

## INVITED REVIEW AND RESEARCH OPINION

**Nitrogen oxides as environmental sensors for seeds****Zlatko Giba<sup>1</sup>, Dragoljub Grubišić<sup>2</sup> and Radomir Konjević<sup>1\*</sup>**<sup>1</sup>Institute of Botany, Faculty of Biology, University of Belgrade, and <sup>2</sup>Institute for Biological Research 'Siniša Stankovic', 29 novembra 142, 11060 Belgrade, Yugoslavia**Abstract**

Nitrates have long been known to promote seed germination in many species, and various proposals have been made regarding the role of nitrates in this process. One hypothesis suggests a possible ecological role for nitrate, providing the seeds with a report of soil nitrogen status and the proximity of already established plants. However, nitrogen oxides (NO, NO<sub>2</sub>), which are present in the soil, also may be information carriers that indicate not only soil nitrate content, but also microbial activity and, therefore, soil quality. Because of annual variation of soil trace gas fluxes, seeds could be provided with information on seasonal and climate changes in their surroundings. Thus, nitrogen oxides would be the outer information carriers providing the seeds with integral data about many important factors required for successful germination and seedling establishment.

**Keywords:** seed ecology, nitrogen oxides, germination, smoke, soil trace gases

**Introduction**

During evolution, plants developed a special organ, the seed, which ensures their dispersion in space and time. The seed is structurally and physiologically equipped for this role. Besides the protective covering, the seed is generously provided with food reserves to support the emerging seedling until the moment when it becomes a self-sufficient autotrophic organism. In the pre-autotrophic phase of plant life, the seed is the neatest and most easily transportable, self-contained package (unit) ensuring dispersion and perpetuation of the species. There are several

adaptations for this 'distribution in space'. Dispersion of seeds can be mediated by animals (endo- and ectozoochorous seeds), or by different seed coat extensions, such as seed wings, enabling seed movement by wind (anemochorous seeds). In *Paulownia tomentosa*, for example, seed wings have several additional roles. They are an exaptation (secondary adaptation) for water uptake (Vujičić *et al.*, 1993), and also contribute to the reduction of ferric chelates (Giba, 1998) in the surroundings, providing the future seedling with reduced iron.

Seeds of many species enter a state of dormancy upon their shedding from the mother plant. Seeds can remain dormant and not germinate even under optimum external conditions, and must pass through certain special situations before they can germinate. Despite the fact that many researchers study dormancy, there is no unambiguous definition of this phenomenon. This is probably due to the fact that dormancy is manifested and broken in various ways in different species (Bewley and Black, 1982; Nikolaeva *et al.*, 1985). However, dormancy is often considered as an adaptive trait that optimizes the 'distribution in time' of seed germination. Commonly, seed dormancy is regarded as an incapability of intact, viable seeds to complete germination under otherwise favourable conditions.

In this paper we review the effects of nitrogen oxides, known from smoke-stimulated species, and discuss their possible broader role as germination triggers and environmental sensors in nature.

**The role of inorganic nitrates**

Nitrogenous compounds, such as potassium nitrate, promote germination in many seeds, especially in light-sensitive, i.e. photoblastic seeds. Nitrates significantly increase the light sensitivity and decrease the light requirement of seeds (Toole *et al.*,

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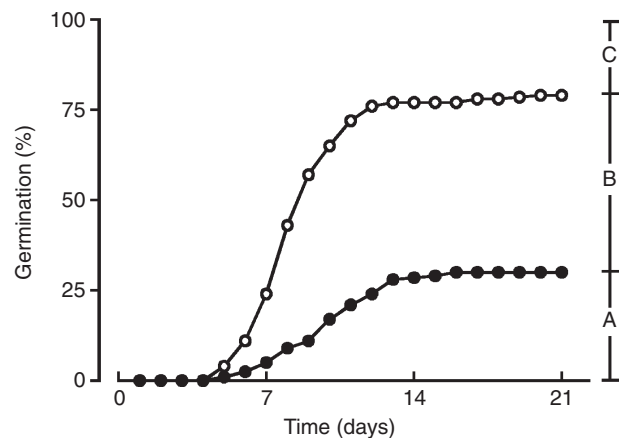
1955). International Seed Testing Association (ISTA) rules recommend the application of  $\text{KNO}_3$  in more than 50% of germination tests of various species. Although this effect of nitrates and nitrites has been known for a long time (Lehmann, 1909), and several hypotheses of their interaction with phytochrome have been formulated (Hilton, 1985; Grubišić and Konjević, 1990), almost nothing is known about the mechanism of action. The proposed connection between nitrogen compounds, the pentose phosphate pathway and seed dormancy (Roberts, 1973) has not been confirmed experimentally (Adkins et al., 1984; Cohn, 1987). Nitrate activation of a postulated phytochrome receptor has been proposed to explain the germination of *Sisymbrium officinale* seeds (Hilhorst and Karssen, 1989). Inorganic nitrates also modulate the phytochrome-controlled germination of *Spirodela polyrhiza* turions (Appenroth et al., 1992), as well as phytochrome-controlled germination of fern spores, where their effectiveness was explained on the basis of the electron-accepting properties of nitrates (Haas and Scheuerlein, 1991).

A possible ecological role for the nitrate requirement in seed germination has been proposed. The nitrate requirement would provide seeds with information on the nitrogen status in the soil and the proximity of already established plants (Pons, 1989). Assuming that nitrate dependence of dormant seeds allows them to sense the nitrogen status of the ecosystem before germination starts (Fig. 1), this capacity may be considered as a part of the survival strategy for plants (Goudey et al., 1988; Hsiao and Quick, 1996; Bell et al., 1999). Additionally, Thanos and Rundel (1995) suggested that increased soil nitrate content after wildfires (Polglase et al., 1986) could stimulate germination from the soil seed bank, thus triggering repair of a fire-damaged ecosystem.

### Fire-triggered germination

During fires in savannahs and steppes, air temperatures can range between 200 and 400°C, while fires in European coniferous forests can generate temperatures between 800 and 900°C. Yet, soil temperatures at 3 cm depth did not exceed 90°C, and were only between 60 and 80°C at 7 cm below the soil surface (Larcher, 1995; see also Walter, 1983).

For some species with fire-triggered germination, a brief heat shock between 80 and 120°C is sufficient to induce germination. This heat shock may enhance germination by allowing water uptake in localized regions, such as the hilum, chalazal cap and strophliolar plug (Keeley and Fotheringham, 1998), or by causing destruction of chemical inhibitors present in the seeds (Bell et al., 1993). Besides the fact that the seed coat may be ruptured by the influence of high



**Figure 1.** Light-induced seed germination of one of the species from the genus *Gentiana*. Seeds were irradiated, for the indicated time, with continuous white light in the presence of 1 mM potassium nitrate (○) or in distilled water (●). Germination was scored every day. The results obtained could be extrapolated to the field conditions. After dispersal under optimal external conditions, only part of the seed population, denoted as A, germinates. Parts denoted as B and C remain in the soil seed bank. If the seeds contact nitrate-rich soil, only part of the population, denoted as C, remains in the soil seed bank.

temperatures, some authors suggested that removal of seed dormancy by dry heat is not due to changes in the seed coat but in the embryo (Ruge, 1955). In some species the heat shock must be combined with additional stimuli, such as stratification or light (Keeley et al., 1985). Heat-shock stimulated germination is widespread in different families, such as *Fabaceae*, *Rhamnaceae*, *Convolvulaceae*, *Malvaceae*, *Cistaceae*, or *Sterculiaceae*, and is found in different ecosystems (Ballard, 1973; Bewley and Black, 1982; Thanos et al., 1992; Bell et al., 1993).

For substantial numbers of species, heat shock does not affect germination. Rather, dormancy is broken by combustion products or their derivatives (Keeley, 1991). Post-fire growth and abundance of seedlings is greater in burned areas, and some authors attributed this phenomenon, partly, to the ash generated during a fire, since it may release considerable amounts of readily available plant nutrients (Burrows et al., 1990; Chambers and Attiwill, 1994; Thomas and Wein, 1994). However, the stimulative effect of ash on seed germination was not demonstrated under controlled experimental conditions designed to eliminate other possible consequences of fire, such as allelopathic effects, decreased competition for water, light, etc. (Thanos and Skordilis, 1987; Reyes and Casal, 1998).

Charred wood was first shown to stimulate seed germination in the case of *Emmenanthe penduliflora* (*Hydrophyllaceae*), a post-fire annual species (Wicklow,

1977), and it is also effective for many other species (Keeley *et al.*, 1985). On the other hand, smoke evolved during wildfires may be the most important chemical stimulus for germination of 'fire-type' species (Brown and van Staden, 1997). De Lange and Boucher (1990) were the first to report that plant-derived smoke stimulates seed germination. They showed that smoke acts as a cue for breaking dormancy of *Audouinia capitata* (*Bruniaceae*). After this discovery smoke- and smoke-extract-stimulated germination has been reported for many fire- and non-fire-dependent species in different families, such as *Brassicaceae*, *Caryophyllaceae*, *Papaveraceae*, *Lamiaceae*, *Apiaceae*, *Solanaceae*, *Ericaceae*, *Poaceae* or *Asteraceae* (Brown, 1993; Baldwin *et al.*, 1994; Baxter and van Staden, 1994; Dixon *et al.*, 1995; Drewes *et al.*, 1995; Thomas and van Staden, 1995; Brown and van Staden, 1997; Doherty and Cohn, 2000). It was unclear whether the chemicals in charred wood, which are active in triggering germination, are the same as those responsible for smoke-induced germination. Several studies failed to identify the active component in both cases (Keeley and Pizzorno, 1986; van Staden *et al.*, 1995a). It was shown that the promotive effect of smoke is independent of seed size or shape. The promotion of seed germination in response to smoke is widespread and occurs in annual, perennial and herbaceous species (Brown *et al.*, 1995; Brown and van Staden, 1997). A smoke response is observed in species from all plant life forms (trees to herbs) and reproductive strategies (Dixon *et al.*, 1995). Smoke appears to be an almost universal signal for germination. However, in a majority of cases, smoke stimulates light-sensitive seeds, improving germination of both positively and negatively photoblastic seeds (Brown and van Staden, 1997).

Smoke or aqueous smoke extracts alter aspects of plant growth other than seed germination. The stimulation of flowering in some *Watsonia* species and in fire lily (*Cyrtanthus ventricosus*) (Bean, 1962; Keeley, 1993), as well as increased root growth and development in mung bean (Taylor and van Staden, 1996), have been reported. Interestingly, both effects could also be brought about by exogenous application of nitric oxide (NO) (Gouvea *et al.*, 1997; Leshem *et al.*, 1998). In addition, smoke can induce seed protection against microbiological attack (Parmeter and Uhrenholdt, 1975). Generally, nitric oxide is associated with the hypersensitive response in plants (Noritake *et al.*, 1996; Durner *et al.*, 1998; Delledonne *et al.*, 2001).

### Nitrogen oxides are active components of smoke

Nitrogen oxides, especially NO<sub>2</sub>, are active components of smoke responsible for the smoke-stimulated germination of *Emmenanthe penduliflora*

seeds (Keeley and Fotheringham, 1997). The dormancy-breaking effect of nitrogen dioxide was demonstrated for the first time in *Oryza sativa* seed germination (Cohn and Castle, 1984). The physiological basis of the stimulative nitrogen dioxide effect could be explained by the spontaneous generation of nitric oxide (NO), by analogy to animal systems where exogenously applied NO<sub>2</sub> mimics typical NO-mediated effects (Zweier *et al.*, 1995; Davidson *et al.*, 1996; see also Giba *et al.*, 1998b). Nitric oxide may affect steps in the phytochrome signal transduction chain involving cyclic guanosine monophosphate (cGMP) biosynthesis (Bowler *et al.*, 1994). Stimulation of cGMP formation by NO in plants was first reported in spruce needles, where the concentration of cGMP rapidly increased by almost four orders of magnitude after NO exposure (Pfeiffer *et al.*, 1994). Rapid and transient effects of exogenously applied NO on the concentration of cGMP have also been observed in tobacco leaves and isolated cells (Durner *et al.*, 1998).

Gaseous nitric oxide breaks seed dormancy of *E. penduliflora* seeds (Keeley and Fotheringham, 1998). It is also active in solution in light-induced *Paulownia tomentosa*, *Pinus mugo* (Giba *et al.*, 1997a, b) and *Arabidopsis thaliana* seed germination (Batak *et al.*, 2002), as well as in *Lactuca sativa* (Beligni and Lamattina, 2000) and *Stellaria media* seed germination in darkness (Grubišić *et al.*, 1991; Giba *et al.*, 1992). The presence of nitric oxide after administration of different NO donors, such as sodium nitroprusside or nitroglycerine, and its involvement in the germination process of *Paulownia tomentosa*, was demonstrated by spin-trapping techniques combined with electron paramagnetic resonance spectroscopy. In the same experimental system, the stimulative effect of NO-releasing substances was inhibited by using the guanylyl cyclase inhibitor, methylene blue, indicating a possible connection with cGMP formation (Giba *et al.*, 1998a). In addition, it has been shown that plant tissue could easily metabolize nitroglycerine to dinitro- and mononitro-derivatives (Goel *et al.*, 1997). Therefore, the dormancy-breaking activity of nitric oxide (Giba *et al.*, 1998a) may also be the basis for the stimulative effects of NO<sub>2</sub> on dry seeds (Cohn and Castle, 1984; Keeley and Fotheringham, 1997), the effect of acidified nitrite solutions (Cohn *et al.*, 1983; Giba *et al.*, 1998a), and the effect of different types of organic nitrates (Grubišić *et al.*, 1991, 1992).

### Fire-related germination cues are not restricted to fire-prone species

The heat cue for germination is not specific for post-fire environments (Pierce *et al.*, 1995) and is relevant





Mediterranean-type climate. From this point of view, the stimulative effect of nitrogenous compounds present in the smoke could be considered as a special case, i.e. as a part of a general mechanism by which ecosystems regulate the timing and germination sequence of various species.

### Physiological aspects and some examples

Although spontaneous, endogenous production of nitric oxide during seed germination has been reported (Caro and Puntarulo, 1999), there is an apparent lack of evidence for the presence of NO synthases in plants, especially in seeds. This favours the hypothesis that nitric oxide is an external signalling molecule in plants. Enzymes that transform L-arginine into citrulline and nitric oxide (Palmer *et al.*, 1988) are found in practically all kingdoms of the living world (for a review, see Torreilles, 2001), but not in plants. However, some authors suggest such a possibility for plants as well (Leshem, 1996; Corpas *et al.*, 2001). In plants, especially under stress conditions, nitric oxide may be produced non-enzymatically through light-mediated conversion of NO<sub>2</sub> by carotenoids (Cooney *et al.*, 1994), enzymatically by nitrate and nitrite reductases (Dean and Harper, 1988; Yamasaki *et al.*, 1999; Stöhr *et al.*, 2001) and possibly by other nitric oxide synthase-independent mechanisms (Kozlov *et al.*, 1999). Moreover, because of the presence of nitrogen oxides in soil and/or the lower troposphere, it would be reasonable to expect that plants have developed specific mechanisms to protect against elevated concentrations of nitrogen oxides, rather than additional mechanisms for their production. Some authors speculate that the presence of the alternative oxidase in plants, which decreases the probability of superoxide formation, serves additionally for such purposes (Millar and Day, 1996). When the cytochrome pathway is restricted or inhibited by nitric oxide or cyanide ions during terminal oxidation electron flow, operation of the alternative oxidase (which is insensitive to both cyanide ions and nitric oxide) would additionally decrease the formation of the extremely strong oxidant, peroxyxynitrous acid, a product of the superoxide and nitric oxide reaction (Beckman *et al.*, 1990). Recent studies, using microarray techniques, confirm this statement (Huang *et al.*, 2002).

As mentioned previously, in a majority of cases nitrogenous compounds stimulate germination of light-sensitive seeds. Seeds of *Sisymbrium officinale* are not photoblastic if they do not germinate in the presence of potassium nitrate, or if endogenous concentrations of nitrates in seeds are too low (Karssen and Hillhorst, 1992). Moreover, even seeds of some photoblastic plant species (some members of

family *Gentianaceae*, e.g. *Gentiana cruciata*) that are usually not in contact with nitrate-rich soils respond to nitrate treatments. Although these seeds germinate if potassium nitrate is added before or during the irradiation period, they are completely insensitive if nitrates are added later. The sensitivity of *G. cruciata* seeds to exogenous gibberellins is not altered (Grubišić *et al.*, 1995). Very rarely, and we are aware of only one such example, nitrogenous compounds inhibit germination: although light sensitive, *Vaccinium myrtillus* seed germination is strongly inhibited in the presence of potassium nitrate, organic nitrates or sodium nitroprusside (Giba, 1992; Giba *et al.*, 1995). Seeds of some other *Vaccinium* species are just nitrate insensitive (Stushnoff and Hough, 1968).

Even if we accept that the stimulative effects of nitrogenous compounds in light-induced germination are NO-mediated phenomena, the nature of the underlying interaction with the phytochrome pigment system and/or some component of the phytochrome transduction chain is still not clear. Recently, based on elegant experimentation of Shinomura *et al.* (1994, 1996), which physiologically 'dissected' phytochrome A (PHY A)- and phytochrome B (PHY B)-specific induced germination of *Arabidopsis thaliana* seeds, we have obtained a slightly deeper insight into these mechanisms. One can show that the presence of nitrogenous compounds in the incubation medium during light-induced germination of *A. thaliana* seeds affected predominantly PHY A-specific induced germination, while PHY B-specific induced germination was affected to a far lesser extent (Batak *et al.*, 2002). It is important to emphasize that at least two downstream components of NO signalling found in animals, cGMP or ADP-ribose, are functional in plants (Durner *et al.*, 1998; for a review, see Wendehenne *et al.*, 2001). Because cGMP is the only known member of the PHY A transduction chain (Bowler *et al.*, 1994) that is also sensitive to nitric oxide in plants (Pfeiffer *et al.*, 1994), these data establish a potential connection between NO effectiveness, cGMP formation and PHY A activity.

It is believed that PHY A is the most recently evolved phytochrome. The appearance of this new photoreceptor – which is activated by light of practically all parts of visible spectrum, is several orders of magnitude higher in photosensitivity compared with PHY B controlled reactions and has some other unique properties (Shinomura *et al.*, 1996) – was undoubtedly an evolutionary step forward. Under a thin layer of soil, seeds became able to detect surface light conditions (Furuya and Schäfer, 1996). It is not surprising that additional seed sensitivity to the presence of nitrogenous compounds in the surroundings was coupled with phytochrome activity, the basic pigment system by which seeds detect environmental changes. From an ecophysiological

point of view, this provided plants with a new advantage at the seed level.

## Summary

Finally, one must bear in mind that the proposed delicate regulation of germination processes in ecosystems could be easily disturbed. The effect of nitrogenous compounds as air pollutants (Brimblecombe and Stedman, 1982; Barnes and Wellburn, 1998) must be taken into account. For example, more than 80% of nitric oxide emissions worldwide are generated by human activities. Higher levels of pollutants in the lower troposphere may stimulate seed germination when other external conditions are inappropriate for seedling growth. This process would be initiated by the activity of primary nitrogen pollutants, NO<sub>2</sub> and NO, collectively known as NO<sub>x</sub>, and/or by the activity of secondarily generated atmospheric nitrogenous compounds, such as organic nitrates (including alkyl nitrates, aromatic nitrates and bifunctional alkyl nitrates) (Schneider *et al.*, 1998). Organic nitrates (i.e. different nitrate esters such as nitroglycerine, isosorbide mono- and dinitrate, or pentaerytrityl tetranitrate) are very potent stimulators of seed germination. In some cases they exert activity at concentrations almost 100 times lower than inorganic nitrates (Grubišić *et al.*, 1992). Pollutants could trigger the seed germination and, consequently, deplete the soil bank of stored seeds, with irreversible impact upon the future of populations and community structure. On the other hand, carefully controlled germination of seeds in soil seed banks of disturbed land stimulated by 'smoke techniques' is possible. Several attempts have been reported (e.g. Brown, 1994; reviewed by Brown and van Staden, 1997). At least in part, the 'smoke techniques' may substitute for using localized and controlled fires, commonly performed in some countries, to renew ecosystems.

In this review several ideas and explanations based on experimental data and observed germination sequences in different ecosystems and situations are covered. Particular emphasis was given to the possible involvement of nitric oxides as signalling molecules that 'translate' external environmental conditions into physiologically pertinent information for the regulation of seed germination. Clearly, more work is needed to test these hypotheses. Atmospheric chemical processes, as well as chemical and biochemical processes in the soils, are far from adequately understood (e.g. Cattáneo *et al.*, 2002). Although much work has been done, deeper insights into mechanisms underlying the physiological effectiveness of NO and other free radicals in biological systems (e.g. Wentworth *et al.*,

2003) are still required. This represents an open and fruitful field for future investigations.

## References

- Adkins, S.W., Simpson, G.M. and Naylor, J.M. (1984) The physiological basis of seed dormancy in *Avena fatua*. IV. Alternative respiration and nitrogenous compounds. *Physiologia Plantarum* **60**, 234–238.
- Appenroth, K.J., Augsten, H. and Mohr, H. (1992) Photophysiology of turion germination in *Spirodela polyrrhiza* (L.) Schleiden. X. Role of nitrate in the phytochrome-mediated response. *Plant, Cell and Environment* **15**, 743–748.
- Baldwin, I.T., Staszak-Kozinski, L. and Davidson, R. (1994) Up in smoke. I. Smoke-derived germination cues for post-fire annual *Nicotiana attenuata* Torr. ex. Watson. *Journal of Chemical Ecology* **20**, 2345–2371.
- Ballard, L.A.T. (1973) Physical barriers to germination. *Seed Science and Technology* **1**, 285–303.
- Barnes, J.D. and Wellburn, A.R. (1998) Air pollutant combinations. pp.147–164 in De Kok, L.J.; Stulen, I. (Eds) *Responses of plant metabolism to air pollution and global change*. Leiden, Backhuys Publishers.
- Baskin, J.M. and Baskin, C.C. (1985) The annual dormancy cycle in buried weed seeds: A continuum. *BioScience* **35**, 492–498.
- Batak, I., Dević, M., Giba, Z., Grubišić, D., Poff, K.L. and Konjević, R. (2002) The effects of potassium nitrate and NO donors on phytochrome A- and phytochrome B-specific induced germination of *Arabidopsis thaliana* seeds. *Seed Science Research* **12**, 253–259.
- Baxter, B.J.M. and van Staden, J. (1994) Plant-derived smoke: an effective seed pretreatment. *Plant Growth Regulation* **14**, 279–282.
- Bean, P.A. (1962) An enquiry into the effect of veld fires on certain geophytes. MSc thesis, University of Cape Town.
- Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A. and Freeman, B.A. (1990) Apparent hydroxyl radical production by peroxyxynitrite: Implication for endothelial injury from nitric oxide and superoxide. *Proceedings of the National Academy of Sciences, USA* **87**, 1620–1624.
- Beligni, M.V. and Lamattina, L. (2000) Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta* **210**, 215–221.
- Bell, D.T., Plummer, J.A. and Taylor, S.K. (1993) Seed germination ecology in southwestern Western Australia. *Botanical Review* **59**, 24–73.
- Bell, D.T., King, L.A. and Plummer, J.A. (1999) Ecophysiological effects of light quality and nitrate on seed germination in species from Western Australia. *Australian Journal of Ecology* **24**, 2–10.
- Bewley, J.D. and Black, M. (1982) *Physiology and biochemistry of seeds in relation to germination*. 2. *Viability, dormancy and environmental control*. Berlin, Springer-Verlag.
- Blackmer, A.M. and Cerrato, M.E. (1986) Soil properties affecting formation of nitric oxide by chemical reactions of nitrite. *Soil Science Society of America Journal* **50**, 1215–1218.

- Bowler, C., Neuhaus, G., Yamagata, H. and Chua, N.H.** (1994) Cyclic GMP and calcium mediate phytochrome phototransduction. *Cell* **77**, 73–81.
- Brimblecombe, P. and Stedman, D.H.** (1982) Historical evidence for a dramatic increase in the nitrate component of acid rain. *Nature* **298**, 460–462.
- Brown, N.A.C.** (1993) Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**, 575–584.
- Brown, N.A.C.** (1994) First the gas, now instant dehydrated smoke. *Veld and Flora* **80**, 72–73.
- Brown, N.A.C. and van Staden, J.** (1997) Smoke as a germination cue: a review. *Plant Growth Regulation* **22**, 115–124.
- Brown, N.A.C., Botha, P.A. and Prosh, D.** (1995) Where there's smoke. *Garden (London)* **120**, 402–405.
- Burrows, N., Gardiner, G., Ward, B. and Robinson, A.** (1990) Regeneration of *Eucalyptus wandoo* following fire. *Australian Forestry* **53**, 248–258.
- Caro, A. and Puntarulo, S.** (1999) Nitric oxide generation by soybean embryonic axes. Possible effect on mitochondrial function. *Free Radical Research* **31**, S205–S212.
- Cattanio, J.H., Davidson, E.A., Nepstad, D.C., Verchot, L.V. and Ackerman, I.L.** (2002) Unexpected results of a pilot throughfall exclusion experiment on soil emissions of CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O, and NO in eastern Amazonia. *Biology and Fertility of Soils* **36**, 102–108.
- Chambers, D.P. and Attiwill, P.M.** (1994) The ash-bed effect in *Eucalyptus regnans* forest: chemical, physical, and microbiological changes in soil after heating or partial sterilization. *Australian Journal of Botany* **42**, 739–749.
- Christianson, C.B. and Cho, C.M.** (1983) Chemical denitrification of nitrite in frozen soils. *Soil Science Society of America Journal* **7**, 38–42.
- Cohn, M.A.** (1987) Mechanisms of physiological seed dormancy. pp. 14–20 in Frazier, G.W.; Evans, R.A. (Eds) *Seed and seedbed ecology of rangeland plants*. Washington, DC, USDA-ARS.
- Cohn, M.A.** (1996) Chemical mechanisms of breaking seed dormancy. *Seed Science Research* **6**, 95–99.
- Cohn, M.A. and Castle, L.** (1984) Dormancy in red rice. IV. Response of unimbibed and imbibing seeds to nitrogen dioxide. *Physiologia Plantarum* **60**, 552–556.
- Cohn, M.A., Butera, D.L. and Hughes, J.A.** (1983) Seed dormancy in red rice. III. Response to nitrite, nitrate and ammonium ions. *Plant Physiology* **73**, 381–384.
- Conrad, R.** (1996) Soil microorganisms as controllers of atmospheric trace gases (H<sub>2</sub>, CO, CH<sub>4</sub>, OCS, N<sub>2</sub>O, and NO). *Microbiological Reviews* **60**, 609–640.
- Cooney, R.V., Harwood, P.J., Custer, L.J. and Franke, A.A.** (1994) Light-mediated conversion of nitrogen dioxide to nitric oxide by carotenoids. *Environmental Health Perspectives* **102**, 460–462.
- Corbineau, F., Bagniol, S. and Come D.** (1990) Sunflower (*Helianthus annuus* L.) seed dormancy and its regulation by ethylene. *Israel Journal of Botany* **39**, 313–325.
- Corpas, F.J., Barosso, J.B. and del Rio, L.A.** (2001) Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. *Trends in Plant Science* **6**, 145–150.
- Crutzen, P.J.** (1979) The role of NO and NO<sub>2</sub> in the chemistry of the troposphere and stratosphere. *Annual Review of Earth and Planetary Sciences* **7**, 443–472.
- Davidson, C.A., Kaminski, P.M., Wu, M.D. and Wolin, M.S.** (1996) Nitrogen dioxide causes pulmonary arterial relaxation via thiol nitrosation and NO formation. *American Journal of Physiology–Heart and Circulatory Physiology* **39**, H1038–H1043.
- Davidson, E.A.** (1992) Sources of nitric and nitrous oxide following wetting of dry soil. *Soil Science Society of America Journal* **56**, 95–102.
- Dean, J.V. and Harper, J.E.** (1988) The conversion of nitrite to nitrogen oxide(s) by the constitutive NAD(P)H-nitrate reductase enzyme from soybean. *Plant Physiology* **88**, 389–395.
- De Lange, J.H. and Boucher, C.** (1990) Autoecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany* **56**, 700–703.
- Delledonne, M., Zeier, J., Marocco, A. and Lamb, C.** (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proceedings of the National Academy of Sciences, USA* **98**, 13454–13459.
- Derx, M.P.M. and Karsen, C.M.** (1994) Are seasonal dormancy patterns in *Arabidopsis thaliana* regulated by changes in seed sensitivity to light, nitrate and gibberellin. *Annals of Botany* **73**, 129–136.
- Dixon, K.W., Roche, S. and Pate, J.S.** (1995) The promotive effect of smoke-derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* **101**, 185–192.
- Doherty, L.C. and Cohn, M.A.** (2000) Seed dormancy in red rice. XI. Commercial liquid smoke elicits germination. *Seed Science Research* **10**, 415–421.
- Drewes, F.E., Smith, M.T. and van Staden, J.** (1995) The effect of plant-derived smoke extract on the germination of light-sensitive lettuce seed. *Plant Growth Regulation* **16**, 205–209.
- Durner, J., Wendehenne, D. and Klessig, D.F.** (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. *Proceedings of National Academy of Sciences, USA* **95**, 10328–10353.
- Furuya, M. and Schäfer, E.** (1996) Photoperception and signalling of induction reactions by different phytochromes. *Trends in Plant Sciences* **1**, 301–307.
- Galbally, I.E. and Roy, C.R.** (1978) Loss of fixed nitrogen from soils by nitric oxide exhalation. *Nature* **275**, 734–735.
- Giba, Z.** (1992) The effect of white light, growth regulators and temperature on the germination of blueberry (*Vaccinium myrtillus* L.) seeds. MSc Thesis, University of Belgrade (in Serbian).
- Giba, Z.** (1998) Modified phytochrome action in *Paulownia tomentosa* seed germination. PhD Thesis, University of Belgrade.
- Giba, Z., Grubišić, D. and Konjević, R.** (1992) Sodium nitroprusside – stimulated germination of common chick weed (*Stellaria media* L.) seeds. *Archives of Biological Sciences* **44**, 17P–18P.
- Giba, Z., Grubišić, D. and Konjević, R.** (1995) The involvement of phytochrome in light-induced germination of blueberry (*Vaccinium myrtillus* L.) seeds. *Seed Science and Technology* **23**, 11–19.
- Giba, Z., Grubišić, D., Sajc, L., Stojaković, Đ. and Konjević, R.** (1997a) Effect of some nitric oxide donors



- and methylene blue on light-induced germination of *Paulownia tomentosa* seeds. *Archives of Biological Sciences* **49**, 15P–16P.
- Giba, Z., Skordilis, A., Grubišić, D., Thanos, C.A. and Konjević, R. (1997b) Control of seed germination in Mountain Pine. *First Balkan Botanical Congress Abstracts*, Thessaloniki, Greece p. 95.
- Giba, Z., Grubišić, D., Todorović, S., Sajc, L., Stojaković, Đ. and Konjević, R. (1998a) Effect of nitric oxide-releasing compounds on phytochrome-controlled germination of Empress tree seeds. *Plant Growth Regulation* **26**, 175–181.
- Giba, Z., Todorović, S., Grubišić, D. and Konjević, R. (1998b) Occurrence and regulatory roles of superoxide anion radical and nitric oxide in plants. *Iugoslavica Physiologica et Pharmacologica Acta* **34**, 447–461. Available at website <http://www.ippa.bg.ac.yu> (verified May 2003).
- Goel, A., Kumar, G., Payne, G.F. and Dube, S.K. (1997) Plant cell biodegradation of a xenobiotic nitrate ester, nitroglycerin. *Nature Biotechnology* **15**, 174–177.
- Goudey, J.S., Saini, H.S. and Spencer, M.S. (1988) Role of nitrate in regulating germination of *Sinapis arvensis* L. (wild mustard). *Plant, Cell and Environment* **11**, 9–12.
- Gouvea, C.M.C.P., Souza, J.F., Magalhaes, A.C.N. and Martins, I.S. (1997) NO-releasing substances that induce growth elongation in maize root segments. *Plant Growth Regulation* **21**, 183–187.
- Grubišić, D. and Konjević, R. (1990) Light and nitrate interaction in phytochrome-controlled germination of *Paulownia tomentosa* seeds. *Planta* **181**, 239–243.
- Grubišić, D., Giba, Z. and Konjević, R. (1991) Organic nitrates stimulated germination of common chick weed (*Stellaria media* L.) seeds. *Arhiv Bioloških Nauka* **43**, 7P–8P.
- Grubišić, D., Giba, Z. and Konjević, R. (1992) The effect of organic nitrates in phytochrome-controlled germination of *Paulownia tomentosa* seeds. *Photochemistry and Photobiology* **56**, 629–632.
- Grubišić, D., Giba, Z. and Konjević, R. (1995) Seed germination of *Gentiana cruciata* L. *Bulletin de l'Institut et du Jardin Botaniques de l' Université de Beograd* **29**, 93–100.
- Guerinot, M.L. and Yi, Y. (1994) Iron: nutritious, noxious and not readily available. *Plant Physiology* **104**, 815–820.
- Haas, C.J. and Scheuerlein, R. (1991) Nitrate effect on phytochrome-mediated germination in fern spores: Investigation on the mechanism of nitrate action. *Journal of Plant Physiology* **138**, 350–357.
- Hilhorst, H.W.M. and Karssen, C.M. (1989) Nitrate reductase independent stimulation of seed germination of *Sisymbrium officinale* L. (hedge mustard) by light and nitrate. *Annals of Botany* **63**, 131–137.
- Hilton, J.R. (1985) The influence of light and potassium nitrate on the dormancy and germination of *Avena fatua* L. (wild oat) seed stored buried and under natural conditions. *Journal of Experimental Botany* **36**, 974–979.
- Hsiao, A.I. and Quick, W.A. (1996) The roles of inorganic nitrogen salts in maintaining phytochrome- and gibberellin A<sub>3</sub>-mediated germination control in skotodormant lettuce seeds. *Journal of Plant Growth Regulation* **15**, 159–165.
- Huang, X., von Rad, U. and Durner, J. (2002) Nitric oxide induces transcriptional activation of the nitric oxide-tolerant alternative oxidase in *Arabidopsis* suspension cells. *Planta* **215**, 914–923.
- Karssen, C.M. (1982) Seasonal patterns of dormancy in weed seeds. pp. 243–270 in Khan, A.A. (Ed.) *The physiology and biochemistry of seed development, dormancy and germination*. Amsterdam, Elsevier Biomedical Press.
- Karssen, C.M. and Hilhorst, H.W.M. (1992) Effect of chemical environment on seed germination. pp. 327–348 in Fenner, M. (Ed.) *Seeds: The ecology of regeneration in plant communities*. Wallingford, CAB International.
- Keeley, J.E. (1991) Seed germination and life history syndromes in the California chaparral. *Botanical Review* **57**, 81–116.
- Keeley, J.E. (1993) Smoke-induced flowering in the fire-lily *Cyranthus ventricosus*. *South African Journal of Botany* **59**, 638.
- Keeley, J.E. and Fotheringham, C.J. (1997) Trace gas emissions and smoke-induced seed germination. *Science* **276**, 1248–1250.
- Keeley, J.E. and Fotheringham, C.J. (1998) Smoke-induced seed germination in California chaparral. *Ecology* **79**, 2320–2336.
- Keeley, J.E., Morton, B.A., Pedrosa, A. and Trotter, P. (1985) Role of allelopathy, heat, and charred wood in the germination of chaparral herbs and suffrutescents. *Journal of Ecology* **73**, 445–458.
- Keeley, S.C. and Pizzorno, M. (1986) Charred wood stimulated germination of two fire-following herbs of the California chaparral and the role of hemicellulose. *American Journal of Botany* **73**, 1289–1297.
- Kozlov, A.V., Staniek, K. and Nohl, H. (1999) Nitrite reductase activity is a novel function of mammalian mitochondria. *FEBS Letters* **454**, 127–130.
- Larcher, W. (1995) Plants under stress. pp. 340–348 in *Physiological plant ecology – Ecophysiology and stress physiology of functional groups* (3rd edition). Berlin, Springer-Verlag.
- Lehmann, E. (1909) Zur Keimungsphysiologie und -biologie von *Ranunculus scleratus* L. und einigen anderen Samen. *Berichte der Deutschen Botanischen Gesellschaft* **27**, 476–494.
- Leshem, Y.Y. (1996) Nitric oxide in biological systems. *Plant Growth Regulation* **18**, 155–159.
- Leshem, Y.Y., Wills, R.B.H. and Ku, V.V. (1998) Evidence for the function of the free radical gas – nitric oxide (NO) – as an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiology and Biochemistry* **36**, 825–833.
- Millar, A.H. and Day, D.A. (1996) Nitric oxide inhibits the cytochrome oxidase but not the alternative oxidase of plant mitochondria. *FEBS Letters* **398**, 155–158.
- Nielsen, T., Pilegaard, K., Egelov, A.H., Granby, K., Hummelshøj, P., Jensen, N.O. and Skov, H. (1996) Atmospheric nitrogen compounds: occurrence, composition and deposition. *Science of Total Environment* **190**, 459–465.
- Nikolaeva, M.G., Rasumova, M.V. and Gladkova, V.N. (1985) in Danilova, F. (Ed.) *Reference book of dormant seed germination* (in Russian). Leningrad, Nauka Publishers.
- Noritake, T., Kawakita, K. and Doke, N. (1996) Nitric oxide induces phytoalexin accumulation in potato tuber tissues. *Plant and Cell Physiology* **37**, 113–116.
- O'Neill, P. (1985) *Environmental chemistry*. London, George Allen and Unwin.



- Palmer, R.M.J., Ashton, D.S. and Moncada, A.S. (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**, 664–666.
- Parmeter, J.R. and Uhrenholdt, B. (1975) Some effects of pine needle or grass smoke on fungi. *Phytopathology* **65**, 28–31.
- Pfeiffer, S., Janistyn, B., Jessner, G., Pichorner, H. and Ebermann, R. (1994) Gaseous nitric oxide stimulates guanosine-3,5-cyclic monophosphate (cGMP) formation in spruce needles. *Phytochemistry* **36**, 259–262.
- Pierce, S.M., Esler, K. and Cowling, R.M. (1995) Smoke-induced germination of succulents (Mesembryanthemaceae) from fire-prone and fire-free habitats in South Africa. *Oecologia* **102**, 520–522.
- Polglase, P.J., Attiwill, P.M. and Adams, M.A. (1986) Immobilization of soil nitrogen following wildfire in two eucalypt forests of south-eastern Australia. *Acta Oecologica-Oecologia Plantarum* **7**, 261–271.
- Pons, T.L. (1989) Breaking of seed dormancy by nitrate as a gap detection mechanism. *Annals of Botany* **63**, 139–143.
- Powlson, D.S., Saffigna, P.G. and Kragt-Cottaar, M. (1988) Denitrification at sub-optimal temperatures in soils from different climatic zones. *Soil Biology and Biochemistry* **20**, 719–723.
- Remde, A., Slemr, F. and Conrad, R. (1989) Microbial production and uptake of nitric oxide in soil. *FEMS Microbiology Ecology* **62**, 221–230.
- Reyes, O. and Casal, M. (1998) Germination of *Pinus pinaster*, *P. radiata* and *Eucalyptus globulus* in relation to amount of ash produced in forest fires. *Annales des Sciences Forestières* **55**, 837–845.
- Roberts, E.H. (1973) Oxidative processes and the control of seed germination. pp. 189–231 in Heydecker, W. (Ed.) *Seed ecology*. London, Butterworths.
- Ruge, U. (1955) Zur Analyse der Saatguterhitzung von Malvaceen. *Beiträge zur Biologie der Pflanzen* **31**, 409–417.
- Schneider, M., Luxenhofer, O., Deissler, A. and Ballschmiter, K. (1998) C<sub>1</sub>-C<sub>15</sub> alkyl nitrates, benzyl nitrate, and bifunctional nitrates: Measurement in California and South Atlantic air and global comparison using C<sub>2</sub>Cl<sub>4</sub> and CHBr<sub>3</sub> as marker molecules. *Environmental Science and Technology* **32**, 3055–3062.
- Shinomura, T., Nagatani, A., Chory, J. and Furuya, M. (1994) The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A. *Plant Physiology* **104**, 363–371.
- Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M. and Furuya, M. (1996) Action spectra for phytochrome A- and B-specific photoinduction of seed germination in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **93**, 8129–8133.
- Skiba, U., Hargreaves, K.J., Fowler, D. and Smith, K.A. (1992) Fluxes of nitric and nitrous oxides from agricultural soils in a cool temperate climate. *Atmospheric Environment, Part A – General Topics* **26**, 2477–2488.
- Slemr, F. and Seiler, W. (1991) Field study of environmental variables controlling the NO emissions from soil and the NO compensation point. *Journal of Geophysical Research* **96**, 13017–13031.
- Stöhr, C., Strube, F., Marx, G., Ulrich, W.R. and Rockel, P. (2001) A plasma-membrane bound enzyme of tobacco roots catalyses the formation of nitric oxide from nitrite. *Planta* **212**, 835–841.
- Stushnoff, C. and Hough, L.F. (1968) Response of blueberry seed germination to temperature, light, potassium nitrate and coumarin. *Proceedings of the American Society for Horticultural Science* **93**, 260–266.
- Taylor, J.L.S. and van Staden, J. (1996) Root initiation in *Vigna radiata* (L.) Wilczek hypocotyl cuttings is stimulated by smoke-derived extracts. *Plant Growth Regulation* **18**, 165–168.
- Thanos, C.A. and Rundel, P.W. (1995) Fire-followers in chaparral: nitrogenous compounds trigger seed germination. *Journal of Ecology* **83**, 207–216.
- Thanos, C.A. and Skordilis, A. (1987) The effects of light, temperature and osmotic stress on the germination of *Pinus halepensis* and *P. brutia* seeds. *Seed Science and Technology* **15**, 163–174.
- Thanos, C.A., Georghiou, K., Kadis, C. and Pantazi, C. (1992) Cistaceae: a plant family with hard seeds. *Israel Journal of Botany* **41**, 251–263.
- Thomas, P.A. and Wein, R.W. (1994) Amelioration of woody ash toxicity and jack pine establishment. *Canadian Journal of Forest Research* **24**, 748–755.
- Thomas, T.H. and van Staden, J. (1995) Dormancy break of celery (*Apium graveolens* L.) seeds by plant-derived smoke extract. *Plant Growth Regulation* **17**, 195–198.
- Thornton, F.C. and Valente, R.J. (1996) Soil emissions of nitric oxide and nitrous oxide from no-till corn. *Soil Science Society of America Journal* **60**, 1127–1133.
- Toole, E.H., Toole, V.K., Borthwick, H.A. and Hendricks, S.B. (1955) Photocontrol of *Lepidium* seed germination. *Plant Physiology* **30**, 15–21.
- Torreilles, J. (2001) Nitric oxide: one of the more conserved and widespread signaling molecules. *Frontiers in Bioscience* **6**, D1161–D1172.
- Tortoso, A.C. and Hutchinson, G.L. (1990) Contributions of autotrophic and heterotrophic nitrifiers to soil NO and N<sub>2</sub>O emissions. *Applied and Environmental Microbiology* **56**, 1799–1805.
- van Cleemput, O. and Samater, A.H. (1996) Nitrite in soils: accumulation and role in the formation of gaseous N compounds. *Fertilizer Research* **45**, 81–89.
- van Staden, J., Drewes, E.E. and Jäger, A.K. (1995a) The search for germination stimulants in plant-derived smoke extracts. *South African Journal of Botany* **61**, 260–263.
- van Staden, J., Jäger, A.K. and Strydom, A. (1995b) Interaction between plant-derived smoke extract, light and phytohormones on the germination of light-sensitive lettuce seeds. *Plant Growth Regulation* **17**, 213–218.
- Vujičić, R., Grubišić, D. and Konjević, R. (1993) Scanning electron microscopy of the seed coat in the genus *Paulownia* (Scrophulariaceae). *Botanical Journal of the Linnean Society* **111**, 505–511.
- Walter, H. (1983) *Vegetation of the earth and ecological systems of the geo-biosphere*. Berlin, Springer-Verlag.
- Wendehenne, D., Pugin, A., Klessig, D.F. and Durner, J. (2001) Nitric oxide: comparative synthesis and signaling in animal and plant cells. *Trends in Plants Science* **6**, 177–183.
- Wentworth, P., Wentworth, A.D., Zhu, X., Wilson, I.A., Janda, K.D., Eschenmoser, A. and Lerner, R.A. (2003)

- Evidence for the production of trioxxygen species during antibody-catalyzed chemical modification of antigens. *Proceedings of the National Academy of Sciences, USA* **100**, 1490–1493.
- Wicklow, D.T.** (1977) Germination response on *Emmenanthe penduliflora* (Hydrophylaceae). *Ecology* **58**, 201–205.
- Yamasaki, H., Sakihama, Y. and Takahashi, S.** (1999) An alternative pathway for nitric oxide production in plants: new features of an old enzyme. *Trends in Plant Science* **4**, 128–129.
- Yamulki, S., Harrison, R.M., Goulding, K.W.T. and Webster, C.P.** (1997): N<sub>2</sub>O, NO and NO<sub>2</sub> fluxes from a grassland: effect of soil pH. *Soil Biology and Biochemistry* **29**, 1199–1208.
- Ye, R.W., Averill, B.A. and Tiedje, J.M.** (1994) Denitrification: production and consumption of nitric oxide. *Applied and Environmental Microbiology* **60**, 1053–1058.
- Zweier, J.L., Wang, P.H., Samouilov, A. and Kuppusamy, P.** (1995) Enzyme-independent formation of nitric oxide in biological tissues. *Nature Medicine* **1**, 804–809.

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