is possible that these treatments generate other changes in the seed such as altered sensitivity to temperature, light or inhibitors and promoters.

Generally the biotic factors which naturally open the seed coat in doing so additionally aid the germination. Seed which is cracked by being trampled is likely to be at least partially buried in the soil in the process. Seed which has passed through the digestive system of an animal is excreted into a convenient package of nutrients and moisture. Seed which is stimulated to germinate by heat has reduced competition for water, light and nutrients, and more light.

The aim of this experiment is to test methods of breaking dormancy in Acacia seeds and to see which is most efficient.

Hypothesis:

The native Australian plant Acacia falcate grows mainly in the bush fire prone areas of NSW and Victoria, it is hypothesised that bush fires are a vital process in breaking dormancy of the seed, in this experiment methods of breaking dormancy would be tested to find which is most effective.

Materials:

Plastic tray with holes in the underside

180 dormant Acacia falcate seeds

Newspaper

Oven

Sandpaper

Newspaper

Water

Soil

Labels

Light crush covering

Method:

The plastic tray was lined with newspaper and filled with soil.

A row for each variable was marked out in the soil equally spaced apart from each other.

20 seeds were placed in the oven which was set on fan forced at 80 degrees Celsius.

After 30 seconds the seeds were removed from the oven.

Step 3 and 4 were repeated 2 more times, each time the time limit in the oven increased by 30 seconds.

20 seeds were placed into a pot of boiling water.

After 30 seconds the seeds were removed from the boiling water.

Step 6 and 7 were repeated 2 more times, each time the time limit in the water increased by 30 seconds.

20 seeds were scarified with sandpaper so that the surface of each seed was rough and had lost its shine.

20 seed were placed in bowl of room temperature tap water.

After 60 minutes the seeds were removed from the water.

One set of 20 seeds was set aside to be the control for the experiment.

Each set of 20 seeds (9 in total) is dispersed evenly in each row.

The entire tray is then covered with the crush.

Labels at the end each row are placed so that the position if each variable row is identifiable. (Figure 1)

Each day the tray is sprayed with 200mL of water mist.

Results were recorded after 4 weeks.

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Figure . Setup of experiment showing the labelled rows

Results:

Results Table 1

Variables

Germination recorded after 2 weeks

Germination recorded after 4 weeks

Control

0

1

Soaked

0

0

Scarified

0

0

Boiled 30 seconds

7

11

Boiled 60 seconds

4

7

Boiled 120 seconds

3

6

Dry Heat 30 seconds

1

2

Dry Heat 60 seconds

0

0

Dry Heat 120 seconds

0

0

Table . Table of final results collected

Graph of Table 1

Figure . Bar graph of results gathered from experiment which shows the number of germinating seeds occurring of Acacia falcate seeds after each method was tested on each seed

Discussion:

According to many sources methods for breaking dormancy include scarification, dry heating, boiling.

The outcome for the control variable which was seeds planted directly from the packet was to be expected. The seeds in the packet are dormant seeds

and the outcome supports this with the exception of one seed, this seed can be counted as an outlier as it is of such small percentage.

Soaking the seeds for a long period of time should have created a high osmotic gradient for the membrane of the seed. This treatment was not a suggested treatment, it would be expected that this treatment will have no effect on the seed as it still remains difficult for water to enter the seed through the undamaged seed coat.

Scarifying the seed coat should damage the seed coat enough to let water transfuse through to the embryo, however in this experiment this was not the case and scarifying produced no effects on germination of the seed. This unexpected outcome brings into question the method of the experiment; it can also however simply differ from species to species. In further experiments scarification should be included, using different methods of scarification and doing more and less damage to the seed coat.

Placing the dormant seeds in boiling water was a suggested treatment to break dormancy. In this experiment it can be seen that it is an effective method of breaking dormancy. The results support the suggestion from

It is without question that dormancy is regarded as an evolutionary acquired bonus for any plant. The unique combinations of environmental pressures that have occurred in various communities have simultaneously refined a variety of dormancy mechanisms. With sound knowledge about the seed dormancy characteristics of plant populations, seed batches can be manipulated and environmental situations can be engineered to encourage

release from seed dormancy to promote or inhibit certain species in crop and weed management.