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Petiole Elongation in *Rumex* Species During Submergence and Ethylene Exposure: The Relative Contributions of Cell Division and Cell Expansion

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Abstract. Submergence of *Rumex crispus* L. and *R. palustris* Sm. stimulates elongation of the youngest petiole. In *R. palustris* this response can be mimicked partially by exposure to exogenous ethylene. In both species, petiole elongation induced by ethylene and/or submergence is distributed nearly equally over the whole petiole length and is almost completely attributable to increased cell expansion. In *R. acetosa* L., extension of the youngest petiole is inhibited by submergence of the whole plant. The strongest growth inhibition within the youngest petiole was observed in the most distal parts and is probably the result of reduced cell expansion.

River margins in the Netherlands are frequently flooded in both winter and summer months. These inundations are consequences of melting snow and excessive precipitation in the upper and middle reaches of the Rhine and Moselle. The occurrence and location of a plant species in such a floodplain is related to its tolerance to flooding regimes. Plant distributions related to the pattern of flooding is a common phenomenon in various habitats (Kozlowski 1984). The genus *Rumex* is cosmopolitan and various species are widely distributed in Dutch river areas; they are found in nearly permanent flooded parts of the floodplain to very erratic inundated regions of this area. *R. palustris* and *R. crispus* found in the lower, more or less frequently flooded regions of the river area have developed adaptations to survive aeration stress caused by flooding. One of these adaptations is a marked elongation of petioles, which is mediated by increased ethylene concentrations in the flooded shoot (Voesenek and Blom 1989a). This enhanced growth can restore leaf–atmosphere contact if the flooding is not too deep. The diffusion of oxygen through leaves to petioles and roots can relieve aeration problems in the roots (Laan et al. 1989a,b; Voesenek and Blom 1989b). A representative *Rumex* species from infrequently flooded dykes and river levees is *R. acetosa*. This species is poorly adapted to submerged conditions; it does not show enhanced elongation of petioles and is therefore unable to accommodate to submergence. This response is not related to a lack of ethylene production or accumulation after submergence, but to a different sensitivity of cells in the petioles (Voesenek and Blom 1989a).

It is known from other plants that both cell division and cell expansion are involved in enhanced growth of petioles during submergence. The relative contribution of cell division and cell expansion depends on cell location within a petiole and age of the whole petiole (Ridge 1985; Ridge and Amarasinghe 1984).

This article describes the results of a comparative ecomorphological study on petiole growth of three *Rumex* species under submerged and ethylene-enriched conditions. We studied three levels of organization: organ (whole petiole response), tissue (responses of zones within the petiole), and the cell level of organization (cell division and/or cell expansion).

Materials and Methods

Plant Material and Growth Conditions

Seeds of *R. acetosa*, *R. crispus*, and *R. palustris* were collected in 1986 and 1987 in the river area near Nijmegen (The Netherlands). Until use, seeds were stored dry at room temperature in the dark. Experiments were performed with mixtures of seed from several plants. Seeds were germinated in petri dishes with two layers of wetted Schleicher and Schuell filter paper for 7 days in a cabinet with a night temperature of 10°C (12 h) and a daytime temperature of 25°C (photon flux density: 30 μE $m^{-2}s^{-1}$). After this period, germinated seeds were placed singly

in small containers (height, 50 mm; diameter, 55 mm) filled with a mixture of sand and potting compost (prepacked flower soil Jongkind no. 5) (1:1 vol/vol) and grown for 19 days at a constant 20°C and a photon flux density during the light period (16 h) of 350 $\mu E\ m^{-2}s^{-1}$. After this growth period plants were ready for experimental handling; by this time *R. acetosa* and *R. crispus* were developing their fourth leaf, whereas *R. palustris* had developed five leaves.

Growth, Cell Length, and Cell Number of Petioles

To measure whole petiole extension and the increase in length of zones within a petiole, three identical groups of plants per species (n = 5–9) were selected. Zones of 3 mm (*R. crispus* and *R. palustris*) or 6 mm (*R. acetosa*) in length were marked with Indian ink; an older (second oldest petiole) and a younger petiole (fourth or fifth oldest) were used. The initial length of a zone depended on the species-specific morphology. The plants were submerged in glass containers (volume: 18 L) filled with water (depth: 30 cm) or exposed to a static-air–ethylene mixture (0.5 Pa ethylene; renewed every 24 h) in vacuum desiccators or used as controls. The experiments were conducted in a growth chamber with a temperature of 20°C and a photon flux density during the light period (16 h) of 350 $\mu E\ m^{-2}s^{-1}$. Finally, after 7 days of treatment the lengths of the petiole zones were measured again. A small, newly developed zone below the most proximal mark was not measured. In each petiole zone of two plants per species a small segment (1.5–2.0 mm) was excised with a razor blade and used for cell length measurements and estimations of the cell number. Previous studies indicated that these segments were representative samples for the whole petiole zone. After excision, the segments were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer at pH 6.8 for 2 h at room temperature, vacuum extracted during 15 min with a vacuum pump, dehydrated in an ethanol series, and embedded in Spurr's resin (Spurr 1969). Longitudinal sections, 2-μm thick, were cut in the cortical tissue with a Sorvall ultra microtome MT 5000 and stained with toluidine-blue. The lengths of all cortical cells in such sections were measured. The arithmetic mean and the zone length were used to estimate the cell number in one row of cells in a petiole zone. Previous experiments showed that, especially the younger petioles, were capable of cell division under control conditions. Means of the two plants were calculated to enable direct comparisons with the zone elongations. Comparisons of these means were performed with Bonferroni *t* tests, after analyses of variance. Statistical analyses were conducted with a SAS statistical package (SAS Institute Inc. 1985).

Results

The whole petiole response of the three species to the various treatments is presented in Fig. 1. In all species the greatest response to both ethylene and submergence was observed in the youngest petiole. In *R. acetosa*, submergence inhibited the growth of the youngest petiole significantly. Both other *Rumex* species showed an enhanced growth in response to submergence of the old and the young

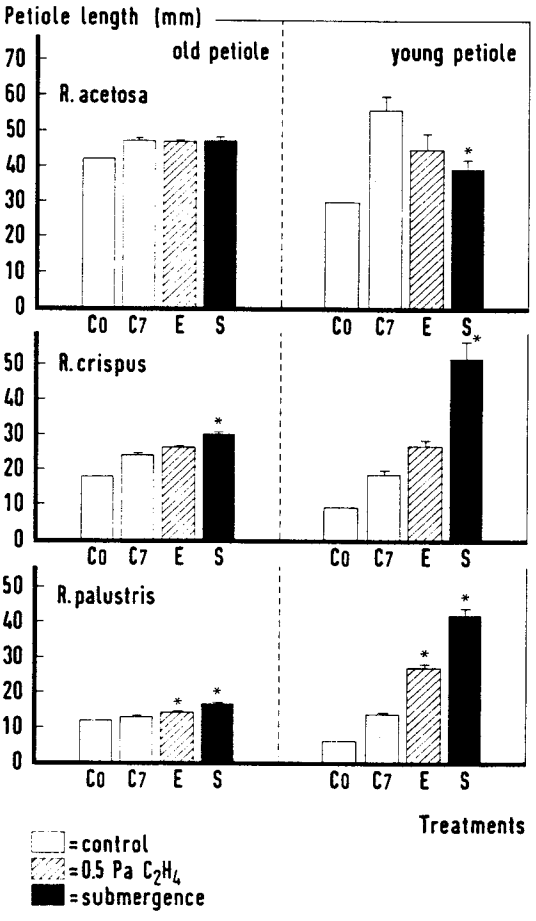

Fig. 1. Length (n = 5–9; +1 SE) of old and youngest petiole of *Rumex acetosa*, *R. crispus*, and *R. palustris* in response to 7-day exposure to three treatments [control (C7); 0.5 Pa ethylene (E); and submergence (S)]. Results can be compared with initial petiole lengths (Co). Treatment means significantly different from C7 are indicated by an asterisk (Bonferroni *t* test; $P < 0.05$).

petioles. Exposure to 0.5 Pa ethylene partially mimicked this growth stimulation in *R. palustris*.

Greatest length increase under control conditions in *R. acetosa* was observed in the most distal petiole zones (Fig. 2A). Growth inhibition under submerged conditions occurs especially in these distal zones in the youngest petiole. No significant differences between the treatments were observed in cell length and cell number of *R. acetosa* petioles (Fig. 2B and C). However, a trend toward inhibited cell expansion was observed in the youngest petiole of this species.

In *R. crispus*, petiole elongation in response to submergence was distributed nearly equally over the whole length of the old and young petiole (Fig. 3A). More than 85% of the elongation of the three zones in the youngest petiole of *R. crispus* can be explained by cell expansion (Fig. 3B and C). A sig-

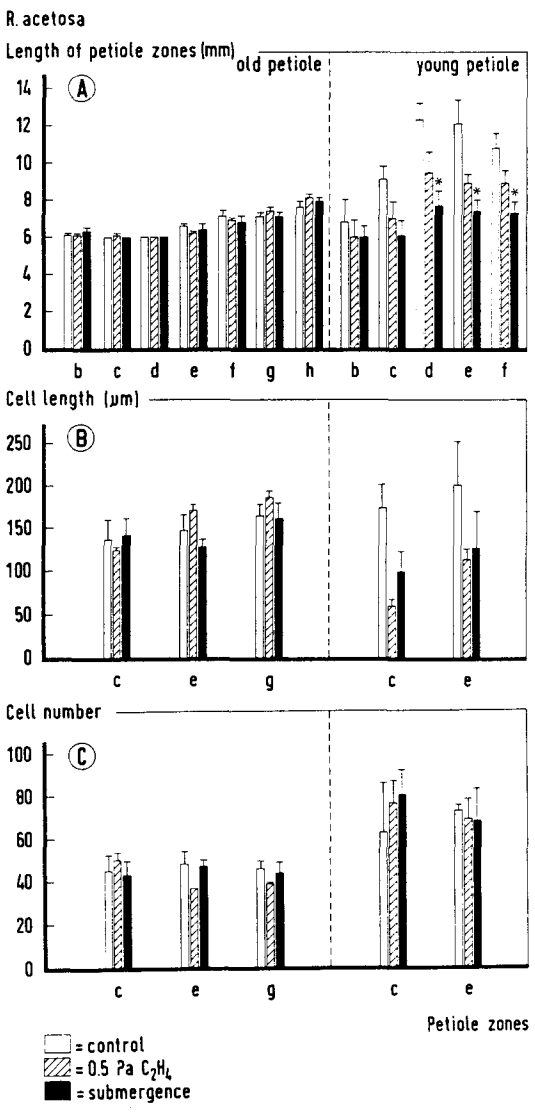

Fig. 2. (A) Length of petiole zones (n = 5–7; +1 SE) (initial length 6 mm), (B) length of cortical cells (n = 2; +1 SE), and (C) an index of the number of cortical cells in one row of a certain petiole zone (n = 2; +1 SE) of *Rumex acetosa* in response to 7-day exposure to three treatments (control, 0.5 Pa ethylene, and submergence). Petiole zones are indicated by letters b–f/h (b, the most proximal zone; f/h, the most distal). Treatment means significantly different from controls are indicated by an asterisk (Bonferroni *t* test; $P < 0.05$).

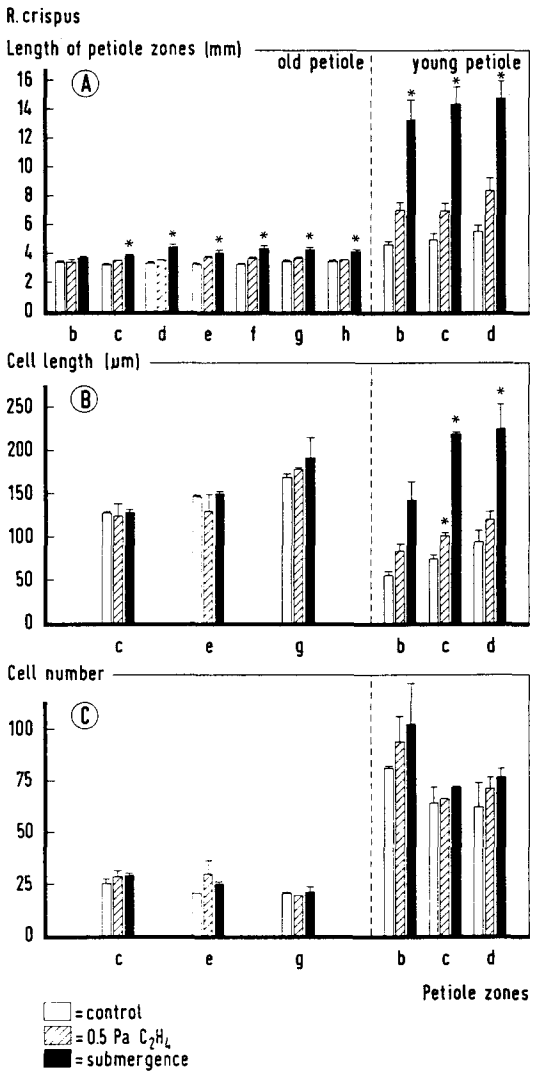

Fig. 3. (A) Length of petiole zones (n = 5–9; +1 SE) (initial length, 3 mm), (B) length of cortical cells (n = 2; +1 SE), and (C) an index of the number of cortical cells in one row of a certain petiole zone (n = 2; +1 SE) of *Rumex crispus* in response to 7-day exposure to three treatments (control, 0.5 Pa ethylene, and submergence). Petiole zones are indicated by letters b–d/h (b, the most proximal zone; d/h, the most distal). Treatment means significantly different from controls are indicated by an asterisk (Bonferroni *t* test; $P < 0.05$).

nificant increase in cell length was observed in the c-zone in response to ethylene.

Petiole elongation in *R. palustris* was not limited to only one part of the youngest petiole; all zones responded nearly similarly to ethylene and to submergence (Fig. 4A). In the youngest petiole, stimulated growth in response to both treatments must be attributed to a significant increase in cell lengths (Fig. 4B); more than 90% of the elongation of both zones can be explained by cell expansion. The estimated number of cells did not significantly change in response to ethylene and submergence treatments (Fig. 4C).

Discussion

The distribution of *Rumex* species in the river area may reflect adaptations developed within a plant

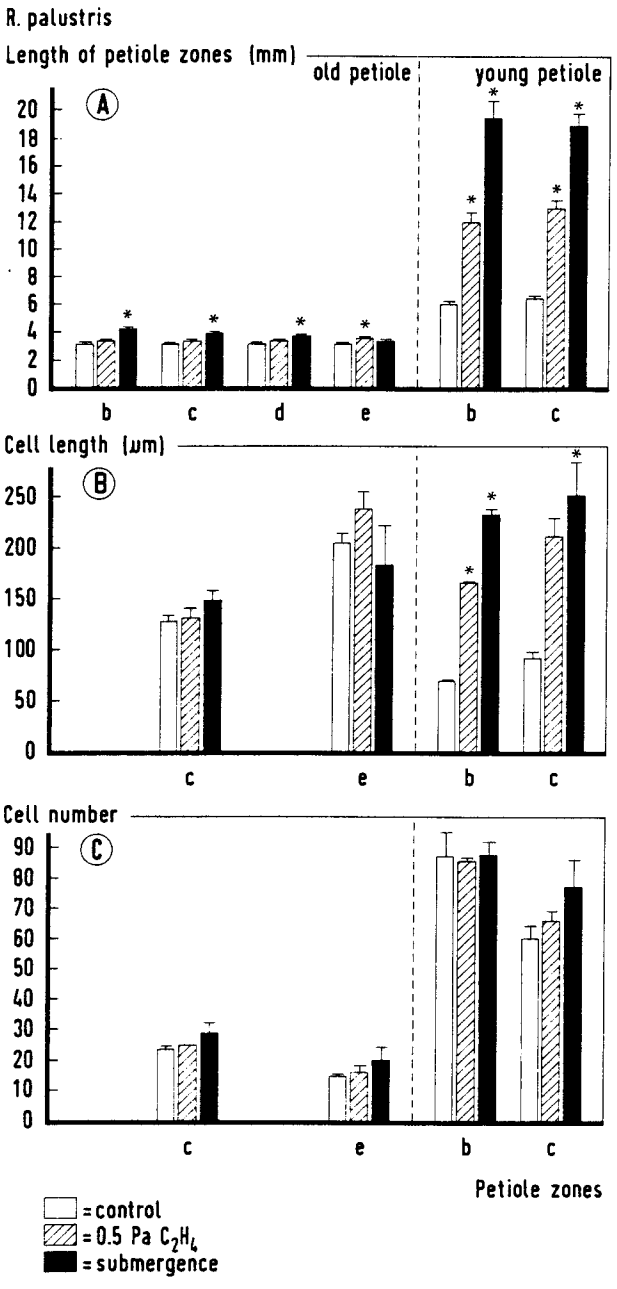

Fig. 4. (A) Length of petiole zones (n = 9; +1 SE) (initial length 3 mm), (B) length of cortical cells (n = 2; +1 SE), and (C) an index of the number of cortical cells in one row of a certain petiole zone (n = 2; +1 SE) of *Rumex palustris* in response to 7-day exposure to three treatments (control, 0.5 Pa ethylene, and submergence). Petiole zones are indicated by letters b–c/e (b, the most proximal zone; c/e, the most distal). Treatment means significantly different from controls are indicated by an asterisk (Bonferroni *t* test; $P < 0.05$).

species to flooding-related environmental factors. The observed differences in the responses of petioles of *R. acetosa*, *R. crispus*, and *R. palustris* to submergence must therefore be interpreted in this context. Ethylene plays a role in the reactions of *Rumex* petioles to submergence. This conclusion is based on the different sensitivity between the species toward this plant hormone as observed in the underlying experiments, and on previous experiments (Voesenek and Blom 1989a). However, a discrepancy between submergence and ethylene responses in *R. crispus* and, to a lesser extent, *R. palustris* is evident. Therefore, it is unlikely that ethylene alone regulates petiole elongation in these species. Other factors, such as the increased carbon dioxide concentration in the shoot (Jackson 1985; Ridge 1987) and the influence of buoyant tension (Musgrave and Walters 1974; Ridge and Amarasinghe 1984), can perhaps also contribute to petiole elongation in *Rumex*.

Table 1. The mean cell surface areas ($\mu m^2 \pm 1$ SE) measured on cross-sections of two old and two young petioles of *Rumex acetosa* after treatment (controls, exposed to 0.5 Pa ethylene, or submerged).

	Old petiole	Young petiole
Control	3444.0 ± 25.6	2781.6 ± 53.3
Ethylene	2923.6 ± 283.0	2364.0 ± 234.7
Submerged	2829.4 ± 246.6	2463.5 ± 496.9

The 2-µm thick sections were cut approximately in the middle of the whole petiole (d-zone) and 81–126 cells were measured in each section.

The inhibited petiole elongation and the reduced cell expansion of *R. acetosa* resemble the classical responses of many plants toward ethylene (Burg 1973; Osborne 1985). This response is mostly accompanied by an enhanced lateral expansion, resulting in short, "swollen" cells (Ridge 1973). To study this phenomenon in *R. acetosa*, we measured cell surfaces in cross-sections of petioles exposed to ethylene, submerged in water, and grown under control conditions. No significant treatment effects (Bonferroni *t* tests) were observed in the lateral expansion of cells of old and young petioles (Table 1).

Both *R. crispus* and *R. palustris* elongated mainly their youngest petiole in response to ethylene and/or submergence. This increased length can to a great extent be attributed to increased cell expansion. The influence of promoted cell division can be neglected in old and young petioles developed before the initation of the treatments. Further study is necessary to elucidate the relative contribution of cell division and cell expansion in petioles which develop entirely during submergence or ethylene treatments. Our results correspond well with the observation that ethylene-mediated petiole elongation in *Hydrocharis morsus-ranae*, *Ranunculus*

sceleratus, and *Regnellidium diphyllum* is caused exclusively by cell expansion (Cookson and Osborne 1978; Musgrave and Walters 1974). In other plant species, such as *Ranunculus repens* and *Nymphoides peltata*, with comparable petiole responses to both ethylene and submergence, a much greater contribution of cell division to the elongation response was demonstrated. In *N. peltata* this especially held for the youngest petioles, whereas in *R. repens* both older and younger petioles predominantly increased their lengths by means of promoted cell division (Ridge 1985; Ridge 1987; Ridge and Amarasinghe 1984). In both *N. peltata* and *R. repens* petiole elongation was completed at the end of the experiments (I. Ridge, personal communication). In *R. crispus* and *R. palustris* extension of the youngest petiole had not ceased by the end of the 7-day period. Therefore, stimulation of cell division later in the growth of the youngest petiole cannot be ruled out. Deep-water rice elongates its internodes in response to ethylene and flooding (Métraux and Kende 1983; Raskin and Kende 1984). This is related to an activation of cell division and longitudinal expansion of the intercalary meristem (Métraux and Kende 1984).

It can be concluded that the response of the petioles of *R. crispus* and *R. palustris* fit best into a model formulated by Ridge (1985), which assumes that ethylene solely promotes cell elongation envisaging that more cells respond to the gas from younger than older petioles; and that cells become increasingly less sensitive to ethylene during the later stages of petiole growth.

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