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Root exudation of organic acids: importance to nutrient availability and the calcifuge and calcicole behaviour of plants

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Many vascular plant species are unable to colonize limestone soils and the floristic composition of adjacent limestone and acid silicate soils differs greatly. Low-molecular organic acids (LOAs) in root exudates may greatly affect plant availability of nutrients and it is hypothesized that contrasting exudation of LOAs is a major mechanism controlling the calcicole and calcifuge behaviour of plants. Rhizosphere soil solution from two calcicole and two calcifuge species, grown in a pH-intermediate soil, was expelled by high-speed centrifugation. The concentrations of LOAs in these solutions were determined by an application of ion chromatography using a supported liquid membrane enrichment technique. Concentrations of dicarboxylic (mainly oxalic) and tricarboxylic (mainly citric) acids were much higher in the soil solution of the calcicole species, whereas there was no difference in monocarboxylic (mainly lactic + acetic) acids between rhizosphere soil solutions of the two species categories. A consistent difference in the relative molar proportion of mono-, di- and tricarboxylic acids was also demonstrated among all species, indicating a species specific exudation of LOAs from plant roots. The solubilizing effect of acetic, oxalic and citric acid and their Na-salts on Fe, Mn and phosphate in two limestone soils and in the pH-intermediate soil was also tested. Citric acid and/or Na-citrate were powerful solubilizers of Fe and Mn and oxalic acid and/or Na-oxalate of phosphate, whereas acetic acid and/or Na-acetate was quite weak in this respect. The results from this study strongly support the view that high exudation rates of di- and tricarboxylic LOAs is a major mechanism controlling calcicole behaviour of plants.

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Calcifuge behaviour, or the inability of many vascular plant species to colonize limestone soils, is a classical field of ecological research. No generally accepted explanation has been found despite appreciable research efforts, mainly during the 1960's and 1970's. One of the possible explanations to calcifuge behaviour of plants is unavailability of phosphate. This was previously demonstrated for an isolated British population of *Viscaria vulgaris* (Jarvis and Pigott 1973) where growth was distinctly increased by addition of phosphate. Phosphate as well as nitrogen limitation, particularly in combination with drought stress has also been suggested as a source of plant growth limitation on calcareous grasslands in Britain (Grime and Curtis 1976). In recent years the general validity of the view that calcifuge behaviour is due to phosphate limitations has been documented. Total P content is often similar in acid and limestone soils, whereas the easily exchangeable and soil solution concentrations of phosphate are very low in limestone soils (Tyler and Olsson 1993). Seed establishment of several calcifuge species was shown to be successful in limestone soils only when additional phosphate was supplied (Tyler 1992, 1994). To develop in such soils plants must be able to mobilize considerable amounts of phosphate from other than these minute soil pools. However, establishment and

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growth of calcifuges on limestone soil seem sometimes to be limited by Fe. *Galium saxatile*, a calcifuge species, was unable to grow on such soil unless Fe (in the form of Fe-citrate) was supplied (Tyler 1994). Lime-chlorosis is a well known feature of plants cultivated on calcareous soils throughout the world. The symptoms are consistent with those caused by Fe deficiency (Bergmann 1988) and it seems possible that lime-chlorosis sometimes excludes calcifuges from limestone soils (Grime and Hutchinson 1967). If these soils are high in organic matter and have a pH > 6.5, Mn deficiency is also likely to occur. Moreover, high concentrations of Ca in the soil will have a negative effect on Mn uptake and transport in the plant (Bergmann 1988). Therefore, it cannot be excluded that calcifuge behaviour in some cases is due to Mn deficiency.

Plant roots exude a variety of organic compounds to the soil, including sugars, amino acids, organic acids and phenols (Vancura 1964, Smith 1976). The low-molecular organic acids (LOAs) in root exudates may play an important role in the solubilization and plant availability of mineral nutrients in the soil (Mench and Martin 1991, Fox and Comerford 1992, Gerke et al. 1994). We have previously proposed that contrasting exudation of LOAs between calcicole and calcifuge species is a major mechanism controlling the calcifuge behaviour of plants. Calcicole species were found to exude much more oxalic and citric acid than calcifuge species, from both roots (Ström et al. 1994) and germinating seeds (Tyler and Ström 1995). Oxalic acid was an effective extractor of phosphate and citric acid of Fe in a limestone soil studied.

In these studies exudates were collected in water, a very nutrient poor matrix which might lead to elevated exudation rates of LOAs (Ratnayake et al. 1978, Kraffczyk et al. 1984, Lipton et al. 1987) and also influence the cell wall properties of the roots. Following further development of the ion chromatographic method used it is now possible to both concentrate and purify the sample immediately prior to analysis using an on-line system (Shen et al. 1996a, b). This enables the analysis of LOAs in more complex matrices, like soil solutions.

The purpose of this study is to grow two calcifuge and two calcicole species in a pH-intermediate soil, offering all four species good growth conditions. Growing all four species in a limestone soil has proven impossible because of the limitations mentioned above. The amounts and composition of LOAs in the rhizosphere soil solution will be analysed. The solubilizing power of LOAs and their salts on Fe, Mn and phosphate in this pH-intermediate soil, as well as in two limestone soils, will also be tested. One monocarboxylic (acetic), one dicarboxylic (oxalic) and one tricarboxylic (citric) acid and the Na-salts of these acids will be considered. It is hypothesized that the calcicole species exude more di- and tricarboxylic LOAs than the calcifuge species and that this difference is measurable in their rhizosphere soil solution. It is further hypothesized that LOAs are efficient in solubilizing Fe, Mn and phosphate from the soils and that di- and tricarboxylic LOAs are more powerful solubilizers than monocarboxylic LOAs.

Materials and methods

Plants

Two calcifuge species, *Deschampsia flexuosa* (L.) Trin. and *Viscaria vulgaris* Bernh., were sown in an acid silicate soil and two calcicole species, *Sanguisorba minor* Scop. and *Gypsophila fastigata* L., were sown in a slightly alkaline (calcareous) soil. The vessels were randomized in a greenhouse, where temperature was kept at 14–16°C during the night and 19–21°C during the day. Additional light was provided for 12 h d^{-1} using 400-W Philips Son-T Agro lamps, yielding 70 W m^{-2} in the 350–800 nm range and 0.12 W m^{-2} in the 250–350 nm range. After eight weeks the plants were replanted in 2-dm^3 plastic pots containing a pH-intermediate soil, described below, and cultivated for 12 weeks using the same temperature and light conditions. Ten pot replicates, each containing four plants of the same species, were run with all four species. After the experiment total dry weights of shoots and roots from the plants in each pot were determined.

Soils

The limestone soils were Rendzic leptosols, derived from Ordovician limestone on the 'alvar' of Öland (56° 40′N, 16° 30′E) and from an outcrop of Archaen limestone (marble) at Marmorbruket (58° 40′N, 16° 30′E). The clay content was low in both cases. The pH-intermediate soil used for cultivation originated from a loamy Eutric Cambisol developed from a slate moraine in Fyledalen, Scania (55° 34′N, 13° 46′E). All soils were sifted (mesh size 6 mm) before use. Soils used for sowing and replanting were loosely packed into freely drained plastic pots and kept at 60% of the water holding capacity by addition of H_2O.

Analytical methods

The soil adjacent to the roots of all four plants in each of the ten replicates, was sampled to expel soil solution, using a specially equipped cooling centrifuge, keeping sample temperature at 4°C, at 12 000 rev. min^{-1}, for 1 h. The centrifugation was done closely after sampling the soil and, after removal of a small amount for pH determination, the centrifugate was directly deep frozen (ca −20°C) to await analysis of LOAs.

The LOAs determined were, in order of appearance in the chromatogram, lactic, acetic, propionic, formic, pyruvic, malic (incl. succinic), oxalic, citric, isocitric and aconitic acid. Because lactic and acetic acid did not always separate in a consistent way, they were assessed together.

Analyses were performed using an ion chromatograph connected on-line to a sample preparation flow system which removed interfering metal ions and inorganic anions using ion exchange columns and a support liquid membrane unit, in which the target analytes were enriched. The sample preparation flow system set-up and operation as well as the equipment of the ion chromatograph, the NaOH gradient used and analytical procedures are described in detail elsewhere (Shen et al. 1996a, b).

The 'total' contents of Fe, Mn, Ca and P in the three soils were determined. Five replicates per soil of 1 g of dry soil were digested (microwave-oven, 2.5 h) in conc. HNO_3 and evaporated to a final volume of 4 cm^3, which was adjusted to 50.0 cm^3 with H_2O. Fe, Mn and Ca (after addition of 0.5% $LaCl_3$) were determined by flame AAS. Phosphate was determined colorimetrically (vanadate method, Rauterberg 1951). The organic matter content of the soils was determined as loss on ignition (600°C for 2 h). pH was determined electrometrically on 15 g of soil at field moisture shaken for 2 h with 50 cm^3 H_2O.

Easily extractable concentrations of Fe, Mn and phosphate were also determined. Used as extractants were 100 mol m^{-3} $BaCl_2$ for Fe and Mn (Tyler 1996) and 50 mol m^{-3} $Na_2SO_4 + 20$ mol m^{-3} NaF for phosphate (Tyler 1992). Five replicates of 10 g of soil at field moisture were shaken for 30 min with 100 cm^3 of extractant and immediately filtered (Munktell OOK). Blanks of the extractant solution were treated the same way as the samples. Fe and Mn of the soil extracts were determined by flame AAS. Phosphate was determined colorimetrically (molybdate/$SnCl_2$ method, Murphy and Riley 1962) using flow injection analysis (FIA).

The solubilizing ability of three LOAs and their sodium salts on soil Fe, Mn and phosphate was investigated. Used as extractants were 30 mol m^{-3} acetic acid and Na-acetate, 15 mol m^{-3} oxalic acid and Na-oxalate and 10 mol m^{-3} citric acid and Na-citrate. The different molar concentrations were chosen to make acids comparable on an equivalent basis. Five replicates of 10 g of soil at field moisture were shaken for 30 min with 100 cm^3 of extractant and immediately filtered (Munktell OOK). Before filtration a small portion of the suspension was removed for pH determination. Filtrates (50.0 cm^3) were evaporated to dryness, treated with conc. HNO_3 for complete oxidation of the organics and evaporated to a final volume of 2 cm^3, which was adjusted to 10.0 cm^3 with H_2O. Blanks of the extractant solution were treated the same way as the samples. Fe and Mn of the soil extracts were determined by flame AAS. Phosphate was determined by ion chromatography using the same equipment as for LOA determination but without the sample preparation flow system and with a slight modification of the NaOH gradient: 0.5 mol m^{-3} increasing to 5.0 mol m^{-3} during 3.0 min, to 26.8 mol m^{-3} during 8 min and to 38.2 mol m^{-3} during the following 1 min. The injection volume was 50 µl. This method to analyse phosphate was found superior to colorimetrical methods on these samples due to a small sample volume, and a low pH.

Statistics

Analysis of variance (ANOVA) followed by Tukey's test was used for multiple comparison between sample means (Zar 1984). For comparison of proportions, all values were logarithm transformed. Only means which differed at the $p < 0.01$ level were considered significantly different.

Results

The total concentrations of LOAs found in the rhizosphere soil solution of the two calcicole species were twice the concentrations found in the soil solution of the calcifuge species (Table 1). This was not due to different concentrations of monocarboxylic acids, but to about fivefold higher concentrations of di- and tricarboxylic acids in the soil solutions of the two calcicole species (Fig. 1). The main monocarboxylic acid in the soil solutions was lactic + acetic, the main dicarboxylic acid was oxalic and the main tricarboxylic acid was citric acid (Table 1). The calcicole *G. fastigata* also had large amounts of other dicarboxylic acids (malic + succinic and tartaric acid) in its soil solution. The concentration of oxalic acid measured in the soil solution of this species was, anyhow, significantly higher than in the soil solution of the two calcifuge species (Table 1). There was no difference between the species in the dry weight of shoot biomass produced, whereas the root dry weight was higher for one of the calcicole species (*S. minor*) than for the other three species (Table 1).

The relative molar proportion of mono-, di- and tricarboxylic acids in the soil solutions was also calculated (Fig. 2). The calcicole plants had a higher proportion of di- and tricarboxylic acids in their soil solutions than the calcifuge plants, which had a higher proportion of monocarboxylic acids. Almost no overlap was, however, found between the four species, showing a consistent difference between species in the composition of mono-, di- and tricarboxylic acids exuded into the soil.

In the limestone soils, pH and 'total' Ca concentration were much higher than in the pH-intermediate soil, whereas the organic matter content and easily extractable concentrations of Fe, Mn and phosphate were

Table 1. Mean LOA concentrations (mmol m^{-3}) in the soil solutions of the two calcifuge (*Deschampsia flexuosa* and *Viscaria vulgaris*) and the two calciole (*Gypsophila fastigata* and *Sanguisorba minor*) species, mean total shoot and root weight (g) of the plants in each pot and pH of the soil solution after 12 weeks' growth. Different letters indicate significant differences ($p < 0.01$) between species. Number of replicates = 10.

				Monocarboxylic			
Species	Shoot weight	Root weight	pH soil sol.	Lactic + Acetic	Propionic	Formic	Pyruvic
D. flexuosa	0.73 a	0.34 b	4.9 b	8.9 a	0.4 a	4.8 a	0.4 a
V. vulgaris	0.67 a	0.49 b	5.3 a	7.9 a	0.3 a	5.1 a	0.3 a
G. fastigata	0.74 a	0.36 b	4.9 b	8.0 a	0.3 a	4.3 a	0.4 a
S. minor	0.75 a	0.76 a	5.2 a	11.9 a	0.3 a	5.9 a	0.4 a

	Dicarboxylic			Tricarboxylic			
Species	Malic + Succinic	Tartaric	Oxalic	Citric	Isocitric	Aconitic	Sum
D. flexuosa	<0.3 b	<0.2 b	1.7 b	1.0 b	<0.2 a	<0.2 b	17.3 b
V. vulgaris	1.4 b	<0.2 b	3.1 b	0.5 b	<0.2 a	<0.2 b	18.7 b
G. fastigata	3.7 a	13.4 a	6.2 a	4.5 a	<0.2 a	<0.2 b	40.8 a
S. minor	1.3 b	<0.2 b	7.9 a	3.8 a	<0.2 a	0.9 a	32.6 a

lower. All soils, however, contained appreciable amounts of 'total' Fe, Mn and P (Table 2).

There were great differences in the solubilizing efficiency of LOAs on Fe, Mn and phosphate (Table 3). For all three soils and all elements the di- and/or tricarboxylic acids were more efficient solubilizers than the monocarboxylic acid. Differences in solubilizing ability were also found between the acids and their salts, especially for the limestone soils. The tricarboxylic citric acid was an efficient solubilizer of both Fe (3–5% of 'total' soil concentration) and Mn (3–35%) in all three soils. In the limestone soils Na-citrate was a less efficient solubilizer of Fe (2%) and Mn (1–2%) than citric acid. In the pH-intermediate soil, however, there was no difference in this respect between citric acid and Na-citrate. Furthermore the dicarboxylic oxalic acid and Na-oxalate were efficient solubilizers of both Fe and Mn in this soil (Table 3).

The dicarboxylic Na-oxalate was an efficient solubilizer of phosphate (5–8%) in all three soils. Again, a pH effect of the extractant on solubilization was only found in the limestone soils, where oxalic acid (1–2%) was much less efficient than Na-oxalate in solubilizing phosphate. In the pH-intermediate soil, however, oxalic acid, Na-oxalate and Na-citrate were equally efficient (Table 3).

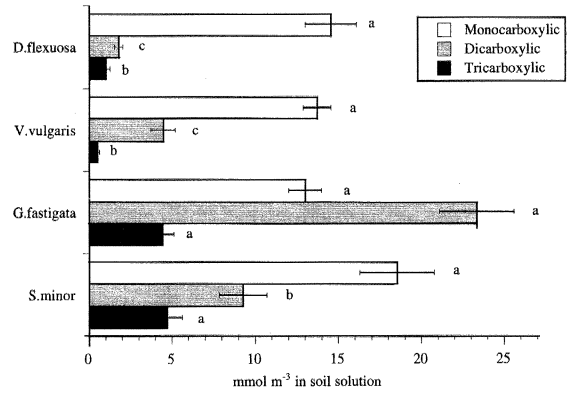

Fig. 1. The concentrations (mmol m^{-3}) of mono-, di- and tricarboxylic acids in the soil solution of two calcifuge (*Deschampsia flexuosa* and *Viscaria vulgaris*) and the two calcicole (*Gypsophila fastigata* and *Sanguisorba minor*) species. Means ± S.E.; $n = 10$. Different letters indicate significant differences ($p < 0.01$) between sample means of the same LOA category.

Discussion

The LOAs exuded by plant roots are at least to some extent present in soil solutions. Large amounts of LOAs were found in the proteoid root layer of *Banksia integrifolia* compared to the surrounding soil and leaf

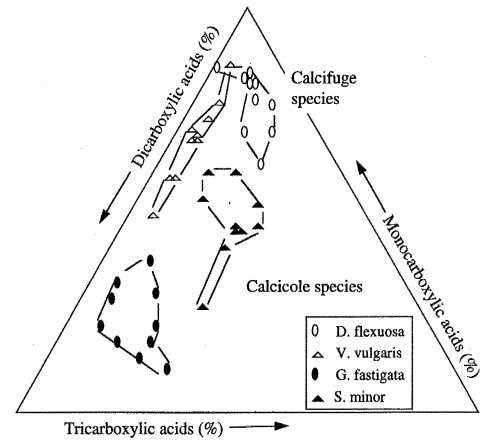

Fig. 2. Relative molar proportion of mono-, di- and tricarboxylic acids in the soil solution of the two calcifuge and the two calcicole species. Every replicate is illustrated.

Table 2. pH(H_2O), % organic matter (O.M.) of soil dry weight, mean 'total' (HNO_3-digestable) concentrations (μmol g^{-1} soil dry weight) of Fe, Mn, Ca and P and extractable concentraions (μmol g^{-1} soil dry weight) of Fe, Mn (100 mol m^{-3} $BaCl_2$) and phosphate (50 mol m^{-3} Na_2SO_4 + 20 mol m^{-3} NaF) in three soils. Different letters indicate significant differences ($p < 0.01$) between sample means. Number of replicates = 5. No detectable concentrations in the sample: <.

Soil	Soil pH	% O.M.	'total' Ca
Silicate	4.9 a	10.9 a	16 c
Archaen limestone	8.3 b	4.9 c	7480 a
Ordovician limestone	8.2 b	6.2 b	2100 b
Soil	**'total' Fe**	**'total' Mn**	**'total' P**
Silicate	652 a	19 c	33 a
Archaen limestone	77 c	24 b	12 b
Ordovician limestone	234 b	38 a	28 a
Soil	**Extr. Fe**	**Extr. Mn**	**Extr. P**
Silicate	0.010 a	0.267 a	0.028 a
Archaen limestone	<b	<b	0.007 b
Ordovician limestone	<b	<b	0.002 c

litter (Grierson 1992). Fox and Comerford (1990) found higher concentrations of LOAs in rhizosphere soil of slash pine (*Pinus elliottii*) than in bulk soil. Higher concentrations of LOAs were also found in the rhizosphere soil solution of five field-layer species in a beech forest than in the soil solution, where field layer species were lacking (Shen et al. 1996b).

The two calcicole species were found to have much higher concentrations and molar proportions of di- and tricarboxylic acids in their rhizosphere soil solution than the calcifuge species (Figs 1, 2). However, there was also a clear difference between all four species in their soil solution composition of mono-, di- and tricarboxylic acids. This indicates a species specific exudation of LOAs from plant roots.

Although shoot dry weights produced were similar in all plants, one of the calcicole species (*S. minor*) had a higher root dry weight than the other species and this might to some extent explain the higher concentrations of LOAs found in the soil solution of this species. The calcicole species with the highest LOA concentration in its soil solution (*G. fastigata*) did, on the other hand, not have a higher root dry weight than the calcifuge species (Table 1). However, explaining the LOA concentrations in soil solutions simply by root dry weight might be misleading since large differences in the substances exuded from different parts of roots are expected (Bowen and Rowira 1991). The exudation of LOAs might be highest from the fine root tips and there might also be differences between the plant species in the main LOA exudation site along the root.

Theoretically, a source of error which might bias the result is the high speed centrifugation if it caused rupturing of tissues and release of cellular contents into the soil solution. However, in a study where roots labelled with ^{14}C was added to the soil prior to high-speed centrifugation, a maximum of 1% of the ^{14}C in the roots was recovered in the soil solution (Zabowski 1989). Higher g-values than those obtained by centrifugation at 12 000 rev. min^{-1} would be required for solute release from root cells. Therefore, the influence on soil solution concentrations of LOAs, measured by this technique, from small remains of roots in the soil should be almost negligible.

Estimation of LOA production and exudation rates from their concentration in soil solutions can, however, be seriously biased by highly variable rates of LOA immobilization in soil (Shen et al. 1996b). The recovery of added LOAs varied greatly between acids in beech forest soils lactic + acetic acid having a recovery of 83–111%, oxalic acid of 13–25%, and citric acid of 13–25%. This indicates that especially the amounts of oxalic and citric acid actually produced by the plants in my experiment might be underestimated. The large relative difference between species in their exudation should, however, not have been seriously biased by varying recoveries of LOAs since the plants were all grown in the same, carefully mixed soil. But the danger of comparing exudation by species grown in their original soils (limestone and acid soil) is apparent, because different soil properties most likely lead to different immobilization rates of LOAs. It would, on the other hand, be of great interest to grow all the species, both calcifuges and calcicoles, in a limestone soil, but failure or poor growth of the calcifuges precludes the use of such a soil. It is also likely that several LOAs would be further immobilized in a limestone soil by Ca precipitation and not recovered in the soil solution. In order to analyse LOAs exuded into limestone soil, it would be necessary to develop a method that sufficiently extracts the LOAs exuded.

The main advantage of my experiment is that the plants were grown in a natural soil, suitable for plant growth of both calcicole and calcifuge species, and not exposed to such artificial conditions as water or nutrient solutions would provide. None of the species developed any signs of nutrient deficiency and all grew well in the pH-intermediate soil of the study (Table 1). Limestone soils are very low in easily exchangeable Fe, Mn and phosphate (Table 2) and many authors have reported an increase in LOA exudation from plants growing under nutrient deficiency (Ratnayake et al. 1978, Kraffczyk et al. 1984, Lipton et al. 1987). Therefore, exudation rates of calcicole plants might actually be even higher in a limestone soil than in the pH-intermediate soil in which the experiment was conducted.

As neither the soils nor the seeds were sterilized and as the seeds were germinated and the plants pre-cultivated in the soil of their origin, the plants would have been enabled to generate their normal rhizosphere microflora. It was not possible in this experiment to distinguish between LOAs produced by plant roots and

Table 3. Fe, Mn and phosphate, as a percentage of total concentration of the element in the soil, extracted from three soils by acetic (30 mol m^{-3}), oxalic (15 mol m^{-3}) and citric acid (10 mol m^{-3}) and the Na-salts of these acids. Different letters indicate significant differences ($p < 0.01$) between sample means of each element, all extractants and soils considered. Number of replicates = 5. Bold figures indicate the highest amount of Fe, Mn or phosphate extracted from each soil.

		Acid Extractant			Salt Extractant		
		Silicate	Archaen limestone	Ordovician limestone	Silicate	Archaen limestone	Ordovician limestone
% Fe	Acetic	0.1 e	0.7 d	0.3 de	0.6 d	0.3 e	0.5 de
	Oxalic	**3.9** b	0.1 e	0.7 d	**3.9** b	2.2 c	1.8 c
	Citric	**3.5** b	**5.2** a	**2.5** c	**3.9** b	2.3 c	1.5 c
% Mn	Acetic	22 d	0.7 h	1.1 h	3.8 f	0.1 k	0.4 i
	Oxalic	**80** a	0.8 h	3.2 f	23 d	0.7 h	1.7 g
	Citric	35 c	**3.5** f	**12** e	54 b	0.9 h	1.7 g
% PO_4	Acetic	3.2 b	0.6 c	0.2 c	1.1 c	0.4 c	0.6 c
	Oxalic	**7.4** a	1.1 c	1.6 bc	**7.9** a	**8.1** a	**5.2** ab
	Citric	0.9 c	0.6 c	0.6 c	**7.4** a	1.6 bc	3.2 b

by microorganisms. No differences were found between the species in the concentration of monocarboxylic acids in their soil solutions (Fig. 1, Table 1). This could be due either to similar amounts exuded by the roots of all species or to another origin of these acids. Monocarboxylic acids are produced during conversion of glucose to pyruvate in glycolysis and, under anaerobic conditions, pyruvate is further transformed to lactate (Stryer 1988). Microbial decomposition of organic matter can also lead to production of several monocarboxylic acids. This tends to occur under wet, anaerobic conditions where fermentative microbes in the soil produce a range of LOAs, including acetic, lactic, formic and propionic acid, from plant residues (Killham 1994). The soils were all kept in open pots at 60% of water holding capacity which essentially would have precluded anaerobic conditions. Anyhow, microbial production as a main source of monocarboxylic acids found in the soil solution of this experiment seems most likely. The di- and tricarboxylic acids might, on the other hand, be more likely to originate from root exudation since their composition and concentration in the rhizosphere soil solution were so clearly different between the two species categories, as well as between all four species (Figs 1, 2, Table 1).

The di- and tricarboxylic acids were found to have a strong solubilizing power of Fe, Mn and phosphate in all the soils tested (Table 3). The proportion of the elements extracted out of the 'total' concentration in the soils was not consistently different between the soil types, except for Mn of which a large proportion was extracted from the pH-intermediate soil. The main difference between the soil types was related to the pH effect of the extractant. In the two limestone soils citric acid (pH 2.7) was a strong extractant of Fe and Mn, whereas Na-citrate (pH 8.1) was weaker. The proportion of phosphate extracted from the limestone soils relative to pH was reversed, Na-oxalate (pH 7.0) being a strong and oxalic acid (pH 2.0) a weaker extractant. In the pH-intermediate soil the same LOAs were effective in solubilizing these elements but, irrespective of pH, oxalic acid had the same solubilizing effect on phosphate as Na-oxalate, and citric acid the same solubilizing effect as Na-citrate on Fe.

These differences are probably related to pH, both in the soil and in the extracts (Table 4) and to pH-related properties of Fe, Mn and phosphate. In a soil with pH > 8 the concentration of Fe^{3+} in the soil solution decreases 1000-fold for each unit increase in pH (Barber 1995). The same relationship is found between Mn and pH. In aerated soils, the Mn^{2+} concentration in the soil solution would theoretically decrease by a factor of 100 for every unit increase in pH (Barber 1995). This clearly shows the importance of lowering the pH to the solubility of Fe and Mn in a limestone soil with a pH of 8. However, since the other extractants of an equally low pH as citric acid had a lower solubilizing effect (Table 3), the importance of Fe and Mn chelation with citrate is also clearly indicated.

The most powerful extractor of phosphate in the limestone soils was Na-oxalate with pH 10 after extraction (Table 4). The pH increase after this extraction should largely be due to the reaction between $CaCO_3$ and the oxalate ion ($C_2O_4^{2-}$) that forms Ca-oxalate and releases CO_3^{2-}, which leads to an increase in pH. Following extraction with oxalic acid the pH was around 6.6 (Table 4). The pKa values of oxalic acid are

Table 4. pH of extractant and after extraction of three soils with acetic (30 mol m^{-3}), oxalic (15 mol m^{-3}) and citric acid (10 mol m^{-3}) and the Na-salts of these acids.

Extractant	pH of extractant	pH after extraction		
		Silicate	Archaen limestone	Ordovician limestone
Acetic acid	3.2	3.6	6.5	6.3
Na-acetate	7.5	5.7	8.7	8.2
Oxalic acid	2.0	2.3	6.7	6.5
Na-oxalate	7.0	6.6	10.3	10.0
Citric acid	2.7	2.9	6.6	6.3
Na-citrate	8.1	6.3	9.7	9.4

1.23 and 4.19 (Weast 1989), which means that oxalic acid was fully dissociated in extraction with both oxalic acid and Na-oxalate. The effect of the oxalate ion in complexing Ca and, thereby, releasing HPO_4^{2-} should therefore be similar in both extractions. However, at pH < 7, $CaCO_3$ starts to dissolve (H_2CO_3; $pKa_1 = 6.38$, $pKa_2 = 10.32$) and the free Ca^{2+} can precipitate HPO_4^{2-} previously released by oxalate. Therefore, it may be hypothesized that the effect of the oxalate ion is equal at extraction with both oxalic acid and Na-oxalate. During the oxalic acid extraction, however, released phosphate is re-precipitated as Ca-phosphate and is therefore not recovered in the extract. The importance of oxalate in solubilizing phosphate is also apparent in the extraction of the pH-intermediate soil (Table 3). In this soil, however, the 'total' Ca concentration was much lower (Table 2) and the re-precipitation of phosphate by Ca was much less important.

The higher concentrations of LOAs in the rhizosphere soil solutions of the calcicole species did not lead to a consistently lower pH in the soil solutions of these two species than of the two calcifuge species (Table 1). This indicates that the higher exudation of LOAs from the calcicoles does not have to be accompanied by a decrease in the rhizosphere pH. It seems, however, that a calcicole species limited by Fe or Mn would benefit from lowering the rhizosphere pH and exude citrate. On the other hand, a calcicole species limited by phosphate would not benefit from lowering soil pH since this would lead to more free Ca^{2+} in the soil solution competing with the plant roots for solubilized phosphate.

Conclusions

We have previously proposed that the calcifuge behaviour of plants may result from a low exudation rate of the dicarboxylic oxalic acid and the tricarboxylic citric acid (Ström et al. 1994, Tyler and Ström 1995). The results from my study, which was performed using soil and plants with an undisturbed root system, strongly support this view. Much higher concentrations of oxalate/oxalic acid and citrate/citric acid were found in the rhizosphere soil solution of the two calcicole than of the two calcifuge species studied. These acids were found to be strong solubilizers of Fe, Mn (citrate/citric acid) and phosphate (oxalacid) in two limestone soils as well as in the pH-intermediate soil in which the study was conducted.

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References

Barber, S. A. 1995. Soil nutrient bioavailability. A mechanistic report. 2nd ed. – Wiley, New York.

Bergmann, W. 1988. Ernährungsstörungen bei Kulturpflanzen. Entstehung, visuelle und analytische Diagnose. – Gustav Fischer, Stuttgart.

Bowen, G. D. and Rovira, A. 1991. The rhizosphere: the hidden half of the hidden half. – In: Waisel, Y., Eshel, A. and Kafkafi, U. (eds), Plant roots: the hidden half. Marcel Dekker, New York, pp. 641–669.

Fox, T. R. and Comerford, N. B. 1990. Low-molecular-weight organic acids in selected forest soils of the southeastern USA. – Soil Sci. Soc. Am. J. 54: 1139–1144.

– and Comerford, N. B. 1992. Influence of oxalate loading on phosphorus and aluminium solubility in spodosols. – Soil Sci. Soc. Am. J. 56: 290–294.

Gerke, J., Römer, W. and Jungk, A. 1994. The excretion of citric and malic acid by proteoid roots of *Lupinus albus* L.; effects on soil solution concentrations of phosphate, iron, and aluminium in the proteoid rhizosphere in samples of an oxisol and a luvisol. – Z. Pflanzenernähr. Bodenkd. 157: 289–294.

Grierson, P. F. 1992. Organic acids in the rhizosphere of *Banksia integrifolia* L.f. – Plant Soil 144: 259–265.

Grime, J. P. and Hutchinson, T. C. 1967. The incidence of lime-chlorosis in the natural vegetation of England. – J. Ecol. 55: 557–566.

– and Curtis A. V. 1976. The interaction of drought and mineral nutrient stress in calcareous grassland. – J. Ecol. 64: 975–988.

Jarvis, S. C. and Pigott, C. D. 1973. Mineral nutrition of *Lychnis viscaria*. – New Phytol. 72: 1047–1055.

Killham, K. 1994. Soil ecology. – Cambridge Univ. Press, Cambridge.

Kraffczyk, I., Trolldenier, G. and Beringer, H. 1984. Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms. – Soil Biol. Biochem. 16: 315–322.

Lipton, D. S., Blanchar, R. W. and Blevins, D. G. 1987. Citrate, malate, and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. – Plant Physiol. 85: 315–317.

Mench, M. and Martin, E. 1991. Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L., *Nicotiana tabacum* L. and *Nicotiana rustica* L. – Plant Soil 132: 187–196.

Murphy, J. and Riley, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. – Analytica Chimica Acta 27: 31–36.

Ratnayake, M., Leonard, R. T. and Menge, J. A. 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. – New Phytol. 81: 543–552.

Rauterberg, E. 1951. Eine colorimetrische Makromethode zur phosphorsäure-bestimmung. – Z. Pflanzenernähr. Düng. Bodenkd. 53: 149–155.

Shen, Y., Obuseng, V., Grönberg, L. and Jönsson, J.-Å. 1996a. Liquid membrane enrichment for ion chromatographic determination of carboxylic acids in soil samples. – J. Chromatogr. 725: 189–198.

–, Ström, L., Jönsson, J.-Å. and Tyler, G. 1996b. Low-molecular organic acids in the rhizosphere soil solution of beech forest (*Fagus sylvatica* L.) cambisols determined by ion chromatography using supported liquid membrane enrichment technique. – Soil Biol. Biochem. 28: 1163–1169.

Smith, W. H. 1976. Character and significance of forest tree root exudates. – Ecology 57: 324–331.

Ström, L., Olsson, T. and Tyler, G. 1994. Differences between calcifuge and acidifuge plants in root exudation of low-molecular organic acids. – Plant Soil 167: 239–245.

Stryer, L. 1988. Biochemistry. 3rd ed. – Freeman, New York.

Tyler, G. 1992. Inability to solubilize phosphate in limestone soils – key factor controlling calcifuge habit of plants. – Plant Soil 145: 65–70.

– 1994. A new approach to understanding the calcifuge habit of plants. – Ann. Bot. 73: 327–330.
– 1996. Cover distributions of vascular plants in relation to soil chemistry and soil depth in a granite rock ecosystem. – Vegetatio 127: 215–223.
– and Olsson, P. 1993. The calcifuge behaviour of *Viscaria vulgaris*. – J. Veg. Sci. 4: 29–36.
– and Ström, L. 1995. Differing organic acid exudation pattern explains calcifuge and acidifuge behaviour of plants. – Ann. Bot. 75: 75–78.
Vancura, V. 1964. Root exudates of plants. I. Analysis of root exudates of barley and wheat in their initial phases of growth. – Plant Soil 21: 231–248.
Weast, R. C. 1989. Handbook of chemistry and physics. 70th ed. – CRC Press, Boca Raton, FL.
Zabowski, D. 1989. Limited release of soluble organics from roots during the centrifugal extraction of soil solutions. – Soil Sci. Soc. Am. J. 53: 977–979.
Zar, J. H. 1984. Biostatistical analysis. 2nd ed. – Prentice-Hall, London.