Temperature regulation of seed dormancy in *Vernonia galamensis*

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Abstract

The seeds of *Vernonia galamensis* (Cass.) Less. contain 35-45% of triglyceride oil rich in vernolic acid, a naturally epoxidized fatty acid with low viscosity. Naturally epoxidized, vernolic acid is a potentially useful raw material for manufacturing non-ozone depleting paints and coatings.

Exploitation of *V. galamensis* as a crop could be potentially hampered by ill understood seed dormancy characteristics. Besides, seed viability assessments via a germination test are not possible in the absence of an appropriate dormancy release protocol. To address this problem, the seeds of two subsp (subsp *nairobiensis* and subsp *afromontana* var. *gibbosa*) harvested at the point of natural dispersal were subjected to chilling and incubation temperature treatments with a view to developing appropriate dormancy release procedure. The results revealed that chilling fully imbibed seeds for at least two weeks at 5^{o}C can be used to overcome seed dormancy prior to incubating at 30/25 or 25/17^{o}C.

Introduction

Dormancy is defined negatively as the inability for viable seeds to germinate under conditions considered adequate for radicle emergency (Amen, 1968; Roberts, 1972, Roberto *et al*., 2004); caused by a block to the process of germination that is as a result of some property of the seed (Baskin and Baskin, 1998). Temperature may influence the release of dormancy either by enhancing permeability of the seed coat or through activating some seed biochemical processes that ultimately enhance the growth potential of the embryo (Baskin, *et al.,* 1998; Pritchard *et al*., 1988; Thanos & Georghiou, 1988; Russi, 1989; Russi *et al*., 1992; Derkx & Karssen, 1993a; Teketay, 1996; Daws *et al*., 2002 Daws *et al*., in press). While some species require warm

stratification to release dormancy, others may respond well to chilling (cold stratification), dry after-ripening, heat-shock or alternating temperatures (Gosling & Rigg. 1990; Gutterman, 1990a; Hilhorst & Karssen, 1992; Hilhorst, 1993; Bewley & Black, 1994; Foley, 1994; Vleeshouwers *et al*., 1995; Hilhorst, 1998;). Besides, the level of seed dormancy has been shown to vary depending on the prevailing temperatures during seed development (Baskin & Baskin, 1998, Nyamongo, 2006).

The seeds of these sub-species of *Vernonia* previously tested for germination by incubating them at 25^{o}C were found to take up to 40 days to germinate, with a large proportion of fresh/firm seeds remaining non-germinated at the end of a germination test, a clear indication of dormancy. Studies carried out by Teketay (1993) confirmed dormancy in *Vernonia galamensis* var. *ethiopica*, one of the closely related varieties. In this study, seeds harvested from two different ecological zones over two seasons were subjected to a variety of temperature treatments with a view to determine optimum dormancy breaking / germination temperature requirements and develop appropriate germination testing protocol for *V. galamensis*. The study also investigated the differences in dormancy levels both between the subspecies, between the seed lots across seasons, and between seed lots across ecological zones.

Materials and methods

Experiments were conducted using seeds harvested from two ecological zones, Muguga in Central Kenya and Maseno in Western Kenya over two seasons {long rains of 2002 (first crop) and short rains of 2003 (second crop)}. The two sites are ecologically different in relation to annual temperature and rainfall. The average maximum and minimum annual temperatures for Muguga and Maseno are 22^{o}C & 16^{o}C and 30^{o} & 18^{o}C respectively. Muguga (altitude: 2200m) receives an average

annual rainfall of *c.* 836 mm while Maseno (altitude: 1500m) receives 1160 mm. (Source: Metrological Department – Nairobi, Kenya).

All seeds were harvested at the point of natural dispersal and initially dried to equilibrium water content (*c.* 5% WC – fresh weight basis) at the National genebank of Kenya drying room maintained at 20^{o}C and 18 – 20% RH. The seeds were then hermetically packaged in laminated foil packets and stored at -20^{o}C until the time of the experiments detailed below.

The effect of incubation temperature on germination

Experiment one

A two-way Thermo-gradient Plate was used to investigate a variety of temperatures. A thermo-gradient plate is a versatile facility that can be manipulated to generate many temperature regimes across the table. The thermo-gradient table was set to run at a 12 hour photoperiod with daytime lowest and highest latitudinal temperature range of 4.8 to 6.7^{o}C and 34.5 to 35.8^{o}C respectively, while the respective night time lowest and highest latitudinal temperature range was 4.8 to 34.2^{o}C and 6.8 and 35.8^{o}C.

Using seed lots from the first crop (long rains 2002), forty-nine samples of 25 seeds were drawn from each seed lot and sown in 7cm Petri dishes containing 1% water agar. The Petri dishes were arranged on the Thermo-gradient table in a specific pattern to allow easy determination of the respective temperature regime for each Petri dish, while ensuring that each seed lot received similar temperature treatments. The germination data were contour plotted to illustrate the optimum incubation temperature regime(s).

Experiment two

The third crop seeds of the two subspecies: *nairobiensis* and *afromontana* var. *gibbosa* from the two sites: Muguga and Maseno were used in this study. From each

seed lot, three samples of 100 seeds were drawn and each subdivided to produce four replicates of 25 seeds before sowing in 7cm Petri dishes containing 1% water agar. A set of the four replicates was incubated at each of the following three temperatures regimes: 25^{o}C, 30/25^{o}C, and 25/17^{o}C with a 12hour photoperiod.

Effect of Chilling (cold stratification)

Experiment one

Seeds used in this experiment were drawn from both the first and second season comprising seed lots of the two subspecies (*nairobiensis* and *afromontana* var. *gibbosa*), and from both Muguga and Maseno. Four samples of 100 seeds each were drawn from each of the four seed lots. Each sample was subdivided to give a set of four replicates of 25 seeds each. Seeds were sown on 1% water agar and chilled for one week at 5^{o}C before incubating a set of each seed lot in each of the following temperature regimes: 12, 25, 25/17 and 30/25^{o}C. A similar set of non-chilled samples was used as a control.

Experiment two: Effect of incubation temperature and chilling period

The experimental design was similar to that for experiment one except that samples were subjected to ten chilling periods (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 weeks).

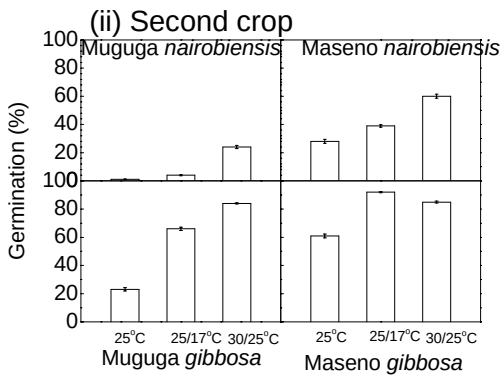
Data analysis

The effects of the factors investigated in the respective experiments were tested by standard ANOVA using the general linear model of SPSS version 12.0. Means were separated by LSD.

Results

Alternating versus constant temperatures

All seed lots recorded highly significant differences ($P < 0.001$) in germination levels in respect to incubation temperatures. Seed lots incubated at alternating temperatures



consistently germinated to higher levels than those incubated at constant temperatures (Figs. 1). However differences in germination between samples incubated at the two alternating temperatures: 30/25°C and 25/17°C were non-significant.

Fig. 1: Germination response (average % ±SE of 4 x 25) of the 2nd crop seeds of *Vernonia galamensis* subsp *nairobiensis* and subsp *afromontana* var. *gibbosa* to constant and alternating temperatures.

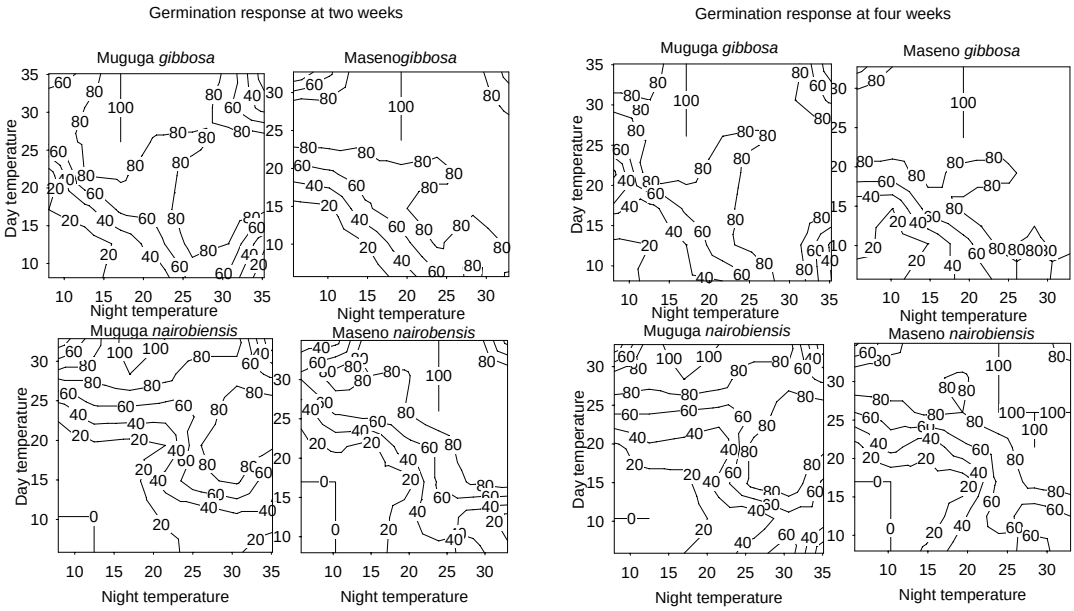


Fig. 2: Germination (%) response of *Vernonia galamensis* subsp *nairobiensis* and subsp *afromontana* var. *gibbosa* seeds to a variety of temperatures on the two-way Thermo-gradient table.

The seeds of subsp *nairobiensis* consistently germinated to significantly lower levels ($P < 0.001$) than those of subsp *gibbosa*, across the incubation temperatures, even when the two seed lots were from the same seed bulking site (Fig. 1). The contour plots also revealed a much broader area for the subsp. *afromontana* var. *gibbosa* over which seeds germinated to 100% Fig. 2). This area is time dependent as it was shown to widen with incubation period (Fig. 2). Similarly, the Muguga seed lots consistently

germinated to significantly lower levels ($P < 0.001$) as compared to the respective seed lots from Maseno (Figs. 1 & 2). The thermo-gradient test results also revealed that seeds of the two subsp. generally germinate better at higher temperatures as compared to lower temperatures (Fig. 2).

Chilling experiments (cold stratification)

The positive effect of one-week of chilling at 5^{o}C was highly significant ($P < 0.001$). The interaction effect of chilling and subsp was similarly significant indicating differences in dormancy levels among seed lots. Thus, while all the seed lots of subsp *nairobiensis* recorded a positive germination response those of subsp *afromontana* var. *gibbosa* exhibited mixed response (Fig. 3). Besides the highly significant ($P < 0.001$) inter-subspecies differences in germination response, highly significant differences in respect to seed source and season were also evident. Maseno seed lots, particularly those of subsp nairobiensis generally recorded higher germination levels than Muguga seed lots (Fig.3). Similarly, both the chilled and non-chilled samples of the second crop generally germinated to lower levels than those of the first crop.

In the second chilling experiment, all samples generally recorded significant improved germination with increased chilling period, picking at some point, before beginning to register a decline (Fig. 4). The interaction effect of chilling period and subsp was highly significant ($P < 0.001$). The interaction effect of incubation temperature and chilling period was also significant ($P < 0.05$).

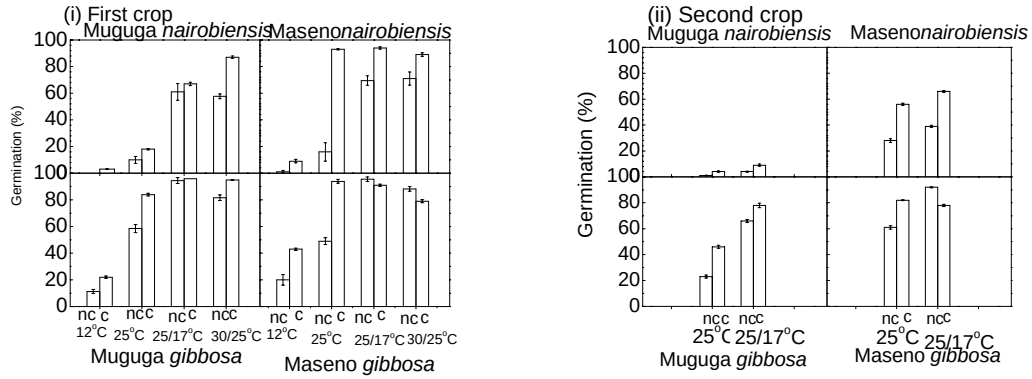


Fig. 3: Germination response (average % ±SE of 4 x 25) to chilling of the first and second crop seeds of *Vernonia galamensis* subsp *nairobiensis* and sub species *afromontana* var. *gibbosa*. Seeds harvested at the point of natural dispersal and dried to *c.* 5% WC ($f_{wt}b$) were sown on 1% water agar and chilled (c) for one week at 5°C before incubating a set of each seed lot in each of the following temperature regimes: 12, 25, 25/17 and 30/25°*C.* A similar set of non-chilled (nc) samples was also incubated under similar conditions as a control

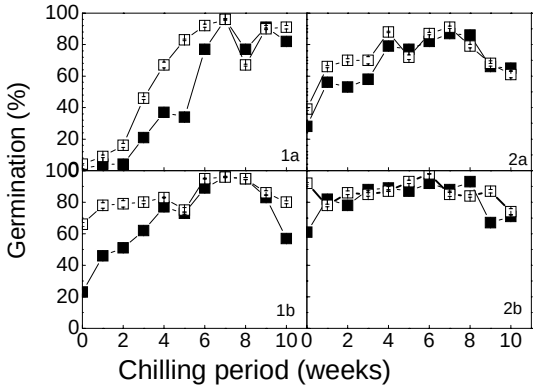


Fig. 4: Germination response (average % ±SE of 4 x 25) of third crop seeds of *Vernonia galamensis* subsp *nairobiensis* (a) and subspecies *afromontana* var. *gibbosa* (b) from Muguga (1) and Maseno (2), to incubation temperature and chilling period. Seeds harvested at the point of natural dispersal and dried to *c.* 5% WC ($f_{wt}b$) were sown on 1% water agar and chilled up to 10 weeks at 5°C. Germination was assessed weekly by incubating a set of each seed lot at either 25 (■) or 25/17°C (□).

Discussion

Seeds of *Vernonia galamensis* are sensitive both to chilling and alternating temperature. The beneficial effects of chilling and alternating temperature on dormancy loss have been recognized for centuries (Stokes, 1965; Lewak & Rudnicki, 1977; Nikolaeva, 1977). Superficially, the postive germination response of *Vernonia* seeds to chilling seems odd considering that it is a tropical species where natural chilling conditions are rarely experienced. It should however be born in mind that the species originates from the highlands of Ethopia that at times experience temperatures below 10°C. In Kenya, the more dormant *Vernonia galamensis* subsp *nairobiensis* has been found to naturally grow in altitudes higher than 2700 m where natural chilling temperature just before the short rains is a common occurance.

The alternating temperature cue is ecologically necessary once seeds are mature and have been dispersed. Such a cue is important particularly to small seeds, which due to their resource limitation must germinate when close to the soil surface for effective seedling establishment (Thompson *et al*., 1977; Vázquez-Yanes and Orozco-Segovia, 1982; Daws *et al*., 2002). Sensitive seeds buried deep in the soil, under leaf litter or below vegetation will not experience alternating temperatures and thus are unlikely to germinate until the soil is disturbed or the vegetation cleared (Daws *et al*., 2002). These ecological attributes perfectly fit *Vernonia galamensis*, which naturally occurs in disturbed areas (Gilbert, 1986) and is small seeded. Therefore the sensitivity of *Vernonia* seeds to alternating temperature is perhaps not surprising.

The germination of just a proportion of the sown sample across the treatments is a reflection of dormancy heteromorphism or the phenotypic plasticity in germination of seeds. Indeed one week chilling substituted for the alternating temperature requirement only in the first crop Maseno *nairobiensis* and Maseno *gibbosa.* Otherwise, in the rest of the seed lots, the chilled samples still required the alternating temperature for enhanced germination. Furthermore, extended chilling was essential in some seed lots to annul the requirement for alternating temperature. This plasticity is ecologically important as it decreases risks to species survival by increasing the diversity of seed germination times.

The seeds of subsp *afromontana* var. *gibbosa* were consistently less dormant than those of subsp *nairobiensis*, consistently so among the seed lots from Maseno and Muguga and across the seasons (Figs 2 & 3). It is therefore plausible that this variation is due to the genetic differences between the subsp. Similarly, the Maseno seed lots were evidently less dormant than the Muguga as revealed by the consistent higher germination levels of both treated and non-treated seeds from Maseno (Figs. 1 & 2). This may be attributed to Maseno's warmer temperature conditions as compared to those of Muguga. Previous work

has shown that dormancy is typically inversely related to the heat sum (^{o}C d) accumulated during seed development (Fenner, 1991; Sharif-Zadeh & Murdoch, 2000; Qaderi *et al*., 2003; Daws *et al*, 2004).

Conclusion and Recommendations

Temperature plays an important role in regulating dormancy in *Vernonia galamnsis*. Seeds of *V. galamensis* originating from warmer environments are likely to be less dormant than those from cooler environments, as the case was for Maseno and Muguga seed lots. The preference for alternating temperature to constant temperature is a clear manifestation of seed dormancy. In testing for seed viability through a germination test, chilling fully imbibed seeds for 1 – 4 weeks before incubating at 30/25^{o}C or 25/17^{o}C is recommended as an appropriate protocol.

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References

Amen, R.D. (1968) A model of seed dormancy. *The Botanical Review* **34**, 1-31.

Baskin, C.C. and Baskin, J.M. (1998) *Seeds: Ecology, biogeography and evolution of dormancy and germination.* San Diego, Academic Press.

Bewley J.D and Black M., (1994). SEEDS: Physiology of Development and Germination. Plenum Press, New York.

Datta, S.C., Evenari, M. and Gutterman, Y. (1972a) Photoperiodic and temperature responses of plants derived from the various heteroblstic caryopses of *Aegilops ovata* L. *Journal of the Indian Botanical Society* **50A,** 546-559.

Daws, M.I., Burslem, D.F.R.P., Crabtree, L.M., Kirkman, P., Mullins, C.E. and Dalling, J.W. (2002) Differences in seed germination responses may promote coexistence of four sympatric *Piper* species. *Functional Ecology* **16**, 258-267.

Daws M.I., Lydall, E., Chmielarz, P., Leprince, O., Mathews, S., Thanos, C.A., Pritchard H.W. (2004). Developmental heat sum influences recalcitrant heat traits in *Aesculus hippocastanum* across Europe. *New Phytologist* **162**, 157-166.

Daws, M.I., Orr, D., Burslem, D.F.R. and Mullins, C.E. (in press) Effect of high temperature on chalazal plug removal and germination in *Apeiba tibourbou* Aubl. *Seed Science and Technology*.

Derkx, M.P.M. and Karssen, C.M. (1993a) Effects of light and Temperature on seed dormancy and gibberellin-stimulated germination in *Arabidopsis thaliana:* studies with gibberellin- deficient and insensitive mutants. *Physiologia Plantarum* **89**, 360-368.

Derkx, M.P.M. and Karssen, C.M. (1993b) Changing sensitivity to light and nitrate but not to Gibberellins regulates seasonal dormancy patterns in *Sisymbrium officinale* seeds. *Plant Cell and Environment* **16**, 469-479.

Fenner, M. (1991) The effects of the parent environment on seed germinability. *Seed Science Research* **1**, 75-84.

Fenner, M. (ed.) (2000) *Seeds*: *The Ecology of Regeneration in Plant Communities* - 2nd edition. CAB International, Wallingford, UK.

Fenner, M. and Thompson, K. (2005) *The Ecology of Seeds.* Cambridge, University Press, UK. Pritchard, H.W., Manger, K.R. and Prendergast, F.G. (1988) Changes in *Trifolium arvense* seed quality following alternating temperature treatment using liquid nitrogen. *Annals of Botany* **62**, 1-11.

Foley, M.E. (1994) Temperature and water status of seed affect afterpre-heating in wild oat (*Avena fatua*). *Weed Science* **42**, 200-204.

Gilbert, M.G. (1986) Notes on East African Vernonieae (Compositae). A revision of the *Vernonia galamensis* complex. *Kew Bulletin* **41,** 19-35.

Gosling, P.G. and Rigg, P. (1990) The effect of moisture content and prechill duration on the effeciciency of dormancy breakage I stika spruce (*Picea sitchenisis*) seeds. *Seed Science and Technology* **18**, 337-343.

Gutterman, Y. (1990a) Do the germination mechanisms differ in plants originating in deserts receiving winter or summer rains? *Israel Journal of Botany* **39**, 355-372.

Hilhorst, H.W.M. and Karssen, C.M. (1992) Seed dormancy and germination: The role of Abscisic acid and gibberellins and the importance of hormone mutants. *Plant Growth Regulation* **11**, 225-238.

Hilhorst, H.W.M. (1993) New aspects of seed dormancy. In: Côme, D. and Corbineau, F. (eds) *Fourth International Workshop on Seeds*. *Basic and Applied Aspects of Seed Biology.* ASFIS, Paris, pp. 571-579.

Hilhorst, H.W.M. (1998) The regulation of seed dormancy: the membrane hypothesis revisited. *Seed Science Research* 8, 77-70.

Lewak, S. and Rudnicki, R.M. (1977) Afterpre-heating in cold-requiring seeds. In: Khan A.A. (ed) *The Physiology and Biochemistry of Seed Dormancy and Germination*. North Holland Publishing, Amsterdam, pp. 193-217.

Nikolaeva, M.G. (1977). Factors controlling the seed dormancy pattern. In *The Physiology and Biochemistry of Seed Dormancy and Germination* (A.A. Khan, ed.) pp 51-74. North Holland, Amsterdam/New York.

Nyamongo D.O, Daws M.I, Nyabundi J.O and Ayiecho P.A (1996) Onset of dormancy, dormancy levels and the appropriate seed production environmental conditions for two subsp of *Vernonia galamensis*. In: Proceedings of the 10th KARI Biennial Conference.

Pritchard, H.W., Manger, K.R. and Prendergast, F.G. (1988) Changes in *Trifolium arvense* seed quality following alternating temperature treatment using liquid nitrogen. *Annals of Botany* **62**, 1-11.

Probert, R.J. (1992) The role of temperature in germination ecophysiology. In: Fenner, M. (ed.) *Seeds: The ecology of Regeneration in Plant Communities*. CAB International, Wallingford, UK, pp. 285-325.

Qaderi, M.M., Cavers, P.B. & Bernards M.A. (2003). Pre- and Post-dispersal factors Regulate germination patterns and structural characteristics of scotch thistle (*Onopordum acantium*) cypselas. *New Phytologist* **159**, 263-78.

Roberto L. and Benech-Arnold (2004). Inception, Maintenance and Termination of Dormancy in grain Crops: Physiology, Genetics and Environmental Control. *Handbook of Seed Physiology* **5**, 169-198.

Roberts, E.H. (1972). Dormancy: A factor affecting seed survival in the soil**.** In: Roberts, E.H. (ed.) *Viability of seeds.* Chapman and Hall, London, pp. 321-359.

Russi, L. (1989) Ecological and Physiological aspects of seeds of annual grasslands in a mediterranean environment. Unpublished PhD. Thesis, Unversity of Reading, UK.

Russi, L., Cocks, P.S. and Roberts, E.H. (1992) Hard-seededness and seed bank dynamics of six pasture legumes. *Seed Science Research* **2**, 231-241.

Sharif-Zadeh, F. & Murdoch, A.J. (2000). The effects of different maturation conditions on seed dormancy and germination of *Cenchrus ciliaris*. *Seed science Research* **10**, 447-57.

Stokes, P. (1965) Temperature and seed dormancy. In: Rhuland, W. (ed) *Encyclopidia of Plant Physiology*. Vol. XV/2. Springer Verlag, Berlin, pp. 746-803.

Teketay, D. (1993) Germination ecology of Thompson, A.E., Dierig, D.A. and Kleiman, R., (1994b) *Vernonia galamensis* (Cas.) Less. Var. *ethiopica* M.G. Gilbert, A new Industrial oilsed crop. *Tropical Ecology* **34**, 64-74.

Thanos, C.A. and Georghiou, K. (1988) Ecophysiology of fire-stimulated seed germination in *Cistus incanus* ssp creticus (L). Heywood and *C. salvifolius* L. *Plant, Cell and Environment* **11**, 841-849.

Thompson, K., Grime, J.P. and Mason G. (1977) Seed germination in response to diurnal fluctuations in temperature. *Nature* **267**, 147-148.

Vázquez-Yanes, C. and Orozco-Segovia, A. (1982) Seed germination of a tropical rainforest pioneer tree (*Heliocarpus donnell simithii*) in response to diurnal fluctuations repons of temperature. *Physiologia Plantarum* **56**, 295-298.

Vleeshouwers, L.M., Boumeester, H.J. and Karssen, C.M. (1995) Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology* **83**, 1031-1037.