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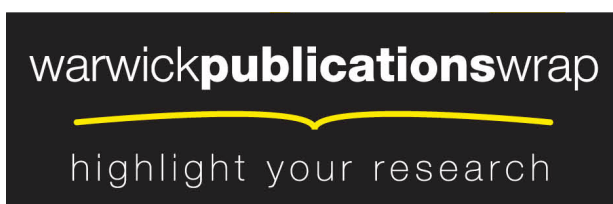
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Research Opinion

To germinate or not to germinate: a question of dormancy relief not germination stimulation.

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A common understanding of the control of germination through dormancy is essential for effective communication between seed scientists whether they are ecologists, physiologists or molecular biologists. Vleeshouwers *et al.* (1995) realised barriers between disciplines limited progress and through insightful conclusions in their paper “Redefining seed dormancy: an attempt to integrate physiology and ecology”, they did much to overcome these barriers at that time. However, times move on, understanding develops, and now there is a case for “Redefining seed dormancy as an integration of physiology, ecology and molecular biology”. Finch-Savage and Leubner–Metzger (2006) had this in mind when they extended and re-interpreted Vleeshouwers *et al.* (1995) definition of dormancy by considering dormancy as a having a number of layers that must be removed, with the final layer of dormancy being synonymous with the stimulation/induction of germination. We write this opinion article to support this unifying understanding, and to counter the opinion expressed by Thompson and Ooi (2010). The latter argued against the view expressed by several speakers at the ISSS Seed Ecology Conference in Utah (2010) “that (physiological) dormancy is broken by light, smoke,

nitrate or diurnal temperature alternations". Finch-Savage and Leubner-Metzger (2006) identified a number of potential sources for this confusion in the literature that "has resulted in part from different views on dormancy, such as whether light terminates dormancy or induces germination". Thompson and Ooi (2010) have perpetuated this confusion. We argue below, using accepted definitions of dormancy and evidence from physiology, ecology and molecular biology, that the regulation of germination is not through separate dormancy relief and stimulation of germination (*sensu* Thompson and Ooi, 2010), but by a dormancy continuum. Our intention is to briefly show the evidence in a form that is accessible to seed scientists from different disciplines.

Thompson and Ooi (2010) state towards the end of their opinion paper that the right questions to be asked are "what factors control dormancy" and "which cues promote germination once seeds are non-dormant". They insist that there is a distinction between cues that are responsible for dormancy alleviation and others that are germination stimulants. They suggest that without this distinction "results from experimental treatments on dormant seeds are often not clear and this can hinder subsequent research, by diverting attention away from developing the right questions" i.e. those above. In contrast, Finch-Savage and Leubner-Metzger (2006) state "A wide range of factors can alter dormancy in primary dormant seeds. However, there is an important distinction in the seeds response to these factors. 1) There are factors that are related to slow seasonal change. These factors (e.g. temperature) are integrated over time to alter the depth of dormancy, and the sensitivity to other factors (e.g. light). 2) There are other factors that indicate in a more immediate way that conditions are suitable for germination (e.g. light), which could be considered to terminate dormancy and therefore induce germination. Each of these factors therefore remove successive blocks to germination, but this process usually needs to be carried out in a set order for it to work, i.e. in the process described light must come last to be effective." Thus a dormancy continuum driven by environmental conditions in both directions is described, and when all layers are removed

germination occurs. This latter interpretation seems to be borne out by subsequent molecular ecophysiological studies of dormancy cycling in the laboratory (Cadman *et al.*, 2006; Finch-Savage *et al.* 2007) and field (Footitt *et al.*, 2011) as we will discuss below.

Interestingly, despite the different views expressed, both these sets of authors support their arguments by quoting Vleeshouwers *et al.* (1995) and that “dormancy is a seed characteristic, the degree of which defines what conditions should be met to make the seed germinate”. Both argue that the key thing is that dormancy should not just be associated with the absence of germination, but rather as a characteristic of the seed that determines the conditions required for germination (Vleeshouwers *et al.*, 1995; Thompson, 2000; Fenner and Thompson, 2005; Finch-Savage and Leubner-Metzger, 2006). On this basis, Finch-Savage and Leubner-Metzger (2006) argue that “any environmental cue that alters the conditions required for germination is by definition altering dormancy. Also by extension, when the seed no longer requires specific environmental cues it is non-dormant” and provide an argument that nitrate, light and alternating temperatures alter the conditions required for germination and so alter characteristics of the seed and therefore dormancy. Apparently in agreement, Thompson and Ooi (2010) state in the concluding paragraph of their opinion paper after Vleeshouwers *et al.* (1995). “Thus a germination cue is a change in the *environment* that aligns that environment with germination requirements of the seed; dormancy breaking is a change in the *seed* that determines what those requirements are”. However, the confusion still remains.

How can this be resolved?

It seems to all boil down to the question: what do we mean when we say “dormancy is a seed characteristic, the degree of which defines what conditions should be met to make the seed germinate”. The authors in both cases agree in this definition by Vleeshouwers *et al.*, (1995) made in point 2 of their summary. The difference comes in point 4 of the summary,

which states “it is argued that the process of dormancy should be clearly distinguished from the germination process itself. It is stated that as yet only temperature has been shown to alter the degree of dormancy in seeds. Factors like light and nitrate are often indispensable for germination, but only by promoting the germination process itself, not by mitigating the requirements for germination”. Perhaps the key phrase here is “as yet only temperature” i.e. in 1995 and another is the phrase in the concluding paragraph of Thompson and Ooi (2010) “dormancy breaking is a change in the seed “. We therefore concentrate below on the following criteria: 1. does light and nitrate “mitigate the requirements for germination” i.e. alter dormancy and 2. does light and nitrate result in a “change in the seed”. If these criteria are satisfied, then by the agreed definitions light and nitrate are breaking dormancy.

Physiological evidence since 1995:

Finch-Savage and Leubner-Metzger (2006) summarised the physiological evidence in the following way:

Nitrate: “exogenous nitrate can affect the requirement for light to promote *A. thaliana* seed germination (Batak *et al.*, 2002), and the initial level of dormancy in the seed is influenced by the nitrate regime fed to the mother plant (Alboresi *et al.*, 2005). Therefore nitrate affects the requirements for germination and so could be said to directly affect dormancy rather than just promote germination.”

Light: “Light has both been considered to stimulate germination (e.g. Vleeshouwers *et al.*, 1995) and to terminate dormancy (e.g. Benech-Arnold *et al.*, 2000; Batlla *et al.*, 2004). To some extent, this depends on where one chooses to draw the line between the processes of dormancy and germination. In this review (i.e. Finch-Savage and Leubner-Metzger, 2006), we have used the definition above that dormancy is a seed characteristic which defines the conditions required for germination and therefore any cue that widens the environmental

requirements for germination should be regarded as a dormancy release factor. Following this argument, exposure to light changes the seed so that it can germinate in darkness and is therefore the last step in the dormancy-breaking process, rather than the first step in the germination process (Bewley and Black, 1994; Pons, 2000; Leubner-Metzger, 2003). This light effect (red light via phytochrome) can also be reversed in some cases by far-red light, until the seed is committed to the process of germination (Casal and Sanchez, 1998; Sanchez and Mella, 2004). In seeds with coat dormancy, it is thought that light and GA can both release (coat) dormancy and promote germination (e.g. Casal and Sanchez, 1998; Leubner-Metzger, 2001; Leubner-Metzger and Meins, 2001; Sanchez and Mella, 2004; Kucera *et al.*, 2005)."

Further to this, dark stratification is often used to alleviate physiological dormancy (e.g. Goggin *et al.*, 2008, 2011; Long *et al.*, 2011) and the presence of white light actively inhibits dormancy alleviation; the quality and quantity of light received is therefore critical for seeds undergoing "ecologically relevant" dormancy alleviation.

Molecular biological evidence:

One of the findings that has come to the forefront relatively recently at the molecular level is that dormancy induction (prevention of germination), and dormancy relief are both under negative regulation. There are proteins PP2Cs and DELLAs that respectively block the downstream gene expression that results in dormancy induction or dormancy relief (e.g. Cutler *et al.*, 2010; Sun and Gubler, 2004, respectively). These negative regulators must be removed before these processes can be completed. A balance of the hormones ABA (inhibits completion of germination) and GA (promotes completion of germination), in response to environmental signals influences these negative regulators. This balance mechanism is summarized in Figure 1.

As we begin to understand this negative form of regulation it becomes clear that there is no distinction between dormancy relief and stimulation of germination. You could try and read

this into the bifurcated mechanism illustrated in Figure 1, but everything seems to suggest that both sides interact. Recently Footitt *et al.* (2011) have shown under ecologically relevant conditions, namely in seeds undergoing dormancy cycling in the soil seed bank, that these processes are seasonally separated over the annual cycle in a way that emphasis on one side or the other of the balance changes with the seasons (see Fig. 6 in Footitt *et al.*, 2011).

Vleeshouwers *et al.* (1995) say “we reserve the term dormancy for a block or blocks within the seed that prevent germination, and distinguish it from the absence of factors required to evoke germination”. Within this definition the negative regulation by PP2Cs and DELLAs can be seen as blocks to dormancy induction and dormancy relief respectively. So what effect does light and nitrate have on these negative regulators (blocks):

Light: the action of light is to promote the expression of *GA3ox1* (e.g. Cadman *et al.*, 2006) the key gene responsible for synthesis of GA. The effect of this in the mechanism summarized in Figure 1 is to increase GA and therefore remove the DELLA repression (Block) of GA signaling and metabolism to relieve dormancy, which results in the completion of germination.

Nitrate: the action of nitrate is to promote the expression of *CYP707A2* (Alboresi *et al.*, 2005; Matakiaadis *et al.*, 2009) the key gene responsible for ABA catabolism. The effect of this in the mechanism summarized in Figure 1 is to reduce ABA and therefore enhance the repression (Block) of ABA signaling and thus reduce dormancy. It was observed in the soil seed bank that nitrate sensitivity only increased following increased expression of the nitrate transporter, *NRT1.1*, which coincided with increased *CYP707A2* expression (Footitt *et al.*, 2011).

Conclusions from physiology and molecular biology:

Physiological studies (post 1995) now clearly show that light and nitrate break dormancy. Molecular biological studies have progressed further to underpin this physiology. Therefore, if we consider the criteria raised above; firstly do light and nitrate “mitigate the requirements for

germination” the answer is clearly yes as the negative repressors PP2cs and DELLAs determine the requirements for germination and these are altered by nitrate and light respectively. For the second question, do they result in a “change in the seed”, the answer is also yes since they are clearly responsible for a cascade of events (gene expression etc.) in the seed (Figure 1). Thus by the agreed definition “dormancy breaking is a change in the seed”, light and nitrate are clearly breaking dormancy. The alternative view of them being a germination cue defined as “a change in the *environment* that aligns the environment with the germination requirement of the seeds” is not now relevant to light and nitrate. We feel these same arguments can be made for alternating temperatures and smoke (Karrikinolide), but wished to keep this opinion article short and focussed. However, the question remains: are there any germination cues or does germination just go to completion when all layers of dormancy have been removed by changes in the *seed*? Possibly water, but not oxygen as this can have specific effects and anoxia is essential for germination in some species (Probert and Benchley, 1999) as pointed out by Thompson and Ooi (2010).

Further discussion:

Thompson and Ooi (2010) make valid points about problems in practice concerning use of applied treatments (such as smoke) in restoration, i.e. they may be applied at the wrong time when seeds are not sensitive due to a lack of understanding by the practitioner. However, we do not think their approach is helpful. The problems are more likely avoided if dormancy is considered as a continuous variable and dormancy breaking cues have to come in the correct order for the completion of germination *sensu* Finch-Savage and Leubner-Metzger (2006). Thompson and Ooi (2010) suggest that the problem is solved when the right questions are asked “What factors control dormancy” and “which cues promote germination once seeds are non-dormant”. However, the problem is not solved by making this distinction between dormancy and germination, but from understanding that the seed germinates in the right season, in a

favourable place under suitable conditions because of a complex set of responses to a range of different environmental signals required to fully relieve dormancy. i.e. smoke has to be applied at the right time when these other criteria have been satisfied.

In conclusion, Thompson and Ooi, (2010) provide an excellently crafted and compelling set of words, but without facts to convince the reader. We hope to have shown that physiological and molecular understanding can help to resolve these otherwise philosophical debates that can lead to confusion. There is no clear distinction between the ecological, physiological and molecular biological understanding of seed behaviour and it is only the separation of these communities that has brought about some of the difficulties. We should embrace the avalanche of new information that makes seed science so interesting at the moment, but interpret it in a framework that is relevant to all disciplines.

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(EDITOR) INSERT FIG. 1 HERE!

Response to Finch-Savage and Footitt's opinion paper "To germinate or not to germinate: a question of dormancy relief not germination stimulation".

Germination and dormancy breaking: two different things

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Finch-Savage and Footitt (2012) take issue with our recent opinion piece (Thompson and Ooi 2010), in which we attempt to explain the crucial distinction between dormancy breaking and stimulation of germination, even though our paper consisted mostly of quotes from other authorities (e.g. Carol and Jerry Baskin) who seem to agree with us. Here is our very brief reply.

Let's start where we can agree, with Finch-Savage and Footitt's (2012) statement, quoting Finch-Savage and Leubner-Metzger (2006), that 'any environmental cue that alters the conditions required for germination is by definition altering dormancy'. Unfortunately, Finch-Savage and Footitt's (2012) appear not to accept the inevitable corollary of this statement, which is that 'conditions required for germination' do actually exist. In Finch-Savage and Footitt's (2012) universe, conditions required for germination do not exist, other than 'possibly water'. We don't know what to make of that 'possibly'.

The argument appears to hinge on the answer to the question: do light and nitrate (for example) result in a 'change in the seed', specifically one that enlarges the range of conditions under which germination will occur? If they do (since we both also seem to agree with Vleeshouwers et al. (1995) that 'dormancy is a seed characteristic, the degree of which defines what conditions should be met to make the seed germinate'), then light and nitrate break dormancy, and cannot by definition be germination cues. But here's the crux of the matter, which we admit owes a lot to looking at seeds from an ecological perspective. Anything that 'changes the seed' is indeed breaking dormancy, if that's all it does; in other words, if the result of that change is still a seed. A seed that is one step nearer germination, but still a seed nevertheless. On the other hand, anything that persuades the seed that here is the place and now is the time to germinate is a germination cue.

The distinction is profound, and transcends any similarity in the underlying molecular events. Changes to the seed (dormancy breaking) may well fine-tune its response to light, to nitrate or to karrikinolide, but it's the light that tells the seed it is near the surface of the soil, the nitrate that tells the seed it's in a competition-free gap, and the karrikinolide that tells the seed there has just been a fire. It's that final, crucial cue that tells the seed that now is the time to take the most important step it will ever take. And that is why a germination cue is fundamentally, qualitatively different from dormancy breaking, and why the distinction is worth preserving.

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