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Ethylene and flooding responses of *Rumex* species

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ABSTRACT

Enhanced growth of the youngest petiole was observed in *Rumex crispus* and *Rumex palustris* in response to submergence. A third species, *Rumex acetosa*, did not show this "supergrowth" reaction. Evidence is found that ethylene has a regulatory role in the growth responses in relation to submergence of all *Rumex* species studied. In the course of time variation in endogenous ethylene concentration may be caused by interaction of accumulation, fluctuating production and dilution by increased shoot porosity. The growth responses of the three species are highly correlated to the positions of the plants in the flooding gradient in the field.

INTRODUCTION

Rumex species are distributed in flooding gradients of river areas in the Netherlands. Three of these species were selected for this study, according to their distribution in the gradient. *Rumex palustris* is found on frequently flooded mudflats along old river beds, whereas *R. acetosa* is located mainly on seldom flooded dykes. *Rumex crispus* has an intermediate position in that gradient (Voesenek and Blom, 1987). It is hypothesized that the resistance to flooding mainly determines this zonation. An important adaptation to aeration stress in the soil and submergence of the shoot is enhanced growth of petioles or stems, mediated by increased ethylene levels (Jackson, 1983, 1985a; Osborne, 1984; Ridge, 1987). This "supergrowth" restores contact of leaves with the atmosphere (Jackson, 1985b; Ridge, 1985). It consequently relieves aeration stress of the roots in the anaerobic soil by oxygen diffussion through aerenchymous tissue and radial oxygen loss. The ca. 10,000 times slower diffussion rate of ethylene in water compared to air is, at least partly, responsible for the increased ethylene concentration in submerged plant tissue (Musgrave *et al.*, 1972). In rice both accumulation and increased production of ethylene are responsible for the enhanced concentrations in the shoot during submergence (Raskin and Kende, 1984).

This paper presents the results of experiments concerning

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the regulatory role of ethylene in the growth responses of petioles and laminae of *R. acetosa, R. crispus* and *R. palustris* under flooded conditions.

MATERIALS AND METHODS

Submergence and ethylene experiments

Experiments were performed to study the influence of submergence, exogenous applied ethylene and $AgNO_3$, a known inhibitor of ethylene action in plants (Beyer, 1976; Liebermann, 1979; Drew *et al.*, 1981), on the growth of petioles and laminae of *Rumex* species. The plants used for these experiments were grown out of seed. The seeds were sown in petridishes on moisted filter paper and incubated for 7 days in a germination cabinet with a temperature of 25°C. during the light period (12 hours) and 10°C. during darkness. After germination, seedlings were transplanted in small pots (height: 50 mm; diameter: 55 mm) filled with a mixture of sand and potting compost (1:1 v/v). All pots were placed in a growth chamber of 20°C., a day/night regime of 16/8 hours and a light intensity of 350 µE $m^{-2}s^{-1}$. After 19 days of growth plants were ready for experimental handling. All submergence and gas mixture experiments lasted 4 days and were conducted with twelve replicates per treatment.

The endogenous ethylene concentration in *Rumex* plants was determined with the vacuum extraction method of Beyer and Morgan (1970). Gas samples of 1 ml extracted from 6-15 shoots (extraction time: 2.30 min; 10 KPa) were injected directly in a Chrompack Packard gas chromatograph, model 438 A, with a packed Poropack-Q-column (length 100 cm), filled at a density of 0.34 g cm^{-3}, used at 60°C. In one experiment distinction was made between the endogenous ethylene concentration in the two youngest and the two oldest leaves. For this experiment 12-30 shoots were separated under water (to minimize ethylene escape) and vacuum extracted as mentioned before.

Growth rate of leaves

To investigate the growth rate of leaves (petioles + laminae), plants were grown under identical conditions as applied in the before mentioned experiments. During the growth experiments length increase of the youngest leaf (petiole + lamina) under submerged and non-submerged was measured. For the submergence experiments *Rumex* plants and pot were placed in a glass cylinder (diameter:6 cm; height: 45 cm) filled with distilled water. The youngest leaf was pushed in a small glass tube (diameter: 10 mm) with open ends. The tube was fixed in the soil of the pot and it prevented horizontally movement of the leaf. The growth of this leaf was measured every five hours by means of a traveling microscope with a horizontally oculair. The growth of the non-submerged leaves (controls) was measured with a marking gauge.

RESULTS

Submergence and ethylene

Figure 1A-D presents the growth responses of the youngest petioles and laminae, to submergence, exogenous applied ethylene and submergence in a $AgNO_3$-solution. The growth of petioles and laminae in air emphasis the different morphology and growth of *R. acetosa* compared to the other two species (Fig. 1A). This species has relative long petioles. Submergence results in a significant enhanced growth of the petioles of *R. crispus* and *R. palustris* (Fig. 1B). Complete flooding results in *R. palustris* also in a significant growth increase of the youngest laminae. In *R. crispus* and *R. palustris* the lamina/ petiole ratio changes dramatically, in favor of the petiole. No significant changes in length and ratio were observed in *R. acetosa*. The submergence results can be mimicked with *Rumex* plants grown in gas tight vacuum desiccators with a gas mixture of 5 ppm ethylene in medical air (Fig. 1C). Figure 1D presents the growth of petioles and laminae submerged in a non-toxic $AgNO_3$ solution (10^{-6}M). The growth of petioles of *R. crispus* and *R. palustris* is significantly reduced compared to petioles submerged in water. The lamina/petiole ratio in these two *Rumex*

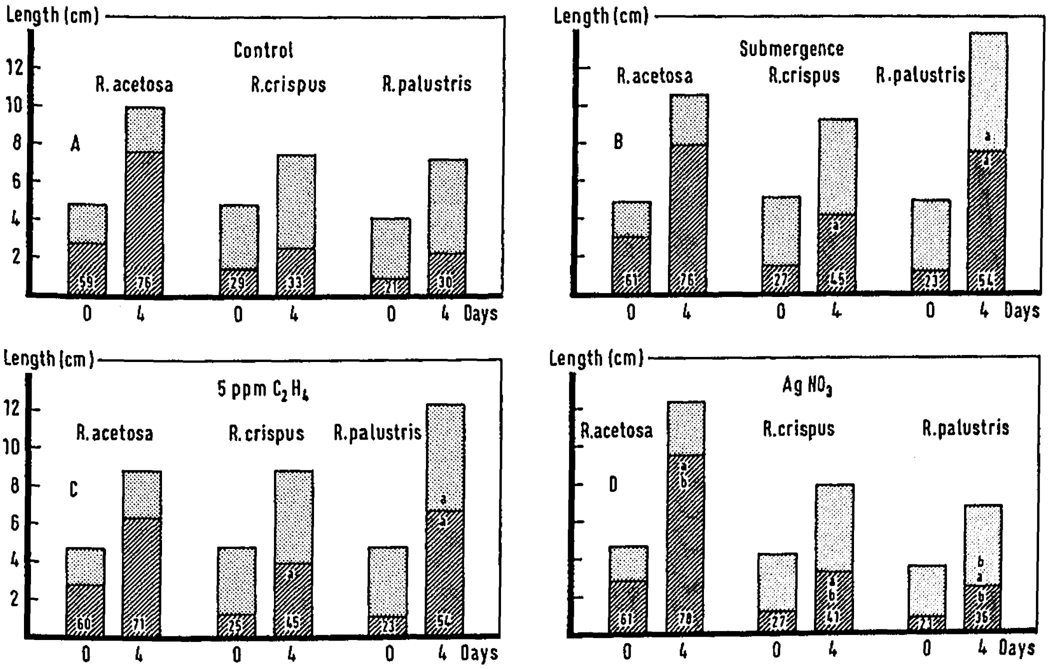

FIGURE 1. The mean length of the youngest petioles (lower part) and laminae (upper part)(n=8-12) of three *Rumex* species before (day 0) and after the treatments submergence, exogenous ethylene and $AgNO_3$ (day 4). Included are the percentages of the total length. Differences between treatments were assessed using the least significant difference (LSD) after an analysis of variance (a:significantly different from control level; b: significantly different from submergence level).

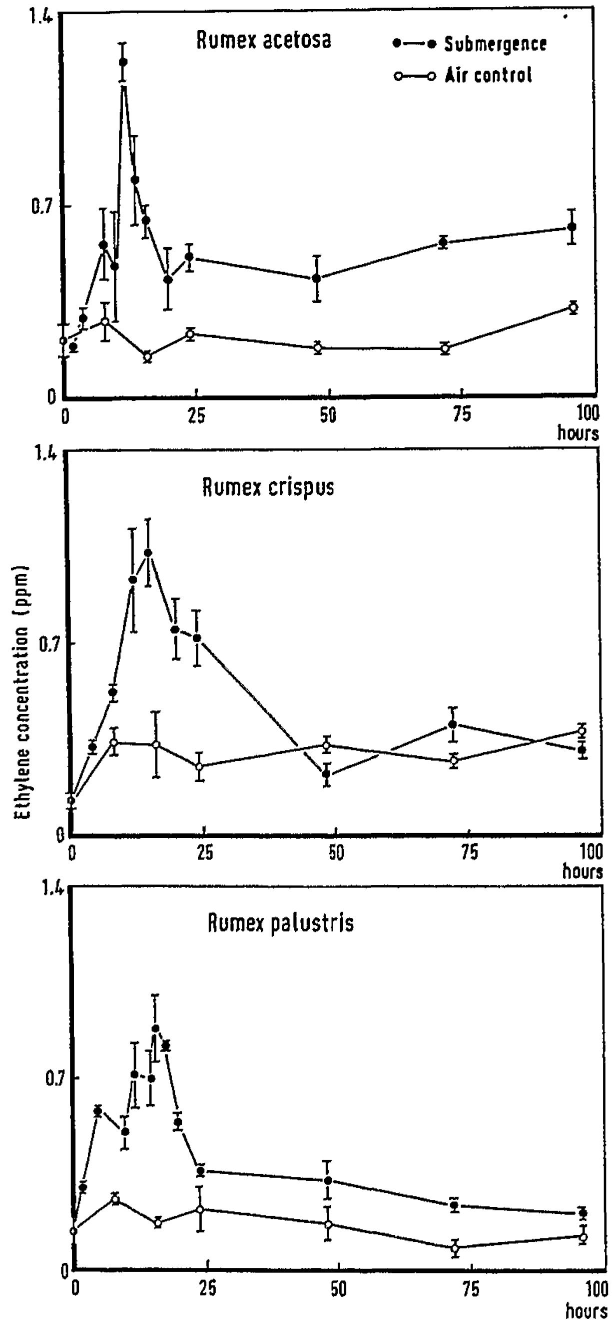

Figure 2. The mean ethylene concentration (n=2-3;±1SE) in gas samples vacuum extracted from shoots of submerged and non-submerged (air controls) *Rumex* plants.

species is also infuenced by the $AgNO_3$ treatment and has an intermediate position between growth in air and growth under submerged (water) conditions. Growth of *R. acetosa* petioles is significantly increased by submergence in a $AgNO_3$ solution. The endogenous ethylene concentration in non-submerged and submerged shoots is given in figure 2. In all species submergence results in a sharp increase of the internal ethylene concentration followed by a decrease of the concentration after approximately 20 hours of submergence. The internal ethylene concentration is not equal in all leaves (petioles + laminae). The highest concentrations were in all species found in the youngest leaves (Table 1).

TABLE 1. The mean endogenous ethylene concentration (± 1SE) in the oldest and youngest leaves (ppm). Means were calculated from measurements after 16, 24, 48, 72, and 96 hours of submergence.

	R. acetosa	*R. crispus*	*R. palustris*
Two oldest leaves	1.33±0.32	0.81±0.11	0.82±0.08
Two youngest leaves	1.91±0.22	1.94±0.49	1.63±0.16

Growth rate of leaves

After a few hours of submergence the growth rate of the youngest petioles and laminae of *R. crispus* and *R. palustris* was increased approximately 3-6 fold and remained higher during the total observation period (96 hours). In submerged *R. acetosa* plants growth was not different from air grown controls.

DISCUSSION

The results indicate that two of the three *Rumex* species studied show enhanced growth under submerged conditions, which in literature is often defined as "depth accommodation" (Ridge, 1987) or "supergrowth" (Osborne, 1984). In *R. crispus* and *R. palustris* this enhanced growth of the leaf in response to submergence and exogenous applied ethylene is located mainly in the petioles. This consequently results in a change of the lamina/petiole ratio, in favor of the petiole. The growth responses to exogenous ethylene, $AgNO_3$ and the observed increased endogenous ethylene concentrations in the shoot, indicate that in these *Rumex* species the plant hormone ethylene plays a central role in the growth process leading to "depth accommodation". In the third species, *R. acetosa* , growth of petioles was not enhanced in response to submergence or exogenous

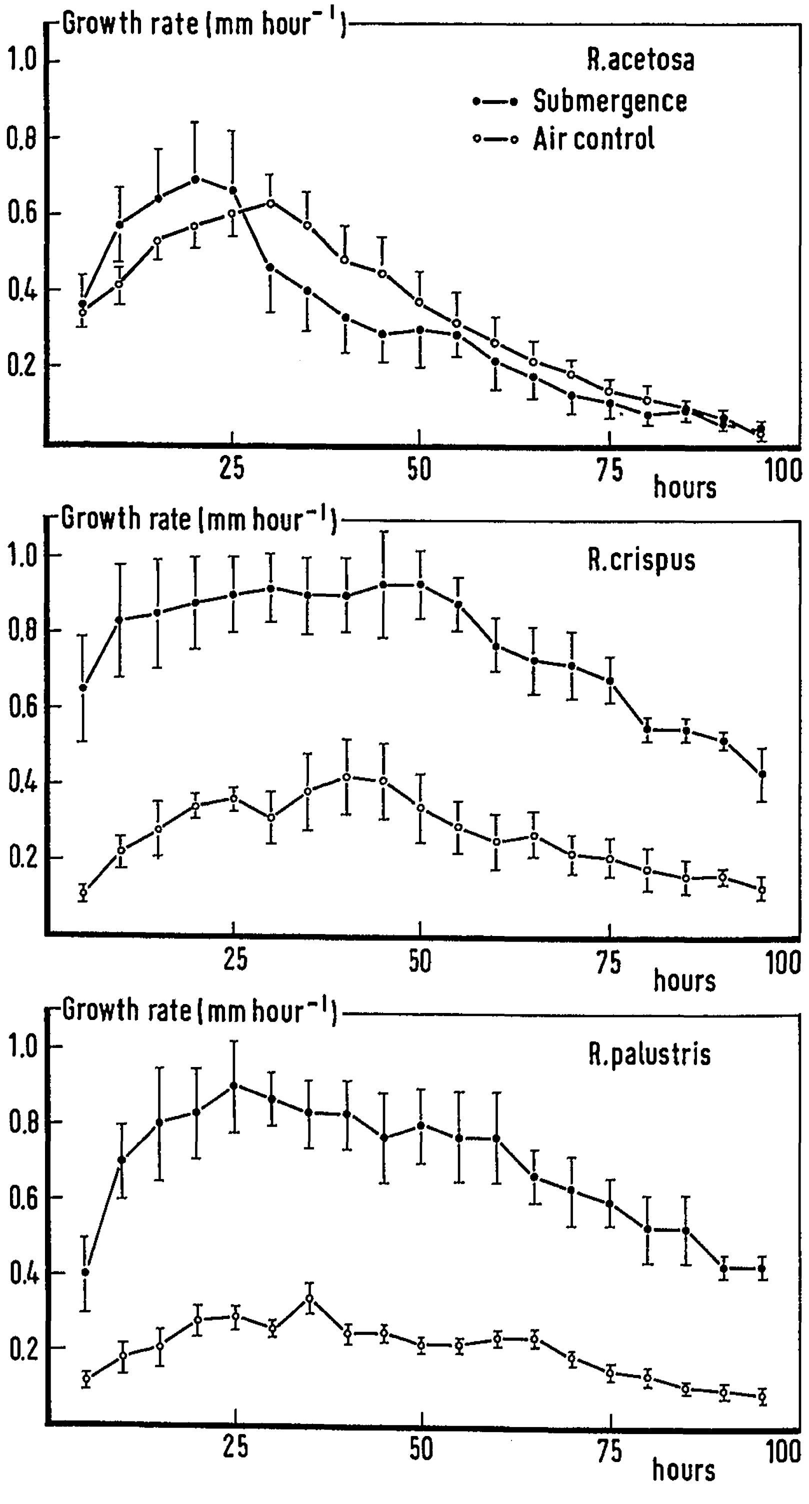

FIGURE 3. The mean growth rate (n=3-6;±1SE) of the youngest leaf (petiole + lamina) of submerged and non-submerged *Rumex* plants.

ethylene, although the endogenous concentration of this hormone increased to the same extent as in the other species. Dose-response curves indicate that the growth of petioles of *R. acetosa* is even inhibited by high ethylene concentrations (data not shown). The total length of the *R. acetosa* leaf (petiole + lamina) after 4 days of submergence exceeds that of *R. crispus*. Despite this difference, *R. crispus* can better overgrow a certain flooding depth. Responsible herefore is another growth response observed in this species within a few hours after submergence. The angle between the petioles and the soil surface changes from approximately 15-25 degrees to 80-90 degrees. *Rumex acetosa* is able to change its petiole orientation only slightly, this to a maximum of 45 degrees.

If ethylene plays a central role in "depth accommodation" the rate of ethylene accumulation, should match the timing of the submergence response (Ridge, 1987). The endogenous ethylene concentration in *R. crispus* and *R. palustris* increases sufficiently rapidly (Fig. 2) to explain the increase in the growth rate within a few hours after submergence (Fig. 3). However, after 24 hours leaf growth remains enhanced, while the internal ethylene concentration drops in the same period to control levels. These apparently contradictory results can be explained by the fact that gas was extracted from the whole shoot, whereas growth of only the youngest leaf was observed. Young tissue produces and therefore accumulates more ethylene (Table 1). Enhanced ethylene levels in the youngest leaves will therefore influence growth for a relative long period.

Musgrave *et al.* (1972) explained "supergrowth" responses under submerged conditions with the "entrapment hypothesis" (Ridge, 1987). Ethylene is produced continously by the plant tissue and accumulates in the intercellular air spaces because it diffuses only slowly in water (ca. 10,000 times slower than in air). The accumulated ethylene can easily dissipate into the atmosphere when a leaf overgrows the water surface. In the view of this "entrapment hypothesis" the found endogenous ethylene concentrations in *Rumex* shoots are unexpected. How can the internal concentration decrease, while the leaves are still submerged? We suggest that two phenomena are involved in the development of the internal ethylene concentration in submerged *Rumex* shoots. The first explanation is based on preliminary results from waterlogging experiments which indicated that flooding of the root environment of *Rumex* plants resulted in a stress induced increase of the ethylene production during the first 4-10 hours. Hereafter production decreased to control levels. It is assumed that this stress (flooding) induced increase of the ethylene production also occurs during the first hours of complete submergence. The second phenomena is the development of air spaces (aerenchyma) in the petioles and laminae during growth under submerged conditions. Measurements of the shoot porosity with a pycnometer method (Jensen *et al.*, 1969) indicate that the porosity increases in all species with approximately 50% during a 4 day submergence period. This increase of intercellular air volume causes a dilution of the concentration ethylene.

Based on these two phenomena we suggest that the initial fast increase of the endogenous ethylene concentration is the result of stress (flooding) induced increase of production and accumulation. The decline in the internal ethylene concentration is probably related to the decrease of the production to control levels and the increase of the porosity in petioles and laminae.

From the experiments can be concluded that the three *Rumex* species show different growth responses to submergence. These growth responses are related to the sensitivity of the plant tissue to enhanced ethylene concentrations during flooding. The growth responses are highly correlated to the positions of the species in the flooding gradient in the field. *Rumex crispus* and *R. palustris*, distributed in regions with frequent floodings are able to restore leaf/atmosphere contact during a flooding with restricted depth. *Rumex acetosa*, seldomly flooded, is not able to change the growth of petioles in response to flooding.

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