

Fire-followers in chaparral: nitrogenous compounds trigger seed germination

C. A. THANOS* and P. W. RUNDEL

Laboratory of Biomedical and Environmental Sciences and Department of Biology, University of California Los Angeles, Los Angeles, CA 90024, USA

Summary

1 Application of nitrates (optimal concentration *c.* 10 mM) promoted seed germination in the fire annuals *Emmenanthe penduliflora* and *Phacelia grandiflora* and to a lesser extent in the fire-adapted shrub *Salvia mellifera*. Ammonium ions, although virtually inactive in *E. penduliflora*, were as effective as nitrate in both *P. grandiflora* and *S. mellifera*.

2 Diurnal illuminations and a chilling pretreatment, though of little effect by themselves, significantly enhanced the nitrate-mediated promotion of germination in the two fire annuals.

3 Nitrate was the principal factor inducing germination in *E. penduliflora* (nitrate or ammonium in *P. grandiflora*) whereas in *S. mellifera* light was the principal agent. It was also shown in *E. penduliflora* that nitrates are not required during chilling, i.e. seed sensitisation by chilling and nitrate-mediated induction of germination are entirely different mechanisms.

4 In the three species tested, the effect on seed germination caused by nitrogenous substances was nearly identical to that produced by an extract of charred wood, although chemical analysis of the extract showed that the combined concentrations of nitrates, ammonium ions and free amino acids could not account for the promotive action of charate. However, the nitrate and ammonium concentrations required to induce germination are very close to the increased values encountered after a fire in the otherwise nitrogen-poor chaparral soil. Therefore, in addition to the possible effect of charred wood, the postfire germination flush observed in chaparral may be induced by the increased levels of available nitrogen as well.

Keywords: ammonium, nitrate, *Emmenanthe penduliflora*, *Phacelia grandiflora*, *Salvia mellifera*

Journal of Ecology (1995) **83**, 207–216

Introduction

Wildfires are an integral component of ecosystem structure and function in the two major scrub vegetation types (chaparral and coastal sage scrub) which dominate the Mediterranean-type environment of central and southern California. Fires typically kill aboveground biomass in chaparral vegetation, although below-ground organs survive in resprouting species. In the first postfire year, in addition to vigorous vegetative regeneration in numerous shrub species, a flush of seed germination takes place, which is, in turn, followed by abundant seedling growth of

herbaceous and suffrutescent plants (Keeley & Keeley 1988). This postfire herbaceous flora is dominated by annuals, some of which are classed as 'fire annuals' or 'fire endemics' because, although they may dominate a site in the first year after fire, they virtually disappear within a few years, persisting only as dormant seeds in the soil until the next fire. These plants are seldom encountered in unburned disturbed sites and prominent among them are *Emmenanthe penduliflora* and several *Phacelia* species.

The soils of California are low in both total nitrogen and total phosphorus concentrations (Rundel 1982). From the differential ratios of nutrient concentrations in both leaf and stem tissues it was hypothesised that nitrogen availability might be the limiting factor for plant growth in California soils. It was further proposed that natural selection would favour

*Present address and correspondence: Department of Botany, University of Athens, Athens 15784, Greece. Fax: +30-1-7234136; E-mail: cthanos@atlas.uoa.ariadne-t.gr

adaptations for efficient uptake and utilisation of available nitrogen (Rundel 1982). Moreover, nitrate-nitrogen is of particular interest because of its apparent deficiency in many chaparral soils, in sharp contrast to its comparatively high concentrations after burning (Christensen 1973). The direct chemical changes during soil heating and combustion of soil organic matter lead to a massive volatilisation of simple nitrogenous compounds (mainly nitrate and ammonium). At the same time, fire transforms soil nitrogen bound in organic substances into ammonium, a form readily available for either plant growth or subsequent microbial nitrification (DeBano *et al.* 1979; Dunn *et al.* 1979).

During the first postfire year diverse soil measurements have shown an increase of ammonium and nitrate concentrations in many California ecosystems [e.g. in the surface of chaparral soil (Sampson 1944); in a dense chaparral dominated by chamise (Christensen 1973); in surface soils of a sequoia-mixed conifer forest (St. John & Rundel 1976); in a prescribed burn of a chamise chaparral (Rundel 1983)], as well as in other Mediterranean-type regions [e.g. in Greek phrygana (Arianoutsou-Faraggitaki & Margaritis 1982); in South African coastal fynbos (Stock & Lewis 1986); in south-eastern Australia eucalypt forests (Polglase *et al.* 1986)].

Wicklow (1977) and Jones & Schlesinger (1980) demonstrated that *Emmenanthe penduliflora*, one of the most widespread postfire chaparral herbs, is stimulated to germinate in the presence of charate, the water extract of charred wood. This germination behaviour has been further documented for nearly fifty species of chaparral herbs and shrubs (Keeley 1991), among them *Phacelia grandiflora* and several other *Phacelia* species, as well as *Salvia mellifera*. The latter is a small summer-deciduous shrub widely distributed in southern California; it is often dominant in the semi-woody coastal sage vegetation or successional to chaparral vegetation on disturbed sites (Keeley 1986). It can regenerate after a wildfire both by resprouting and reseedling; in addition, seedlings can readily establish in gaps within the unburned chaparral. This latter ability should be attributed to the marked promotion of seed germination by light, observed in laboratory experiments (Keeley 1986); buried seeds would remain dormant until after fire when water leaching through charred wood could stimulate germination (Keeley 1991).

Under the nearly closed canopy conditions in a mature chaparral, low quantity and poor quality of light, limited water availability, insufficiency of nutrients and high predation risk may have acted, singly or in various combinations, as selective forces against seedling establishment on unburned sites. Therefore, it seems that many species of annuals have evolved mechanisms that ensure seed dormancy until the canopy is removed. In particular, heat scarification of a water-impermeable seed coat and charred wood

requirement would cue germination and subsequent establishment to the postfire environment. With the exception of only a few hard-seeded species, the majority of fire-followers have seeds that can imbibe promptly; thus their germination inability is clearly due to some kind of embryonic dormancy (Sweeney 1956). The chemical conditions in the micro-environment of a seed may be a determining factor in promoting or inhibiting germination. Nitrate ion is the most common soil chemical known to promote germination in laboratory conditions. In addition to the high soil concentrations following fire, nitrate-nitrogen concentration in temperate climate fields has been shown to fluctuate seasonally, due to variations in the activity of soil micro-organisms, particularly nitrifying bacteria (Karssen & Hilhorst 1992). We therefore considered it of interest to investigate the potential role of nitrates and other simple nitrogenous compounds as postfire cues for the relief of seed dormancy and the subsequent success of seedling establishment in burned chaparral. To this end, seed germination has been tested in the charate-promoted fire annuals *Emmenanthe penduliflora* and *Phacelia grandiflora* as well as for the charate- and light-promoted, fire-adapted shrub *Salvia mellifera*.

Materials and methods

Seeds of *Emmenanthe penduliflora* Benth. (whispering bells, Hydrophyllaceae), *Phacelia grandiflora* (Benth.) Gray (large-flowered phacelia, Hydrophyllaceae) and *Salvia mellifera* Greene (black sage, Labiatae) were purchased from Theodore Payne Foundation, Sun Valley, California. During the relatively short experimental period, seeds were kept at room conditions without any alteration of their germination characteristics. The average, air dry seed weight for the three species was 0.30, 0.075 and 0.99 mg, respectively.

Germination experiments were conducted in plastic Petri dishes (5 cm in diameter), lined with two filter paper discs and moistened with 1.2 mL (1.5 mL for *S. mellifera*) of deionized water or other imbibition medium. Criterion of germination was the visible protrusion of the radicle (for the very small seeds of *P. grandiflora* a stereomicroscope was used); germination was scored regularly (daily for *E. penduliflora* and at least twice weekly for the other species) and germinated seeds were discarded promptly. Germination was expressed as a percentage from at least five samples of 20 seeds each (except for the experiments on *P. grandiflora*: in five dishes, 30–50 seeds were sown per dish and the precise number was determined at the end of the experiment). Germination was scored in a dark room with a dim flash-light filtered through layers of dark green gelatine.

Most experiments were performed in a walk-in growth chamber (A) with a constant temperature, $20 \pm 1^\circ\text{C}$ and a 12-h daily photoperiod. An incubator (B) was also used with a similar daily photoperiod of

12 h, but with a fluctuating temperature between 13 and 15 °C during night and day, respectively. Both facilities were equipped with cool white fluorescent tubes. In certain experiments some dishes were also placed outdoors (C), in a place illuminated only by diffuse skylight; during the experimentation period, temperature varied widely between 10 and 25 °C. Dark controls at all three locations were dishes placed in light-proof metal containers. The total light fluence rate in the PAR range (400–700 nm) was measured at the level of the seeds with a LI-185B quantum radiometer (LI-COR, USA) and found to be 15, 30 and 12 (a gross average) for A, B and C, respectively, compared with 1 $\mu\text{E m}^{-2} \text{s}^{-1}$ while scoring germination. Stratification of seeds was carried out at 3 ± 2 °C, in dishes within metal containers.

Chemicals of at least reagent grade (KNO_3 , NH_4Cl , NH_4NO_3 , NaNO_2 , D-glycine, D-glutamic acid, N-aceto-glucosamine and components of Hoagland solution) were used for making solutions. The pH of the solutions varied over the range 4.1–5.5 with no apparent effect on germination.

Charred wood was obtained by burning dry *Adenostoma fasciculatum* stems (0.5 cm in diameter) with a butane torch in the laboratory. For the preparation of water extracts of charate and uncharred wood the procedure described by Keeley & Nitzberg (1984) was followed (pH were 6.0 and 4.9, respectively). The determinations of $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and free-amino-acid-N were made with the steam distillation methods, adapted from Bremner (1965a,b).

Statistically significant differences were assessed using the two-sample *t*-test (for two means) and the Newman-Keuls multiple range test (for more than two means); percentage values were arcsine transformed prior to statistical tests (Zar 1974).

Results

Maximum seed germination of *Emmenanthe penduliflora* required both light and nitrates (Table 1).

Table 1 Mean (\pm SE) germinability of *Emmenanthe penduliflora* seeds (7 days after onset of imbibition) under daily light and dark alternations (L/D) or in continuous darkness (D). (A) 20 °C; (C) outdoors (10–25 °C). KNO_3 : 10 mM. Statistically different ($P < 0.05$) means within a column are followed by different letters. Parentheses denote statistical differences within rows: *** $P < 0.001$; NS, not significant

Treatment	Germination (%)	
	L/D	D
Treatment A		
H ₂ O	21.0 \pm 7.6 ^b	*** 0.0 \pm 0.0 ^b
KNO ₃	64.0 \pm 6.2 ^a	*** 7.0 \pm 3.4 ^a
Treatment C		
H ₂ O	3.0 \pm 1.2 ^c	NS 6.0 \pm 2.4 ^a
KNO ₃	30.0 \pm 7.6 ^b	*** 9.0 \pm 1.9 ^a

Nitrates alone were relatively ineffective at either temperature while illuminations alone promoted germination only at the relatively more favourable temperature regime of 20 °C and even then only for a small fraction of seed population. Maximum promotion of germination was obtained with a concentration of 10-mM KNO_3 under a daily light/dark alternation (Fig. 1). A considerable enhancement of germinability was also obtained at 20–50 mM and even at 1 mM while a concentration of 100 mM was ineffective. In a comparative experiment with various simple inorganic nitrogenous compounds, germination of *E. penduliflora* was again shown to be promoted by nitrates (NH_4NO_3 being slightly more effective than KNO_3) while nitrites and ammonia had a minimal and statistically insignificant effect (Fig. 2). Nevertheless, the presence of the latter two ions resulted in significant ($P < 0.001$) promotion of germination in combination with one or two subsequent stratification treatments (the latter being only slightly effective in water). It should be further noted that full promotion of germination was obtained in 10-mM nitrate solution after 1 week of stratification.

Light and nitrate interaction in seed germination induction was also observed in *Phacelia grandiflora* (Fig. 3). The temperature used was lower in this case since these seeds did not germinate at 20 °C; also the rate of germination was considerably lower and nearly a month was necessary for completion of germination. In this species nitrate and ammonium ions were statistically equally effective in the presence of light when

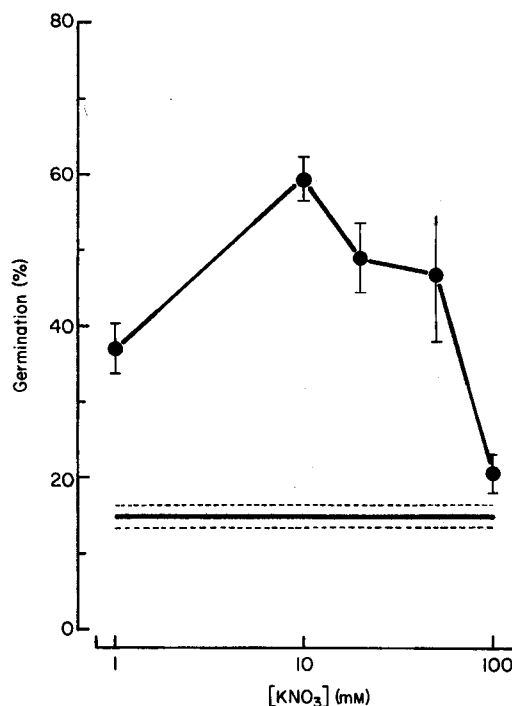


Fig. 1 Final germination (7 days after onset of imbibition under L/D at 20 °C) of *Emmenanthe penduliflora* seeds as a function of potassium nitrate concentration. The horizontal line represents final germination in water (stippled lines: \pm SE). Vertical bars: \pm SE.

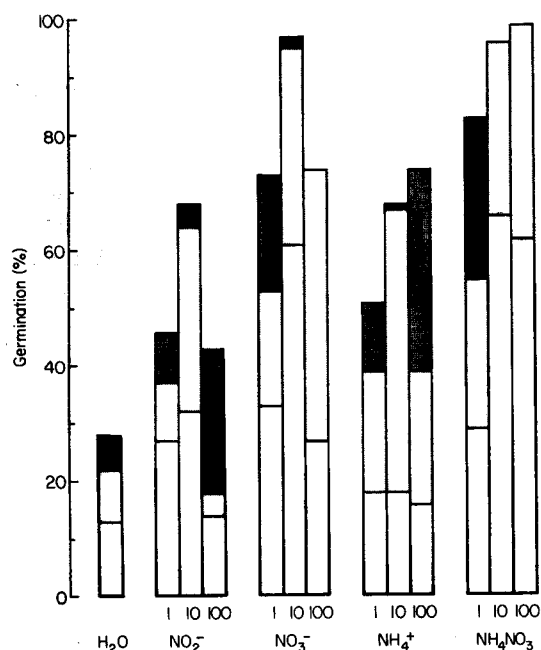


Fig. 2 The effect of various concentrations (in mM) of nitrite, nitrate and ammonium ions on the germinability of *Emmen-anthe penduliflora* seeds. Initial germination, 10 days after the onset of imbibition, under L/D at 20 °C, is shown as white bars. The ungerminated seeds were subjected to a 7-day-long stratification treatment in darkness and were subsequently transferred for 2 days at 20 °C (light grey bars); those still ungerminated were stratified for a second time (14 days) and their germination was scored after an additional, final 2-day period at 20 °C (dark grey bars).

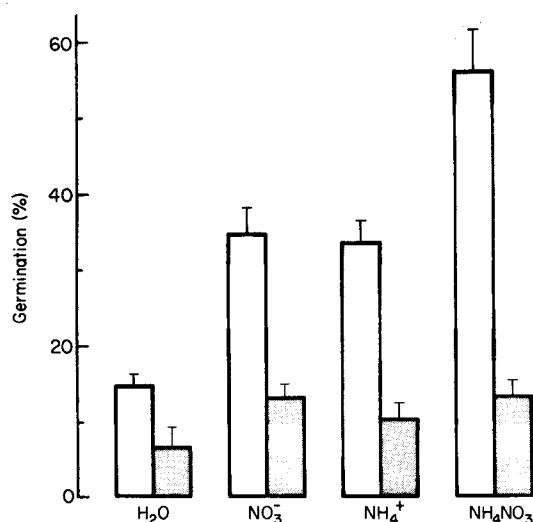


Fig. 3 The effect of light (12-h daily photoperiod) in relation to the presence of nitrate and/or ammonium ions (each at 10 mM) on the final germination (31 days after onset of imbibition, at 15/13 °C; incubator B) of *Phacelia grandiflora* seeds. Dark controls: grey bars; vertical lines: SE.

compared with water controls ($P < 0.001$) while germination in the combined presence of the two ions was statistically higher ($P < 0.001$) than with either one of them and nearly additive.

The interaction of stratification and nitrates was further investigated in *E. penduliflora* (Fig. 4). Seeds

were subjected to a 1-week- (Fig. 4A) or 2-week-long (Fig. 4B) stratification treatments in the dark before being transferred for germination under light/dark alternations at 20 °C. Induction of germination was evidently due to the presence of nitrates during the latter 'warm' period while nitrates present only during the stratification treatment were ineffective. Additional, subsequent stratification treatments (one 7-day long, Fig. 4A or two 2-day long, Fig. 4B) led to further increases of germination only in the presence of ammonium nitrate.

Seed germination of both *E. penduliflora* and *P. grandiflora* was subsequently tested under a sequence of temperature and light conditions that might roughly simulate the natural environment in the field (Fig. 5). Thus, during an initial 11-day period alternating temperatures were used to simulate late autumn conditions, then a 7-day-long stratification treatment simulated low temperatures during winter, followed by a final warm, 'spring' period at 20 °C. Final germination for both species reached very high levels (95 and 80%), particularly for seeds imbibed in potassium or ammonium nitrate while ammonium chloride was somewhat less effective (70 and 65%, respectively).

Similar experiments on the role of light and nitrates were performed with seeds of *Salvia mellifera*, at various temperature conditions (Table 2). Although both light and nitrates alone resulted in a statistically significant promotion of germination, the combined presence of these factors was most inductive. In addition, ammonium ions were found to be as effective as nitrates.

The effect of Hoagland nutrient solutions (com-

Table 2 Mean (\pm SE) germinability of *Salvia mellifera* seeds (14 days after onset of imbibition) under daily light and dark alternations (L/D) or in continuous darkness (D). (A) 20 °C; (B) 15/13 °C; (C) outdoors (10–25 °C). Statistically different ($P < 0.05$) means for each set of conditions within a column are followed by different letters. Parentheses denote statistical differences within rows: *** $P < 0.001$; ** $P < 0.01$; NS, not significant

Treatment	Germination (%)	
	L/D	D
Treatment A		
H ₂ O	64.0 \pm 3.3 ^b	*** 2.0 \pm 2.0 ^c
KNO ₃ (1 mM)		10.0 \pm 2.2 ^b
KNO ₃ (10 mM)	75.0 \pm 2.2 ^a	*** 18.0 \pm 2.5 ^b
KNO ₃ (100 mM)		7.0 \pm 2.0 ^b
NH ₄ Cl (10 mM)	81.0 \pm 4.0 ^a	*** 15.0 \pm 1.6 ^b
NH ₄ NO ₃ (10 mM)	83.0 \pm 2.0 ^a	*** 46.0 \pm 7.3 ^a
Treatment B		
H ₂ O	49.0 \pm 3.7 ^a	*** 7.0 \pm 2.0 ^c
KNO ₃ (10 mM)	63.0 \pm 5.8 ^a	** 28.0 \pm 5.4 ^b
NH ₄ NO ₃ (10 mM)	64.0 \pm 7.3 ^a	NS 49.0 \pm 3.7 ^a
Treatment C		
H ₂ O	4.0 \pm 2.4 ^b	NS 2.0 \pm 2.0 ^b
KNO ₃	48.0 \pm 8.0 ^a	NS 52.0 \pm 8.6 ^a

Fig. 4 The interaction of ammonium nitrate (10 mM) and dark stratification on the promotion of seed germination in *Emmenanthe penduliflora*, under L/D at 20 °C. The seeds were transferred (■) from H₂O to NH₄NO₃ and (▲) from NH₄NO₃ to H₂O immediately after the first stratification period, i.e. at day 7 (A) and at day 14 (B). Seeds imbibing in (▼) H₂O and (●) NH₄NO₃ throughout the experiment. Vertical lines: 2 SE. The horizontal bars at the bottom illustrate the incubation conditions; stippled areas: darkness at 3 °C; white and black bars: 12-h long, light and dark periods, respectively, at 20 °C.

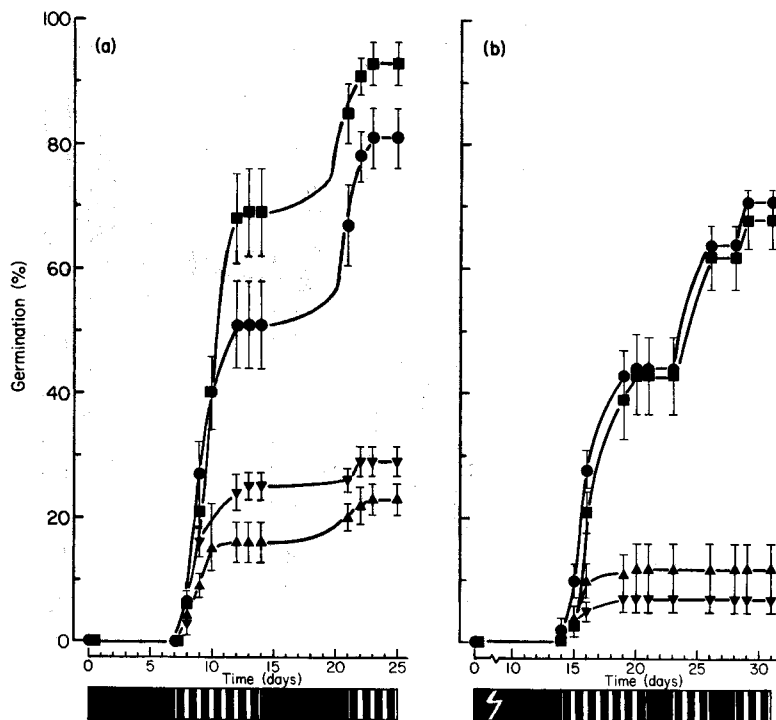
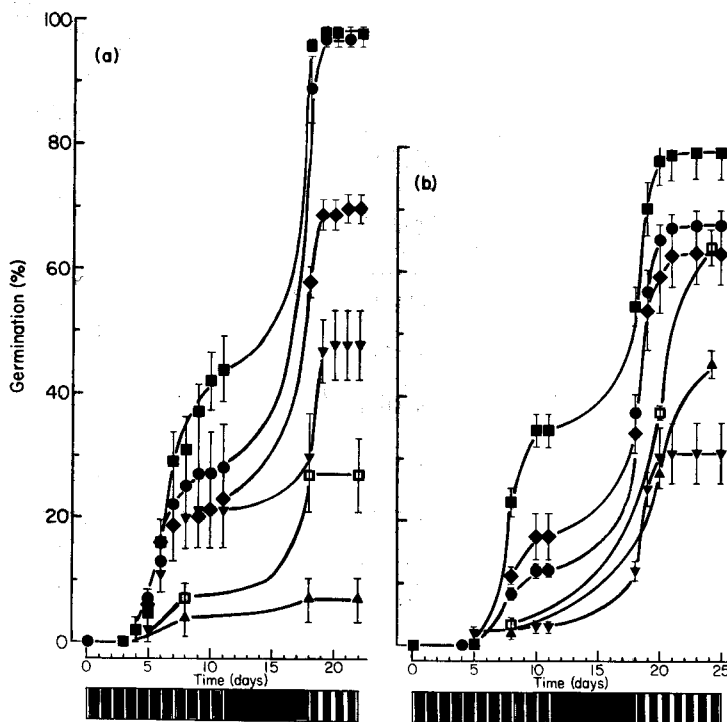


Fig. 5 The effect of nitrate and/or ammonium ions (each at 10 mM) on the germination of *Emmenanthe penduliflora* (A) and *Phacelia grandiflora* (B) seeds, under a regime of diurnally alternating temperatures (8 h 20 °C/16 h 3 °C, light during the 'warm' period), followed by a 7-day dark stratification treatment and a final period at 20 °C, L/D. Seeds imbibing in (▼) H₂O and 10-mM (●) KNO₃, (◆) NH₄Cl and (■) NH₄NO₃; and dark controls in (▲) H₂O and (□) NH₄NO₃. Vertical lines: 2 SE. The horizontal bars at the bottom illustrate the incubation conditions; stippled areas: darkness at 3 °C; white and black bars: photo- and skotoperiods, respectively, at 20 °C.



plete or nitrate deficient) on the germination of *E. penduliflora* seeds was further investigated (Fig. 6) under experimental conditions similar to those of Fig. 2. Induction of germination by a complete nutrient solution (containing 10-mM nitrates) was comparable to that obtained with a 10-mM potassium nitrate solution; similarly, a nutrient solution with only 1-mM nitrates was as effective as the 1-mM potassium nitrate solution (Fig. 1). Nutrient solution without nitrates was ineffective and showed germination similar to that in water.

The germination of *E. penduliflora* seeds was greatly enhanced in the presence of a water extract from charred wood of chamise, *Adenostoma fasciculatum* (Table 3). However, it is interesting that uncharred wood was also effective, although to a much less extent than charate. In addition to the two extracts, the effect on seed germination of three common compounds with an amino group was tested; glutamic acid was found particularly effective while glycine and glucosamine resulted in germination levels similar to the water control. The chemical analysis of the water

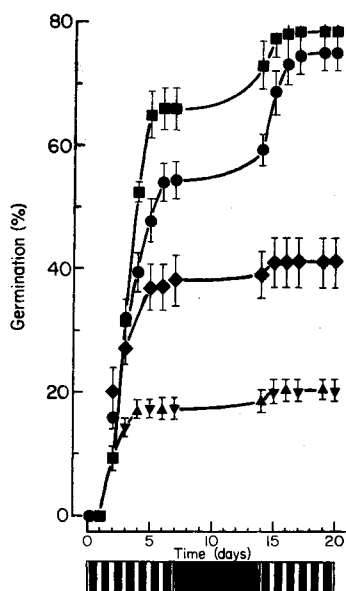


Fig. 6 The effect of a half-strength Hoagland solution with (◆) 1-mM or (■) 10-mM nitrate ions or (▼) without nitrate ions and its interaction with light and stratification on the germination of *Emmenanthe penduliflora* seeds. Controls imbibing in (▲) H₂O and (●) KNO₃ (10 mM). Vertical lines: 2 SE. The horizontal bar illustrates the incubation conditions; stippled areas: darkness at 3 °C; white and black bars: 12-h long, photo- and skotoperiods, respectively, at 20 °C.

Table 3 Mean (\pm SE) germinability of *Emmenanthe penduliflora* seeds (7 days after onset of imbibition) in various water solutions, at 20 °C under L/D. Statistically different ($P < 0.05$) means are followed by different letters

Imbibition medium	Germination (%)
Charred wood extract	81.0 \pm 6.8 ^a
Wood extract	44.0 \pm 1.9 ^b
D-glutamic acid (10 mM)	54.0 \pm 9.7 ^b
D-glycine (10 mM)	23.0 \pm 4.1 ^c
N-acetyl-D-glucosamine (10 mM)	15.0 \pm 4.5 ^c
H ₂ O	21.0 \pm 7.6 ^c

extracts of charred and uncharred wood (Table 4) revealed a significant total concentration (> 3 mM) of the three groups of nitrogen compounds (nitrate, ammonium and free amino acids) in uncharred wood; in charate the total concentration was below 1 mM with both ammonium ions and free amino acids substantially less than in uncharred wood.

Table 4 Concentration (in mM) of nitrate ions, ammonium ions and free amino acids (fAA) in water extracts from charred and uncharred wood of *Adenostoma fasciculatum*

	NO ₃ ⁻	NH ₄ ⁺	fAA
Wood extract	0.2	0.6	2.5
Charred wood extract	0.2	0.1	0.4

Discussion

SEED DORMANCY AND GERMINATION REQUIREMENTS

In the present work, as in previous reports (Sweeney 1956; Wicklow 1977; Jones & Schlesinger 1980; Keeley & Nitzberg 1984; Keeley *et al.* 1985; Keeley & Pizzorno 1986), seeds of *E. penduliflora* were found to be deeply dormant. No germination was scored at 20 °C in the dark; however, nearly 100% germination was obtained with a chilling pretreatment (1–2 weeks) and a subsequent transfer to 20 °C under diurnal light illuminations and in the presence of 10-mM nitrate. A similarly high final percentage (almost 90%) of germination has previously been observed only with seeds imbibing in a charred wood extract during a 3-week-long stratification period in the dark (Keeley & Pizzorno 1986).

A similar set of conditions (a pretreatment of alternating temperatures followed by one week of chilling and a subsequent transfer to 20 °C under diurnal light illuminations and in the presence of 10-mM nitrate and ammonium ions) were found to induce almost 80% germination in the notoriously deeply dormant seeds of *P. grandiflora*. The highest level of germination reported previously for this species is only 21% and was obtained with charate after 20 days of chilling and a subsequent dark incubation (Keeley *et al.* 1985). Similarly low enhancement of germinability by charate was also observed with several additional chaparral species of *Phacelia* (Keeley *et al.* 1985) while stratification has been suggested for seed germination of certain *Phacelia* species of California (Emery 1988). Imamura (1971) reported that scarified *P. grandiflora* seeds remained dormant under high (summer) temperatures but germinated after being exposed to alternating high and low temperatures. Nevertheless, light seems to be an important factor for the germination of the relatively small seeds of *Phacelia*. In particular, after-ripened seeds of *Phacelia ranunculacea*, a mesic woodland winter annual, germinate better in light than in darkness (Baskin *et al.* 1993) while, in contrast, *Phacelia tanacetifolia* seed germination is inhibited by light (Schulz & Klein 1963).

Seed germination of *Salvia mellifera*, a plant which normally regenerates mainly by seeds but is not a fire specialist in the way the two other species are, was not investigated in detail. However, the results obtained confirm the conclusion of Keeley (1986) concerning with the importance of light for germination induction. In addition, ammonium nitrate was found to enhance germination further although its effect was clearly of lesser magnitude than that of light, as was the previously reported effect of charate (Keeley 1986). Prechilling and, to a lesser extent, light, has been suggested for most *Salvia* species (Emery 1988; ISTA 1993), while for *Salvia mellifera* in particular,

germination may be improved by either 3 months of stratification or 1 h presoaking in 400 p.p.m. GA_3 (Emery 1988).

Sweeney (1956) found that seeds of virtually all fire specialists tested (including *E. penduliflora* and *Phacelia heterophylla*) could withstand temperatures similar to those a shallow buried seed might experience in a field fire; moreover, they could be stimulated to germinate by seed coat scarification, although imbibition measurements clearly showed that their seed coats are not water-impermeable. An alternative hypothesis that the seed coat acts as a barrier to oxygen uptake was not supported by incubation of *E. penduliflora* seeds in atmospheres with 90% oxygen; the seeds remained dormant (Keeley 1991). Thus, it is presently suggested that the seed coat may act as a mechanical barrier that restrains radicle expansion and eventually the manifestation of germination; the disruption of the seed coat integrity brought about by scarification, results in a very rapid radicle elongation and consequent seedling growth (Sweeney 1956; Jones & Schlesinger 1980; C. A. Thanos, unpublished).

THE ROLE OF LIGHT AND STRATIFICATION

Light requirement of seed germination is considered as a potentially significant mechanism for gap detection (Pons 1992) and this suggestion could well also extend to burned areas (as shown previously with Mediterranean plants: Roy & Arianoutsou-Faraggitaki 1985; Thanos & Skordilis 1987). From seedling emergence experiments with soil from mature or burned chaparral (Keeley 1984), light was considered a significant factor for seed germination induction in postfire chaparral ecosystems. Changes of light quantity and, more importantly, quality can be encountered in the postfire environment as a result of soil disturbance and/or removal of the 'far-red-enrichment filters' of the canopy and the litter. Nevertheless, in the present work illumination of all three species was an important inducing agent of germination but clearly not the critical one for the two fire annuals. In the ecological context this result is consistent with the fact that, throughout the interfire period, seeds of fire annuals are known to accumulate in a persistent soil seed bank; thus, seeds would eventually be placed well below the soil surface, in virtual darkness, where the fire event could not be perceived, as a general rule, in terms of a light quality change.

Stratification by itself or in combination with light, resulted in only a minimal (10–30%) enhancement of germination for both *E. penduliflora* and *P. grandiflora*. It seems that the role of chilling is to 'sensitize' these seeds, rendering them more responsive to an ensuing enhancing agent. An interesting conclusion from the ecological standpoint (Fig. 4) is that seeds can be stratified repeatedly in the dark, while buried in the soil; the stratification 'advantage' is known to persist during desiccation so after a fire they may be

already responsive to light as well as to relatively large temperature fluctuations and various promoting chemicals. It was also shown clearly in *E. penduliflora* that nitrates are not required during chilling. The finding (Goudey *et al.* 1988) that nitrates are moving passively in and out of the seed, depending on the external concentration, is consistent with the present finding that transfer of seeds stratified in nitrate to water had no effect on germination; this implies that the ions were 'excreted' upon transfer to water and therefore were not present when required to induce germination.

NITRATE AND AMMONIUM AS GERMINATION PROMOTERS

Compared with light and stratification, nitrates were by far more potent inducing factors for the two annuals tested. Ammonium ions, though virtually inactive in *E. penduliflora*, were as effective as nitrates in both *P. grandiflora* and *S. mellifera*. In all three cases, the effect on seed germination caused by nitrate and ammonium is nearly identical to that produced by charate in laboratory experiments. It is therefore suggested that nitrogenous compounds, either alone or in combination with charate, are the inducing agents of postfire seed germination in the field; the ecological plausibility of such a postulate will be discussed later.

According to Mayer & Poljakoff-Mayber (1989) the effect of potassium nitrate on germination was discovered when it was noted that Knop's nutrient solution promoted germination of some seeds. The stimulatory effect of nitrates and other simple nitrogenous compounds (thiourea, ammonium salts, nitrites, hydroxylamine) on seed germination of numerous, mostly weedy, plant species is, nowadays, well documented (e.g. Roberts & Smith 1977; Vincent & Roberts 1977). In addition, both the American and the International Associations for seed testing have, since 1954, adopted officially the systematic use of 0.2% (about 20 mM) KNO_3 in their suggested germination protocols for many species (AOSA 1981, ISTA 1993). A curve of seed germination induction as a function of nitrate concentration similar to that obtained with *E. penduliflora* has been observed in numerous cases (a nearly identical response was reported for *Sinapis arvensis* by Goudey *et al.* 1987). Promotion by nitrates is obtained in the range 0–50 mM (optimum around 10–20 mM) while higher concentrations are gradually less efficient and eventually inhibitory when compared with water controls (Karssen & Hilhorst 1992). Light, temperature shifts and various nitrogenous compounds (a mixture of nitrate and ammonium being most effective) result in full induction of germination when applied together while they have little or no promotive effect on seed germination when given singly.

Early hypotheses on the role of nitrates as a dormancy breaking agent suggested nitrate involvement

in various alternative metabolic processes. However, in most relevant works, nitrate stimulation of germination showed additive effects or positive interactions with light and in many cases with chilling and alternating temperatures as well (e.g. Vincent & Roberts 1977; Bewley & Black 1982). Therefore, an integrative model has recently been formulated (Hilhorst 1990a,b), according to which all these factors interact at a common site, the plasma membrane.

GERMINATION INDUCTION BY NITRATE AND CHARATE IN THE POSTFIRE ENVIRONMENT

Christensen (1973) and Christensen & Muller (1975) analysed the ash of *Adenostoma fasciculatum* plants and found $127 \mu\text{g g}^{-1}$ of exchangeable ammonium and $1.3 \mu\text{g g}^{-1}$ of water-soluble nitrate. On the basis of a soil water holding capacity value of around 35% (Herman 1987), these concentrations correspond to about 20 and 0.06 mM of ammonium and nitrate, respectively. It has previously been shown that completely ashed wood has no effect in inducing seed germination of *E. penduliflora* (Sweeney 1956; Wicklow 1977; Jones & Schlesinger 1980); this ineffectiveness could be readily explained by the present results since nitrates are virtually absent in ash and ammonium cannot promote either seed germination or shoot growth of *E. penduliflora*. On the other hand, germination of *P. grandiflora* and *S. mellifera* which was found to be equally promoted by ammonium and nitrate would be expected to be induced even by ash, a matter that needs further investigation.

The effect of Hoagland solutions (complete or nitrate-deficient) on the germination of *E. penduliflora* seeds (Fig. 6) showed clearly that a complete Hoagland solution behaves as if it contained only nitrates. Previous experiments involving application of nutrients, either within Petri dishes in laboratory experiments (with seeds of the charate-promoted species *Chaenactis artemisiaefolia* and *Eriophyllum confertiflorum*; Keeley *et al.* 1985) or by watering greenhouse pots (Christensen & Muller 1975) and field plots (McPherson & Muller 1969; Christensen & Muller 1975) showed no effect on germination. Nevertheless, these negative results can be attributed to the absence of one or both of the other two factors needed for germination induction (i.e. light and suitable temperatures).

The active component of charate was found to be water soluble (Keeley & Nitzberg 1984) and an oligosaccharin-type molecule (a product of heated xylan and other hemicelluloses) was postulated as the triggering factor for seed germination induction (Keeley & Pizzorno 1986). Nevertheless, recent studies (Keeley 1991) have found that the active compounds are also extractable in non-polar solvents; it was therefore concluded that more than a single compound may be involved. In the present study, the germination of *E. penduliflora* was dramatically

enhanced by a water extract from charred *Adenostoma fasciculatum* wood. It is interesting though that even uncharred wood had some effect, in agreement with a previously reported slight promotion by wood extracts and even by leachates from living *Adenostoma fasciculatum* foliage (Keeley *et al.* 1985). Three other compounds with an amino group were tested and glutamic acid was found significantly active while glycine and glucosamine had no effect. Glutamic acid and other amino acids have only rarely been investigated as potential stimulators although certain results showed a promoting action (e.g. Ogawara & Ono 1955). Therefore, in order to test the possible presence of nitrogenous compounds in the charate, a chemical analysis of its constituents was undertaken. The combined concentrations of nitrates, ammonium ions and free amino acids found in the extract of uncharred wood could explain the moderate effect of the latter. However, both ammonium and amino acids were lower in charred wood, in contrast to the expectation that free amino acids, at least, would increase considerably (as a result of the heat degradation of wood proteins). Since it is still probable that the promotive effect of charate is due to a single or a combination of nitrogenous compounds, more rigorous analytical work on charate is needed; however, the postfire flush of germination could be readily attributed to the increased nitrate and ammonium concentration in the burned chaparral soils.

It is interesting to note that the critical nitrate concentration range for germination induction, revealed from laboratory experiments, is spectacularly close to that encountered in both natural and agricultural ecosystems. For instance, nitrate concentrations likely to exist under fertilised conditions range between 1 and 10 mM (Young & Aldag 1982). Several measurements in various ecosystems have shown both a seasonal fluctuation of nitrate concentration (e.g. Popay & Roberts 1970) and a depletion of available nitrates in the presence of actively growing plants (Pons 1989). Thus, a dormancy relief mechanism triggered by an appropriate level of nitrates would serve as either a seasonal timer [e.g. *Capsella bursa-pastoris* (Popay & Roberts 1970); *Cynoglossum officinale* (Freijsen *et al.* 1980); *Sinapis arvensis* (Goudey *et al.* 1988)] or a competition-avoiding, gap detector (e.g. *Plantago lanceolata*, Pons 1989).

Successive measurements of ammonium-N and nitrogen-N in the soil from burned and unburned chaparral sites have revealed that in the top soil (0–2 cm) ammonium concentrations were much higher in the burned soil throughout the 18-month-long study period; also nitrates in the burned soil increased considerably after the first autumn rains (Christensen 1973; Christensen & Muller 1975). By using a water holding capacity value of 35% [the average value of those measured by Herman (1987) in burned and unburned chaparral soils, in Sequoia National Park, California], we obtained estimates of actual con-

centrations: ammonium concentration range, 0.5–4 mM (unburned) and 12–40 mM (burned soil); nitrates, almost always below 1 mM (unburned) and 2–7 mM (burned). In four alternate sample sites, where measurements were also carried out, the absolute values varied from site to site but the same general trends were clearly observed. Thus, ammonium concentrations varied between 5 and 7 mM and 10–20 mM in unburned and adjacent burned sites, respectively, while the corresponding nitrate concentration ranges were 0.2–0.5 mM and 3–6 mM (Christensen & Muller 1975). In soils of a Sierra Nevada sequoia-mixed conifer forest, sampled to a depth of 10 cm, it was found (St. John & Rundel 1976) that nitrate levels were initially higher in the burned plot and remained higher through most of the study (October values were about 1.5 and 2.5 mM, for unburned and burned sites, respectively). The increase in ammonium was again dramatic (18 mM as opposed to only 3 mM in unburned plots). Additional results by Rundel (1983) for a prescribed burn in a chamise chaparral, showed similar trends. Ammonium and nitrate concentrations (soils sampled to a depth of 5 cm) ranged between 4.5–9 mM and 2.5–4 mM, respectively, in burned sites while the corresponding values in the unburned plots remained rather constant: 2 mM and 1.5 mM, respectively. Therefore, on the basis of the above data, it might be concluded that the postfire nitrate or nitrate + ammonium concentrations can fully satisfy the germination requirements of the three species studied. On the other hand, in mature chaparral sites, the scarcity of germination could be attributed to the considerably lower concentrations in combination, particularly, with the unfavourable light environment.

CONCLUSIONS

In the evolutionary and ecological context selection may have favoured seeds whose usable internal reserves complement those in the soil, thus facilitating the initial growth of the seedling. An alternative would be the substitution of stored nutrients by a physiological mechanism able to monitor the environmental concentrations of the essential nutrient and trigger or suppress germination and subsequent growth as appropriate. Such a mechanism would bear an obvious ecological advantage as far as the plant energy budget is concerned, since such a detection and trigger mechanism would have a negligible cost; plants could take advantage of the savings by either producing more seeds or investing extra reserves on the protective structures of seeds.

It is concluded that the postfire chaparral environment is exploited by the fire annuals *E. penduliflora* and *P. grandiflora* and, to a lesser extent, by the fire-adapted shrub *S. mellifera* through seed germination strategies which monitor the chemical composition of soil (especially the nitrogenous compounds and/or

other substances produced by charred wood), in addition to detecting light quality and temperature conditions.

Acknowledgements

We thank Wade Berry, Don Herman, Cheryl Swift and Rasoul Sharifi for help and advice. This research was supported in part by the Ecological Research Division of the Office of Health and Environmental Research of the US Department of Energy.

References

- Arianoutsou-Faraggitaki, M. & Margaris, N.S. (1982) Decomposers and the fire cycle in a phrygane (East Mediterranean) ecosystem. *Microbial Ecology*, **8**, 91–98.
- Association of Official Seed Analysts (1981) Rules for testing seeds. *Journal of Seed Technology*, **6**, 1–125.
- Baskin, C.C., Baskin, J.M. & Chester, E.W. (1993) Seed germination ecology of two mesic woodland winter annuals, *Nemophila aphylla* and *Phacelia ranunculacea* (Hydrophyllaceae). *Bulletin of the Torrey Botanical Club*, **120**, 29–37.
- Bewley, J.D. & Black, M. (1982) *Physiology and Biochemistry of Seeds in Relation to Germination*, Vol. 2. *Viability, Dormancy, and Environmental Control*. Springer-Verlag, Berlin, Heidelberg, New York.
- Bremner, J.M. (1965a) Inorganic forms of nitrogen. *Methods of Soil Analysis*, Part 2. *Chemical and Microbiological Properties*. *Agronomy* 9 (eds C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger & F.E. Clark), pp. 1179–1237. American Society of Agronomy, Madison.
- Bremner, J.M. (1965b) Inorganic forms of nitrogen. *Methods of Soil Analysis*, Part 2. *Chemical and Microbiological Properties*. *Agronomy* 9 (eds C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger & F.E. Clark), pp. 1238–1255. American Society of Agronomy, Madison.
- Christensen, N.L. (1973) Fire and the nitrogen cycle in California chaparral. *Science*, **181**, 66–68.
- Christensen, N.L. & Muller, C.H. (1975) Effects of fire on factors controlling plant growth in *Adenostoma* chaparral. *Ecological Monographs*, **45**, 29–55.
- DeBano, L.F., Eberlein, G.E. & Dunn, P.H. (1979) Effects of burning on chaparral soils: I. Soil nitrogen. *Soil Science Society of America Journal*, **43**, 504–509.
- Dunn, P.H., DeBano, L.F. & Eberlein, G.E. (1979) Effects of burning on chaparral soils: II. Soil microbes and nitrogen mineralization. *Soil Science Society of America Journal*, **43**, 509–514.
- Emery, D.E. (1988) *Seed Propagation of Native California Plants*. Santa Barbara Botanic Garden, Santa Barbara.
- Freijsen, A.H.J., Troelstra, S.R. & Van Kats, M.J. (1980) The effect of soil nitrate on the germination of *Cynoglossum officinale* L. (Boraginaceae) and its ecological significance. *Acta Oecologica/Oecologia Plantarum*, **1**, 71–79.
- Goudey, J.S., Saini, H.S. & Spencer, M.S. (1987) Seed germination of wild mustard (*Sinapis arvensis*): factors required to break primary dormancy. *Canadian Journal of Botany*, **65**, 849–852.
- Goudey, J.S., Saini, H.S. & Spencer, M.S. (1988) Role of nitrate in regulating germination of *Sinapis arvensis* L. (wild mustard). *Plant Cell and Environment*, **11**, 9–12.
- Herman, D.J. (1987) *Nitrogen isotope fractionation in burned and unburned chaparral soils*. MSc Thesis, University of California, Riverside.

- Hilhorst, H.W.M. (1990a) Dose-response analysis of factors involved in germination and secondary dormancy of seeds of *Sisymbrium officinale* I. Phytochrome. *Plant Physiology*, **94**, 1090–1095.
- Hilhorst, H.W.M. (1990b) Dose-response analysis of factors involved in germination and secondary dormancy of seeds of *Sisymbrium officinale* II. Nitrate. *Plant Physiology*, **94**, 1096–1102.
- Imamura, E. (1971) *Floristic and ecological studies of chaparral communities in the Santa Barbara area, California*. MA thesis, San Francisco State University.
- International Seed Testing Association (1993) International rules for seed testing. Rules & Annexes 1993 *Seed Science and Technology, Supplement*, **21**, 1–288.
- Jones, C.S. & Schlesinger, W.H. (1980) *Emmenanthe penduliflora* (Hydrophyllaceae): further considerations of germination response. *Madroño*, **27**, 122–125.
- Karssen, C.M. & Hilhorst, H.W.M. (1992) Effect of chemical environment on seed germination. *Seeds. The Ecology of Regeneration in Plant Communities* (ed. M. Fenner), pp. 327–348. CAB International, Wallingford, UK.
- Keeley, J.E. (1984) Factors affecting germination of chaparral seeds. *Bulletin of Southern California Academy of Sciences*, **83**, 113–120.
- Keeley, J.E. (1986) Seed germination patterns of *Salvia mellifera* in fire-prone environments. *Oecologia*, **71**, 1–5.
- Keeley, J.E. (1991) Seed germination and life history syndromes in the California chaparral. *Botanical Review*, **57**, 81–116.
- Keeley, J.E. & Keeley, S.C. (1988) Chaparral. *North American Terrestrial Vegetation* (eds M.G. Barbour & W. D. Billings), pp. 165–207. Cambridge University Press, Cambridge.
- Keeley, J.E., Morton, B.A., Pedrosa, A. & 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, J.E. & Nitzberg, M.E. (1984) Role of charred wood in the germination of the chaparral herbs *Emmenanthe penduliflora* (Hydrophyllaceae) and *Eriophyllum confertiflorum* (Asteraceae). *Madroño*, **31**, 208–219.
- Keeley, S.C. & 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.
- Mayer, A.M. & Poljakoff-Mayber, A. (1989) *The Germination of Seeds*, 4th edn. Pergamon Press, Oxford.
- McPherson, J.K. & Muller, C.H. (1969) Allelopathic effects of *Adenostoma fasciculatum*, 'chamise', in the California chaparral. *Ecological Monographs*, **39**, 177–198.
- Ogawara, K. & Ono, K. (1955) Effects of various nitrogen compounds and respiratory intermediates on the germination of the light-favored tobacco seeds. *Bulletin of School of Education, Okayama University*, **1**, 97–104.
- Polglase, P.J., Attiwill, P.M. & 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.
- Pons, T.L. (1992) Seed responses to light. *Seeds. The ecology of regeneration in plant communities*. (ed. M. Fenner), pp. 259–284. CAB International, Wallingford, UK.
- Popay, A.I. & Roberts, E.H. (1970) Ecology of *Capsella bursa-pastoris* (L.) Medik. and *Senecio vulgaris* L. in relation to germination behaviour. *Journal of Ecology*, **58**, 123–139.
- Roberts, E.H. & Smith, R.D. (1977) Dormancy and the pentose phosphate pathway. *The physiology and biochemistry of seed dormancy and germination* (ed. A. A. Khan), pp. 385–411. North-Holland, Amsterdam, New York, Oxford.
- Roy, J. & Arianoutsou-Faraggitaki, M. (1985) Light quality as the environmental trigger for the germination of the fire-promoted species *Sarcopoterium spinosum* L. *Flora*, **177**, 345–349.
- Rundel, P.W. (1982) Nitrogen utilization efficiencies in mediterranean-climate shrubs of California and Chile. *Oecologia*, **55**, 409–413.
- Rundel, P.W. (1983) Impact of fire on nutrient cycles in mediterranean-type ecosystems with reference to chaparral. *Mediterranean-type ecosystems* (eds F.J. Kruger, D.T. Mitchell & J.U.M. Jarvis), pp. 192–207. Springer-Verlag, Berlin.
- Sampson, A.W. (1944) *Plant succession and burned chaparral lands in northern California*. Bulletin 685, Agricultural Experiment Station, University of California, Berkeley.
- Schulz, M.R. Sr & Klein, R.M. (1963) Effects of visible and ultraviolet radiation on the germination of *Phacelia tanacetifolia*. *American Journal of Botany*, **50**, 430–434.
- St. John, T.V. & Rundel, P.W. (1976) The role of fire as a mineralizing agent in a Sierran coniferous forest. *Oecologia*, **25**, 35–45.
- Stock, W.D. & Lewis, O.A.M. (1986) Soil nitrogen and the role of fire as a mineralizing agent in a South African coastal fynbos ecosystem. *Journal of Ecology*, **74**, 317–328.
- Sweeney, J. (1956) Responses of vegetation to fire. A study of the herbaceous vegetation following chaparral fires. *University of California Publications in Botany*, **28**, 143–250.
- Thanos, C.A. & 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.
- Vincent, E.M. & Roberts, E.H. (1977) The interaction of light, nitrate and alternating temperature in promoting the germination of dormant seeds of common weed species. *Seed Science and Technology*, **5**, 659–670.
- Wicklow, D.T. (1977) Germination response in *Emmenanthe penduliflora* (Hydrophyllaceae). *Ecology*, **58**, 201–205.
- Young, J.L. & Aldag, R.W. (1982) Organic forms of nitrogen soils. *Nitrogen in Agricultural Soils* (ed. F. J. Stevenson), pp. 43–66. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison.
- Zar, J.H. (1974) *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, N.J.

Received 27 April 1994

Revised version accepted 5 August 1994