From Forest Nursery Notes, Summer 2009

18. Germination and dormancy breaking requirements for *Vernonia galamensis* (Asteraceae). Nyamongo, D. O., Nyabundi, J., and Daws, M. I. Seed Science and Technology 37:1-9. 2009.

NOTICE: THIS MATERIAL MAY
BE PROTECTED BY COPYRIGHT
LAW (TITLE 17, U.S. CODE)

Nyamongo, D.O., Nyabundi, J. and Daws, M.I. (2009), *Seed Sci. & Technol.*, **37**, 1-9

Germination and dormancy breaking requirements for *Vernonia galamensis* (Asteraceae)

D.O. NYAMONGO[1], J. NYABUNDI[2] AND M.I. DAWS[3]*

[1] Kenyan Agricultural Research Institute, National Genebank of Kenya, P.O. Box 00100, 30148 Nairobi, Kenya
[2] Maseno University, Private Bag, Maseno, Kenya
[3] Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly
(E-mail: matthew.daws@ewlsciences.com.au)

(Accepted September 2008)

Summary

The effects of a range of dormancy breaking treatments and germination cues on two subspecies of *Vernonia galamensis*, *V. galamensis* subsp. *nairobiensis* and *V. galamensis* subsp. *afromontana* var. *gibbosa* were tested. Exposure to dry-after-ripening treatments, cold stratification and GA_3 all resulted in significant increases in germination for both subspecies suggesting the presence of non-deep physiological dormancy. Seed also responded positively to alternating temperatures, the presence of light and KNO_3, all of which are disturbance related germination cues; these responses are consistent with *V. galamensis* occurring in disturbed habitats, e.g. along roads. In addition, secondary dormancy was induced by incubation in the dark: this could be alleviated by cold stratification for 14 days.

Introduction

Vernonia galamensis (Cass.) Less., (*Asteraceae*) is a new potential industrial oil crop that originated in eastern and south-eastern parts of Ethiopia and is endemic primarily to East African countries (Gilbert, 1986). On a dry mass basis, *Vernonia* seeds contain 35-45% of a triglyceride oil which is rich in vernolic acid, a naturally epoxidized fatty acid. Such fatty acids are normally manufactured by chemical epoxidation of fats and vegetable oils and are used in the formulation of oil-based paints to reduce emissions of volatile organic compounds (Roseberg, 1997). In addition, the meal left after oil extraction is a valuable source of crude protein, carbohydrate and major minerals (calcium, potassium, magnesium and phosphorus) (Harborne and William, 1977) and therefore has potential for use as animal feed (Ologunde *et al.*, 1990). However, to facilitate further investigation of the suitability of this species as a crop it is necessary to understand the regulation of seed germination. There appears to be only a single report of germination in this species (using var. *ethiopica*; Teketay, 1993). This study found that germination at constant temperatures was low, but was facilitated by acid scarification and pericarp removal (Teketay, 1993), techniques which are either not ecologically relevant or particularly amenable for simple and rapid propagation of this species.

* Author for correspondence

Studies on Asteraceae have found that many require light for germination (Merritt *et al.*, 2006; Tobe *et al.*, 2006; Karlsson and Milberg, 2007), which is likely a function of their typically small seed mass (Milberg *et al.*, 2000), and / or respond positively to alternating temperature regimes (Wood *et al.*, 2005; Honda and Katoh, 2007). In addition, species of Asteraceae from diverse habitats have been found to respond positively to dormancy breaking treatments including dry-after-ripening (Schutz *et al.*, 2002; Vivar-Evans *et al.*, 2006; Hoyle *et al.*, 2008) and cold stratification (Hamze and Jolls, 2000; Baskin and Baskin, 2002): the induction of secondary dormancy in the dark has also been reported for some species (Banovetz and Scheiner, 1994; Doucet and Cavers, 1997; Brandel, 2004).

Species are likely to exhibit adaptations that ensure seed germination occurs in suitable habitats or at suitable times of the year for onward seedling growth. *V. galamensis* occurs naturally as a species of disturbed habitats, including road margins (Gilbert, 1986). Species adapted to disturbed habitats often germinate in response to environmental cues that signal a disturbance. Such cues include an increase in the quantity and quality of irradiance reaching the soil surface (Vázquez-Yanes *et al.*, 1990), increasing amplitude of soil temperature fluctuations (Daws *et al.*, 2002; Pearson *et al.*, 2002) and elevated NO_3^- levels as a result of the death of plant material in the disturbance process (Pons, 1989; Denslow *et al.*, 1998).

V. galamensis originates in East Africa with a centre of diversity in the Ethiopian highlands (Bhardwaj *et al.*, 2000; Baye *et al.*, 2001). Thus, seeds in the soil are likely to be variously exposed to both low and high temperatures that may result in cold stratification and dry-after-ripening as well as one or more disturbance related germination cues. Consequently, we tested the hypotheses that *V. galamensis* seeds are (1) dormant, (2) require cold stratification and/or dry-after-ripening for dormancy release as well as (3) responding to light, alternating temperatures and KNO_3^- as germination cues. We also tested whether secondary dormancy can be induced by incubation in the dark. We tested these hypotheses using seeds from two sub species of *V. galamensis*, *V. galamensis* subsp. *nairobiensis* and *V. galamensis* subsp. *afromontana* var. *gibbosa* grown at two distinct locations in Kenya.

Materials and methods

Seed material

The initial sample of *Vernonia galamensis* subsp. *nairobiensis* was collected in Nairobi, Kenya (1° 16' S, 36° 48' E), while that of subsp. *afromontana* var. *gibbosa* was collected from eastern Kenya (0° 07' N 37° 36' E). For both sub species, seeds were collected at the point of natural dispersal from over 100 individual plants. The samples were grown out in experimental fields in Muguga (1° 14' N 36° 40' E) in Central Kenya and Maseno (0° 00' N 34° 35' E) in Western Kenya. The two bulking sites were selected because of their differences in annual temperature and rainfall. Muguga has *ca.* 836 mm average annual rainfall, Maseno 1160 mm. The average maximum and minimum annual temperatures for Muguga and Maseno are 22 and 16°C and 30 and 18°C respectively (Source: Metrological Department, Nairobi, Kenya).

Seed germination

Seeds from each seedlot (4 replicates of 25 seeds each per treatment) were sown on the surface of 1% agar in distilled water in 55 mm Petri dishes and germinated at 25/25, 25/17 or 30/25°C with a 12 h photoperiod. In the two alternating temperature regimes, the elevated part of the alternating temperature treatments coincided with the 12 h photoperiod. In the cold stratification treatment, seeds were sown on the surface of 1% agar and chilled at 5°C in the light (12 h photoperiod) for 14 d. Subsequently, dishes (4 replicates of 25 seeds each per treatment) were transferred to each of 25/25, 25/17 and 30/25°C for germination. In the dry-after-ripening treatment, seeds at 20% RH were dry-after-ripened by being hermetically sealed in laminate aluminium foil bags and then held at room temperature (20°C) for 12 months. After 12 months, seeds were sown at each of the three germination temperatures in the light.

Seeds were also germinated, at each of the three temperatures, in the presence of either 0.7 mM GA_3 or 1 mM KNO_3. These potential germination stimulants were incorporated into the agar germination media, i.e. seeds were continuously exposed to these compounds during germination. The concentrations of KNO_3 and GA_3 were chosen because they have been shown to be effective in stimulating germination in other studies (e.g. Daws *et al.*, 2002; Kepczynski *et al.*, 2006; Daws *et al.*, 2007b).

Germination in all experiments was scored daily and assessed as visible radicle emergence by greater than 1 mm. Germination was monitored for up to six weeks by which time germination had ceased to increase.

Induction of secondary dormancy

To test whether secondary dormancy could be induced, seeds of each seedlot (4 × 25 seeds per treatment) were sown in either the light (12 h photoperiod) or dark (wrapped in two layers of aluminium foil) at each of the three germination temperature regimes. Germination in the light treatments had reached a maximum after 8 days: after 11 days of dark incubation (during which these seeds were not scored), the dark treatment seeds were exposed to light (12 h photoperiod) for an additional 18 days. At the end of this period these dishes were moved to 5°C for 14 days followed by re-transfer to the germination temperatures in the light.

Statistical analyses

To test for differences in germination percentages between treatments, One-way ANOVA on arc-sine transformed data was implemented in Minitab 13 (Minitab Inc., PA, USA) followed by Tukey's pair-wise comparisons.

Results

Seed germination responses

Compared to the control treatment, dry-after-ripening, cold stratification, GA_3 and KNO_3 all resulted in a significant increase in germination percentage for all four seedlots (One-way ANOVA, $P < 0.05$; figure 1). Control germination was lowest at a constant 25°C (range 10 to 58.5%; dependent on seedlot) and highest at the two alternating temperature

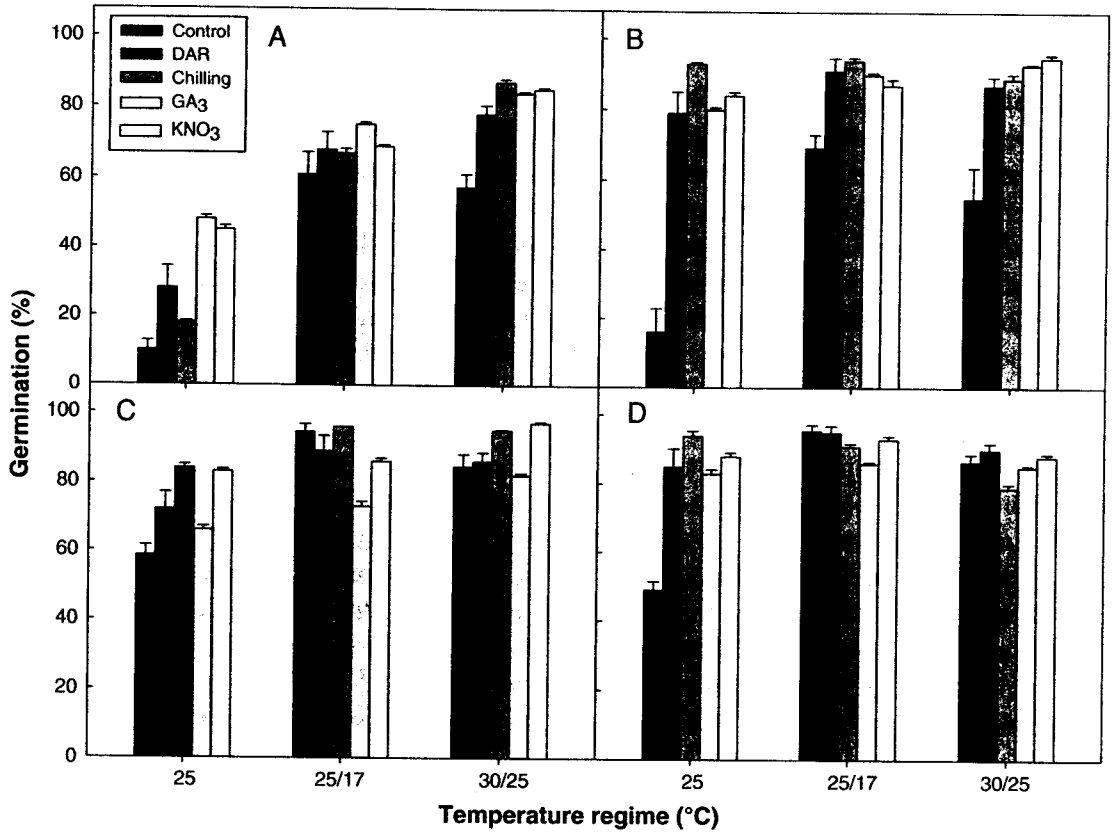

Figure 1. Effect of different temperature regimes as well as dormancy breaking (dry-after-ripening [DAR] and cold stratification [chilling]) and germination stimulant treatments (GA_3 and KNO_3) on seed germination of (A) *Vernonia galamensis* subsp. *nairobiensis* from Muguga (B) *V. galamensis* subsp. *afromontana* var. *gibbosa*, from Muguga (C) *V. galamensis* subsp. *nairobiensis* grown at Maseno and (D) *V. galamensis* subsp. *afromontana* var *gibbosa* grown at Maseno. Error bars are +1SE of the mean.

regimes (range 55 to 99.5%; dependent on seedlot). At a constant 25°C, germination was lowest for subsp. *nairobieneis* from Muguga (10%) and highest in the two seedlots harvested from Maseno (49 and 58.5%; figure 1). The dormancy breaking and stimulant treatments enhanced germination at all temperatures although the effect was most apparent at 25°C: alternating temperatures increased germination to a similar level to the additional treatments, particularly for the Maseno seedlots (figure 1).

Effects of light

In all germination temperature regimes (constant and alternating) seeds of both sub-species from both locations germinated to significantly lower levels in the dark than light (One-way ANOVA, $P < 0.05$; figure 2). At a constant 25°C, maximum germination in the dark was 28% (subsp. *afromontana* var. *gibbosa* from Maseno) and maximum germination in the light was 85% (subsp. *afromontana* var. *gibbosa* from Maseno). At 25°C germination was lowest for subsp. *nairobiensis* grown at the coolest site Muguga (3 and 28% in the dark and light, respectively) and highest for subsp. *afromontana* var. *gibbosa* from the warmest site, Maseno (28 and 85% in the light and dark, respectively). At alternating

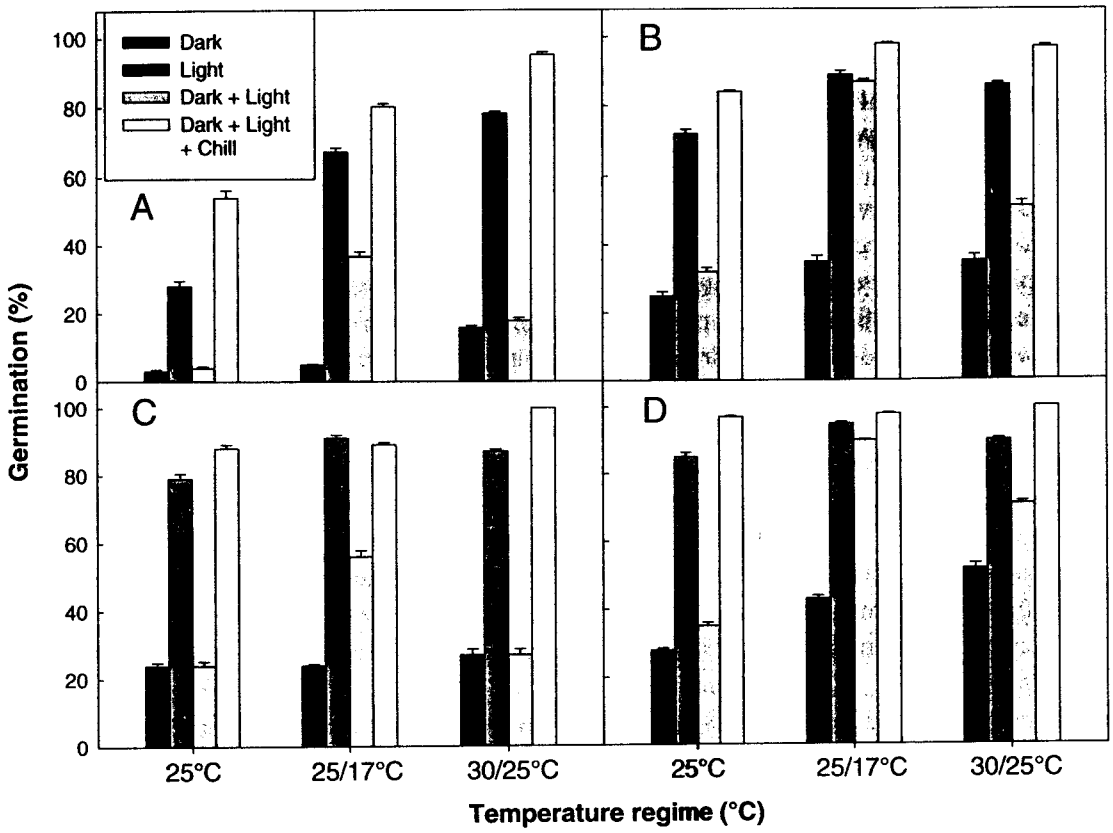

Figure 2. The effect of light on germination of (A) *Vernonia galamensis* subsp. *nairobiensis* from Muguga (B) *V. galamensis* subsp. *afromontana* var. *gibbosa*, from Muguga (C) *V. galamensis* subsp. *nairobiensis* grown at Maseno and (D) *V. galamensis* subsp. *afromontana* var. *gibbosa* grown at Maseno. Seeds were sown in both the dark and the light. Seeds held in the dark were sequentially transferred to the light after 11 days followed by cold stratification at 5°C for 14 days before being re-transferred to the original germination temperature. Error bars are +1SE of the mean.

temperatures, germination in the dark was higher than at a constant 25°C. However, although significant for three of the seedlots (One-way ANOVA, $P < 0.05$; figure 2) this effect was small (maximum increase was from 28% at 25°C to 52% at 30/25°C [subsp. *afromontana* var. *gibbosa* from Maseno]).

Dormancy induction

Upon transfer to the light, seeds at 25°C that had previously been incubated in the dark exhibited only a limited increase in germination although this was significant for two seedlots (One-way ANOVA, $P < 0.05$; figure 2). However, seeds that were incubated in the dark at alternating temperatures and then transferred to the same alternating temperatures in the light exhibited a significant increase in germination percentage (e.g. subsp. *nairobiensis* from Muguga; One-way ANOVA, $P < 0.05$; figure 2). Furthermore for dark incubated seeds at both constant and alternating temperatures that failed to germinate after transfer to the light, germination was significantly increased (to close to maximum germination, see figure 1) by subsequent cold stratification for 14 days followed by re-incubation at the germination temperatures.

Discussion

Dormancy break and level

Seed germination percentage was significantly increased by short-term cold stratification, dry-after-ripening and GA_3 suggesting that seeds of this species have non-deep physiological dormancy (see Baskin and Baskin, 2004). Similarly, seeds of *Actinobole uliginosum* (Asteraceae) from tropical dryland in Australia have been shown to have non-deep physiological dormancy since seeds respond positively to dry-after-ripening and GA_3 (Hoyle *et al.*, 2008). While it may seem surprising that a 'tropical' species such as *V. galamensis* responded to both dry-after-ripening and cold stratification, it should be noted that this species occurs in Kenya and Ethiopia at a wide range of altitudes up to 2,200 m (Bhardwaj *et al.*, 2000; Baye *et al.*, 2001) where temperatures can, at least in the short-term, drop close to 0°C (Source: Meteorological Department, Nairobi, Kenya).

Dormancy can be characterised by the range of conditions over which germination can occur. For *V. galamensis* this is expressed in terms of germination being limited at a constant 25°C although germination could occur at alternating temperatures. However, after the two dormancy-breaking treatments (dry-after-ripening and cold stratification), seeds germinated to high levels at a constant 25°C, i.e. the range of conditions over which germination could occur had widened.

Seedlots were generated at two sites and it appeared that the two seedlots from Maseno were the least dormant (in terms of germination at 25°C). This effect is likely to reflect climatic conditions at the growing sites as opposed to genetic effects since seeds from the same initial bulk collection were sown at the two sites. Maseno has a higher average temperature than Muguga and elevated temperatures during development have also been shown to result in higher germinability / lower dormancy levels in a wide range of species (Fenner, 1991; Wulff, 1995).

Germination requirements and ecological relevance

V. galamensis is a species of open, disturbed habitats. In disturbed habitats solar gain at the soil surface is increased resulting in a greater amplitude of diurnal temperature fluctuations. While seeds can target open micro-sites for germination by detecting light quality (Red: Far Red light), this mechanism is typically restricted to small seeded species (Milberg *et al.*, 2000; Pearson *et al.*, 2002; Jankowska-Blaszczuk and Daws, 2007) since light only penetrates soil to ca. 5 mm depth (Tester and Morris, 1987) and small seeds can only emerge from a few mm depth (Bond *et al.*, 1999; Daws *et al.*, 2007a). In contrast, larger seeds can emerge from greater depths than light can penetrate: a light requirement would unnecessarily limit the germination opportunities (with respect to burial depth) for larger seeded species. Although *V. galamensis* has a comparatively large seed size (1000-seed weight of ca. 4 g) for a light responsive species, it is of note that Thompson and Grime (1983) reported a number of 'intermediate' sized seeds of United Kingdom natives that responded to both light and alternating temperatures for germination presumably as a mechanism for maximising opportunities of germination in suitable micro-sites. Since soil KNO_3 levels typically increase following disturbance, the positive germination response to KNO_3 coupled with the light and alternating temperatures effects suggest that *V. galamensis*

has a strategy of responding to multiple gap related cues to ensure germination occurs in suitable germination micro-sites. Similarly, multiple dormancy breaking / germination stimulating treatments have been found to be effective for stimulating germination of several species. For example, *Clematis vitalba* (Ranunculaceae) germinates in response to light, cold chilling and KNO_3 (Bungard *et al.*, 1997) while *Epacris stuartii* (Epacridaceae) geminates in response to darkness, heat shock and smoke (Keith, 1997).

In addition to a light requirement for germination, incubation in the dark resulted in a change in germination requirements: seeds subsequently transferred to the light germinated to lower levels (even in the presence of alternating temperatures) than seeds that were initially germinated in the light (figure 2). However, cold stratification overcame this inducible 'secondary' dormancy. Secondary dormancy has been reported in other Asteraceae (Banovetz and Scheiner, 1994; Doucet and Cavers, 1997; Brandel, 2004) and presumably functions to increase the temporal spread of germination thereby reducing the risk of this high-risk stage in the life-cycle of a plant.

Conclusions

Seed germination of two sub-species of *V. galamensis* is affected by temperature during seed development with non-deep physiological dormancy of these light and nitrate sensitive seeds being broken by dry-after-ripening and cold stratification.

Acknowledgements

Financial support for this study was kindly provided by the Millennium Seed Bank Project which is supported by the Millennium Commission, The Wellcome Trust and Orange plc. The Royal Botanic Gardens, Kew is supported grant-in-aid from Defra.

References

Banovetz, S.J. and Scheiner, S.M. (1994). Secondary seed dormancy in *Coreopsis lanceolata*. *American Midland Naturalist*, **131**, 75-83.

Baskin, C.C. and Baskin, J.M. (2002). Achene germination ecology of the federally threatened floodplain endemic *Boltonia decurrens* (Asteraceae). *American Midland Naturalist*, **147**, 16-24.

Baskin, J.M. and Baskin, C.C. (2004). A classification system for seed dormancy. *Seed Science Research*, **14**, 1-16.

Baye, T., Kebede, H. and Belete, K. (2001). Agronomic evaluation of *Vernonia galamensis* germplasm from Eastern Ethiopia. *Industrial Crops and Products*, **14**, 179-190.

Bhardwaj, H.L., Hamama, A.A., Rangappa, M. and Dierig, D.A. (2000). *Vernonia* oilseed production in the mid-Atlantic region of the United States. *Industrial Crops and Products*, **12**, 119-124.

Bond, W.J., Honig, M. and Maze, K.E. (1999). Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia*, **120**, 132-136.

Brandel, M. (2004). The role of temperature in the regulation of dormancy and germination of two related summer-annual mudflat species. *Aquatic Botany*, **79**, 15-32.

Bungard, R.A., McNeil, D. and Morton, J.D. (1997). Effects of chilling, light and nitrogen-containing compounds on germination, rate of germination and seed imbibition of *Clematis vitalba* L. *Annals of Botany*, **79**, 643-650.

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., Ballard, C., Mullins, C.E., Garwood, N.C., Murray, B., Pearson, T.R.H. and Burslem, D.F.R.P. (2007a). Allometric relationships between seed mass and seedling characteristics reveal trade-offs for neotropical gap-dependent species. *Oecologia*, **154**, 445-454.

Daws, M.I., Davies, J., Pritchard, H.W., Brown, N.A.C. and Van Staden, J. (2007b). Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. *Plant Growth Regulation*, **51**, 73-82.

Denslow, J.S., Ellison, A.M. and Sanford, R.E. (1998). Treefall gap size effects on above- and below-ground processes in a tropical wet forest. *Journal of Ecology*, **86**, 597-609.

Doucet, C. and Cavers, P.B. (1997). Induced dormancy and colour polymorphism in seeds of the bull thistle *Cirsium vulgare* (Savi) Ten. *Seed Science Research*, **7**, 399-407.

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

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

Hamze, S.I. and Jolls, C.L. (2000). Germination ecology of a federally threatened endemic thistle, *Cirsium pitcheri*, of the Great Lakes. *American Midland Naturalist*, **143**, 141-153.

Harbone, J.B. and Williams, C.A. (1977). Vernonieae: chemical review. In *The Biology and Chemistry of Compositae* (eds. B.L. Heywood, and B.L Turner), pp. 523-535. Academic Press, London.

Honda, Y. and Katoh, K. (2007). Strict requirement of fluctuating temperatures as a reliable gap signal in *Picris hieracioides* var. *japonica* seed germination. *Plant Ecology*, **193**, 147-156.

Hoyle, G.L., Steadman, K.J., Daws, M.I. and Adkins, S.W. (2008). Physiological dormancy in forbs native to south-west Queensland: Diagnosis and Classification. *South African Journal of Botany*, **74**, 208-213.

Jankowska-Blaszczuk, M. and Daws, M.I. (2007). Impact of red: far red ratios on germination of temperate forest herbs in relation to shade tolerance, seed mass and persistence in the soil. *Functional Ecology*, **21**, 1055-1062.

Karlsson, L.M. and Milberg, P. (2007). Comparing after-ripening response and germination requirements of *Conyza canadensis* and *C-bonariensis* (Asteraceae) through logistic functions. *Weed Research*, **47**, 433-441.

Keith, D.A. (1997). Combined effects of heat shock, smoke and darkness on germination of *Epacris stuartii* Stapf., an endangered fire-prone Australian shrub. *Oecologia*, **112**, 340-344.

Kepczynski, J., Białecka, B., Light, M. and Van Staden, J. (2006). Regulation of *Avena fatua* seed germination by smoke solutions, gibberellin A3 and ethylene. *Plant Growth Regulation*, **49**, 9-16.

Merritt, D.J., Kristiansen, M., Flematti, G.R., Turner, S.R., Ghisalberti, E.L., Trengove, R.D. and Dixon, K.W. (2006). Effects of a butenolide present in smoke on light-mediated germination of Australian Asteraceae. *Seed Science Research*, **16**, 29-35.

Milberg, P., Andersson, L. and Thompson, K. (2000). Large seeded species are less dependent on light for germination than small-seeded ones. *Seed Science Research*, **10**, 99-104.

Ologunde, M.O., Ayorinde, F.O. and Shepard, R.L. (1990). Chemical evaluation of defatted *Vernonia galamensis* meal. *Journal of the American Oil Chemists Society*, **67**, 92-94.

Pearson, T.R.H., Burslem, D.F.R.P., Mullins, C.E. and Dalling, J.W. (2002). Germination ecology of neotropical pioneers: interacting effects of environmental conditions and seed size. *Ecology*, **83**, 2798-2807.

Pons, T.L. (1989). Breaking of seed dormancy by nitrate as a gap detection mechanism. *Annals of Botany* **63**, 139-143.

Roseberg, R.J. (1997). Herbicide tolerance by *Vernonia* grown in the temperate zone. *Industrial Crops and Products*, **6**, 89-96.

Schutz, W., Milbert, P. and Lamont, B.B. (2002). Seed dormancy, after-ripening and light requirements of four annual Asteraceae in south-western Australia. *Annals of Botany*, **90**, 707-714.

Teketay, D. (1993). Germination ecology of *Vernonia galamensis* (cass.) Less. var *ethiopica* M.G. Gilbert, a new industrial oilseed crop. *Tropical Ecology*, **34**, 64-74.

Tester, M. and Morris, C. (1987). The penetration of light through soil. *Plant, Cell and Environment*, **10**, 281-286.

Thompson, K. and Grime, J.P. (1983). A comparative study of germinating responses to diurnally fluctuating temperatures. *Journal of Applied Ecology*, **20**, 141-156.

Tobe, K., Zhang, L. and Omasa, K. (2006). Seed germination and seedling emergence of three *Artemisia* species (Asteraceae) inhabiting desert sand dunes in China. *Seed Science Research*, **16**, 61-69.

Vázquez-Yanes, C., Orozco-Segovia, A., Rincon, E., Sanchez-Coronado, M.E., Huante, P., Toledo, J.R. and Barradas, V.L. (1990). Light beneath the litter in a tropical forest: effect on seed germination. *Ecology*, **71**, 1952-1958.

Vivar-Evans, S., Barradas, V.L., Sanchez-Coronado, M.E., de Buen, A.G. and Orozco-Segovia, A. (2006). Eco-physiology of seed germination of wild *Dahlia coccinea* (Asteraceae) in a spatially heterogeneous fire-prone habitat. *Acta Oecologica-International Journal of Ecology*, **29**, 187-195.

Wood, C.B., Miles, S., Rix, C., Terry, J. and Daws, M.I. (2005). Tetrazolium staining on seeds of 171 species indicates a confounding effect of oil on the accuracy of viability assessment. *Plant Genetic Resources Newsletter*, **143**, 17-23.

Wulff, R.D. (1995). Environmental maternal effects on seed quality. In *Seed Development and Germination* (eds. J. Kigel and G. Galili), pp. 491-505. Marcel Dekker Inc, New York, USA.