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PLANT HORMONES REGULATE FAST SHOOT ELONGATION UNDER WATER: FROM GENES TO COMMUNITIES

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Abstract. Flooding affects the abundance and distribution of plant species worldwide. Many plants are damaged or even killed by flooding events due to the associated oxygen deprivation in cells. Stimulated shoot elongation is an important adaptive mode that can restore contact of leaves with the atmosphere above the water surface. This strongly improves inward diffusion of oxygen and the rate of photosynthesis. Fast elongation of submerged petioles of the model plant *Rumex palustris* involves the integrated action of the plant hormones ethylene, auxin, gibberellin, and abscisic acid. The closely related *Rumex acetosa* is unable to switch on petiole elongation when submerged. In a comparative study of these two *Rumex* species, we found that the response to the gaseous phytohormone ethylene, which accumulates in plant tissues during submergence, explains their contrasting elongation behavior. In order to study the importance of this shoot elongation response in the distributional patterns of plants in natural floodplains, we quantified the ethylene-induced elongation response of 22 plant species occurring in the Rhine River floodplain. These results were compared with the results of a multivariate analysis based on 84 vegetation surveys performed in the same area. The species compositions of the surveys were grouped along two environmental gradients: flooding duration and soil dehydration after the floodwater subsided. If we superimpose the ethylene-induced elongation capacity on these vegetation data, it becomes clear that the capacity to elongate upon exposure to ethylene positively correlates with flooding duration and negatively with soil dehydration. Based on this analysis, we conclude that the capacity to elongate is an important selective trait in field distribution patterns of plants in flood-prone environments. Fast shoot elongation under water seems to be a favorable trait only in environments with shallow and prolonged flooding events, while costs associated with this response prevent its expression in sites with deep floods, sites with floods short in duration, or in sites in which flood water recedes rapidly. The approach outlined in this paper may be more widely applicable in ecological studies that aim to understand the functional relationship between plant traits and species distributions along environmental gradients.

Key words: abscisic acid; auxin; cost–benefit analysis; elongation growth; ethylene; flooding duration; gibberellin; plant species distribution; Rumex; *soil dehydration; submergence; upscaling.*

INTRODUCTION

Flooding can occur in many ecosystems worldwide, including river floodplains, salt marshes, bogs, dune slacks, and swamp forests (Maltby 1991). A typical feature of these environments is their episodically anaerobic nature. Plants need molecular oxygen to synthesize large amounts of ATP. Shortage of oxygen is, therefore, considered to be a major factor that negatively affects plant growth in flood-prone habitats. Flooding also leads to accumulation of toxic soil components, reduction in light availability when plants are completely submerged, and the production of free radicals upon reaeration when floodwater subsides, all of which hamper plant performance (Voesenek et al. 1992*b*, Crawford et al. 1994).

Adaptations to flooding

Some plants species avoid flooding stress through life history tactics and survive a period of flooding as dormant seeds or quiescent perennating organs. Concomitantly, growth and reproduction are facilitated within the window of favorable circumstances (Menges and Waller 1983, Blom and Voesenek 1996). Other species exploit amelioration and true tolerance tactics to survive the flooding stress. Amelioration implies that plants are exposed to environmental disturbances, but the adverse effects are alleviated. Examples in relation to flooding include (1) fast elongation of shoot organs to allow contact of a ‘‘snorkel’’ (often leaf blades) with the atmosphere to reestablish gas exchange (Kende et al. 1998, Voesenek and Blom 1999, Peeters et al. 2002; see Plate 1). (2) development of longitudinally inter-

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connected gas-filled channels to facilitate interorgan gas diffusion (Visser et al. 1997, Jackson and Armstrong 1999, Colmer 2003), (3) development of a barrier to radial oxygen loss to prevent excessive loss of diffusing oxygen to the anaerobic soil (Colmer et al. 1998), (4) initiation and outgrowth of adventitious roots (Visser et al. 1996), and (5) continuation of photosynthesis under water that generates oxygen and carbohydrates (He et al. 1999, Vervuren et al. 1999, Rijnders et al. 2000). True tolerance refers to situations in which oxygen deprivation is not dispelled, but adaptive metabolic pathways are switched on to ensure enhanced survival rates. These comprise (1) the generation of energy without oxygen via continued glycolysis and fermentation (Perata and Alpi 1993, Vartapetian and Jackson 1997), (2) a reduction of metabolic rates to levels that just sustain maintenance (so-called anaerobic dormancy), (3) avoidance of cytoplasmic acidosis (Saglio et al. 1999), and (4) protection against post-anoxia injury via antioxidants or enzymes that scavenge oxygen radicals (Smirnoff 1995).

Temporary floods differ considerably in seasonal timing, duration, depth, and frequency (Vervuren et al. 2003). Variation in these factors creates a continuum of flooding regimes in flood-prone habitats. The ability of some plant species to elongate petioles and/or stems upon submergence correlates with the type of flooding regime in their natural environment, because there are costs involved with underwater elongation and because the benefits will not always be apparent. In this paper, we focus on stimulated shoot (petiole or stem) elongation as a trait in which plant hormone sensitivity strongly interacts with the ecology of plant species in flood-prone environments. This flooding-induced elongation response, also described as ‘‘depth accommodation’’ (Ridge 1987), returns the shoot to the atmosphere above the water surface, needed for long term survival.

Costs of elongation

Increased cell division rates and the synthesis of new cell walls during fast cell elongation require substantial amounts of energy and carbohydrates. This is illustrated by work of Setter and Laureles (1996) in which elongating rice varieties had a lower submergence tolerance compared to nonelongating varieties. When the elongation capacity was reduced artificially by the gibberellin biosynthesis inhibitor paclobutrazol, survival increased significantly (Setter and Laureles 1996), suggesting that the resources saved by refraining from elongation were available for survival. Other costs of submergence-induced elongation may become apparent when floodwaters subside. Elongated petioles and leaves of some species (e.g., *Ranunculus repens*, *Caltha palustris*) are brittle and weak, desiccate rapidly compared to nonsubmerged control leaves (Ridge 1987), and the plant parts typically have high mortality rates after desubmergence (Voesenek and Blom 1989*a*).

PLATE. 1. Several *Rumex* species have the capacity to stimulate petiole elongation once submerged. This growth response reestablishes contact of leaves with the atmosphere and thus enhances plant survival. Photo credit: F. Millenaar.

Fast elongation will only persist as a strategy if these costs are outweighed by benefits such as improved aeration and restored rates of photosynthesis (Ridge 1987). Enhanced elongation rates during submergence increase plant survival, only if contact between leaf blades and the atmosphere is restored. Thus, fast elongation under water will be a high-risk strategy when flooding events are of short duration and when the submergence depths go beyond the elongation capacity of the plant (Ridge 1987). We therefore hypothesize that plant traits associated with submergence-induced shoot elongation predominantly occur in plant species from flood-prone habitats characterized by prolonged, but shallow, submergence.

To test this hypothesis we formulated two research questions:

1) Which mechanisms are responsible for shoot growth stimulation in certain species upon submergence and lack of this response in others?

2) Can we relate these opposing elongation responses of plant species upon submergence to their distributional patterns in natural flood-prone environments?

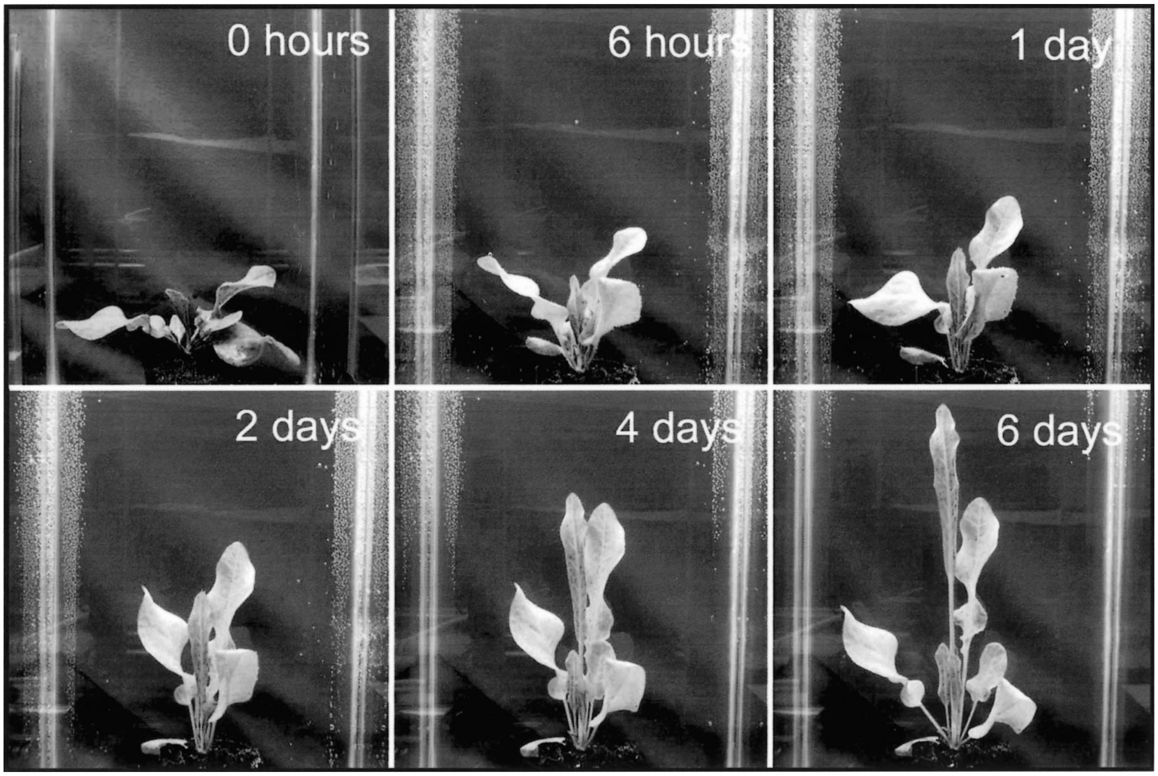

FIG. 1. Submergence-induced hyponastic growth and petiole elongation in *Rumex palustris*, shown as a time series.

To answer the first question, we unraveled the signal perception and transduction cascade involved in submergence-induced petiole elongation in the dicot *Rumex palustris*. Fast elongation of submerged petioles of *R. palustris* involves the integrated action of at least four plant hormones: ethylene, auxin, gibberellin, and abscisic acid. These hormones are main players in the signal perception and transduction network. It is our aim to shed light on key steps in the transduction network that make the difference between species that are able to ''switch on'' fast elongation (e.g., *R. palustris*) and those that are not (e.g., the closely related species *Rumex acetosa*).

In order to answer the second question, we scaled up from a plant trait (enhanced shoot elongation) to the level of the river floodplain community. The elongation capacity of 22 river floodplain species was experimentally tested and the results were superimposed on a vegetation–environment relationship using 84 vegetation surveys from floodplains along the Rhine River in the Netherlands. By this approach, we quantify the relationship between the capacity to elongate and the plant species distribution along an environmental gradient.

SUBMERGENCE-INDUCED SHOOT ELONGATION IN *RUMEX PALUSTRIS*

In natural river floodplains, *Rumex palustris* has a biennial or short-lived perennial life cycle. Shortly after germination it develops a rosette. Vegetative rosettes as well as bolting plants can be exposed to natural floods and both life cycle phases have the capacity to respond to submergence with enhanced elongation of petioles and stems, respectively (Voesenek and Blom 1989*a*, Van der Sman et al. 1991, 1992).

Most work on *Rumex* has focused on submergence-induced elongation of rosette leaves. Rosette leaves assume a horizontal position, close to the substrate under nonsubmerged conditions. The orientation of leaves changes dramatically into an almost vertical position after ~6 h of submergence (Fig. 1; Peeters et al. 2002, Cox et al. 2003). This so-called hyponastic growth is caused by elongation of several cells located at the basal abaxial side of the petiole (M. C. H. Cox, *unpublished data*). When the angle between the petiole and the horizontal is larger than 40–50°, the entire petiole starts to elongate (Cox et al. 2003). This elongation growth is distributed equally over the petiole (Rijnders et al. 1996) and is responsible for 80% of the whole-leaf (petiole + blade) elongation response, indicating that the contribution of the leaf blade in underwater elongation is very restricted (Voesenek and Blom 1989*b*). Elongation of existing petioles in *Rumex* is predominantly driven by cell elongation; cell division is not important (Voesenek et al. 1990). Submergence-induced elongation in *R. palustris* varies between leaves, with the strongest elongation responses in the youngest leaves (Fig. 1; Voesenek and Blom 1989*a*).

SENSING SUBMERGENCE

Ethylene

The gaseous phytohormone ethylene plays a key role in the perception of environmental changes induced by submergence. Nearly all cells of higher plants produce ethylene in low amounts (Abeles 1992). Endogenous levels are controlled by a subtle balance between production rates, conjugation rates, and diffusion to the outside environment (Voesenek and Blom 1999). When a plant is surrounded by air, ethylene is vented off quite rapidly, due to the relatively high diffusion coefficients for ethylene in air and the steep concentration gradient from the interior of the cell to the air outside the plant. The situation changes dramatically when a plant is submerged completely in water. Gases diffuse ~10 000 times slower in water than in air, resulting in fast increase of the endogenous ethylene concentration, assuming that the production rate is unchanged (Jackson 1985, Ridge 1987).

A 20-fold increase in ethylene concentration in shoots of *R. palustris* has been observed within 1 h of submergence (Banga et al. 1996), resulting in an endogenous ethylene concentration (1 μL/L) that is high enough to saturate ethylene-induced elongation in *Rumex* under nonsubmerged conditions (Voesenek et al. 1996). The importance of ethylene accumulation is further illustrated by the observation that silver ions, which interfere with ethylene perception and thus inhibit the action of this hormone, prevent hyponastic growth and stimulated petiole elongation (Voesenek and Blom 1989*a*).

Ethylene binds specifically to members of a family of high affinity receptor proteins belonging to the so-called two-component regulators (Bleecker and Kende 2000). Mutants of *Arabidopsis thaliana* containing one amino acid substitution at position 65 in the ethylene receptor molecule are unable to bind ethylene and are insensitive to applied ethylene in a broad spectrum of responses (Bleecker and Kende 2000). A cDNA clone (*Rp-ERS1*) similar to an ethylene receptor gene of *Arabidopsis thaliana* has been isolated for *R. palustris* (Vriezen et al. 1997). The expression of this gene is strongly up regulated by submergence, ethylene, and low oxygen concentrations; down-regulation occurs upon desubmergence (Voesenek et al. 1997, Vriezen et al. 1997). These data suggest that *R. palustris* plants respond to submergence by increasing the number of their ethylene receptors. Since ethylene receptors are active in the absence of ethylene and negatively regulate ethylene responses, more receptor molecules result in a desensitization of the cells to ethylene (Hua and Meyerowitz 1998). The loss of sensitivity to ethylene during submergence has no effect on the elongation response of *R. palustris*. This is probably related to the over saturating concentrations of ethylene inside plants that are submerged for several hours (Voesenek et al. 1993*a*).

Low oxygen

In addition to ethylene, hyponastic growth and enhanced petiole elongation in *R. palustris* can also be induced by subambient oxygen concentrations of 2–10% (Voesenek et al. 1997). A similar observation has been made for coleoptiles of rice (Horton 1991). When applied together (low oxygen + ethylene), a stronger elongation response was observed than under separate treatments with ethylene and low oxygen. Low levels of oxygen exert their effect on petiole elongation in *Rumex* not independent of ethylene. When low oxygen is applied together with L-alpha-(2-aminoethoxyvinyl)-glycine (AVG), an inhibitor of ethylene biosynthesis, the elongation response is completely abolished. Addition of 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene, restores the low oxygen response, indicating that the effect of AVG is specific (Voesenek et al. 1997). Low oxygen levels do not affect the ethylene production rate, but cause an increase in the sensitivity of the petiole towards ethylene. The exact nature of this shift is still unknown.

We suggest that the very fast elongation that occurs in *R. palustris* when low oxygen and elevated ethylene levels are both present, functions as a mechanism to ensure quick leaf blade emergence. The oxygen-limiting condition can occur in nature when plants are submerged in turbid water, and therefore, produce little photosynthetic oxygen. Under less turbid conditions, photosynthetically derived oxygen improves not only the oxygen status of the whole plant (Rijnders et al. 2000), but also increases its chances of survival considerably (Nabben et al. 1999). A lower petiole elongation rate driven only by ethylene under these conditions would be less harmful in terms of survival.

TRANSDUCTION OF SUBMERGENCE SIGNALS

Auxin and gibberellins

Thirty years ago, Musgrave and colleagues (1972) stated that at least one other plant hormone in addition to ethylene is required for fast underwater elongation. These authors studied stem elongation in *Callitriche platycarpa* and demonstrated that gibberellin was the additional hormone. A similar ethylene–gibberellin requirement is also described for deepwater rice (Kende et al. 1998). In other plant species (e.g., *Ranunculus sceleratus*) that extend rapidly when submerged, the second plant hormone was auxin. It was even suggested that all three hormones mentioned, ethylene, gibberellins, and auxin, are required for sustained elongation under water. Auxin might play a role in the initiation of rapid elongation, whereas gibberellins might be responsible for longer-term maintenance of fast growth (Ridge 1987).

Auxin is predominantly produced in shoot apices and in young expanding leaf blades (Ljung et al. 2001). This plant hormone is transported in a polar manner to petioles, stems, and roots, mediated by influx and efflux carrier proteins located in the membrane (Muday and DeLong 2001). Removal of leaf blades, the putative source of auxin, abolishes hyponastic growth in submerged *R. palustris* plants for many hours. Similarly *N*-naphthylphthalamic (NPA), an inhibitor of auxin efflux, delays hyponastic growth. The response to leaf blade removal could be rescued by 1-naphthaleneacetic acid (1-NAA), an artificial auxin that freely diffuses into cells (M. C. H. Cox and L. A. C. J. Voesenek, *unpublished data*). We conclude that polar auxin transport from the leaf blades to the basal side of the petioles is required for submergence-induced hyponastic growth. These results further point toward an interaction between ethylene that accumulates in submerged petioles and auxin. The precise nature of this plant hormone interaction is not yet known.

Inhibitors of gibberellin (GA) biosynthesis inhibit partially submergence-induced petiole elongation in *R. palustris*. This inhibition could be rescued completely upon application of various GAs, indicating that the effect of the inhibitors is specific and nontoxic (Rijnders et al. 1997). We conclude that, as for deepwater rice, a certain endogenous concentration of GA is required for underwater shoot elongation in *Rumex*. GA levels and the sensitivity of petiole tissues of *R. palustris* towards GA are regulated by submergence. The concentrations of both GA_1 and its precursor GA_{20} is increased during 24 h of submergence (Rijnders et al. 1997). Furthermore, submergence induces an increase in the sensitivity of *Rumex* petioles towards GA. The effects of submergence on GA concentration and sensitivity can by mimicked completely by exposing plants to elevated ethylene levels, indicating that ethylene interacts with GA in this response (Rijnders et al. 1997).

Abscisic acid (ABA)

ABA plays a major role in seed maturation and germination, as well as in adaptations to abiotic stress (Buchanan et al. 2000). Besides its important role in drought avoidance ABA also seems to be important in the regulation of shoot elongation in submerged plants. In both deepwater rice and *Scirpus micronatus*, ABA inhibits submergence-induced shoot elongation. Furthermore, a fast decline in endogenous ABA concentrations in shoots is observed when these species were submerged or exposed to elevated levels of ethylene (Hoffmann-Benning and Kende 1992, Lee et al. 1996). It has been assumed that the sensitivity to GA of deepwater rice internodes is decreased by ABA and that an ethylene-induced down-regulation of ABA increases this sensitivity. In this respect, ABA can be seen as a negative regulator of GA action (Hoffmann-Benning and Kende 1992, Ritchie and Gilroy 1998).

An inhibition of submergence-induced shoot elongation by ABA is also observed in the dicot *R. palustris*. The ABA concentration in this species declined 80% within 1 h of submergence or exposure to elevated ethylene levels. Submergence of *R. palustris* plants pretreated with 1-MCP, an inhibitor of ethylene perception, prevented this fast down-regulation of ABA (J. J. Benschop and L. A. C. J. Voesenek, *unpublished data*). Taken together, all these results suggest that down-regulation of ABA, mediated by ethylene, is a prerequisite for fast shoot elongation under water in both monocots and dicots.

VARIATION IN UNDERWATER ELONGATION: AN ANALYSIS WITH A BOOLEAN NETWORK

The signaling network resulting in enhanced petiole elongation and hyponastic growth in *R. palustris* when completely submerged is summarized in Fig. 2. We applied the Boolean language, which is a digital (numeric) formalism that allows a qualitative description of signal transduction networks (Genoud and Metraux 1999, Genoud et al. 2001, Cox et al. 2003). The Boolean language converts signaling elements into binary state elements, that either have an ‘‘on’’ (1) or an ‘‘off’’ (0) state. Interfering signals can be seen as ‘‘inputs’’ that join in a Boolean gate or operator. The combination of signals results in a specific ‘‘output’’ signal (Genoud and Metraux 1999). Complete submergence of *R. palustris* always results in an enhanced endogenous ethylene concentration. Furthermore, when flooding coincides with reduced light levels it can induce a strong decline of the endogenous oxygen concentration in the petioles. Subsequently, low oxygen conditions increase the sensitivity of petioles towards ethylene. The submergence-oxygen-ethylene interaction is characterized by two NOT gates, indicating that in both cases the output signal is negatively regulated by the input. The oxygen–ethylene pathway joins with the ethylene-alone pathway in an OR gate, indicating that an input signal generated by at least one of the pathways is required to induce an output signal. This output signal downregulates the ABA concentration, upregulates both the concentration of and the sensitivity to GA, and acts as an input signal leading to hyponastic growth. However, this differential growth response will only take place when the petiole angle is smaller than 80°. The ethylene output signal and the angle $<80°$ signal join in a so-called AND operator, indicating that both signals are absolutely required to evoke an output. Preliminary evidence indicates that this output signal modifies local auxin concentrations and/or auxin sensitivities, leading to a differential growth stimulus with a hyponastic curvature as a result. Enhanced petiole elongation takes place when the signal associated with the reduced ABA level and the GA signal join in an AND operator. However, it will only lead to elongation growth when the petiole angle is above 40–50°, demonstrating that hyponastic growth interacts with petiole elongation. From this, we hypothesize that the petiole angle generates a signal that is required as an input signal for both hyponastic growth and petiole elongation. The nature of this signal is yet unknown.

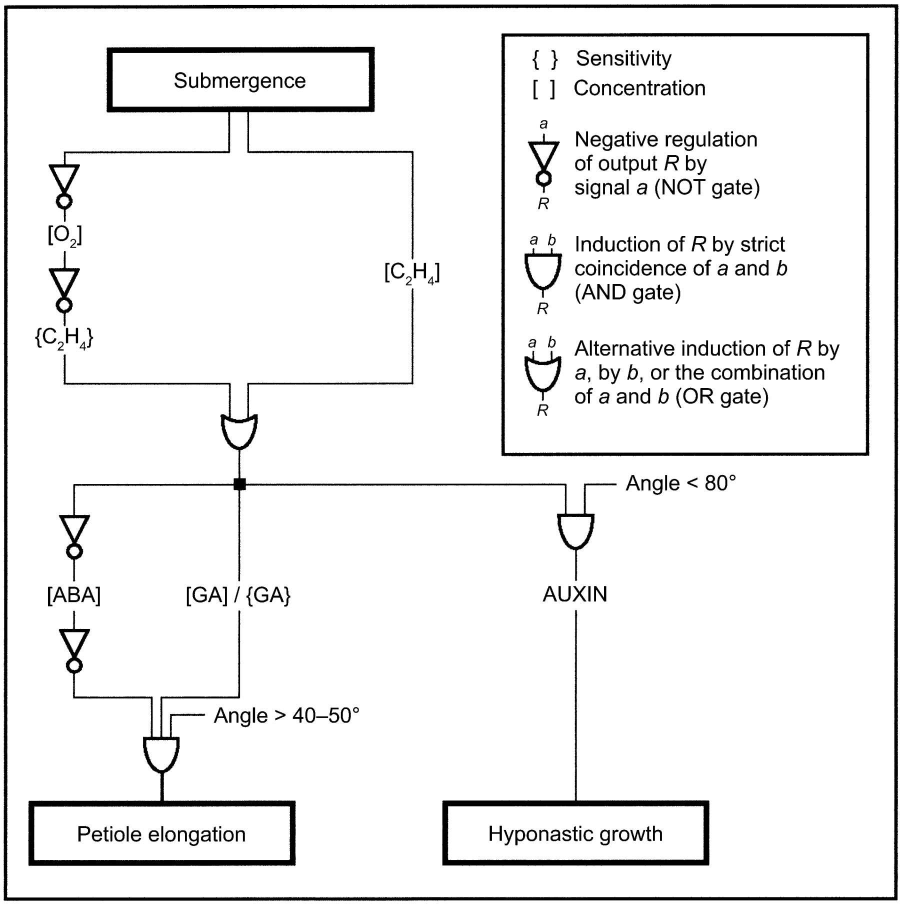

FIG. 2. Model of signal-transduction network involved in submergence-induced hyponastic growth and petiole elongation of *Rumex palustris*.

Given the signaling network as revealed to date, we can now begin to answer one of the central questions of this paper: why are certain species able to elongate and others not? Is it possible to trace back a fundamental difference in growth response to certain steps or even single genes in the signal-transduction cascade? To answer these questions, we compared the signal transduction network of *R. palustris* with that of the closely related species *Rumex acetosa*. *Rumex acetosa* L. is a perennial species from grasslands. In river floodplains, it rarely occupies flooded sites. Under experimental conditions it is less tolerant to complete submergence than *R. palustris* is. This is true for seeds (Voesenek et al. 1992*a*), seedlings (Voesenek et al. 1993*b*) and adult plants (Voesenek 1990). Petioles of *R. acetosa* do not demonstrate hyponastic growth and fast rates of petiole elongation when exposed to complete submergence (Voesenek and Blom 1989*a*). Despite the absence of an elongation response, ethylene does accumulate to concentrations that induce responses in *R. palustris* (Voesenek et al. 1993*a*). Moreover, an inhibition of petiole elongation is observed when *R. acetosa* was exposed to gas mixtures containing elevated ethylene concentrations (Voesenek and Blom 1989*a*).

In contrast to *R. palustris*, the GA and ABA concentrations do not change when *R. acetosa* is submerged or exposed to elevated ethylene levels (Rijnders et al. 1997; J. J. Benschop and L. A. C. J. Voesenek, *unpublished data*). Furthermore, this species exhibits a decrease in GA sensitivity during submergence and in the presence of ethylene, rather than an increase (Rijnders et al. 1997). Based on these data, we conclude that the decline in the ABA concentration and the increase in the GA concentration/sensitivity typical for submerged *R. palustris* plants is absent in submerged shoots of *R. acetosa*. Interestingly, if we expose *R. acetosa* to elevated GA concentration in a nonsubmerged (air) environment, a significant stimulation of petiole elongation is observed (Rijnders et al. 1997).

We conclude that in *R. acetosa* the downstream signal transduction pathway from GA to petiole elongation is intact and that it can be switched on by the appropriate signal. However, from our results, it is clear that the phytohormone ethylene is unable to switch on this downstream cascade. We speculate that in *R. acetosa* other input signals than ethylene will be able to induce enhanced elongation. One such signal may be low light and/or decreased red to far red ratios. These signals, perceived by phytochrome proteins, generate longer stems and petioles in a large number of plant species as part of the shade avoidance response (Schmitt et al. 1999, Smith 2000).

The main difference explaining the variation in submergence-induced petiole elongation between *R. palustris* and *R. acetosa* is now reduced to essentially one step in the network: the interaction between ethylene-generated signals with downstream targets such as the growth promoting hormone GA. We hypothesize that ethylene in *R. palustris* interacts with genes whose products are able to regulate ABA and GA in such a way that elongation growth is stimulated. This specific interaction is different or absent in *R. acetosa*.

In order to scale up this result to riverine plant communities, we must ask the question whether the differences in response between the two *Rumex* species are indicative for a larger number of plant species, and whether the essential difference in a plant trait is predictive for the distribution of the species in the field. To this aim, we now first analyze the species composition of herbaceous plant communities along the lower river Rhine in the Netherlands, as an example of a particular gradient along which flooding intensity varies strongly. Next, for a group of 22 species from these communities, including both *Rumex* species, we examine the shoot elongation response to elevated levels of ethylene.

METHODS

Field survey of plant species distribution and hydrological characteristics

Floodplains in The Netherlands are characterized by multiple variations in elevation, both natural and human made, resulting in a variety of habitats including river banks, natural levees, floodplain channels, back swamp areas with floodplain channels, summer flooding protecting embankments, and flood preventing dikes. Elevational variation in combination with runoff pattern (free or obstructed) results in the formation of flooding gradients perpendicular to the river and a clear vegetation zonation (Sykora et al. 1988, Blom et al. 1990, 1996).

We examined variation in floodplain vegetation, and its underlying hydrological factors, by a set of 84 relevees (vegetation surveys) from floodplains along river Rhine. Most relevees were from the Waal distributary, the major branch of the Rhine River delta system in The Netherlands. Hydrologically, this part of the river belongs to the upper, nontidal part of the Rhine River system, characterized by large water-level fluctuations of several meters. Main flooding events occur in winter and early spring. Surveys were made between Rhine kilometers 863 to 950 (measured from Konstanz, Germany) at different positions (sites) along the flooding gradient. The relevees date from the period 1972–1991.

Vegetation relevees were made according to the technique of the Zürich-Montpellier School (see Shimwell 1971) using species cover estimates with plot sizes between 4 m^2 and 25 m^2. The scores for species abundance were transformed according to Van der Maarel (1979) to an ordinal scale of 1 through 9, reducing the effect of dominant species. Ordinal species abundances were processed following standardized ordination techniques. A detrended correspondence analysis (DCA; Hill and Gauch 1980), using the CANOCO program package (Ter Braak 1987), generated scores for sites and species at a first and a second ordination axis. For classification of the sites, the TWINSPAN package (Hill 1979) was used.

The elevations of the sites at the places of the relevees were determined by leveling or estimated from detailed topographical maps (scale 1:25 000). Measured NAP-levels (+ Normal Amsterdam Level) were transformed to Lobith levels by correcting for the decline of the river water level (Survey Department of the Dutch Ministry of Public Works). At Lobith, a gauging station records daily water levels of the Rhine River. Data for the period 1981–1990 (Waterdata desk, Dutch Ministry of Public Works, Lelystad, The Netherlands), together with the Lobith-level elevation data, were used to compute the mean flooding incidence per year for each of the sites.

The geomorphology of the Rhine River system is complex and has lead to the formation of riverine dunes, depressions in old branches, dikes, embankments, and the like. Soil types vary concomitantly, from coarse river sand on dunes to heavy clay in blocked depressions. Due to the dynamic nature of the river, the hydrology of the sites may have altered in history so that combinations of sand and clay may be found. For soil dehydration rate, two factors are relevant. First, the soil texture, with clay drying out slowly and sand drying out fast. Second, the drainage conditions of the site, i.e., whether free runoff of flood water is prevented as is the case behind small artificial embankments or in depressions. Based on these considerations and the variation encountered in the field, each of the sites was assigned to one out of five ordinal classes for dehydration rate: very quick (sand, free runoff), quick (sand on clay, free runoff), intermediate (clay, free runoff), slow (clay, blocked), and very slow (mud, blocked).

Ethylene-induced shoot elongation

Species were selected for ethylene-induced shoot elongation when they were generally common in the Waal River floodplains and when they occurred at restricted elevations within the flooding gradient. These criteria led to a selection of 22 grass and herbaceous dicot species. Seeds of all screened species were collected in Waal River floodplains and germinated on moist filter paper in Petri dishes for 10 d in a germination cabinet (16 h photoperiod, day/night temperature of 25°/10°C, photosynthetic photon flux density, PPFD, of 30 $\mu mol\cdot m^{-2}\cdot s^{-1}$ from fluorescent lamps). Seedlings were planted in plastic pots (60 mL) filled with a mixture (1:1 by volume) of sand and potting compost and grown for 4 wk in a growth chamber (16 h photoperiod, day/night temperature of 21°/15°C, PPFD of 95 $\mu mol\cdot m^{-2}\cdot s^{-1}$ from fluorescent lamps).

For the first experiment, similar sized 4-wk-old plants of 11 species were selected and placed in 500-mL pots with a mixture (1:1 by volume) of sand and potting compost. Replicate plants were randomly distributed over two treatments (ethylene exposure and control, $n = 8$–9 per treatment–species combination). In a second experiment, plants of 22 species were selected and were allowed to grow for an additional period of 3 wk after repotting. Thereafter, three to four replicate plants per species were randomly assigned to one of the two treatments.

Plants were treated for 5 d with air or air containing 10 μL/L ethylene in a gas-tight compartment (2.3 m^3, 16 h photoperiod, day/night temperature of 25°/20°C, PPFD of 95 $\mu mol\cdot m^{-2}\cdot s^{-1}$ from fluorescent lamps). At this concentration ethylene-induced shoot elongation is saturated in all plant species so far studied (Ridge 1987, Voesenek and Blom 1999). At the beginning and the end of the experimental period, for each plant, the longest leaf, youngest leaf, youngest petiole, and the youngest internode were marked and measured. For monocots, the youngest internode, longest and youngest leaf, and leaf sheath were measured. The result of the most responsive organ to the ethylene treatment was used in subsequent analyses.

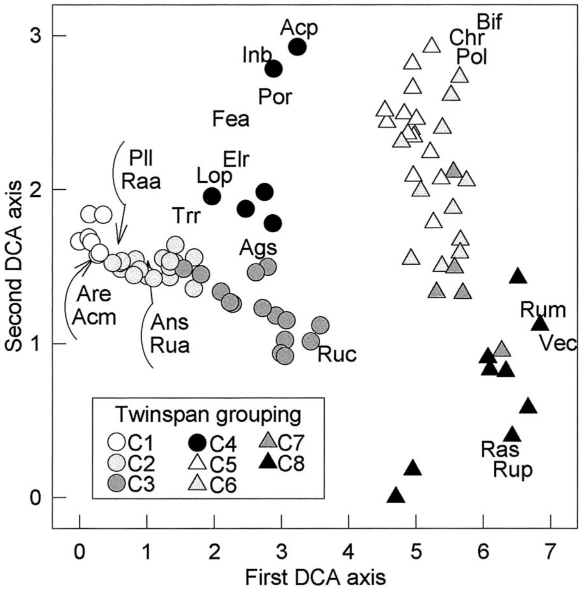

FIG. 3. Two-axis ordination diagram of the species composition of 84 sample sites in Rhine River floodplains in The Netherlands, based on a detrended correspondence analysis (DCA). A TWINSPAN classification of the site data produced eight classes (C1–8), indicated with different symbols. The species screened for ethylene-induced shoot elongation (see Fig. 4) are superimposed according to their position in the ordination. Abbreviated species names: Acm, *Achillea millefolium*; Acp, *A. ptarmica*; Ags, *Agrostis stolonifera*; Ans, *Anthriscus sylvestris*; Are, *Arrhenatherum elatius*; Bif, *Bidens frondosa*; Chr, *Chenopodium rubrum*; Elr, *Elymus repens*; Fea, *Festuca arundinacea*; Inb, *Inula britannica*; Lop, *Lolium perenne*; Pll, *Plantago lanceolata*; Pol, *Polygonum lapathifolium*; Por, *Potentilla reptans*; Raa, *Ranunculus acris*; Ras, *R. sceleratus*; Rua, *Rumex acetosa*; Ruc, *R. crispus*; Rum, *R. maritimus*; Rup, *R. palustris*; Trr, *Trifolium repens*; Vec, *Veronica catenata*.

PLANT COMMUNITIES AND HYDROLOGY IN RHINE RIVER FLOODPLAINS

The results of a multivariate analysis of the species composition of the 84 sample sites are shown in Fig. 3. The first two ordination axes could be explained from two major environmental variables. The first variable is flooding duration that correlated strongly with the first axis site scores ($r = 0.93$, Pearson product–moment coefficient, $P < 0.001$). The second variable, soil dehydration rate, was significantly negatively correlated with the first axis ($r = -0.61$, Spearman rank coefficient, $P < 0.001$), and positively with the second axis ($r = 0.61$, $P < 0.001$).

The ordination diagram of Fig. 3 suggests that two environmental gradients can be distinguished that both run from the seldom-inundated sites on dike slopes towards the long flooded sites. These gradients were further apparent from a TWINSPAN classification of the field survey data resulting in eight different vegetation classes depicted in Fig. 3. At the left side of the ordination diagram, both gradients start with classes C1 and C2, for which flooding was nearly absent (on average less than 2 d/yr). These vegetations include seldom-flooded sites on sand or sandy clay, dominated by perennial grasses like *Festuca rubra*, *Poa pratensis*, and *Arrhenaterum elatius*, and the dicot *Rumex acetosa*.

From these high-elevated sites one gradient directs towards open and well-drained sites (the upper right corner in the ordination diagram). Vegetation class C4 is an intermediate, consisting of sites on very quickly drying sandy substrate exposed to floods of short duration (mean 35 d/yr), and characterized by *Potentilla reptans* and *Achillea ptarmica* as well as several grass species. With means of about 120 d of flooding per year, flood duration is substantial for the extreme sites (classes C5 and C6) along this gradient. These vegetations are situated at riverbanks with very quickly drying sandy soils dominated by short-lived plant species such as *Chenopodium rubrum*, *Atriplex prostata*, and *Polygonum lapathifolium*. Many of these species avoid these flooding events through life cycle timing.

The other gradient extends towards more blocked (free runoff is restricted) and slowly drying depressions on mudflats (the lower right corner of Fig. 3; classes C7 and C8), with even longer flood durations of around 170 d/yr. These sites were dominated by annuals, biennials, and ephemeral perennials, but the species composition was very different from the classes C5 and C6. Typical species include *Rumex palustris, Limosella aquatica,* and *Rorippa amphibia*. For this gradient, the moist clay sites of class C3 are intermediate, characterized by short flooding (mean 33 d/yr) but dehydrating more slowly than the sites of class C4. A typical species for class C3 is the perennial grass *Agrostis stolonifera*.

ETHYLENE-INDUCED SHOOT ELONGATION IN RELATION TO PLANT SPECIES DISTRIBUTION

Species from the floodplain communities described above differ markedly in flooding induced shoot elongation. For example, *Rumex acetosa* from high elevations did not significantly increase in shoot height under submerged as compared to drained conditions, whereas low-elevated *Rumex palustris* increased threefold after 4 d of submergence (Voesenek and Blom 1989*a*). A qualitatively similar difference exists between two *Ranunculus* species. *R. acris* from high-elevated sites hardly increased its petioles upon submergence (1.2-fold as compared to drained controls), while flood-tolerant *R. sceleratus* increased 3.5-fold (He et al. 1999). Ridge (1987) showed that for 27 herbaceous species with marked elongation underwater, the hormone ethylene was involved in the response in 21 cases. For the remaining six species, ethylene sensitivity had not been studied at that time. This information is consistent with our current knowledge on the regulation of elongation given above, and highlights ethylene as the key plant hormone needed to initiate and continue fast shoot elongation under water. It calls for a systematic investigation of ethylene-induced shoot elongation in relation to species distribution in the field.

The results of a comparative screening showed that species differ markedly in response to ethylene, both quantitatively and qualitatively (Fig. 4). From the 11 species tested in the first experiment, the four species from rarely inundated sites, at the left side of the first ordination axis (Class C1 and C2 in Fig. 3), produced on average shorter shoots upon exposure to ethylene and for two of these species (*Rumex acetosa, Trifolium repens*) this response was significant (Fig. 4A). The three species from long-flooded and slowly dehydrating sites (*Rumex palustris, Rumex maritimus,* and *Ranunculus sceleratus*; class C8) were characterized by a significant ethylene-induced increase in stem elongation. The intermediate four species produced either shorter or longer shoots in response to ethylene, but none of these responses were statistically significant. Notably, the two species that tended to form shorter shoots (*Potentilla reptans* and *Chenopodium rubrum*) were from sites with relatively good dehydration (classes C4 and C5 in Fig. 3), while the two species that marginally increased in length (*Agrostis stolonifera* and *Rumex crispus*) were typically from more slowly drying sites (class C3).

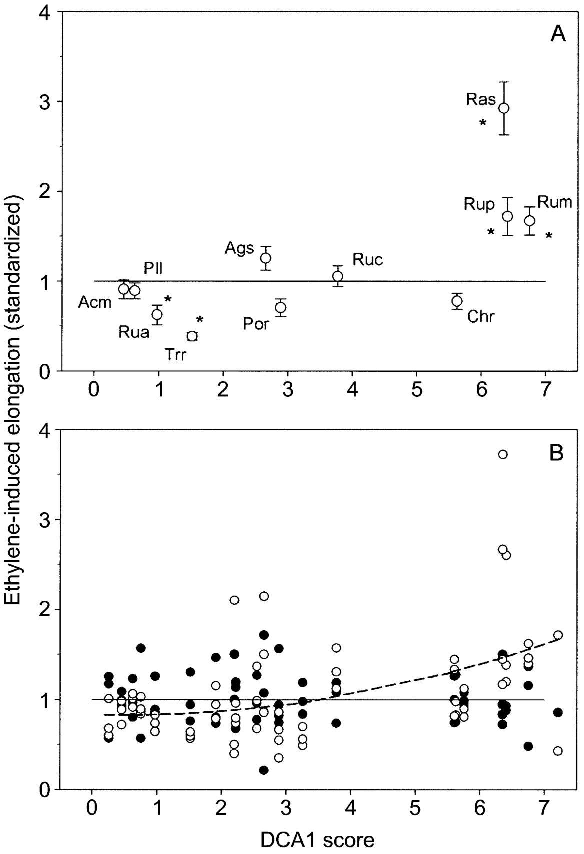

FIG. 4. Ethylene-induced shoot elongation in herbaceous species from floodplains of the Rhine River. (A) Mean shoot elongation rates (±1 SE, $n = 8$–9) of 11 species relative to the mean elongation under control conditions (standardized at 1). Species abbreviations are as in Fig. 3. Asterisks indicate significant differences ($P < 0.05$) between ethylene and control treatments. (B) Shoot elongation rates of individual plants of the 22 species listed in Fig. 3, under ethylene exposure (open symbols) and control conditions (closed symbols), relative to the mean elongation rates under control conditions (standardized at 1). The nonlinear regression line (dashed) is statistically significant at $P < 0.001$ ($F_{2,63} = 9.51$). In both panels, species are ordered on the abscissa according to their position on the first DCA axis of Fig. 3.

The results of the second experiment confirmed the results of the first experiment (Fig. 4B). Over all 22 species, there was a significant positive correlation between ethylene-induced elongation values and the scores on the first ordination axis ($r = 0.481$, $P < 0.001$). Nonlinear regression analysis showed a tendency for plants from rarely inundated sites at the left side of the diagram to produce shorter shoots upon ethylene exposure, while the shoots of plants from long-flooded sites may double or more in length under ethylene as compared to controls. The mid group of species in Fig. 4B showed a variable response, which may be explained by the negative correlation between ethylene-induced elongation and the position of the species along the second ordination axis ($r = -0.624$, $P < 0.001$). As in the first experiment, species of riverbanks with relatively good dehydration (classes C4 and C5) tended to produce shorter shoots upon ethylene exposure while the reverse was true for species of the more slowly drying sites of classes C3 and C8. Hence, ethylene-induced elongation was negatively related to soil dehydration rate scores of the species.

We suggest that the match between elongation capacity and the distribution of the species along the flooding gradient is an expression of the costs and benefits associated with fast shoot elongation underwater. The results are consistent with the hypothesis that elongation is only beneficial in areas characterized by long-term, but shallow, flooding. The blocked, slowly draining sites, where shallow, temporary pools are formed at which the plants have a fair chance of reaching the water surface, contain the species with the most pronounced ethylene-induced shoot elongation capacity. These sites retain floodwater much longer than the quickly drying exposed sites. For plant species in these latter sites the increase in shoot elongation was small or absent. In species of dry grassland sites, the response to ethylene exposure was even the opposite (i.e., shoot length reduction), suggesting that there are costs involved with a rapid elongation upon flooding.

The results of the screening also suggest that the critical difference in elongation response between the contrasting species couple *Rumex palustris* and *R. acetosa*, as outlined earlier in this paper, may well apply to a large group of herbaceous species, both monocots and dicots. Sensitivity to ethylene varies widely among species and may largely explain the large differences in shoot elongation capacity under submergence. Interestingly, no phylogenetic patterns are apparent. Large differences in response occur for species of the same genus, while species of different families may respond similarly, suggesting that the ability to elongate underwater has easily changed in the course of evolution. In addition, the two *Rumex* species occupy contrasting positions along one of the major environmental gradients identified in the vegetation surveys, i.e., from rarely flooded river dike grasslands (*R. acetosa*) to long-flooded, slowly drying mudflats (*R. palustris*), and may therefore serve as model species for the plant communities in which they occur.

PERSPECTIVES AND CONCLUSION

Our vegetation analysis suggests that flood duration and soil dehydration are major environmental variables shaping the species composition of the plant communities along flooding gradients of the Rhine River. Rapid shoot elongation, regulated at least by four plant hormones, is an effective remedy to counteract the problems of flooding, but only when flooding is prolonged and shoots can reach the water surface. Species growing under such conditions in the field have a high shoot elongation capacity, but not the species from more elevated sites that are rarely flooded or species from riverine dunes where the water resides rapidly, probably because of the major costs involved with the elongation response. The signal transduction pathway analysis of two *Rumex* species suggests that hormone sensitivity to ethylene may be a key factor explaining the range in elongation responses. In interaction with the environmental gradients, this single trait and its associated costs and benefits may thus act as an important structuring force for plant species distribution and community composition in the riverine floodplain.

Knowledge of the crucial components that regulate submergence-induced shoot elongation allows direct experimentation into the occurrence of the trait among genotypes, populations, or species. Next, with knowledge of the regulation mechanism, original tests of the hypotheses generated by these comparisons may be developed. Once the genes have been discovered that are responsible for the elongation, transgenic plants with altered expressions may be constructed allowing for a direct experimental test of the costs and benefits. The nature of these costs can be further typified with field transplant experiments and thus it may be tested to what extent they are responsible for structuring the floodplain species composition. We suggest that the approach outlined in this paper may be more widely applicable to research aimed at a functional understanding of species distributions along environmental gradients.

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