

Hydrophyllaceae Seeds and Germination

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ABSTRACT

Germination of members of the Hydrophyllaceae family has been extensively studied, to shed light on basic germination mechanisms and to determine the evolved reproductive strategies that enable species to adapt to different environmental conditions. The effect of environmental factors on seed development and on seed soil permanence has been studied in *Hydrophyllum appendiculatum*, *Phacelia secunda* and *Wigandia urens*. Metabolic changes of osmotically conditioned *W. urens* were also investigated. The effect of fire on seed germination has been studied mainly in *Emmenanthe penduliflora* and several *Phacelia* species. These studies include the effect of smoke products on germination of different Hydrophyllaceae species. The mechanisms involved, and the effect of the putative main promoter of smoke have also been determined in several Hydrophyllaceae. The basic mechanisms of dormancy in relation to light and temperature inhibition have been determined in seeds of *Nemophila insignis* and *Phacelia tanacetifolia*.

Keywords: *Emmenanthe*, negative photoblastism, *Nemophila*, *Phacelia*, smoke

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GENERAL ASPECTS OF THE HYDROPHYLLACEAE FAMILY

The Hydrophyllaceae family is distributed mainly in West America, with 300 herbs and shrubs included in 18 genera (*Codon*, *Draperia*, *Ellisia*, *Emmenanthe*, *Eriodictyon*, *Eucrypta*, *Hesperochiron*, *Hydrolea*, *Hydrophyllum*, *Lemmonia*, *Nama*, *Nemophila*, *Phacelia*, *Pholistoma*, *Romanzoffia*, *Tricardia*, *Turricula*, *Wigandia*). These are grouped in the tribes Hydrophyllaeae, Phacelieae, Nameae and Hydroleae (Constance 1963). The genus *Phacelia* is the largest and most diverse member of the family Hydrophyllaceae. The members of this family are mostly annuals as *Nama dichotomum*, *Nemophila aphylla* and *Phacelia ranunculaceae* (Baskin *et al* 1993; Ochoa-Gaona 2001), biennials such as *Hydrophyllum appendiculatum* (Constance 1942) and perennial herbs such as *Phacelia secunda* and *Nama lobbii* (Nord and Leisser 1974; Cavieres and Arroyo 2001); occasionally they are shrubs such as *Eriodictyon* spp. (Abrams and Smiley 1915), or trees such as *Wigandia urens* (Ochoa-Gaona 2001).

California is the centre of diversity of this family, where 13 genera and 139 species are native. There is one genus endemic to southern Africa (*Codon*); another is distributed pantropically (*Hydrolea*). Evolutionary relationships within the angiosperm family Hydrophyllaceae have been analyzed (Ferguson 1999). Mature seeds of the Hydrophyllaeae tribe,

which includes the five genera *Hydrophyllum*, *Ellisia*, *Nemophila*, *Pholistoma*, and *Eucrypta*, are mainly light brown to black, 1-3.5 mm in diameter and 0.8 to 4 mm long, and lack a cucullus (**Table 1**). The seeds of *Nemophila* species have a cucullus that may be conspicuous. Primary dispersal is generally autochorous, as in *Nemophila menziesii*, or anemochorous as in *W. urens* (**Fig. 1**, Cruden 1974, pers. obs.), but secondary dormancy is related to the presence of an elaiosome, as in *Nemophila* species, which may be significant in seed dispersal by ants (Werker 1997). The elaiosome may be the result of outgrowth of the exostome; this structure has been also reported as a caruncle (exostome aril), by Chandler (1902). In the other four genera, the seed coat surfaces are distinctly reticulate, with hexagonal or pentagonal chambers (Chuang and Constance 1992). The seed coat is important for systematic analysis in Hydrophyllaceae, as in *Nama* (Chance and Bacon 1984). Also, Hydrophyllaceae seeds have a copiously oily endosperm, which may be: mononuclear (as in *Phacelia malvifolia*), three-cellular (helobial endosperm is reported only for *Phacelia parryi*), of the *Phacelia* type (as in *Romanzoffia*), and of the *Nemophila* type (as in *Nemophila* species); embryos may be chlorophyllous or achlorophyllous (Svensson 1925 cited in Berg 1985; Werker 1997; Watson and Dallwitz 2007). In this family the origin and the timing of endosperm development distinguishes the Hydrophyllaceae tribes (Berg 1985). The family includes dwarf, linear and spatula

Table 1 Characteristics of Hydrophyllaceae seeds.

Genus	Species	Seeds/ capsule	Seed size (mm)	Colour	Other traits	Reference
<i>Draperia</i>	---	4	---	---	---	Hitchcock 1933
<i>Ellisia</i>	---	---	1-3.5 L, 0.8-4 D	light brown-black	no cucullus	Constance 1939
	<i>nyctelea</i>	4	2-3 D	dark brown	---	Chuang and Constance 1992
<i>Eriodictyon</i>	<i>angustifolium</i>	---	---	black	---	Abrams and Smiley 1915
	<i>californicum</i>	6-12	---	brown	---	Abrams and Smiley 1915
	<i>sessilifolium</i>	---	---	dark brown-black	---	Abrams and Smiley 1915
	<i>trichocalyx</i>	----	---	black	---	Abrams and Smiley 1915
<i>Eucrypta</i>	<i>chrysanthemifolia</i>	8	1-1.5	dark brown-black	no cucullus	Constance 1938; Chuang and Constance 1992
	<i>micrantha</i>	7-15	>1	dark brown-black	no cucullus	Constance 1938; Chuang and Constance 1992
<i>Hydrolea</i>	---	10-50	---	---	---	Coleman 2008
<i>Hydrophyllum</i>	---	1-3	2-3.5 D	---	no cucullus	Chuang and Constance 1992
	<i>appendiculatum</i>	1	2 D	reddish- brown	no cucullus	Constance 1942
	<i>canadense</i>	1-2	3-4D	light-dark brown	no cucullus	Constance 1942
	<i>capitatum</i>	2	2-3 D	light brown	no cucullus	Constance 1942
	<i>fenleri</i>	1-3	2.5-3 D	light brown	no cucullus	Constance 1942
	<i>occidentale</i>	1-2	3	brown	no cucullus	Constance 1942
	<i>tenuipes</i>	1	3.5 D	yellowish-reddish- brown	no cucullus	Constance 1942
	<i>virginianum</i>	2	2-3.5 D	light brown	no cucullus	Constance 1942
<i>Lemmonia</i>	---	4	---	dark brown-black	---	Bacon 1987, Hitchcock 1933
<i>Nama</i>	<i>aretioides</i>	10-35	---	---	---	Hitchcock 1933
	<i>biflorum</i>	40-100	---	brown	---	Hitchcock 1933
	<i>densum</i>	14-16	~0.6 L	dark brown	---	Hitchcock 1933
	<i>dichotomum</i>	20-70	0.3-0.8	brown	---	Ochoa-Gaona 2001
	<i>havardii</i>	40-60	~0.6 L	brown	---	Hitchcock 1933
	<i>hirstum</i>	4-12	~0.7 L	brown	---	Hitchcock 1933
	<i>hispidum</i>	---	0.3-1.5 D	yellow	---	Chance and Bacon 1984
	<i>jamaicense</i>	---	---	brown	---	Chance and Bacon 1984
	<i>lobbii</i>	8-14	1.4-1.5 L	dark brown	---	Chance and Bacon 1984
	<i>organifolium</i>	20-60	0.4-0.1, L, 0.1 W	brown-black	---	Hitchcock 1933; Ochoa-Gaona 2001
	<i>palmeri</i>	12-40	~1 L	brown	---	Hitchcock 1933
	<i>purpusii*</i>	80	---	---	---	Hitchcock 1933
	<i>rothrockii</i>	16-20	1.5 L, 1.4 D	brown	---	Hitchcock 1933; Chance and Bacon 1984
	<i>rotundifolium</i>	~150	0.3 L	brown	---	Hitchcock 1933
	<i>sandwicense</i>	---	0.3-1.5 D	grey white	---	Chance and Bacon 1984
	<i>sericeum</i>	100-130	0.5-0.7 L	brown	---	Hitchcock 1933
	<i>spathulatum</i>	40-50	~1 L	brown	---	Hitchcock 1933
	<i>stenocarpum</i>	120-250	0.3 L	straw colored	---	Hitchcock 1933
	<i>stenophyllum</i>	30-40	---	light brown	---	Hitchcock 1933
	<i>stevensii</i>	---	0.3-1.5 D	yellow	---	Chance and Bacon 1984
	<i>turneri</i>	---	0.3-1.5 D	yellow	---	Chance and Bacon 1984
	<i>undulatum</i>	80-260	0.4-0.6 L, 0.2 W	light yellow	---	Ochoa-Gaona 2001
	<i>xylopodum*</i>	~150	0.5 L	yellow	---	Hitchcock 1933
<i>Nemophila</i>	<i>aphylla</i>	1-4	2-4 L	---	cucullus	Chuang and Constance 1992
	<i>atomaria</i>	---	---	---	cuculus	Cruden 1974
	<i>aurita</i>	4	1.2-3 D	---	no caruncle	Chandler 1902
	<i>breviflora</i>	1	2-3.5 D	blood red	caruncle	Chandler 1902
	<i>exilis</i>	2-5	---	---	caruncle	Chandler 1902
	<i>heterophylla</i>	1-4	2-4 L	---	cuculus	Chuang and Constance 1992
	<i>integrifolia</i>	---	---	---	cuculus	Cruden 1974
	<i>kirtleyi</i>	2-4	2-3 L	---	caruncle	Chandler 1902
	<i>maculata</i>	5-12	1.5-3 L	---	---	Chandler 1902
	<i>menziesii</i>	5-25	1.2 L	---	caruncle	Chandler 1902
	<i>microcalyx</i>	1-2	---	---	---	Chandler 1902
	<i>parviflora</i>	4, 2-5	---	---	caruncle	Chandler 1902
	<i>pedunculata</i>	6-8	1.5-2.5 L	---	caruncle	Chandler 1902
	<i>phacelioides</i>	4	---	dark	cuculus	Chandler 1902; Chuang and Constance 1992
	<i>sepulta</i>	4	2.3 L	---	caruncle	Chandler 1902
	<i>spatulata*</i>	5-20	2-4 L	---	cuculus	Chuang and Constance 1992
<i>Phacelia</i>	<i>adenophora*</i>	8-14	1-1.5 L	black-brown	---	Eastwood 1944; Halse 1981
	<i>alba</i>	---	2.4-3 L, 1.4-1.5 W	light-dark brown	---	Atwood 1975
	<i>amabilis</i>	2-4	3-4 L	---	---	Atwood 1975
	<i>ambigua</i>	---	3.3 L, 1.5 W	---	---	Atwood 1975
	<i>anelsonii</i>	---	2.7-3.4 L, 1-1.3 W	---	---	Atwood 1975
	<i>argillacea</i>	4	2.4 L	brown	---	Atwood 1975

Table 1 (Cont.)

Genus	Species	Seeds/ capsule	Seed size (mm)	Colour	Other traits	Reference
<i>Phacelia</i>	<i>arizonica</i>	4	1.8-1.9 L		---	Atwood 1975
	<i>bakeri</i>		2.7-3 L, 1.3-1.6 W	brown		Atwood 1975
	<i>barnebyana</i> *	18-20	0.7-1 L	blackish	---	Howell 1943
	<i>bombycina</i>	---	2.2-2.5 L, 1-1.4 W	dark brown	---	Atwood 1975
	<i>cephalotes</i> *	8-12	1.5 L	---	---	Howell 1943
	<i>coerulea</i>	---	---	dark brown	---	Atwood 1975
	<i>congesta</i>	4	2.6-3.2 L, 1.2-1.4 W	brown	---	Atwood 1975
	<i>constancei</i>	4	2.5-2.8 L, 1.1.2 W	black	---	Atwood 1975
	<i>corrugata</i>	---	3.1-4 L, 1.3-1.7 W	light brown	---	Atwood 1975
	<i>covillei</i>	2-4	2-2.5 L	brown	---	Sewell and Vincent 2006
	<i>coulteri</i>	4	1.8-2 L, 0.8 W	---	---	Ochoa-Gaona 2001
	<i>crenulata</i>	4	2-3.6 L, 1-2 W	dark		Atwood 1975
	<i>demissa</i> *	10-16	1-1.5 L	brown	---	Howell 1943
	<i>denticulata</i>	4	4 L, 1.7 W	brown		Atwood 1975
	<i>filiformis</i> *	20-24	0.6-1 L	brown	---	Howell 1943
	<i>formosula</i>	4	2.5-3 L, 1.2-1.4 W	dark brown	---	Atwood 1975
	<i>geraniifolia</i> *	50-100	0.5-0.7 L	black	---	Howell 1943
	<i>glaberrima</i> *	7-10	1.1-1.5 L	---	---	Eastwood 1944, Halse 1981
	<i>glandulosa</i>	---	2.4-3.3 L, 1.1-1.4 W	reddish brown	---	Atwood 1975
	<i>glechomifolia</i> *	36-43	0.5 L	black brown	---	Howell 1943
	<i>heterophylla</i>	1-5	1.5-2.5 L, 0.8-1.3 W	brown	---	Ochoa-Gaona 2001
	<i>howelliana</i>	4	3.2-4 L, 1.4-1.8 W	brown	---	Atwood 1975
	<i>hydrophyloides</i>	3-16	---	---	---	Hoffman 1999
	<i>incana</i> *	23-37	0.6 L	brown	---	Howell 1943
	<i>indecora</i> *	~40	0.6 L	---	---	Howell 1943
	<i>integrifolia</i>	---	3.1-4.5 L, 1.7-2.2 W	dark brown-black	---	Atwood 1975
	<i>intermedia</i>	---	2.7-2.9 L, 1.5-1.6 W	dark brown	---	Atwood 1975
	<i>inundata</i> *	20-26	1.5-1.7L	---	---	Eastwood 1944, Halse 1981
	<i>inyoensis</i> *	18-20	1 L	---	---	Eastwood 1944
	<i>inyoensis</i> *	16-21	0.5-1L	---	---	Halse 1981
	<i>laxiflora</i> *	~220	0.5-0.7 L	black	---	Howell 1943
	<i>lemmonii</i> *	70-140	0.5 L	brown	---	Howell 1943
	<i>lutea</i> *	10-17	1-1.5 L	brown	---	Eastwood 1944, Halse 1981
	<i>mammillarensis</i>	4	3 L, 1.5 W	brown	---	Atwood 1975
	<i>monoensis</i> *	7-10	1.1-1.7L	---	---	Halse 1981
	<i>mustelina</i> *	40-70, 80	0.5-0.7 L	dark brown	---	Howell 1943
	<i>neomexicana</i>	4	3.2 L, 1.1-1.5 W	brown	---	Atwood 1975
	<i>nevadensis</i>	18-24	> 1 L	brown	---	Howell 1943
	<i>palida</i>	4	2.5 L, 1.5 W	black	---	Atwood 1975
	<i>parishii</i> *	30-43	1-1.3 L	black brown	---	Howell 1943
	<i>pedicelata</i>	4	3 L, 1.1-1.8 W	---		Atwood 1975
	<i>peirsoniana</i> *	40-50	1-1.3 L	brown-black	---	Howell 1943
	<i>perityloides</i> *	~200	0.5 L	brown	---	Howell 1943
	<i>platycarpa</i>	8-19	1.3 L, 0.5-2 W	reddish brown	---	Ochoa-Gaona 2001
	<i>popei</i>	4	1.8 L, 1.4 W	brown		Atwood 1975
	<i>pulchella</i> *	30-50	0.5-1 L	brown	---	Howell 1943
	<i>rafaelensis</i>	4	3.5-4 L, 1.5-2 W	---	---	Atwood 1975
<i>ranunculacea</i>	2-4	1.8-2.8 L	light brown	---	Sewell and Vincent 2006	
<i>robusta</i>		2.9-3.7 L, 1.1-1.7 W	reddish brown	---	Atwood 1975	
<i>rotundifolia</i> *	60-100	0.5 L	brownish	---	Howell 1943	
<i>rupestris</i>	4	2.1-2.7 L, 1 W	brown	---	Atwood 1975	
<i>salina</i> *	7-9	1.5-2 L	brown	---	Eastwood 1944, Halse 1981	
<i>saxicola</i> *	60	0.3-0.4 L	blackish	---	Howell 1943	
<i>scariosa</i>	4	3 L, 1-1.3 W	dark brown	---	Atwood 1975	
<i>scopulina</i> *	11-15	1.3-1.8 L	---	---	Eastwood 1944	
<i>suavolens</i> *	8-16	1-2 L	blackish	---	Howell 1943	
<i>submutica</i> *	10-11	1.5-2	---	---	Eastwood 1944	
<i>tanacetifolia</i>	4	2.7 L, 1.5 W	---	---	Serrato <i>et al.</i> 1998, Hoffman 1999	
<i>tetramera</i> *	20	1 L	---	---	Eastwood 1944, Halse 1981	
<i>utahensis</i>	4		dark	---	Atwood 1975	
<i>vossii</i>	4	2.5-3.1 L, 1-1.4 W	---	---	Atwood 1975	
<i>welshii</i>	4	2.8-3.4 L, 1.3-1.5 W	brown	---	Atwood 1975	
<i>Pholistoma</i>	<i>auritum</i> *	4	2-3 D	---	---	Berg 1985
	<i>lilja</i>	1-4	2-3 D	---	cucullus none	Constance 1939
	<i>membranaceum</i>	1-2, 1-4	2-3D	---	cucullus none	Constance 1939; Berg 1985
	<i>racemosum</i>	1, 4-8	1-2 D	---	cucullus none	Constance 1939; Berg 1985
<i>Wigandia</i>	<i>urens</i>	---	0.7 L 0.5 W	brown-black	---	Ochoa-Gaona 2001

* Ovule data

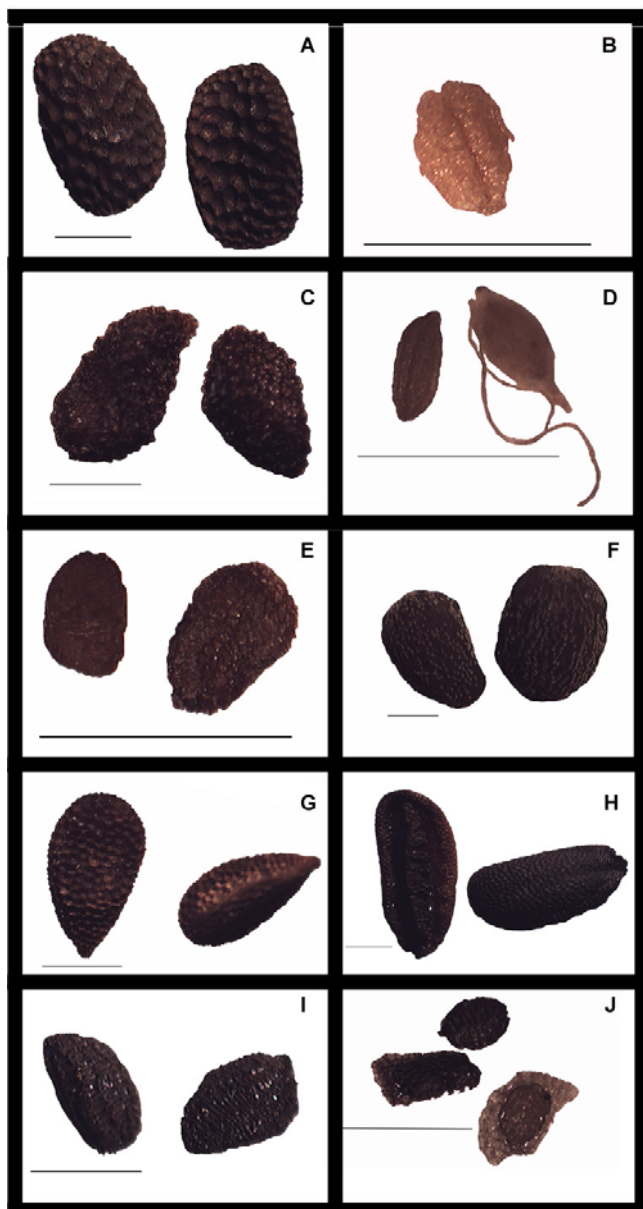


Fig. 1 Micrographs of mature Hydrophyllaceae seeds. (A) *Emmenanthe penduliflora* (Nevada, USA). (B) *Eriodictyon trichocalix* (Baja California, Mexico). (C) *Hesperochiron* (USA). (D) *Hydrolea quadrivalvis* (USA). (E) *Nama dichotomus*. (F) *Nemophila spatulata* (Nevada, USA). (G) *Phacelia secunda* (Argentina). (H) *Phacelia magallaniza* (Argentina). (I) *Turricula parryi* (California, USA). (J) *Wigandia urens* (México DF, Mexico). The seeds were obtained from the Hydrophyllaceae collection (Herbario Nacional de México). Bars = 5 mm.

types of embryo. Seeds of *Eriodictyon*, *Hydrolea* and *Nama* genera have dwarf embryos and a soft-fleshy endosperm, whereas *Pholistoma* has a linear embryo (Berg 1985), and *Hydrophyllum* and *Phacelia* have representative species of both linear and spatulate embryo types and a fleshy endosperm (Martin 1946). Hard endosperm has been reported only for *Hydrophyllum canadense*. In Hydrophyllaceae, seed germination is phanerocotylar, as in *Phacelia campanularia*, *Phacelia crenulata*, *Phacelia tanacetifolia* and *Eriodictyon* spp (Abrams and Smiley 1915; Juhren *et al.* 1956). Dormancy, germination requirements and growth habitats suggest that seed storage behaviour in members of this family is orthodox. Few reports exist about seed longevity and seed storage behaviour. Viabilities of 5-21 years for *N. lobbii*, *N. menziesii* subsp. *atomaria*, *Phacelia ramosissima*, *Phacelia viscida* and *P. tanacetifolia*, have been reported (Hong and Ellis 1996).

Hydrophyllaceae species grow in a wide range of

Table 2 Hydrophyllaceae species cited as weeds in Holm *et al.* (1979).

Genus	Species	Country	Ranking of importance	
<i>Ellisia</i>	<i>nycteles</i>	USA	unknown	
<i>Eriodictyon</i>	<i>angustifolium</i>	USA	unknown	
	<i>californicum</i>	USA	unknown	
	<i>crassifolium</i>	USA	unknown	
<i>Hydrolea</i>	<i>graminifolia</i>	Nigeria	principal weed	
	<i>glabra</i>	Nigeria	principal weed	
	<i>macrocephala</i>	Nigeria	principal weed	
	<i>quadrivalvis</i>	USA	unknown	
	<i>spinosa</i>	Trinidad	unknown	
		Indonesia	present	
	<i>uniflora</i>	USA	unknown	
<i>Phacelia</i>	<i>zeylanica</i>	Ceylan	unknown	
		Brazil	present	
		Indonesia	present	
		India	present	
		Philippines	present	
	<i>Nama</i>	<i>jamaicense</i>	Argentina	common weed
	<i>Phacelia</i>	<i>purshii</i>	USA	unknown
<i>tanacetifolia</i>		Guatemala	unknown	
		Honduras	unknown	
		Salvador	unknown	

environments. *Phacelia* and *Nama* species grow mainly in arid or in helophytic environments (Howell 1943; Bacon 1987), whereas *Hydrophyllum* and *Pholistoma* species are mainly mesophytic. Species of the genus *Hydrophyllum* growing in arid environments are always found in the shade, adjacent to rocks for example (Constance 1942); whereas in *Pholistoma*, species growing in open grasslands such as *Pholistoma auritum* establish themselves under the shade of trees (Parker and Muller 1979). There are also species growing in mesic forest, such as *Ellisia nyctelea* (Struik 1965), and in hydrophilous habitats such as *Hydrolea zeylanica*, which has been reported growing in wet seeded rice systems (<http://www.biologie.uni-hamburg.de/b-online/delta/angio/www/hydrophy.htm>). Several Hydrophyllaceae species have been reported as weeds or potential weeds (Table 2). Species of several genera (e.g. *Nama*, *Phacelia*, *Wigandia*) have weed traits, such as abundant seed production, small seed size, and complex dormancy favouring their presence in permanent seed banks. Several are halophytes and grow in poor soils with low water availability, such as *P. campanularia* and *Nama demissum* which live in deserts and are able to germinate and grow after rainfall of 23-98 mm (Went and Wetergaard 1949; Juhren *et al.* 1956), *Phacelia distans* and *Emmenanthe rosea* which grow on serpentine soils (Zammit and Zedler 1994; Taylor and Levy 2002), and *W. urens* on volcanic rock (González-Zertuche *et al.* 2001).

ENVIRONMENTAL FACTORS AFFECTING SEED DEVELOPMENT AND SEED SOIL PERMANENCE

The production of heterogeneous seeds is an important strategy of higher plants for perpetuating themselves under adequate ecophysiological conditions. This heterogeneity is related to morphological characteristics such as seed colour, size and shape, and to physiological properties that relate to the extent of dormancy, dispersion and persistence within the seed bank (Matilla *et al.* 2005). The architectural distribution of flowers in the inflorescence has no effect on seed size or other reproductive structures, and an important ontogenic component has therefore been proposed to explain variation in certain reproductive traits of *H. appendiculatum* (Wolfe 1992). In this species, seed size heterogeneity within a population is a result of pollination effects, abiotic environment, and developmental constraints. Seed size was significantly influenced by light level, but no effect was observed due to soil type, fertilizer addition, or defoliation (Wolfe 1995). A maternal component of variation in seed mass has also been demonstrated in this species, and

there is a temporal decline in seed size; seeds formed earlier are larger. Large seeds of this species develop into larger seedlings, which have higher survival and show a selective advantage over small seeds (Wolfe 1995). Seed size variation in this species has been also attributed to a male-female trade off; higher pollen production results in smaller seed size. Additionally, inbreeding and the presence of hermaphroditism reduce seed production (females produce more seeds) in *N. menziesii* and in *Phacelia linearis* (Eckhart 1992; Diggle 1995; Byers *et al.* 1997; Gómez 2006). In other *Phacelia* species no correlation was found between seed size and flowering in seeds and fruit traits (Primak 1987). A relation between seed size and the number and position of the ovules present in the ovary has been established in *Nama* species (Chance and Bacon 1984). Seed size variation can be also related to competence in *N. menziesii*, which causes a reduction in seed size and increases seed dormancy (Platenkamp and Shaw 1993). Also, fungal disease reduces seed mass in three species of *Hydrophyllum* (Marr and Marshal 2006). In annual Hydrophyllaceae such as *N. menziesii*, seed heterogeneity can also result in functional heterogeneity, which may disperse germination through at least three years, favouring a seed bank (Baskin and Baskin 1973; Cruden 1974; Baskin *et al.* 1993). A relation between seed heterogeneity and the year of production has been demonstrated in *Phacelia dubia* var. *dubia*. Dormancy loosing and germination depend on the production year in a range of 56.7 to 100% (Baskin and Baskin 1973). Germination dispersal in a particular germination period is also related to seed heterogeneity. A fraction of *P. dubia* seeds are able to germinate at 15-20°C immediately after dispersal but the optimal germination age is 3-5 months; 90% of this population germinates in a wide range of temperatures (5 to 30°C, Baskin and Baskin 1985). Thus the afterripening period required to germinate could be variable among seed population.

Studies of the relation between seed germination patterns that maximize seedling establishment to local environments have been conducted with *N. menziesii* and *P. secunda*.

N. menziesii is endemic to California and Oregon, and grows in many communities from sea level to altitudes over 1515 m, in high habitat diversity. The germination response of local populations to temperature and water availability varies with altitude and latitude. Seeds from more northern latitudes and higher altitudes require more water for germination at a given temperature than seeds from hotter-drier habitats. Water requirement for germination was tested using different concentrations of mannitol solutions (0.8-0.08 M, Cruden 1974).

Many Hydrophyllaceae species are able to grow at a wide range of heights, such as *Hydrophyllum occidentale* (75-2900 m asl) and *Hydrophyllum capitatum* (350-3000 m asl, Constance 1942). The effect of environmental variation with height has been studied in *P. secunda* and *W. urens*. These species grow from sea level to 3400 and 2600 m asl, respectively. They are also widely distributed in latitude; *P. secunda* grows from the south of the United States to southern South America, and *W. urens* from Mexico to Colombia. *P. secunda* is a herb, whereas *W. urens* is a shrub or a tree. In Chile, *P. secunda* has a wide distribution in latitude and altitude; its germination was studied at its various altitudes. This species is physiologically dormant and requires stratification to germinate, avoiding precocious germination under autumn conditions so as to prevent damage to young seedlings by freezing temperatures. *P. secunda* seeds taken from lower elevations require a shorter period of cold stratification (two months) to begin germination, whereas those from higher elevations require longer periods (five months, Cavieres and Arroyo 2000). In contrast, *W. urens* seeds show dormancy only in a small fraction of the seed population (<10%). Seeds germinate from 10°C to 35°C and do not have limiting dormancy or light requirements. Germination of this species was studied at a range of altitudes from 1260 to 2500 m asl. The germination capacity of this spe-

cies conformed to a normal distribution along the gradient. The highest germination capacity was found at 2345 m asl, and the lowest at 1260 m asl. The five populations distributed over height all germinate in the same temperature range, but the thermal-time to reach a given percentile germination increased with elevation (Reyes-Ortega 2001). *P. secunda* and *W. urens* seeds cope with temperature variations along the altitudinal gradient using different strategies. Also, it has been found that in *Hydrophyllum fendleri* and *H. capitatum* growing at 2987.04 and 1895.6 m asl respectively, the deepness of dormancy increases with elevation. Although both species had viable seed, *H. capitatum* did not germinate after several stratification and scarification treatments. About 31.5% of *H. fendleri* germinated at 18°C after exposure to the same treatments (Pelton 1956). In *N. menziesii*, stratification of imbibed seed at low temperatures ($\leq 10^\circ\text{C}$) induced germination, but stratification at warm temperatures $\geq 26^\circ\text{C}$ inhibited germination; at high altitudes the effect of cold stratification is related to the environmental conditions, but in annual species germination is related to the seasonality of the species, either winter or summer plants (Baskin and Baskin 1971, 1973; Cruden 1974; Baskin and Baskin 1983, 1985).

Persistent seed banks are those in which the seeds of a species remain viable in the soil for at least 1 year after production. The formation of a persistent seed bank as a selected life history trait depends on dormancy or environmental requirements (light and temperature). In these alpine environments, persistent seed banks might be related to seed dormancy or to favorable conditions for seed preservation. In alpine environments, 14% of the persistent seed bank was represented by *P. secunda* (Arroyo *et al.* 1999). In *P. secunda* there is a difference in viability between different populations. Seeds collected from lower elevations were not viable after 1 year of burial at any elevation, whereas most of the seeds collected from higher elevations remained viable. Seeds from higher elevations show a stronger innate dormancy that is possibly related to an accumulation of phenolic compounds (Cavieres and Arroyo 2001).

Seed heteromorphism and microenvironment heterogeneity maintain permanent seed banks of the Hydrophyllaceae winter annual plants from different habitats. The individual seeds viability and the heterogeneity in germination timing were determined by the germination requirements such as a population difference in the dormancy deepness (physiological dormancy for Hydrophyllaceae) and the secondary dormancy induction by environmental and microenvironmental conditions in non dormant seeds. A cyclic pattern of dormancy induction by winter low temperatures and dormancy breaking by warm temperatures is observed during several years on different seed populations. This dormancy-non dormancy cycle has been described for *P. dubia*; 30-60% of the seeds germinate during the first year and winter temperatures induce dormancy in the remaining seeds that germinate during autumn (Baskin and Baskin 1973). Some remaining seeds re-enter in dormancy the following winter and new seeds are added to the seed bank maintaining it over time. Cycles of secondary dormancy are not present in all Hydrophyllaceae species. *N. aphylla* and *P. ranunculacea* seeds remain in seed bank because primary dormancy has not been overcome or germination requirements were not covered (Baskin *et al.* 1993). Dormancy cessation is also determined by the variation on environmental conditions. In *Hydrophyllum macrophyllum*, 2 to 4 weeks of warm stratification are necessary to break root dormancy.

Burial has effects on seedling emergence, and on the cessation of dormancy and the coverage requirements of seeds. For example, seeds of *P. tanacetifolia* must be buried 3-6 cm (1/8-1/4 inches) deep to prevent exposure to light during germination (Gilbert 2003). Buried seeds may also undergo hydration (natural priming), regulated by the soil and seed water potential. The relation between laboratory priming and the persistence of the seeds in the soil has been studied in the Hydrophyllaceae species *W. urens*. The phy-

biological and ecological consequences for germination, establishment, survival and growth of the natural priming have also been investigated in *W. urens* (González-Zertuche *et al.* 2001). Controlled imbibition of seeds in order to improve germination, seedling vigour, emergence and establishment is known as priming. This procedure has been extensively used with commercial seeds, and the biochemical and metabolic changes that occur in the primed seeds are well documented in the literature (Bray 1995). In general, the controlled hydration of seeds promotes pregerminative metabolic events such as an increase in RNA content, protein synthesis of enzymes related to endosperm weakening, and mobilization of stored compounds and proteins. Mobilization of globulins and sucrose was observed in natural primed *W. urens* seeds, suggesting that the metabolic processes that occur during priming also take place in buried seeds (González-Zertuche *et al.* 2001; Gamboa-deBuen *et al.* 2006). In *W. urens*, synchronous and rapid germination when rains are well established have been related to natural priming. Rapid germination after the first rains has also been reported for *Pholistoma* (Searcy 1970).

DORMANCY AND GERMINATION REQUIREMENTS

Smoke regulation of germination

Many studies of the relation between germination and fire have been carried out in “fire recruiter” species of the genera *Phacelia* and *Emmenanthe*. “Fire-recruiters” are characterized by prolonged deep seed dormancy in a soil seed bank, whose germination is promoted by smoke or charred wood. They are always established in the first spring after a fire. Smoke and charred wood are considered to be an environmental cue for germination in Hydrophyllaceae and many other plant families (van Staden *et al.* 2000). In turn, these plants recharge the persistent soil seed bank until the next fire, so that the population is maintained over time. Seeds of Hydrophyllaceae fire-recruiters can remain in the soil for several years; *Phacelia brachyloba* seeds have been found in the seed bank 85 years after the last fire, and their germination was induced only by natural fires (Zammit and Zedler 1988; Moreno and Oechel 1991; Keeley and Fotheringham 1998a). After fire, some species remain growing in the burned area for 20-25 years, such as *Eriodictyon californicum* (Biswell and Gilman 1961), while others remain only a short time, such as *Pholistoma auritum*, *Emmenanthe penduliflora* and *E. rosea* (Wicklow 1966; Guo 2001); there is therefore a sequential replacement of Hydrophyllaceae species post-fire (Zammit and Zedler 1994; Guo 2001). The effect of field fires is not the same for all Hydrophyllaceae species growing in these areas. Germination and establishment of *P. brachyloba*, *P. viscida*, and *E. penduliflora* is increased after fires, but *Eriodictyon crassifolium* and *Turricula parryi* have little fire dependence (Keeley and Pizorno 1986a; Moreno and Oechel 1991; Zammit and Zedler 1994; Tyler 1995). *Eucripta chrysanthemifolia* is common in fire-prone Mediterranean areas; although smoke induces its germination, its establishment does not increase after fires (Keeley and Soderstrom 1986; Zammit and Zedler 1994; Tyler 1995; Keeley *et al.* 2005). Establishment of Hydrophyllaceae after fires may also be determined by the topography. *E. penduliflora*, *Phacelia cicutaria* and *Phacelia grandiflora* can establish themselves on north and south facing slopes, but *Phacelia auritum* is established only on north facing slopes (Guo 2001). After fires, Hydrophyllaceae can also be facultative seeders or resprouters in fire-prone habitats, as in *Eriodictyon trichocalyx* and *E. crassifolium* (Keeley *et al.* 2006).

The mechanisms involved in germination of fire recruiters have been studied in Hydrophyllaceae. Sweeney (1956) attributed the requirement of fire for germination to physical mechanisms that impose seed dormancy. In *Phacelia* seeds and other Hydrophyllaceae, structures exist adjacent to the embryo that impede embryo growth and root protrusion, so that removal of the cap endosperm and/or

acid scarification induces germination (0 to 69%, Jones and Schlensinger 1980). In the case of *E. penduliflora*, smoke erodes the external waxy cuticle, modifying its selective permeability so as to promote the leakage of inhibitors (polyphenols) present in the seed. This mechanism also occurs in *N. lobbii* (Nord and Leisser 1974; Egerton-Warburton 1998). *Phacelia minor*, *P. brachyloba* and *P. grandiflora* and *E. penduliflora* seeds contain high levels of sucrose linoleate, a potent inhibitor to seed germination; this compound was not detected in fire independent species such as *P. tanacetifolia* and *P. campanularia*. A significant difference in the yield of this compound between species correlates with life history traits (fire-dependent versus -independent) (Egerton-Warburton and Ghisalberti 2001). Sucrose linoleate may contribute to the control of dormancy in Hydrophyllaceae, extending longevity of the soil seed bank by direct inhibition of germination. Tests indicate that sucrose linoleate extracts from *E. penduliflora* seeds can totally inhibit germination of *P. tanacetifolia* at an appropriate concentration (5 mg/mL, Egerton-Warburton and Ghisalberti 2001). Sucrose esters may limit seed herbivory or the deterioration of seeds via the action of soil microbes because of their selective insecticidal properties (Chortyk *et al.* 1996) and antibacterial and antimicrobial activities (Chortyk *et al.* 1993). The presence of inhibitors in seeds is related to the reduction in oxygen available for germination (Atwater 1980); this may explain the induction of germination of *E. penduliflora* and *Nemophila insignis* by H₂O₂ (and under media with high oxygen availability), at a concentration of 1 kmol m⁻³ d⁻¹ (0 to 88%; Keeley and Fotheringham 1998b) or 100% of oxygen tension in *P. tanacetifolia* (80% germination, Chen 1970). Woody seed coats with a semipermeable layer have also been reported in Hydrophyllaceae (Silvertown 1984). The presence of a waxy outer cuticle may explain this impermeability, as in *E. penduliflora* (Egerton-Warburton 1998). In contrast, for smoke-stimulated seeds a relevant seed trait is the presence of poorly developed outer cuticle adjacent to a highly textured seed coat (Keeley and Fotheringham 1998b). In fact, *E. penduliflora*, *E. chrysanthemifolia*, *P. grandiflora* and other fire recruiting Hydrophyllaceae species have permeable seeds and are fully imbibed a few hours after sowing on a wet surface (Sweeney 1956; Christensen and Muller 1975; Keeley and Fotheringham 1998b). As a result, Hydrophyllaceae seed recruiters and others are physiologically dormant; and, in contrast to legume fire recruiters with impermeable seeds that have physical dormancy (Baskin and Baskin 2004), in the Hydrophyllaceae impermeable seed coats have not been reported (Baskin and Baskin 2000). Smoke increases sensitivity to gibberellic acid and facilitates *Nicotiana attenuata* seed germination (Schwachtje and Baldwin 2004). However, no effect of different gibberellic acid concentrations (1, 5, and 10 mmol m⁻³) was observed on the germination of *E. penduliflora* or *P. grandiflora* (Keeley and Fotheringham 1998b).

The effect of several fire products (charred wood, heat, ash, smoke) and the consequent environmental changes (increases in temperature, light and nutrients and decreases in soil moisture and allelopathic products coming from stand vegetation) have been studied in Hydrophyllaceae. In *E. crassifolium*, germination response to charate (concentrated charred wood) can occur independently of fire heat, whereas in other species there is an interaction between the different fire products (Keeley 1987). Heat itself does not induce germination of *E. penduliflora*, *E. chrysanthemifolia*, or *P. grandiflora*. These species did not germinate after treating seeds with high temperatures such as 80, 100, 120, 140, or 160°C during 10 min, or 60, 80, and 100°C for 1 h, or 60 and 80°C for 2 h (Christensen and Muller 1975). The germination of *Phacelia fremontii*, *P. brachyloba*, *P. minor*, *P. parryi*, *P. viscida*, and *E. crassifolium* pretreated with high temperatures at different exposure time was very low (<10%, Keeley *et al.* 1985; Keeley 1987; Keeley and Keeley 1987). Germination of *E. californicum* is partially induced by heat (0 to 29%, Le Fer and Parker 2005). At dif-

ference, heat promotes germination of fire recruiter plants from different families such as *Lotus scoparius* (Leguminosae) and *Phytolacca ericoides* (Rhamnaceae) (Christensen and Muller 1975; Keeley and Bond 1997). Simultaneously exposure to heat and smoke, charred wood or charate induced germination in *E. crassifolium* (0 to 30%, 33 to 82%), *E. penduliflora* (0 to 100%), *P. cicutaria* (5 to 15%, 6 to 57%) and *P. grandiflora* (1 to 75%) indicating that seeds are viable after heat shock (Keeley 1987; Keeley and Keeley 1987; Keeley 1991; Keeley *et al.* 2005). *E. californicum* and *P. cicutaria* are among the few species in which only partial germination is induced by heat (Keeley *et al.* 1985; Le Fer and Parker 2005). Temperatures of 50°C during two weeks incubation or heat shock at 80°C (120 min) or at 120°C in combination with charred wood increase the germination of *P. grandiflora* (2 to 10%), *P. parryi* (0 to 4%) and *P. viscida* (4 to 19%) (Keeley *et al.* 1985). *E. crassifolium* germination is also increased by heat shock and smoke; seeds are more responsive to these factors when subsequently ripened during the next year (Keeley *et al.* 2005). Heating of chaparral soil at 195°C (temperatures registered during fires) induces germination of seeds contained in the seed bank, and the addition of charate has a positive effect on germination. The presence of fire products in soils burned a considerable time before has been related to their permanence and their slow lixiviation (Keeley and Nitzberg 1984).

In some species the effect of moist and dry heat has been tested. *E. penduliflora* and *E. californicum* have good tolerance to dry heat shock (150°C), but not to moist heat shock (Parker 1987; Le Fer and Parker 2005). The effect of fire seasonality on seed germination has also been studied using Hydrophyllaceae plants. In California chaparral, wet conditions in spring cause high mortality of the soft coated seeds of *Emmenanthe*, so that fire-stimulated seed germination occurs mainly following autumn fires (Le Fer and Parker 2005). The effect of heat on imbibed seeds can also result in a change of composition and structure of vegetation after deliberate fires; some Hydrophyllaceae appear if fire is applied during the rainy season (*P. brachyloba*) or at its end (*P. cicutaria*, *P. grandiflora*, *P. viscida*, *E. penduliflora*, *E. chrysanthemifolia*), or alternatively during the dry season (*E. penduliflora*, Beyers and Wakeman 1997).

Nitrates induce germination of several species. Germination of *E. penduliflora* seeds is increased from 38 to 585 by 10 mM KNO₃ (Pons 1989; Karssen and Hilhorst 1992; Thanos and Rundel 1995). However, ammonium and nitrates do not increase germination of several "fire recruiters" (Keeley 1991). Also, nitrates, ammonium, phosphates, potassium and sulphates do not increase germination of *P. grandiflora* and *E. penduliflora* seeds (Christensen and Muller 1975; Keeley and Fotheringham 1998b). The contradictory findings about the effect of NO₃⁻ are apparently due to pH-dependent stimulating effect of nitrates (Keeley and Fotheringham 1998b).

Germination after fire has also been related to the elimination of inhibitors present in the plants growing in the area. Leaf leachate of *Adenostoma fasciculatum*, which is a dominant shrub growing in the Californian chaparral, inhibited germination of several species (not Hydrophyllaceae), but did not inhibit germination of postfire species such as *E. penduliflora*, *E. chrysanthemifolia*, *P. grandiflora*, and *P. fremontii*. In this case, *E. grandiflora* and *E. chrysanthemifolia* should be scarified to induce germination with and without the leachate (Christensen and Muller 1975; Keeley *et al.* 1985; Keeley and Fotheringham 2000). In combination with heat shocks (80-120°C), the leachate of *A. fasciculatum* increases germination of *P. cicutaria* and *P. grandiflora* (Keeley *et al.* 1985). Hydrophyllaceae such as *Pholistoma auritum* also produce allelopathic substances (Parker and Muller 1979) that may be destroyed by fire.

Stimulation of germination by fire also could be related to chemicals from combustion products. Charred wood was first shown to stimulate *E. penduliflora* germination (0 to 57%) (Wicklow 1977; Jones and Schlesinger 1980), an effect subsequently reported for many other chaparral spe-

cies (Keeley 1991). In other species, such as *P. minor*, germination is increased only slightly by charred wood (0 to 13%) (Keeley and Keeley 1987). Promotion of germination by charred wood was also observed in non "fire recruiter" species, although germination of *P. brachyloba* is not induced by charred wood (Zammit and Zedler 1988; Minor-sky 2002). Smoke stimulates germination of "fire recruiter" species directly or indirectly, by binding to soil particles in an aqueous or gaseous form (Keeley and Fotheringham 1997). Smoke induced a highly significant increase in germination in *E. penduliflora* (0 to 100%), *E. chrysanthemifolia*, *E. crassifolium* (0 to 80%), *P. grandiflora* (1 to 60-90%) and *P. minor*, but *P. brachyloba* did not germinate (Keeley and Fotheringham 1998a; Keeley *et al.* 2005).

There exist some controversial data relating to the smoke component involved in this stimulation. Thanos and Rundel (1995) concluded that nitrate and other nitrogenous ions were responsible for fire-stimulated germination of *E. penduliflora* seeds. Keeley and Fotheringham (1998b) found that nitrate ions alone failed to induce germination of *E. penduliflora* seeds, and suggested that nitrogen oxides (NO_x) were the compounds responsible for smoke-stimulated germination. They proposed that these chemicals act as internal signals that activate enzymes or the production of growth regulators to mediate germination (Keeley and Fotheringham 1997; Fotheringham and Keeley 2005). Nitrogen oxides can not be the main germination signal after fire, because no effect on germination was observed when dormant seeds of *E. penduliflora* were treated with solutions of sodium nitroprusside (SNP) and S-nitroso-N-acetylpenicillamine, which release oxides of nitrogen (SNAP, Preston *et al.* 2004; Baldwin *et al.* 2005). NO_x could also be a signal that provides seeds with information on soil quality and microbial activity, since levels of nitrogen oxides in soil change as a result of bacteria metabolism (Giba *et al.* 2003). Preston *et al.* (2004) suggested that an unidentified cellulose combustion factor is the ecologically relevant germination signal in fire habitats. Which increases germination of *E. penduliflora* seeds from 0 to >70%. Oligosaccharin, a hormone which stimulates seed germination, could be released as a consequence of heating alterations in xylan or glucuronic acid by fire (Keeley and Pizzorno 1986b). The butenolide 3 methyl-2H-furo (2,3-c) pyran-2-one has been found to be a key germination stimulant present in smoke (Flematti *et al.* 2007). The effect on germination of this compound has been demonstrated in many smoke responsive plant species, including *E. penduliflora* (10 to 60%) (Flematti *et al.* 2004). In nature, this compound is relevant for Hydrophyllaceae germination and subsequent establishment, but it is also effective in stimulating germination of economically important plant species that include the cultivated Hydrophyllaceae (Stevens *et al.* 2007). Promotion by fire of seed germination could be a consequence of the integration of different signals resulting from either fire products or environmental changes.

The germination response to charred wood and smoke has also been studied in species from several plant families growing in the California chaparral (including the Hydrophyllaceae *E. penduliflora*) and South African fynbos. It was found that, in Hydrophyllaceae and other plant families from fire prone seasonal environments, there is a similar germination response to fire products (Keeley and Bond 1997). The effect of smoke on germination is common in phylogenetically distant plant families, suggesting a convergent evolution of the signalling mechanism (Keeley and Fotheringham 1997).

Negative photoblastism

Light plays an important role in seed germination and subsequent steps in the life cycle of plants (Neff *et al.* 2000). Positive photoblastic seeds have an absolute requirement for light for germination, as reported in a wide number of angiosperms; germination can be either a low fluence response (LFR), or a very low fluence response (VLFR) medi-

ated by phytochrome B and A (Smith 1995). Negative photoblastism has been reported in a few species, but has been studied much less than positive photoblastism (Baskin and Baskin 2001). In such seeds, germination is inhibited by light; this is a high irradiance response (HIR) regulated by phytochrome A (Casal and Sánchez 1998; Takaki 2001). In the Hydrophyllaceae, other morphogenetic responses have been discovered that are regulated mainly by phytochrome A: the effect of length of day on flowering and growth form in *P. ranunculacea* (short day plant) and in the long day plant *P. tanacetifolia* (Allard 1940). In other species, flowering is not regulated by light, as in the 'neutral day' plants *N. menziesii* and *P. campanularia* (Mattson and Erwin 2005). Winter annual species of Hydrophyllaceae are expected to be short day flowering plants, but the present study indicates that no generalization is possible.

In *P. tanacetifolia* (negative photoblastic), the main wavelengths that inhibit germination in the visible are far red (25%), red (50%) and blue (27%), as in other angiosperms, the inhibitory effect of these wave lengths increases if irradiation is preceded by a heat shock (37°C, 2 h). For this species, inhibition has also been reported by UV-A and UV-B (64%; Schultz and Klein 1963). Inhibition by far red and blue light has also been found in *N. insignis* (from 88 to 12 and 49% respectively), in which germination is indifferent to light at some temperatures (5-19°C), but inhibited at 21-30°C (Black and Waering 1960). Several of the negative photoblastic species studied belong to the Hydrophyllaceae, including *E. penduliflora*, *N. menziesii* and *P. tanacetifolia*. These germinate rapidly and require short light exposure periods for inhibition or reversion (24-48 h), making them excellent for the study of photoinhibition (Black and Waering 1960). In these species, inhibition of germination by light occurs at relatively high temperatures (> 26°C), as in *P. tanacetifolia* (at 30°C germination drop to zero; Pirovano *et al.* 1999), or at a wide range of temperatures (22-27°C, from 92 to 77-0% in *N. insignis*) (Chen 1968). Darkness for germination may similarly be a requirement for all seeds of a batch or for some of them at particular temperatures as in *N. menziesii* (Cruden 1974), in which both light and temperature inhibition of the germination process have been studied.

Darkness is not an absolute requirement for germination at certain temperatures. In *N. insignis*, germination in darkness or light in the interval 22-30°C is reduced as the temperature increases. In this species, short photoperiods (4 h light) induce higher germination (from 20 to 88%), and the duration of both light and dark periods are relevant for germination (Black and Waering 1960). On the other hand, seeds exposed to light-hard treatments (seeds exposed to light at inhibitory temperatures, 21°C, and then transferred to darkness at the same or other temperatures) increased germination with temperature, but decreased after light-hard treatments from 18-3°C. Also, seeds exposed to an inhibitory temperature (21°C) in darkness (thermodormant) increased their germination at progressively lower temperatures (Black and Waering 1960). Finally, germination of other species from these genera are also non-negative photoblastic, including *Nemophila brevifolia*, *N. aphylla*, *Phacelia sericea*, *Phacelia purshii*, *P. dubia*, and *P. ranunculacea* (Baskin *et al.* 1993). Light responses mediated by phytochrome act in concert with other environmental cues (Franklin and Whitelam 2004); negative photoblastism occurs mainly at relatively high temperatures (> 20°C), and at high photon fluxes and long exposures to light (in relation to germination time); at lower temperatures, inhibition of germination is partially expressed in the seed sample as low germination and/or reduced germination rate, as in *P. tanacetifolia* (Black and Wareing 1957; Schultz and Klein 1963; Macchia *et al.* 2000). Additionally, negative photoblastism is commonly expressed at low water potential, as in *N. menziesii* (Cruden 1974; Niedzwiedz-Siegien and Lewak 1989).

The study of signal perception mechanisms that promote or inhibit germination is important in understanding the adaptations of these plants to their environments. For

negative photoblastic seeds, darkness may be a cue for seeds to remain in a dry environment in which moisture availability is sufficient for germination and seedling establishment, as when seeds are buried in the soil, and by which the seed detects inadequate autumn conditions and awaits further germination in spring (Thanos *et al.* 1989, 1991; Pons 2000). Temperatures, photon fluxes and water potentials that induce negative photoblastism support this hypothesis; to learn more, further studies are necessary (Koller 1957; Pons 2000). The negative photoblastic species *E. penduliflora*, *N. insignis* and *P. tanacetifolia* grow in sharply seasonal Mediterranean chaparral relevant to this hypothesis. Nevertheless, not all the Hydrophyllaceae or other species that grow in dry environments have negative photoblastic seeds, indicating that species with a wide diversity of functional or morphological traits can handle the same environment. In these species, negative photoblastism regulates the physiological and biochemical processes related to the removal of mechanical constraint on embryo growth (cuticle and cap endosperm) (Chen and Thimann 1966; Egerton-Warburton 1998; Pirovano *et al.* 1999). Gibberellic acid (1 mM to 0.5 mM) promotes germination of *P. tanacetifolia* and *N. insignis* seeds, from 11 to 29% and from 8 to 78%; respectively) inducing changes in the mechanical properties of the endosperm (Chen 1968; Safran and Galsky 1974; Coccuci *et al.* 1981). When germination is inhibited by light in these Hydrophyllaceae, abscisic acid does not play a role as it does in positive photoblastic seeds with physiological dormancy, in which there is an antagonist effect to gibberellic acid (Kucera *et al.* 2005).

Mature seeds of *P. tanacetifolia* are boat-shaped with a length of 2.7 mm and a width of 1.5 mm. There is a coat of integument with no cellular organization, and a well-formed endosperm that surrounds a curved embryo (Serrato-Valenti *et al.* 1998). The transition from dormancy to mobilization is rapid, and radicle emergence takes place within 24 hours after the onset of imbibition. In mature seeds, proteins are the major storage substances, although starch is the main reserve during seed development. Protein bodies were not present in the micropylar region after 4 h of seed imbibition; this suggests that protein digestion begins in this region of the endosperm in the early stages of germination (Serrato-Valenti *et al.* 2000). Localization of endo- β -mannanases near cell walls during germination of *P. tanacetifolia* seeds could be related to endosperm weakening, suggesting its possible role during the early phases of germination (Piana *et al.* 2003). The activity of these hydrolases is enhanced by gibberellic acid in many solanaceous species seeds (Nonogaki *et al.* 2000). The effect of gibberellic acid in enhancing germination of light-inhibited *P. tanacetifolia* seeds has been described by Safran and Galsky (1974) and Coccuci *et al.* (1981), and could be related to stimulation of the embryo growth through an increase of endo- β -mannanase activity. The effect of gibberellic acid on testa and endosperm rupture to end dormancy has been studied in tobacco seeds (Leubner-Metzger 2002, 2003).

Basic mechanisms involved in photodormancy have also been studied in *Phacelia tanacetifolia* seeds. The start of germination is characterized by activation of respiration, H⁺ extrusion and K⁺ uptake at the membrane level, and macromolecule synthesis. During germination of dormant and nondormant seeds, the cytoplasmic pH is kept constant by a H⁺/K⁺ exchange mechanism that couples K⁺ influx to H⁺ efflux. Cytoplasm alkalization leads to activation of macromolecular synthesis and the development of germination (Pardi *et al.* 1980; Pirovano *et al.* 1996, 1997). Inhibited light conditions promote cytoplasmic acidification, suggesting the involvement of pH in photodormancy (Coccuci *et al.* 1989; Espen *et al.* 1995). Germination of *P. tanacetifolia* seeds in light is promoted by the maintenance of constant cytoplasmic pH with fusicoccin, a phytotoxin which stimulates the H⁺ pump (from 0 to 80%); also, cytoplasmic acidification caused by butyric acid inhibits germination in the dark. The inhibiting effect of high temperatures on germination could be related to a decrease of potassium uptake

(Cocucci *et al.* 1985).

The calcium sensor protein calmodulin (CaM) is involved in the germination of *P. tanacetifolia* seeds. There is a parallel increase in CaM and seed germination; this increase does not occur during light inhibition or high temperature inhibition (Cocucci and Negrini 1991). Further studies suggest that calmodulin is involved in maintaining the Ca^{++} concentration within a particular range (Negrini *et al.* 1995; Duval *et al.* 2002). Dark germination of *P. tanacetifolia* seeds generally depends on respiration activation, transport activities and macromolecular synthesis, which are the basic mechanisms involved in the germination process in non-dormant seeds.

Other requirements for germination and dormancy breaking

Physiological dormancy is the most common dormancy among Hydrophyllaceae. As well as the negative photoblastic seeds and fire recruiters described above, it has been reported in *N. aphylla*, *Phacelia bipinnatifida*, *Phacelia compacta*, *Phacelia vallicola*, *Phacelia notabilis*, *Phacelia quickie*, *P. lemmonii*, *P. ranunculacea* and *P. sericea*, (Quick 1947; Baskin and Baskin 2001). In *P. bipinnatifida*, *P. compacta* and *P. notabilis*, dormancy breaking requires cold stratification, unlike *N. aphylla* and *P. ranunculacea* which require warm stratification (Baskin and Baskin 2001). Requirement of warm or cold stratification for germination has been related to the life cycle of these species as winter or summer annuals, or biennial as is *P. bipinnatifida* (Baskin and Baskin 2001). The requirement of low or warm temperatures to break dormancy is common in species growing in sharply seasonal environments including rocky zones, deserts and serpentine soils, such as *P. dubia* (Taylor and Levy 2002).

P. purshii and *P. dubia* are winter annuals that require incubation at relatively high summer temperatures during afterripening to break dormancy meanwhile low winter temperatures (5-10°C) induce dormancy. In seasonal environments, this adaptation guarantees the germination at the end of autumn by preventing germination in spring and the formation of a seed bank (Baskin and Baskin 1971, 1973). *P. purshii* requires a double environmental cue to break its dormancy. Dry-wet soil cycles induce high germination if seeds were incubated at summer temperatures (35/20°C and 35/25°C) and were then transferred to autumn temperatures (20-10°C) (Baskin and Baskin 1973).

Serpentine soils are characterized by a combination of low calcium, heavy metals, nutrient deficiency and low water availability. *E. rosea* is found only in serpentine soils or serpentinized rocks in California. *E. rosea* seeds were indifferent to scarification, but after germination their radicles and hypocotyls showed signs of rooting. Seeds of *E. penduliflora* can germinate only after scarification and seedlings did not show signs of decay. However, *E. rosea* seedlings were more tolerant than *E. penduliflora* seedlings to low calcium, potassium, magnesium and phosphorus concentrations (Tadros 1957). *E. penduliflora* was unable to grow in such conditions.

Hydrophyllum macrophyllum is an herbaceous plant growing in mesic deciduous forests that shows an interesting mechanism of dormancy reported in few other species (*Quercus* is one). Seed maturation and dispersal of *H. macrophyllum* takes place in mid- to late July, and seeds exhibit both root and shoot dormancy. As has been described in winter annual Hydrophyllaceae species, summer temperatures break dormancy (30/15°C), seeds germinate in early autumn and the shoot remains dormant. Winter temperatures (5°C) are required to break shoot dormancy. A delay in germination of several years has also been described in this species. Other species of the same genus, *H. capitatum*, *H. flenderi* and *H. appendiculatum* are also dormant and some of them may present the double dormancy observed in *H. macrophyllum* (Baskin and Baskin 1983, 1985).

Germination treatments

The best strategies to germinate Hydrophyllaceae species are related mainly to dormancy broken procedures and fulfil germination requirements.

All Hydrophyllaceae species have physiological dormancy. The methods to break this can be divided into the following plant groups:

1) Fire recruiters: physiological dormancy can be broke with a smoke solution or charate added to the germination substrate.

2) Non-fire recruiters: For species distributed at high latitudes or altitudes, the pre-germination treatments of cold stratification at 5°C in moist substrate and light can be effective to promote germination meanwhile for winter annuals the exposure to warm stratification is determinant to break dormancy. In conclusion, the method to break dormancy depends on plant ecology.

3) Species with double dormancy mechanism (root and cotyledonary): It is adequate warm stratification to favour root emergence, while cold stratification it is necessary to favour cotyledon emergence.

The use of smoke to break dormancy can be tested in all Hydrophyllaceae species because it promotes germination of recruiters and non-recruiters species from different plant families.

Germination requirements of Hydrophyllaceae species:

Hydrophyllaceae species could be negative photoblastic, positive photoblastic or indifferent to light but, as a first approach, germination can be tested at low temperatures (10-19°C) in light or higher temperatures in darkness (20-30°C). Some species as *W. urens* are thermophilous and indifferent to light and germinates between 11 and 33°C. For *Phacelia* species is recommended germination in darkness at 16°C (*P. campanularia*, *P. distans*, *P. grandiflora*, *P. minor*, *P. parryi*, *P. sericea*, *P. tanacetifolia* and *P. viscida*).

It is important to remark that these general procedures are only general recommendations for the induction of germination of Hydrophyllaceae species but do not provided any particular knowledge of the Hydrophyllaceae species germination. There is an important variability between species and also between genera and this heterogeneity could be a result of the effect of the environmental variability including abiotic and biotic factors.

CONCLUSIONS

Dormancy in Hydrophyllaceae is physiologic. Dormancy is broken by environmental cues related to habitat. Fire products break the dormancy of species growing in fire prone habitats. The most studied of these species are *E. penduliflora* and some *Phacelia* species. For dry environment species, negative photoblastism prevent seeds from germinating in moist conditions adverse to seedling establishment and growth. In both cases, whether "fire recruiters" or negative photoblastic species, the mechanism involved in dormancy involves an immature and weak embryo surrounded by structures that mechanically constrain embryo growth. This is the same situation as in winter annual species, which requires the high temperatures of summer to end physiological dormancy. Study of the mechanisms involved in breaking dormancy, and the germination requirements of Hydrophyllaceae seeds, is important, because they are peculiar Angiosperms in these respects. There are only a few studies of different Hydrophyllaceae species, and information about germination conditions in most species is buried in ecological studies or technical bulletins. To learn more, systematic studies of germination are required for the majority of the genus of this amazing plant family.

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