ROOT MORPHOLOGY AND AERENCHYMA FORMATION AS INDICATORS OF THE FLOOD-TOLERANCE OF RUMEX SPECIES

P. LAAN,* M. J. BERREVOETS,* S. LYTHE,† W. ARMSTRONG† AND C. W. P. M. BLOM*

* Department of Experimental Botany, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands and † Department of Applied Biology, University of Hull, Hull, North Humberside HU6 7RX

SUMMARY

(1) *Rumex* species are zoned along a gradient of elevation in the river ecosystem in The Netherlands.

(2) Plants of *R. thyrsiflorus*, *R. acetosa*, *R. obtusifolius*, *R. crispus*, *R. conglomeratus* and *R. maritimus* were flooded to identify and quantify any relevant adaptive features and to test whether their distribution might be caused by a differential response to flooding in the growing season.

(3) Most *Rumex* species have a tap-root from which the laterals originate. As a response to flooding, new laterals are formed.

(4) The number, place of origin, growth direction and formation rate of new laterals differed between the species.

(5) The number and formation rate of new roots were associated with the elevational distribution of the species: as a response to flooding, low-elevation species formed more new roots, and faster, than high-elevation species.

(6) The high-elevation species had root porosity values lower than 10%; the intermediate- and low-elevation species had values higher than 10%.

(7) Schizogenous aerenchyma was constitutively formed by the low-elevation and flood-tolerant *R. maritimus*, and not by the high-elevation and flood-intolerant species *R. thyrsiflorus*. In the intermediate-elevation species *R. crispus* it was induced in stagnant hypoxic solution cultures.

(8) The results indicate that aerenchyma formation is closely connected with the growth rate of new roots. It appears that development of aerenchyma in the new roots is the main determinant in the flood-tolerance of *Rumex* species.

INTRODUCTION

Flooding induces a number of responses in plant roots, of which aerenchyma formation is one of the most obviously adaptive (Arber 1920; Sculthorpe 1967; Armstrong 1979; Konings & Verschuren 1980; Crawford 1982; Jackson & Drew 1984; Justin & Armstrong 1987). Aerenchyma is formed either by some cell wall separation and cell collapse (lysigeny) or by cell separation without collapse (schizogeny). Both forms result in larger longitudinal channels in the root cortex. This structure enhances the diffusion of atmospheric or photosynthetic oxygen via, or from, the shoot to the roots so that aerobic respiration and growth can be maintained (Armstrong & Gaynard 1976; Lambers, Steingröver & Smakman 1978; Armstrong 1979; Armstrong & Webb 1985; Drew, Saglio & Pradet 1985).

Changes in root morphology also occur after flooding and this is true for both wetland and non-wetland species. There may be: (i) an increase in branching of the roots (Vose
Flood-tolerance of *Rumex* species

1962; Geisler 1965); (ii) the development of new, adventitious roots (Bergman 1920; Kramer 1951; Alberda 1953; Arikado & Adachi 1955; Jackson 1955; Drew, Jackson & Gifford 1979; Wenkert, Fausey & Waters 1981; Hook 1984); and (iii) superficial rooting (Alberda 1953; Armstrong & Boatman 1967; Sheikh & Rutter 1969; Schat 1984).

In most cases newly formed roots are more porous than roots of the primary root system (Arikado & Adachi 1955; Luxmoore & Stolzy 1969; Schat 1984; Justin & Armstrong 1987) and development of new, porous roots is thought to be beneficial to the whole root system and thus to plant growth and development (Jackson 1955; Stanley, Kaspar & Taylor 1980). Development of such newly formed roots may be especially advantageous in situations of transient flooding or in plant species which are unable to form aerenchyma in the primary root system.

In order to test whether such features might accord with plant distribution within the genus *Rumex*, a number of species was subjected to various flooding regimes and the responses compared. Most of the species used occur as a zonal pattern along an elevational gradient in the river ecosystem in The Netherlands; this is predominantly caused by transient flooding in the growing season (Van de Steeg 1984). At the lowest elevations *R. maritimus* L. dominates. At slightly higher elevations *R. crispus* L. and *R. obtusifolius* L. are found, whilst *R. acetosa* L. and *R. thyrsiflorus* Fingerh. occur above high flood levels. *R. conglomeratus* Murr. occurs in periodically wet, low elevation sites behind the river flood-banks.

All the species except *R. acetosa* produce a tap-root from which the laterals originate and, in response to flooding, secondarily formed laterals are developed to different extents. In *R. acetosa* new roots are formed on the older primary roots or near the root–shoot junction.

In this study, the morphology and gas-space development of the root systems are described and the differences considered in relation to the differential flood-tolerance and distribution of the species.

**MATERIALS AND METHODS**

*Plant growth*

Seeds of *Rumex thyrsiflorus*, *R. acetosa*, *R. obtusifolius*, *R. crispus*, *R. conglomeratus* and *R. maritimus* were collected from natural populations in the river area near Nijmegen (The Netherlands).

*Sand-culture*

Seeds of all species were sown in Petri dishes on wet Whatman filter paper and left to germinate at 25 °C (day), 15 °C (night), 16 h light at 60–100 μmol m⁻² s⁻¹ PAR, 8 h dark. After a week a batch of seedlings was transferred to river sand (organic matter content 0.5 ± 0.1%) in 3.3-litre pots (height 18 cm), and allowed to grow for three to four weeks in a glasshouse (c. 19 °C, relative humidity 70%, 16 h light at minimum 100–150 μmol m⁻² s⁻¹ PAR, 8 h dark). Half of these plants were then subjected to soil flooding by placing the pots in 50-litre plastic containers, which were slowly filled with quarter-strength Hoagland’s solution (Hoagland & Arnon 1950) until the water level was 1–2 cm above the soil surface. Black polyethylene grains (low density grains, British Petroleum, Grangemouth, U.K.) floating on the water suppressed growth of algae. The water level was checked daily. Control, non-waterlogged plants were watered every two or three days with quarter-strength Hoagland’s solution. At various times during the treatment,
drained and flooded plants, eight per treatment, were harvested at random and carefully washed on a sieve to remove the sandy soil. The roots were washed twice to remove the remainder of the adhering particles, and could then be used to determine root length, distribution of laterals over the tap-root, and root porosity.

Another batch of seedlings was transferred to vertical PVC-tubes (76 cm long, diameter 7.5 cm) filled with river sand. A metal grid covered with a piece of nylon was mounted on the bottom of the tubes allowing free contact between the nutrient solution and the substrate. The plants were allowed to grow for seven weeks, after which the tubes were flooded to 1–2 cm above the soil surface with quarter-strength Hoagland’s solution. The water was subsequently maintained at this original level. After three weeks of flooding, the plants were harvested by carefully pushing out the sand core complete with root system. Root morphology was described and maximal depth of the different root types was recorded.

Hydroculture

Five-week-old plants of *R. thyrsiflorus*, *R. crispus* and *R. maritimus* were grown either in aerated hydroculture, using quarter-strength Hoagland’s solution (aerated plants), or in perspex containers with quarter-strength Hoagland’s solution in anaerobic 0.05% agar (stagnant plants). Stagnant plants developed new lateral roots with a length of 4–8 cm within one to two weeks. Primary lateral roots of the aerated plants and newly formed laterals of the stagnant plants were used for examination of root anatomy.

Root porosity by pycnometry

After seven, fourteen and twenty-one days of flooding, three plants were harvested at random and the root systems carefully washed on a sieve. The apical 20–30 cm of the different types of new roots were cut off with a razor-blade. Root porosity was measured with a pycnometer (Jensen *et al.* 1969); gas was not removed by maceration, but by evacuation on a freeze-dryer.

Root length

Total root length of secondarily formed roots (i.e. roots formed after flooding) was measured with a ruler or, when length exceeded 1 m, with a root length scanner (Comair, accuracy c. 0.1 m).

Distribution of laterals

Plants which had been flooded for three weeks were separated into shoots and roots. Tap-roots were divided into segments and, when necessary, the shoot was divided into segments of about 1 cm. Secondarily formed laterals were counted per segment; they were not woody and were thus distinguishable from primary laterals.

Root anatomy

The apical first centimetres of the primary lateral roots and 1-cm segments from the apical 4–5 cm of the newly formed lateral roots were fixed in 3% (w/v) K$_2$MnO$_4$ (30 min), washed and stored in distilled water at 3 °C. The segments were dehydrated in an acetone series (30, 50, 70, 90 and 100%, 10 min each step) and, after rinsing twice in 100% acetone, placed in propylene oxide for 45 min. The root segments were then embedded in a resin mixture (Epon 812: Araldite 6005: DDSA = 3:3:8; Frances Allen method, modified from Coffey, Palevitz & Allen 1972) by gradual replacement of the propylene oxide by the resin.
Flood-tolerance of Rumex species

Rumex thyrsiflorus  Rumex acetoșa  Rumex obtusifolius  Rumex crispus  Rumex conglomeratus  Rumex maritimus

Fig. 1. Root morphology and root depth of Rumex species after three weeks of flooding in river-sand (age of the plants at the start of the experiment six weeks; original lateral root system not shown). Root type: 1, downward-growing laterals; 2, horizontally-growing laterals; 3, thick downward-growing laterals; 4, adventitious roots.

mixture. The resin was allowed to polymerize for 24 h at 70 °C. Sections of 1 μm from halfway along the blocks were cut with a Cambridge ultra-microtome. The sections were stained with 1% (v/v) toluidine blue in 1% (w/v) borax and photographed on a Zeiss photomicroscope. Gas-space cross-sectional area as a proportion of total root cross-sectional area was determined using an area-meter (MOP-system, Kontron, GMBH).

RESULTS

Changes in root morphology after flooding

The species showed considerable differences in root morphology after three weeks of flooding in river-sand (Fig. 1). In R. thyrsiflorus the primary laterals died within a week and few new roots were formed after flooding: some very thin and unbranched horizontally growing laterals, growing on or just below the water surface, and some downwardly growing laterals which soon ceased growth. After three weeks the apical 3–5 cm of the longest new downward-growing laterals had a transparent appearance, indicating that they were fully watersoaked and had ceased to function.

In R. acetosa the primary laterals, as in R. thyrsiflorus, died within one or two weeks and even fewer new laterals were formed after flooding, most of which were short and concentrated in the superficial zone. Here too, newly-formed laterals ceased growth and the tips died after some time, but before this occurred, new superficially growing roots were formed. Most of these roots originated above the root–shoot junction (adventitious roots), were very thick and unbranched, and also remained short (type 4; Fig. 1).

In R. obtusifolius, R. crispus and R. conglomeratus the primary laterals did not die when flooded, but the root system of each of these species was extended considerably by the formation of different types of new laterals. In particular, large numbers of horizontally growing laterals were formed on or just below the water surface, and in R. conglomeratus these formed a compact root mat after three weeks (type 2; Fig. 1). All three species also
formed adventitious roots and, in addition, *R. obtusifolius* developed some very thick and unbranched downwardly growing laterals (type 3).

*R. maritimus* showed the most extensive root outgrowth in response to flooding. Not only was there a rapid development of large numbers of downward and horizontally growing laterals (types 1 and 2), but also of adventitious roots, formed on the nodes of the flower stalk, which penetrated the soil within only one week (type 4; Fig. 1). Most of the primary laterals continued to grow.

**Course of events leading to changed root morphology and differential responses to flooding**

Four types of response, which more or less characterize the different species, could be distinguished.

**Type 1**

In *R. thyrsiflorus* only a few new roots (on average twenty-nine per plant) were more or less uniformly distributed over the tap-root (Fig. 2a); the same type of response was found in *R. acetosa*, which also developed few new roots. Growth of these new roots was slow and hardly significant (Fig. 2c). After two weeks of flooding the accumulated total length of new roots, with very low porosity (3–6%, Fig. 2b), was 3.0 m and 3.5 m in *R. acetosa* and *R. thyrsiflorus*, respectively, less than 10% of the total length at the start of the treatment (Fig. 2c).

**Type 2**

In *R. crispus* and *R. obtusifolius* more new laterals developed than in *R. thyrsiflorus* or *R. acetosa* (forty-seven and twenty-eight per plant, respectively), and these too were more or less uniformly distributed over the tap-root. Due to the formation of aerenchyma, however, high porosity values were reached (15–22%). As a consequence, growth of the new roots was significant within a week. This resulted in an accumulated total length of 14 m and 16 m in *R. obtusifolius* and *R. crispus*, respectively, after two weeks; this was 26% and 32% of the total length of the roots at the start of the treatment (Fig. 2c).

**Type 3**

In *R. conglomeratus* many new laterals were formed (on average 108 per plant), most of them originating at the tap-root apex (Fig. 2a). Root porosity was high (exceeding 20%) and the final accumulated length of newly formed roots was 43 m after two weeks of flooding; hence, the extent to which the original root system was replaced was very high (44%, Fig. 2c). This high final rate of growth was not only caused by the large number of new roots in combination with a high root porosity, but also by regrowth of the original root system and the formation of secondary laterals on the old roots.

**Type 4**

*R. maritimus* developed as many new roots as *R. conglomeratus* (115 per plant) but the distribution pattern was opposite: most of the new roots were formed on the shoot and the basal parts of the tap root (58% of total in segments 1–3; Fig. 2a). The initial growth rate of the new roots, with a very high porosity (25–35%, Fig. 2b), was high and an acceleration of growth could be observed after outgrowth of the root primordia of the adventitious roots on the first stem node (i.e. after five days). This resulted in a final accumulated total length of 24 m, which equals 23% of the initial root length (Fig. 2c).
Flood-tolerance of Rumex species

Fig. 2. Effects of flooding on the root systems of some Rumex species growing in river sand in a glasshouse. (a) Position of origin of newly formed roots. Segments 1 and 2: 1-cm root segments above the root–shoot junction; segments 3–9: 1-cm root segments below the root–shoot junction; arrows indicate the position of the root–shoot junction; values are means ± 1 S.E. (n = 8); (b) root porosity of newly formed lateral roots: values are means ± 1 S.E. (n = 3); (c) extension rate of primary root system, i.e. the total length of the newly formed root system as a percentage of the length of the primary root system at the start of the flooding treatment; values are means ± 1 S.E. (n = 8); initial lengths of primary root systems were: R. thyrsiflorus 38.2 m; R. crispus 61.5 m; R. conglomeratus 99.3 m; R. maritimus 104.6 m. All plants were twelve weeks old at the start of the treatment.

Root anatomy

An explanation for the observed differences between the Rumex species might be found in a differential ability to form aerenchyma. Such differences were already suggested by the root porosity data (Fig. 2).
Fig. 3. Cross-sections at 0.5 cm behind the apex of lateral roots of *Rumex thyrsiflorus* (a), (b), *R. crispus* (c), (d) and *R. maritimus* (e), (f), formed under aerobic (a), (c), (e) and stagnant anaerobic (b), (d), (f) hydroculture conditions. Bars represent 100 μm.
Plants precultured in aerated hydroculture and then transferred to a stagnant anaerobic agar solution developed new laterals within one week (\textit{R. maritimus} and \textit{R. crispus}) or two weeks (\textit{R. thyrsiflorus}). The diameter of these laterals (2 cm behind the apex) varied from $0.62 \pm 0.04 \text{ mm}$ (\textit{R. thyrsiflorus}) to $0.72 \pm 0.02 \text{ mm}$ (\textit{R. crispus}) and $0.84 \pm 0.04 \text{ mm}$ (\textit{R. maritimus}). There were clearcut differences in gas-space development between the investigated species (Figs 3 & 4). \textit{R. thyrsiflorus} was apparently unable to form aerenchyma in anaerobic conditions (Fig. 3), even at the basal ends of the new roots (Fig. 4). At 0.5 cm behind the root apex the cross-sectional area occupied by the intercellular spaces in the root cortex was 7.7\% in the primary roots (percentage of whole root segment, Fig. 3) and, due to some expansion of the intercellular spaces, there was an increase to 10–15\% in the newly formed roots. The other species developed a schizogenous aerenchyma (‘honeycomb’-like aerenchyma structure). \textit{R. crispus} was non-aerenchymatous in aerobic conditions and the cross-sectional area occupied by intercellular spaces was similar to that in \textit{R. thyrsiflorus} (7.8\% at 0.5 cm behind the apex; Fig. 3) but the stagnant anaerobic conditions caused a four- to five-fold increase to 30–40\% due to the formation of aerenchyma. In \textit{R. maritimus}, aerenchyma was formed in both primary and newly formed laterals (Fig. 3). This was reflected in the high cross-sectional area of the primary laterals (15.7\% at 0.5 cm behind the apex, Fig. 3) increasing to 40–50\% in the new roots (Fig. 4).

DISCUSSION

In those \textit{Rumex} species studied, their elevational distribution in the river ecosystem appears to be related to the type and degree of new root formation and gas-space development. After two weeks of flooding, the accumulated total length of the new root system was much higher in \textit{R. obtusifolius}, \textit{R. crispus}, \textit{R. conglomeratus} and \textit{R. maritimus} than in \textit{R. acetosa} and \textit{R. thyrsiflorus} (Fig. 2). These new laterals are supposed to take over the function of the old root system (Hook 1984; Jackson & Drew 1984) and in the low- and intermediate-elevation species more than 20\% of the old root system was replaced by the new one after ten to fourteen days (Fig. 2). Formation of this new root system was probably beneficial to the root system as a whole, for regrowth of the old root system and/
or development of new laterals on the older roots occurred after ten to fourteen days of flooding in sand in *R. obtusifolius*, *R. crispus*, *R. conglomeratus* and *R. maritimus*. The number of horizontally growing roots, floating on or just below the water surface (Fig. 1) and as high in porosity as the downward growing roots, seemed to be correlated with the elevational distribution of the species: the lower the elevation, the higher the total length of horizontally growing laterals. Not much is known about their function, but the fact that they occur on or just below the water surface suggests that they serve functions other than improvement of the oxygen status of the root system, e.g. ion uptake.

Growth rate of the new root system depended on both number and porosity of the roots. Although the total number of new roots formed in *R. obtusifolius* and *R. crispus* was not much higher than in *R. acetosa* and *R. thyrsiflorus*, their high growth rate and concomitant replacement of the old root system was apparently a result of aerenchyma formation (Fig. 3). In both *R. maritimus* and *R. conglomeratus* a large number of new roots with high porosity was formed. As a consequence, the old root system was extended considerably in both species after two weeks (Fig. 2). *R. maritimus*, however, developed twice as much length of new root as *R. conglomeratus* after three days (1-23 m v. 0-60 m); together with the opposite distribution pattern (Fig. 2), this may help to explain the differences in habitat of the two species: *R. conglomeratus* predominantly occurs behind the main dam in stagnant waters, while *R. maritimus* is frequently confronted with fluctuating water levels.

Root growth is very sensitive to the oxygen concentration near the root apex (Armstrong 1979) and roots of *R. thyrsiflorus*, *R. crispus* and *R. maritimus* of comparable length differed significantly in their apical oxygen pressure, correlated with porosity (Laan et al. 1989). Development and outgrowth of new laterals were well correlated with the capability of a species to form aerenchyma (Figs 3 & 4). The high porosity values of the primary roots of *R. maritimus* (Fig. 3) could also help to explain the observation that these roots keep growing when flooded. Aerenchyma formation often distinguishes flood-tolerant from flood-intolerant plants; Smirnoff & Crawford (1983) distinguished between tolerant and intolerant species at 10% porosity; Justin & Armstrong (1987) concluded that most non-wetland species have porosities lower than 7%. In both *R. thyrsiflorus* and *R. acetosa* porosity values were generally lower than 7-10%; in all other species the values generally exceeded 10% (Fig. 2). It should be noted, however, that a small number of wetland species do not form aerenchyma, and survive the wetland habitat by means of shallow rooting (Justin & Armstrong 1987). This phenomenon may also be important for *Rumex* species (Fig. 1).

Compared to the porosity values obtained from the anatomical preparations (Fig. 4), the porosity values of new roots of *R. acetosa* and *R. thyrsiflorus*, determined by pycnometry (Fig. 2), were very low (3-6%). Such low values might be due to a proportion of dead roots in the samples and to the use of older (newly formed) root material, which contains more woody tissue and, in addition, shows progressive branching.

Fractional root porosity values showed that the area occupied by intercellular spaces was already 7-8% under aerobic conditions in both *R. thyrsiflorus* and *R. crispus* (Fig. 3). However, only *R. crispus* formed aerenchyma in the new roots (Figs 3 & 4). This suggests that in these *Rumex* species aerenchyma formation is of more importance in determining flood-tolerance than porosity per se. As a wetland-species, *R. maritimus* develops aerenchyma constitutively (i.e. both in the primary and the new roots) but an increase in porosity as high as in *R. crispus* resulted in very high porosity values in the new roots (Figs 3 & 4).
A study of the morphology and the anatomy of the tap-roots showed clearcut differences between the species. All developed primary lateral roots in three rows on the tap-root. The roots, formed in response to flooding, grew close to the primary roots, except in *R. thyrsiflorus*, in which these new roots were distributed at random over the tap-root. This random growth habit could be a disadvantage when it results in laterals far from aerenchyma and other transport-channels, so that oxygen transport via the tap-root to the laterals is hampered. On the other hand, in *R. maritimus* three big lacunae could be observed in the basal parts of the tap-root, and more than 50% of the new roots originated from the basal 2 cm of the tap-root and from the shoot (Fig. 2). This anatomy correlates well with the very high rate of oxygen transport from shoot to roots under hypoxic conditions (internal aeration) found in this species (P. Laan and B. W. Veen, unpublished data).

The results obtained from the sand cultures should be interpreted with care in relation to the actual flood-tolerance of the species in the river ecosystem. Although it is stated that the oxygen concentration is low in flooded sand culture (Smirnoff & Crawford 1983), the reducing power of clay substrates, as found in the river ecosystem, can be greater than that of sand. However, experiments performed on clay substrate revealed responses of the *Rumex* species to flooding comparable to those reported here, with the exception that there were no clearcut differences between *R. crispus* and *R. maritimus* in their growth rate and biomass production upon flooding (Laan et al. 1989). In that case, factors other than root morphology and aerenchyma formation alone apparently determine the elevational distribution of these species.

In conclusion, in those *Rumex* species studied, aerenchyma formation is closely connected with the growth rate of new roots upon flooding, and the total number of lateral roots, plus their distribution over the tap-root, at least partly explain the differential responses towards transient flooding of the intermediate- and low-elevation species. To explain the distribution of these species in the river ecosystem fully, however, other aspects will need to be considered, e.g. the age of the plants, the flowering stage at the onset of flooding, and the responses to total submergence.

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