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Germination and emergence of *Rumex* in river flood-plains. II. The role of perianth, temperature, light and hypoxia

L. A. C. J. Voesenek, M. C. C. de Graaf and C. W. P. M. Blom

Department of Ecology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

**SUMMARY**

The germination responses of *Rumex acetosa*, *R. crispus* and *R. palustris* were studied in relation to perianth-imposed primary dormancy, the temperature and light control of germination and response to hypoxia. The perianth-imposed primary dormancy in *R. crispus* and *R. palustris* is related to light filtering through the perianth and/or inhibited water uptake due to a water repulsive layer around the perianths. *Rumex acetosa* is able to germinate at a constant rate over a wide range of temperatures, whereas both the other species are characterized by fast and maximal germination at regimes with higher upper temperatures; in this sense *R. palustris* was the most extreme species. In *R. acetosa*, germination in light and dark is independent of diurnal fluctuating temperatures. Dark germination in *R. crispus* is stimulated by alternating temperatures. *Rumex palustris* needs both light and fluctuating temperatures to induce germination. Differences in germination behaviour in relation to light and temperature are discussed in relation to the phytochrome regulation of *Rumex* germination. In contrast to *R. acetosa*, imbibed achenes of both *R. crispus* and *R. palustris* are able to survive a prolonged hypoxic incubation. The results are discussed in relation to the distribution of the three *Rumex* species in a flooding gradient of a river area.

**Key-words:** germination, perianth-imposed dormancy, phytochrome, *Rumex*, seedbank.

**INTRODUCTION**

The timing of germination and seedbank characteristics are important life-history traits, at least partly explaining the field location of *Rumex* species in river flood-plains (Voesenek & Blom 1992). *Rumex acetosa*, a species from rarely flooded grasslands, is characterized by early autumn germination, a transient type of seedbank and flood-intolerant achenes. *Rumex crispus* and *Rumex palustris*, both of which occur in parts of river areas with harsh predictable winter floods and erratic catastrophic summer floods, are characterized by delayed germination until the following spring, a persistent type of seedbank, flood-tolerant achenes and multiple germination cohorts after summer floods. Differentiation in timing of germination (autumn vs. spring) can be regulated by mechanisms of primary dormancy, red/far-red ratios of the germination site, the phytochrome photoequilibrium required for germination and the amount of overlap between...
required and actual temperatures for germination (Bewley & Black 1982; Egley & Duke 1985; Pons 1991). There is evidence that both *R. crispus* and *R. palustris* delay germination to the following spring as a consequence of perianth-imposed primary dormancy. *Rumex acetosa* lacks this kind of primary dormancy as most perianths separate easily from the achenes during achene drop (Voosenek & Blom 1992). Structures surrounding the embryo such as seed coats or perianths may prevent germination in several ways (Taylorson & Hendricks 1977; Bewley & Black 1982). Integrated in this study are the effects on germination of chemical inhibitors in the perianths, interference with water uptake and light filtering.

An important factor stimulating seedbank formation is inhibited dark germination regulated by the phytochrome system (Van Baalen 1982; Pons 1991). Diurnally fluctuating temperatures can, however, interact with this phytochrome control and stimulate dark germination (Thompson *et al.* 1977; Takaki *et al.* 1981). This mechanism may be functionally related to gap-detection (Thompson & Grime 1983). Chilling or stratification can have the same effect leading to widening of the germination requirements (Van der Woude & Toole 1980; Bewley & Black 1982). *Rumex* species are characterized by contrasting types of seedbanks as described in Voosenek & Blom (1992). It is therefore expected that these species differ in the levels of the active far-red absorbing form of the phytochrome \( P_{fr} \) and/or the threshold of \( P_{fr} \) required to stimulate germination. In other words it is expected that achenes of *R. acetosa* have a different light-sensitivity than achenes of *R. crispus* and *R. palustris*.

If germination of achenes in the seedbank is inhibited, survival mechanisms become relevant. In frequently flooded areas soil hypoxia seems to be an important environmental constraint (Hook 1984). It is hypothesized that imbibed achenes of species from wet sites in river flood-plains (e.g. *R. crispus* and *R. palustris*) are more tolerant to oxygen deficiency than species from more elevated field sites.

This paper describes the mechanisms underlying perianth-imposed primary dormancy, the temperature and light (phytochrome) control of germination and the impact of hypoxic conditions on survival of imbibed achenes. All experiments were performed with three *Rumex* species, e.g. *R. acetosa*, *R. crispus* and *R. palustris*, and had a comparative approach.

**MATERIALS AND METHODS**

**General germination procedure**

Some common characteristics of the *Rumex* species under study and specific information about their natural habitats in Dutch river areas have been reported in Voosenek & Blom (1992). Mean achene sizes and weights are described in Voosenek & Blom (1987). Germination experiments were performed with achenes collected in the Nijmegen river area region (The Netherlands) in 1986, 1987 and 1988. Achenes of several plants belonging to one population were used in all experiments; they were stored under dry and dark conditions at room temperature.

In all germination tests, 40 or 50 achenes (four replicates) were incubated on two layers of Schleicher and Schüll filter paper in glass petri-dishes (diameter 70 mm). Achenes were considered to have germinated after radicle emergence. To test germination under dark conditions petri-dishes were wrapped in two layers of aluminium foil. Germination was recorded under dim green safelight (TL 33; 20 W; wrapped in several layers of green
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No germination occurred when achenes were exposed continuously to these green-light conditions (see Blom 1978). In experiments with diurnal fluctuating temperatures, the lower temperature was always given during the night period of 12 h. All germination tests were performed in cabins illuminated at a photosynthetic photon-flux density of 15–30 μEm⁻² s⁻¹. To simulate storage of achenes in a flooded soil they were incubated in 30 ml flasks (40 achenes per flask) filled with hypoxic water (24 h bubbled with N₂; oxygen concentration: 0.20 ± 0.02 mg l⁻¹; see Drew & Robertson 1974). These flasks were stoppered with serum-vial caps and placed in 1.5 l glass pots filled with a reducing solution of cysteine HCl (0.1 g l⁻¹) and an oxygen indicator resazurine (1 ml l⁻¹) of 0.01% solution. These pots were closed airtight and wrapped in two layers of aluminum foil. After termination of all experiments, the non-germinated achenes were placed at a temperature regime of 10/25°C in light for a 2-week period. In previous experiments these conditions resulted in nearly 100% germination in all species. Achenes which did not germinate after this period and were also unable to stain red in a 1% tetrazoliumchloride solution (Sigma; Moore 1972) were assumed to be dead. Non-parametrical Wilcoxon tests were used to analyse the germination data (Sokal & Rohlf 1981).

Experiments

Perianths. Experiments were designed to test whether the perianth-imposed primary dormancy of R. crispus and R. palustris was related to chemical inhibitors or to the restricted uptake and perception of water and light respectively.

A germination experiment was performed with achenes with and without perianths at two temperature regimes (10/15°C; 10/25°C). Radicle emergence was recorded at 2–3-day intervals over a 14-day period. The germination data were corrected for the time lag (1 day) between actual radicle emergence and radical emergence outside the perianth.

The influence of chemical inhibitors was tested by allowing achenes to germinate on two layers of filter paper with crushed perianths (Cyclotec 1093 Sample Mill [Tecator]) in between. In another test, germination of achenes was recorded on filter paper moistened with a perianth extract (a filtered mixture of water and perianth powder). Both experiments were performed at two temperature regimes (20/20°C; 10/25°C) and with twice the amount of perianth that possibly could interfere with achene germination under natural conditions.

The influence of perianths on water uptake through the existence of a water-repellent layer on the surface of the perianth was tested with a pretreatment in which Rumex achenes with perianths were shaken vigorously with acetone (3 h). In the controls, acetone was replaced by water. Hereafter the achenes enclosed by perianths were allowed to dry for 24 h at 35°C, after which they were imbibed to germinate at a temperature regime of 10/25°C.

The influence of light-filtering properties on germination was tested with experiments under a light filter of perianth powder. This layer of crushed perianths was homogeneously glued (Lero glue) on a cut-away of a dish wrapped in aluminum foil. Germination was recorded under dim green safe-light and compared with controls that had only glue and no perianth powder on the cut-away and controls in which light interference was completely blocked (dark-conditions). Temperature regimes were selected that resulted in a low dark germination and a high light germination (R. crispus: 25/25°C; R. palustris: 10/25°C). Only under these conditions possible light filtering due to the perianth will result in a germination percentage which is lower than that obtained under light conditions.
Temperature, light and stratification. The influence of various temperature regimes (10/10; 10/15; 10/20; 10/25; 10/30; 10/35°C), simulating a range of possible field temperatures, on final germination and germination rate in a dark/light regime was tested. Germination was recorded nearly every day over a 14-day period. The germination rate (% per day) was calculated over the period in which 85% of the germinated achenes had germinated (see Walton 1977; Huiskes et al. 1985).

Alternating temperatures can induce germination in darkness in some Rumex species (Thompson et al. 1977; Takaki et al. 1985). To test whether this is also the case with the species under study, achenes were allowed to germinate at various amplitudes of diurnal fluctuations of temperature below a base temperature of 25°C (25/25; 25/22; 25/19; 25/16; 25/13°C). This experiment lasted 14 days and was performed under 12 h of light and under continuous darkness.

Light responses in seeds are under phytochrome control. This pigment system absorbs red light (the P_r form) and is transformed to a form which stimulates germination and absorbs far-red light (P_f) (Bewley & Black 1982). The phytochrome control interacting with alternating temperature or stratification was studied on imbibed achenes maintained in the dark at a constant temperature of 20°C. Treatments were given as pulses of red light (R) (5 min; Philips TL 33; 20 W; wrapped in several layers of red cellophane; light intensity: 1 W m⁻²) and as pulses of far-red light (FR) (5 min; Philips Infrared light [150 W] above a water layer [3 cm], a red filter [plexiglass nr. 501] and a blue filter [plexiglass nr. 627]; light intensity: 1 W m⁻²). All treatments were applied after 24 h of imbibition. The alternating temperature treatment, simulated by a single 35°C temperature pulse (30 min) given after 24 h of imbibition, was conducted by submergence of the tested achenes, placed in a small perforated tube, in a water bath. This treatment was performed under dim green safe-light. Stratification was achieved by placing imbibed achenes in a dark growth-chamber with a temperature of 4 ± 1°C for a 37-day period.

Hypoxia. Achene survival after a hypoxic pretreatment of 0, 2, 4, 8, 12, 20 and 26 weeks was tested at two pretreatment incubation temperatures: 4 and 20°C. After the hypoxia period achene vitality was tested by aerobic germination under a temperature regime of 10/25°C for 14 days.

RESULTS
The influence of perianths on germination
The germination of R. crispus and R. palustris was slowed down by the presence of perianths under both temperature regimes. In nearly all cases perianths induced a lower final germination. The relatively strongest reduction in germination rate was observed at the low temperature regime (10/15°C) (Fig. 1).

Both perianth powder and extract had no effect on either germination rate or on total germination of R. crispus and R. palustris (data not shown).

The acetone-pretreatment stimulated the germination rate of R. crispus achenes enclosed by perianths. No effect was observed in R. palustris (Fig. 2). In both species the acetone-pretreatment had no influence on the germination of achenes without perianths (data not shown).

Light filtering through a layer of perianth powder reduced the total germination of R. crispus; in R. palustris perianth light-filtering reduced the germination rate only slightly (Fig. 3).
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Temperature in relation to light and dark germination

To establish the relation between germination and temperature in a dark/light regime the use of a range of constant temperatures was deliberately avoided. This procedure was selected in order to make more realistic comparisons with germination under field conditions, thereby accepting difficulties to discriminate between pure temperature effects and alternating temperature effects.

Under light conditions considerable differences between the Rumex species were observed in the responses of achenes exposed to various temperature regimes (Fig. 4). In R. acetosa, maximum germination and high germination rates were observed during 10/10, 10/15, 10/20 and 10/25°C treatments. The temperature regime with the highest day temperature (35°C) resulted in a reduced maximum germination. Both other species, however, showed maximum germination rates and a short pre-germination period during regimes with high day temperatures. In this sense R. palustris was most extreme with a 15-fold increase in germination rate when changing the temperature regime from 10/15°C to 10/25; 10/30°C.

In R. acetosa both light and dark germination are independent of the amplitude of diurnal fluctuating temperatures (Fig. 5). Rumex crispus showed a stimulation of
Fig. 2. The effect of a washing with acetone and water prior to germination (±1 SE) of achenes enclosed by perianths of *Rumex crispus* and *R. palustris* at a temperature regime of 10/25 °C. The results are compared with germination percentages (±1 SE) of achenes without perianths. Seed ripened 1988; experiment carried out 5/89.

germination by fluctuating temperatures in darkness. A stimulation of light germination by a few degrees of temperature alternation was observed in *R. palustris*; dark germination was hardly affected by fluctuating temperatures (Fig. 5).

**Phytochrome control and interactions with temperature and stratification**

*Rumex acetosa* showed a relatively high level of germination in darkness. An even higher level of germination was induced by red light (R), red light followed by 35 °C (R-35) and stratification-R treatments. The promotive effect of R light was significantly reversed by 5 min far red light (FR) (Fig. 6).

*Rumex crispus* is characterized by a low level of germination in the dark at 20 °C. Both short pulses of R and 35 °C exerted a promotive effect. The induction of germination by R can be reversed by exposure to 5 min FR. Treatment combinations (R-35; stratification-R) resulted in a germination up to 100%. FR pulses only slightly reversed the stratification-R germination, indicating that stratification alone also strongly promotes germination (Fig. 6).

In *R. palustris* no germination in darkness was observed, whereas R and 35 °C pulses promoted germination in the dark at 20 °C only slightly. However, the small promotion by
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Fig. 3. The influence of light filtered through a layer of perianth powder on achene germination (± 1 SE) of *Rumex crispus* (25/25°C) and *R. palustris* (10/25°C). The final germination is compared with dark germination (± 1 SE). Seed ripened 1986; experiment carried out 6/89.

R light was reversed by FR. Germination was significantly promoted when R pulses were combined with 30 min exposure to 35°C or stratification. FR completely reversed the stimulation of stratification-R promotion. As FR can reverse R-induced germination, it is likely that stratification alone does not stimulate germination in *R. palustris* (Fig. 6).

*Achene survival under hypoxic conditions*

In *R. acetosa*, achene survival after a hypoxia pretreatment depended on both incubation temperature and duration of the treatment (Table 1). The highest mortality was observed during the 20°C pretreatment; after 26 weeks nearly all achenes of *R. acetosa* were dead. In both *R. crispus* and *R. palustris* no mortality at all was observed at both incubation temperatures after a hypoxic pretreatment of 26 weeks (data not shown).

**DISCUSSION**

Primary dispersal of *Rumex* achenes is consistently accompanied by achene drop on the soil surface. Until the achenes are buried in the seedbank both the influence of perianths (not in *R. acetosa*) and environmental conditions near the soil surface determine the fraction of the achene population that germinates. Achenes of *R. crispus* and *R. palustris*, not enclosed by perianths, germinate fast when exposed to alternating temperatures in the light. Slower germination rates and often lower final germination percentages of achenes
Fig. 4. The effect of various temperature regimes given in a dark/light rhythm on final germination, germination rate (calculated over the period in which 85% of the germinated achenes germinated) and number of days (± 1 SE) until the onset of germination of *Rumex acetosa*, *R. crispus* and *R. palustris*. Seed ripened *R. acetosa*: 1987; *R. crispus; R. palustris*: 1986; experiment carried out 8–10/87.

Enclosed by perianths in both species are probably related to light filtering properties of the perianths. It is not yet clear whether this is related to a modified spectral composition (Spence *et al.* 1971; Bewley & Black 1982) and/or to a reduction of the light fluence rate. We do know that a thin layer of perianth powder of both species resulted in an increase in
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Fig. 5. The influence of various amplitudes of diurnal fluctuation of temperature below a base temperature of 25°C on light and dark germination (± 1 SE) of *Rumex acetosa*, *R. crispus* and *R. palustris*. Seed ripened 1988; experiment carried out 5–6/89.

light absorption in the small wavelength regions (data not shown). Therefore, light filtering through this layer results in a reduced R/FR ratio. In *R. crispus*, a promotive effect on germination of achenes with perianths was also observed after washing with acetone. This suggests that acetone removes a water-repulsive layer covering the perianths, leading to water uptake and germination of the achene. Dormancy related to interference with water uptake has been described for various plants by Taylorson & Hendricks (1977) and Bewley & Black (1982).

According to their observations on germination under various temperature regimes (Fig. 4) the *Rumex* species under study can be classified into two groups:

(a) *R. acetosa* which germinates at low temperatures as well as over a wide range of temperatures at a fairly constant rate,

(b) *R. palustris* and to a lesser extent *R. crispus* which are both characterized by fast and maximal germination, whenever the upper temperatures are high (≥ 20°C).

Germination at low temperatures (≤ 7°C) as well as over a wide range of temperatures is a common feature in grassland plants, whereas high upper-temperature limits and relatively small ranges of germination temperatures are characteristic for plants from wet sites (Grime *et al.* 1981).

The responses of *R. acetosa*, *R. crispus* and *R. palustris* respectively towards diurnal fluctuating temperatures (Fig. 5) fit into the three groups which were first distinguished by Thompson & Grime (1983).

1. Species with a high level of ultimate germination in light and dark, independent of the amplitude of temperature alternations. Plants belonging to this group (predominantly grasses) have relatively large seeds or achenes, which are mostly unable to accumulate in a buried seedbank.
Fig. 6. The effects of 5 min red light (R) and far-red light (FR), 30 min 35°C and 37 days of stratification (Strat.) (4°C) on dark germination (±1 SE) of *Rumex acetosa*, *R. crispus* and *R. palustris* at 20°C. Seed ripened *R. acetosa*: 1987; *R. crispus*/*R. palustris*: 1986; experiment carried out 12/87 and 3/88.

Table 1. The effect of hypoxia pretreatments with a variable length given at two temperatures on survival (%) (±1 SE) of *Rumex acetosa* achenes. Achenes ripened 1987; experiment carried out 4/88.

<table>
<thead>
<tr>
<th>Hypoxia period (weeks)</th>
<th>Temperature 4°C</th>
<th>Temperature 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>99 ± 1</td>
<td>99 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>99 ± 1</td>
<td>97 ± 0</td>
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<tr>
<td>4</td>
<td>98 ± 1</td>
<td>83 ± 3</td>
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<tr>
<td>8</td>
<td>90 ± 4</td>
<td>39 ± 6</td>
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<tr>
<td>12</td>
<td>94 ± 2</td>
<td>23 ± 5</td>
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<tr>
<td>20</td>
<td>62 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>26</td>
<td>70 ± 4</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>
Species which require both light and fluctuating temperatures for germination. Many wetland species with small seeds or achenes belong to this group.

The reversibility between R and FR irradiation indicates that germination in all three species is under control of the phytochrome system. These results correspond fairly well with those obtained by Gambi (1966). High dark germination of *R. acetosa* is probably related to high levels of the active form of phytochrome (Pfr) or 'trapped' intermediates of the transformation Pfr to Pfr, which are conversed to Pfr during imbibition (Bewley & Black 1982; Frankland & Taylorson 1983). R-FR treatments led to a reduction of the germination below the level in the dark, indicating an initial high level of pre-existing Pfr (Probert & Smith 1986). Failure of FR to inhibit germination of *R. acetosa* completely is probably due to continued germination at low Pfr concentrations established by FR. The lower ultimate level of germination of *R. acetosa* during the 10/35°C treatment (Fig. 4) might be attributed to reversion of Pfr to the inactive form Pfr, which can occur rapidly at higher temperatures (Bewley & Black 1982).

In *R. crispus* one temperature treatment of 35°C for 30 min stimulated germination up to 81-2%. Analogous to the results obtained by Taylorson & Hendricks (1972) we observed a reduced final germination level (48-5%) when FR irradiation preceded the temperature pulse (data not shown). This indicates that lowering of the Pfr levels by FR reduces germination induced by 35°C pulses. The 35°C pulse promotes germination by decreasing the threshold of Pfr necessary to stimulate germination (Hand et al. 1982; Takaki & Zaia 1984). Recently, however, Takaki et al. (1985) reported that high dark-germination after 35°C treatment in *R. obtusifolius* does not result from increased sensitivity to Pfr; they suggest that a non-phytochrome-related process is responsible for temperature-stimulated dark germination.

Only a very slight stimulation of germination was observed when imbibed achenes of *R. palustris* were exposed to R irradiation. Repeated pulses and longer irradiation times also failed to promote germination in this species under constant temperature (Fig. 5). When a 30 min exposure to 35°C or a chilling period of 6 weeks (4°C) was combined with R treatment, germination of *R. palustris* was significantly stimulated. Both temperature treatments probably sensitized achenes to Pfr (Van der Woude & Toole 1980; Bewley & Black 1982). This increased sensitivity alone is obviously not enough to stimulate action of the low level of pre-existing Pfr as observed in *R. crispus*. Additional R irradiation is needed to increase the concentration of Pfr above the threshold necessary to stimulate germination. A stimulating effect of stratification and a 35°C pulse is also observed in *R. crispus* and *R. acetosa*. In *R. crispus*, stratification alone will probably also stimulate germination (see stratification-FR; Fig. 6). On the other hand in *R. palustris* combination with a light treatment is a necessity for germination. No germination was observed when chilling was combined with a 35°C pulse (data not shown). In contrast to *R. crispus* and *R. acetosa*, germination of *R. palustris* shows obligatory light dependence.

Under field conditions, *R. acetosa* is characterized by early autumn germination (Voosenek & Blom 1992). The ability of *R. acetosa* to germinate at relatively low temperatures is advantageous for autumn germination. Light filtered through the leaf canopy of a grassland will not influence the germination of *R. acetosa*, as its germination is
fairly independent of the red/far-red ratio of the germination site. It can be concluded that the degree of autumn germination of *R. acetosa* in time and place will depend solely on the soil moisture content. In *R. acetosa*, achene germination occurs in the absence of light and achenes show a lack of resistance towards hypoxia. These characteristics hamper both accumulation and survival in a buried seedbank in the river area.

The light filtering and/or the inhibited water uptake through perianths enclosing achenes of *R. crispus* and *R. palustris*, delay and prevent germination after achene release late in the autumn (October). Fast and maximal germination of these species is attained during conditions with high temperatures (Fig. 4); relatively low autumn temperatures will therefore also restrict germination. The small achene size of *R. palustris*, the dark inhibition of germination in *R. crispus* and *R. palustris* and the flooding resistance of their achenes promote their accumulation and survival in a buried seedbank in the river area. Dark germination of *R. crispus* from the seedbank during the spring is stimulated by winter chilling and the amplitude increase of alternating temperatures after subsidence of the winter flood. Later during the growing season, when the vegetation canopy is closed in the natural zone of *R. crispus*, temperature alternations are dampened down (e.g. Thompson *et al.* 1977). As dark germination of *R. crispus* under constant temperatures is low, germination and seedling emergence during the remaining growing season is restricted to gaps. Due to the obligatory need for light in combination with alternating temperatures or stratification, the germination of *R. palustris* on mud-flats in the river area in spring or summer is mainly restricted to cracks in the clay which develop after subsidence of the flood water.

Within the life cycle of *Rumex* species, the achene phase is important for the distribution of the species in the river area. Timing of germination and seedbank type, both related to specific achene and germination characteristics of the three species, can be linked to their growing sites in the flooding gradient.

REFERENCES


