MAXIMUM GROWTH RATE OF SUGAR BEET AS A RESULT OF NUTRIENT SUPPLY, pH AND OTHER ENVIRONMENTAL FACTORS

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ABSTRACT

Growth rate, defined as biomass increase rate, was studied in sugar beet plants, in an aeroponics system, in order to establish non-limiting proportions of nutrient elements. In our experiments we used methods with continuous control of nutrient supply and uptake rates under controlled environmental conditions. The nutrients were supplied at constant relative addition rate and the plants were maintained under steady-state conditions, i.e. the plants were acclimatized. Through systematic elimination of different limitations we succeeded in obtaining a maximum stable growth rate of 0.50 (+/-0.04) g biomass per g biomass and day. This means a capacity to double the biomass in one day and 9 hours. We found that the first and most severe type of limitation that had to be minimized was low and unstable pH in the rhizosphere. This low and unstable pH was observed to be caused by the plants themselves. Sugar beet was found to strongly acidify the rhizosphere in both ammonium and nitrate based nutrient solutions. To maintain stable and non-limiting pH conditions, the nutrient solution had to be continuously titrated with sodium hydroxide. An optimum pH range of 5.2 to 6.2 was established. Under stable pH conditions (approx. +/- 0.1 pH unit) non-limiting proportions of nutrient elements could be established (N:100%, K:145%, P:21%, S:9%, Mg:23%, Ca:24%, Fe:0.7%, Mn:0.4%, Zn:0.09%, B:0.065%, Cu:0.03%). Other environmental factors that we optimized in our experiments were concentration of nutrient solution, pH range, nitrogen source, day length, light intensity and constant and shifted temperature. In experiments with constant temperature, pH activity parallelled growth (the highest growth rate was established at 24°C). When temperatures were shifted from periods of low temperature to 18°C, growth was acclimatized within a few hours to 18°C, while the pH activity of the plants took longer to parallel growth.

INTRODUCTION

Carl S. Sprengel (1771-1853) was the first to realize that the deficiency of a single nutrient element could depress growth even if all the other requisite nutrients were available in the soil (cited from Epstein, 1972). As a generalization credited to Justus von Liebig (1803-1873) this phenomenon is known as Liebig's "Law of the Minimum" which still remains a central concept in agriculture. This simplification has led to excess supply of many macro nutrient elements and confusion about the limiting role of micro nutrients. Limitations due

to low amounts of nutrient elements must be corrected by fertilizer, but today there is a demand for minimal and balanced fertilizer with minimal treatment costs and minimal loss of nutrients to the environment. For such a fertilizer strategy to be practically possible, the optimal environment for uptake and plant efficiencies needs to be realized. However, it is questionable whether we have enough knowledge about the nutrient availability and uptake requirements of cultivated species. In a review, Loneragan (1997) concluded that diagnosis and correction of micronutrient deficiencies with minimal fertilizer and treatment costs will play a vital role in future crop production. It will require development of better and simpler techniques of assessing the micronutrient status of soils and plants. It will also require better quality control in the manufacture and marketing of appropriate fertilizers and in the development of cultivars with high nutrient efficiency. Despite adequate amounts of nutrients being present, external and internal insufficiencies of plant uptake efficiency can limit the uptake. This problem can only be solved by relevant treatment of the soil and development of cultivars with higher uptake efficiencies.

Improved plant material as well as elimination of limitations in the soil must characterize both a quantitative and a qualitative development of plant cultivation in agriculture. Sugar beet is no exception. The problems associated with nutrient uptake are often referred to as either a problem of excess of, or access to, nutrient elements. These two problems have a common basis. Neither problem describes the requirements of the plant on balanced nutrient elements and neither accounts for nutrients being available for uptake at the right time and place during development of the plant. The basis for such a time-dependent approach comprises: (1) optimal proportions of nutrient elements, (2) conditions promoting uptake of available nutrient elements and (3) correct amounts of available nutrient elements. Correct amounts refer to the amounts required for a certain increase rate of biomass over a cultivation period.

Inevitable as well as avoidable limitations result in reductions of the maximum growth capacity of plants. This is a well-known fact, which is the reason for different efforts to eliminate limitations (or as it is usually termed - to optimise conditions) in order to increase and secure yield. Most of the research is done in field experiments, which often lead to contradictory and ambiguous results because of lack of control over plant and environmental variables. Demand for increased precision in agriculture requires a type of specification, quantification and prioritisation of limitations and their influence on plant properties that is not possible to obtain with traditional methods. Elimination of limitations requires methods where environmental conditions can be accurately identified and controlled, and where growth rate can be accurately obtained, quantified and verified together with relevant plant state variables.

Most of the research has been occupied with deficiencies as a static imbalance problem rather than a dynamic uptake problem. The difference between strategies has been discussed in several papers (e.g. Ingestad, 1982, 1997; Hellgren and Ingestad, 1996). The traditional "static" strategy is based on the external nutrient concentration as the driving force determining nutrient uptake rate while the "dynamic" is based on the fluxes directly (e.g. addition, uptake and growth rate).

In our experiments we used methods based on fluxes. Nutrient supply and uptake were continuously monitored under controlled environmental conditions. Using this type of method, optimal proportions of nutrient elements have been established for different species (Ingestad et al., 1994a, b).

The aim of our work was to study dynamic uptake conditions for sugar beet during the initial growth period. For this period, the goal was to establish optimal proportions of nutrient elements under non-limiting uptake conditions through systematic elimination of limitations.

METHOD

Our strategy with the laboratory method we used was not to simulate plant-soil interactions. It was to study plant properties alone without interference from soil properties, which are inevitably and interactively coupled to plant properties in a complex soil-plant environmental system. Our strategy was to control, maintain and verify both uptake and growth rate, and plant state under controlled limiting and non-limiting specified conditions.

By definition, the influence of a constraint on plant growth can only be unambiguously identified by its decrease of the growth rate. A growth rate caused by a specific condition can only be fully verified and identified when connected to a relevant state of the plant. Unstable conditions obstruct verification. If uptake rate of an element relative to the content of the element in biomass (relative uptake rate, *RUR*) is not equal to biomass increase rate per unit biomass (relative growth rate, *RGR*), then:

$$\frac{dU_N}{dt}\frac{1}{U_N} <> \frac{dW}{dt}\frac{1}{W} \qquad (1)$$

where U_N is g uptake of any nutrient *N*, *W* is g biomass and *t* is time. The state U_N/W variable value will be :

$$\frac{d\left(\frac{U_N}{W}\right)}{dt} <> 0 \qquad (2)$$

and will change from one state value to another, with a probable modification of plant growth and development as a consequence. This change can only go on for a restricted period of time until the ratio is no longer possible. When uptake rate, *RUR*, equals growth rate, *RGR*:

$$\frac{dU_N}{dt}\frac{1}{U_N} = \frac{dW}{dt}\frac{1}{W} \qquad (3)$$

the result is a stable state, steady-state, condition:

$$\frac{d\left(\frac{U_N}{W}\right)}{dt} = 0 \qquad (4)$$

A constant *RGR* means exponential growth, which is experimentally only required long enough to verify the growth rate.

All our experiments were carried out in controlled environmental rooms in the Biotron in Alnarp, Sweden, with sugar beet plants cultivated in controlled growth units (Biotronic AB, Uppsala, Sweden). In the growth units, shoots were separated by plastic foam around the shoot-root zone from the roots of intact plants. The roots were freely growing in an air compartment. The roots were continuously sprayed with a culture solution containing all necessary nutrient elements. The concentration of nutrient elements in the culture solution was determined and controlled by one setpoint value for conductivity. When nutrient elements were added in free access to the plants, additional nutrient elements were titrated into the culture solution after depletion of nutrients below the setpoint value. When nutrients were added at a fixed relative addition rate, nutrients were titrated based on calculated amounts, with the only restriction that concentration was not allowed to exceed a maximum setpoint value. With these strategies, the concentration of nutrients could be held at low levels. Addition of nutrients accounted for the increasing demand for nutrients with increasing amount of biomass and titration was performed at shorter and shorter time intervals with increasing size of the plants. This growth technique was adopted from Ingestad and Lund (1979, 1986) and Ingestad et al. (1996).

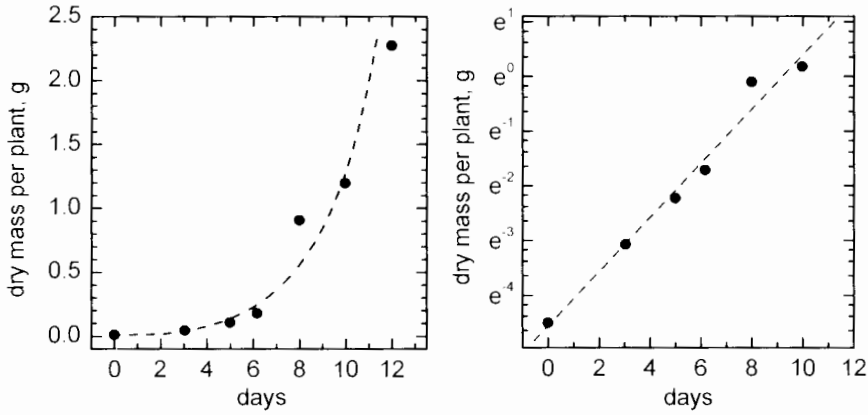
The type of technical system we used to study nutrient uptake and growth rate in sugar beet plants is known as an aeroponic system. The system differs on a number of vital points from the hydroponic system and can be regarded as a development of this system. With the aeroponic system, it is easy to optimize aeration. Circulation velocity of culture solution is high due to the technique of continuous (or intermittent) spraying of culture solution directly on the plant roots. In our experiments, nutrient supply and uptake rates as well as pH and conductivity were continuously monitored and controlled by computer software ("Growth Unit Control", Lund Sweden). The nutrients were supplied either at a constant relative addition rate or as free access and the plants were maintained under steady-state conditions, i.e. the plants were maintained acclimatized during each experiment.

Plant growth and different state variables were obtained by harvests at regular, successive intervals during a steady-state growth period. Measure-ments were made on these occasions of leaf and root fresh and dry weights. Chemical analyses were carried out on plant materials, root and shoot, separately, for nutrient elements. Relative growth rate and state of plants were determined by curve fitting to $y = a\ e^{bx}$ of the harvest measurements. The coefficient of determination r^2 was generally ≥ 0.99 for relative growth rates.

Different limitations were successively minimized or eliminated, which was verified by successively increased growth rates. Through this type of systematic

elimination of different limitations, we succeeded in obtaining a maximum stable growth rate of 0.50 (+/-0.04) g biomass per g biomass and day (**Fig. 1**). This means that a capacity to double the biomass in one day and 9 hours was obtained for sugar beet.

Figure 1. *The left-hand diagram shows data for successive harvests, while on the right the same data are shown on a logarithmic scale indicating the relative growth rate, RGR, by the slope of the curve. In the experiment pH was 5.4. An optimized nutrient solution with 15/85% ammonium/nitrate was used. The plants were grown in 350 µmol m^{-2} s^{-1} light, 24 hours of day and 24°C. RGR was 0.50 ±0.038 g g^{-1} day^{-1}, r^2 = 0.98. This growth capacity means that the biomass was doubled in 1 day and 9 hours.*



An optimized nutritional balance between elements in a nutrient solution requires electroneutrality to be accounted for. This can be solved by mixing elements without excess of any nutrient element. If this is not possible, excess elements should only include well chosen elements, e.g. Ca or Mg. Maximum growth rate requires optimal access to all nutrient elements. Accumulation of elements taken up at a slower rate than the rate of addition would result in an imbalance of nutrients in the culture solution sprayed on the roots. The stock nutrient solution titrated into the culture solution was adjusted until a good balance was reached between supply and uptake according to equation (5), where *A* is g addition of any nutrient element, *N*:

$$\frac{dA_N}{dt}\frac{1}{A_N} = \frac{dU_N}{dt}\frac{1}{U_N} = \frac{dW}{dt}\frac{1}{W} \qquad (5)$$

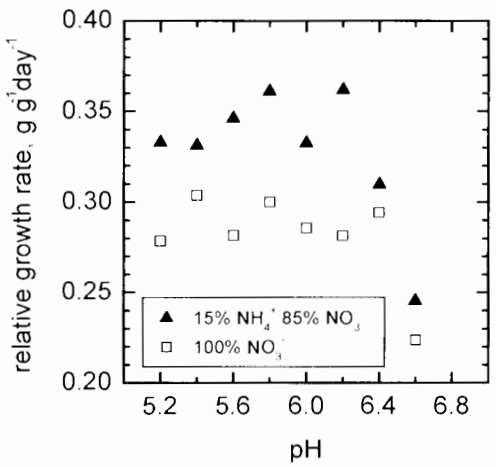
The resulting amounts of the different nutrient elements in the biomass indicate optimal proportions between elements, i.e. an optimal nutritional balance is established.

PREPARATORY EXPERIMENTS TO ESTABLISH OPTIMAL PROPORTIONS OF NUTRIENT ELEMENTS

In a series of experiments the effects of concentration of nutrient solution (conductivity), pH range, nitrogen source, day length and light intensity were studied in order to establish non-limiting conditions.

The first type of limitation that we identified as being crucial to eliminate was low and unstable pH in the rhizosphere, which was caused by the plants themselves. Unlike most other plants, e.g. tomato, oilseed rape, wheat, or barley, sugar beet was shown to strongly acidify the rhizosphere with both ammonium and nitrate as the nitrogen source in the nutrient solution. To maintain stable pH conditions in a non-limiting pH-range, the culture solution had to be continuously titrated with hydroxide. A pH range that was non-limiting was established between 5.2 and 6.2 (**Fig. 2**). In all main experiments two pH setpoint values were used; 5.4 and 6.0. A deviation of +/- 0.1 was possible to maintain in most experiments.

Figure 2. *Relative growth rate as an effect of different fixed pH values for two combinations of nitrogen source. Since sugar beet continuously acidified the nutrient solution, pH was controlled by titration with NaOH.*

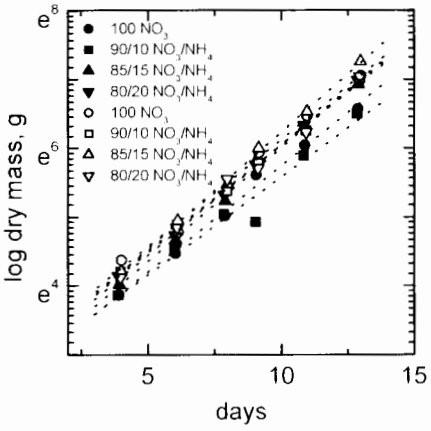


Different combinations of ammonium and nitrate were tested as nitrogen sources. The differences were small (**Fig. 3**). A combination of 85% nitrate and 15% ammonium resulted in the highest growth rates and was chosen for the further experiments.

Plants were grown in continuous light and in different day lengths. The best response was found in continuous light. Therefore, 24 hours day length was chosen for the main experiments. Light intensities, photon flux densities, (quantum sensor, LiCor, USA; fluorescent tubes, CW, 215 W, Sylvania,

Canada) between 50 and 430 μmol m^{-2} s^{-1} were tested. The highest growth rates were determined in 350-430 μmol m^{-2} s^{-1}.

Figure 3. *The effect on relative growth rate of different combinations of nitrate and ammonium as the nitrogen source. The differences were small.*



ROOT-INDUCED ACIDIFICATION/ALKALINIZATION OF THE RHIZOSPHERE

It is well known that plant roots have the capacity to considerably change the rhizosphere pH (e.g. Nye 1986; Römheld, 1986; Hinsinger, 1998). The pH change (which may be up to 2 pH units in the rhizosphere) is said to occur mainly when plants are counterbalancing a net uptake of cations or anions. In many studies, pH changes in the rhizosphere have been linked with N nutrition. It is thus reported that uptake of nitrate results in release of OH^-/HCO_3^- and thereby causes an alkalization of the rhizosphere, while uptake of ammonium results in release of H^+ and acidification (e.g. Römheld, 1986; Gahoonia *et al.*, 1992). It is also known that different species (and different genotypes within a species) respond differently to the same environmental conditions.

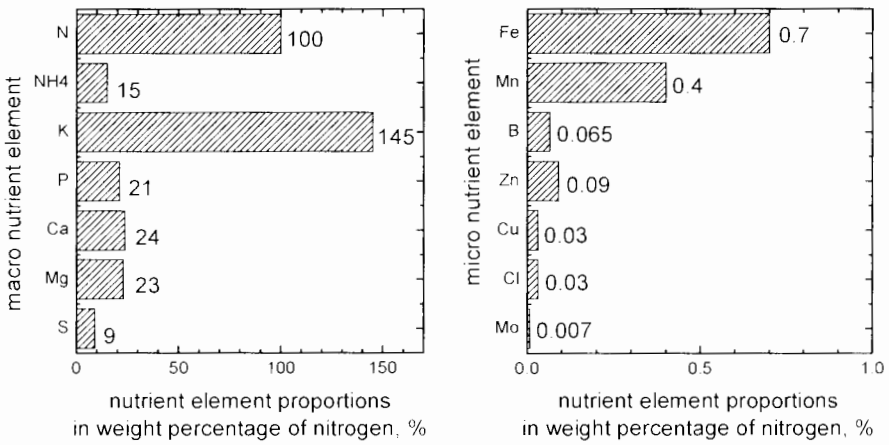
Darrah (1993) reviewed the role of the rhizosphere in plant nutrition, particularly with the focus on quantification of root-mediated changes to the chemical, physical and biological processes of the rhizosphere soil. He concluded that the dynamic and integration of all processes made the effect of a single change very difficult to quantify and evaluate. For example, acquisition of phosphorus by plants may involve combinations of a number of physiological functions, soil pH changes and other soil chemical reactions as well as microbiological activities. A rigorous quantitative approach is needed to answer fundamental questions concerning the role of the rhizosphere and nutrition.

Large and rapid changes in pH have been measured near root surfaces, which is reported to confirm the significance of rhizosphere processes to plant nutrient absorption. Plants have been shown to release P from calcareous soils and also Fe, Mn and Zn, probably by acidifying the rhizosphere (Gardner *et al.*, 1982).

OPTIMAL PROPORTIONS OF NUTRIENT ELEMENTS

Under stable pH conditions (approx. +/- 0.1 pH unit), maintained by rigorously controlled pH titration of the nutrient solution, non-limiting proportions of nutrient elements could be established (**Fig. 4**).

Figure 4. Optimized proportions of nutrient elements relative to nitrogen in a nutrient solution resulting in the highest growth rates.



Potassium is the most abundant essential cation in many higher plants, and sugar beet is no exception to this rule. The uptake of potassium has been concluded to be electrically coupled to proton release (Behl & Raschke, 1987). Increasing external supply of the element usually results in an increase in the total uptake and thereby the proton release also increases. The connection that we observed between uptake of K^+ and release of H^+ was coupled to the increase in growth rate due to optimized supply and uptake of K^+.

TEMPERATURE AND NUTRIENT UPTAKE

The systematic elimination of limitations also included temperature, as constant temperature, which resulted in determination of a peak growth capacity of 0.50 g biomass per g biomass and day for sugar beet as seen in **Fig. 1**. This growth rate was determined at 24°C (**Fig. 5**). In experiments with constant temperature, pH activity was parallel to the growth rate, except during the beginning of the cultivation (**Fig. 6**). At lower temperatures the proton release rate was lower than the growth rate for a longer period than at higher temperatures. The higher the temperature, the sooner the proton release rate came to parallel the growth rate.

When temperatures were shifted from periods of low temperature (2 and 6°C) to 18°C, growth was acclimatized to 18°C rather quickly. The pH activity of the plants took longer to parallel growth (**Fig. 7**).

Figure 5. *Relative growth rate as a response to constant temperature.*

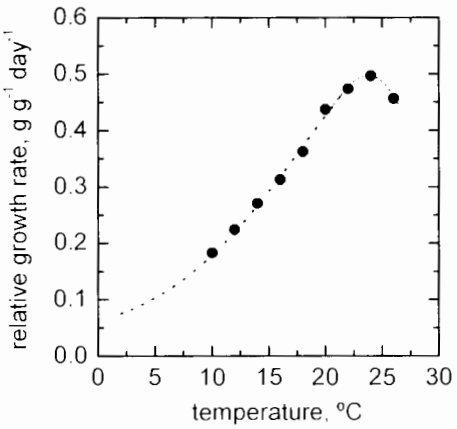


Figure 6. *Biomass increase and accumulated amounts of NaOH titrated to maintain pH 5.4 at a constant temperature of 10°C or 22°C.*

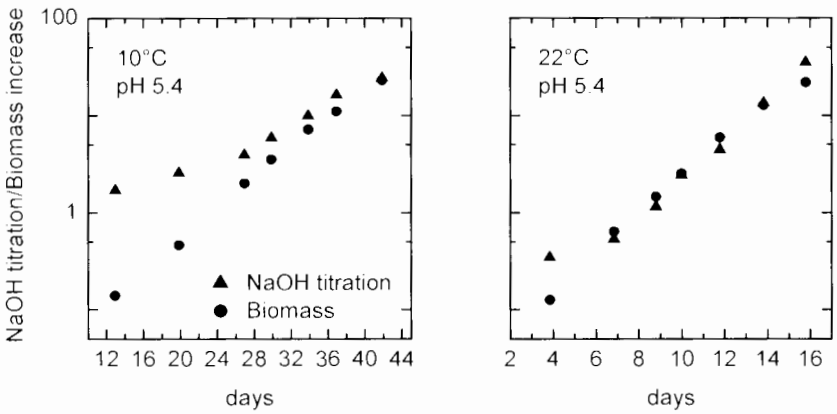
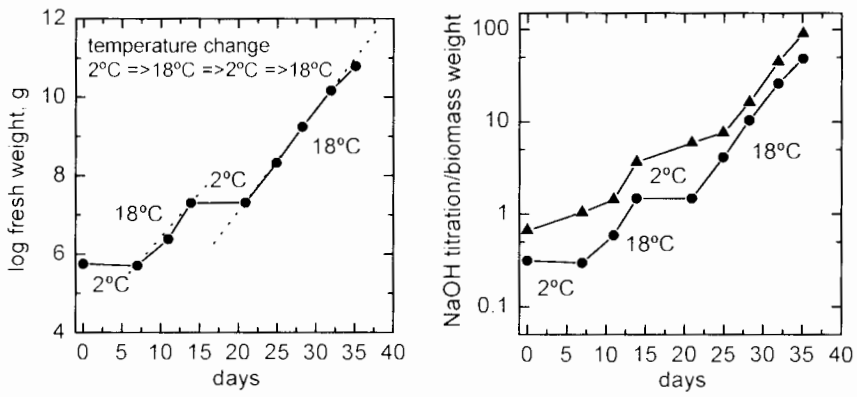


Figure 7. The left-hand diagram shows the almost immediate response to a shift in temperature. The right-hand diagram also shows the response to NaOH titration.



PLANT STATUS AND CONDITIONS FOR NUTRIENT UPTAKE

One of the most commonly observed effects of nutrient deficiency on plant growth is an increase in the root:shoot ratio. This is often reported to be particularly pronounced in fast growing species adapted to sites of high fertility (Chapin, 1980).

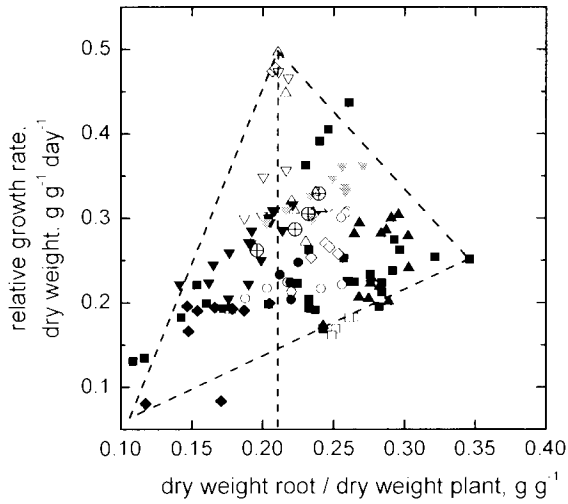
In our experiments root:shoot ratios, expressed as dry weight root per dry weight plant, were also seen to shift under limited conditions compared with those obtained under optimum uptake and growth conditions (**Fig. 8**). Primarily, ratios shifted towards higher values when plants were growing under uptake limiting situations. However, shifts towards lower values could also be observed indicating not only shoot growth limitations, e.g. light limitations.

DISCUSSION AND CONCLUSIONS

In this work sugar beet plants were studied during initial growth. The plants grew exponentially, which made verification of growth rate easy. However, even after the so-called exponential growth phase of the plant, exponential growth can still be identified provided that the amounts of non-productive biomass, e.g. structural and ageing biomass, are quantified and accounted for.

Rhizosphere acidification by the roots of sugar beet, as seen in our experiments, has two significant implications. The first is most likely a strategy of the plant to continuously facilitate or enable uptake of e.g. P, Fe, Mn and Zn. As a consequence of decreased pH, the soil reactions are known to deliver the necessary nutrient elements. The second is very probably a requirement of the plant, that the soil should continuously buffer the decreased pH. If the soil fails to buffer the pH in the rhizosphere it can be physiologically hazardous to the

Figure 8. *At maximum growth rate, the root to plant ratio was approx. 21% of the plant biomass. Increased root to plant fraction cannot be explained solely by limiting conditions of uptake, while decreased root to plant fraction cannot be explained solely by shoot limiting conditions.*



sugar beet plant, according to both the results of our experiments and field experience.

To affect nutrient availability in the soil solution, the magnitude of change and the rate at which it occurs are known to be important features in plant induced H^+ release in the rhizosphere. It is therefore not surprising that acidification by sugar beet parallels growth rate. We were unable to detect a change in acidification due to, for example, different K supply for sugar beet. However, nutrient availability due to the plant strategy of inducing acidification of the rhizosphere has to be further investigated, regarding both macro- and micronutrients.

Calcareous soils are known to rapidly immobilize Fe and Mn. Deficiencies are generally counteracted in practice by foliar applications to the plants. Decreased pH in the rhizosphere as a consequence of plant induced H^+ release has been reported to increase availability of Fe (Lindsay, 1981). Kirk & Bajita (1995) showed that acidification of the rice rhizosphere solubilizes Zn. The strong H^+ release induced by sugar beet roots can have a strong effect on Fe, Zn and Mn availability, especially in calcareous soils. Effects on Fe, Zn and Mn availability in sugar beet need to be investigated.

Of the major nutrient elements phosphorus is regarded as the most limiting for plant growth. This is not due to low amounts of phosphorous in the soil but to its strong retention to different types of soil constituents, resulting in extremely low mobility. Therefore only a small fraction of phosphorus in the soil is present as ions in the soil solution. The high reactivity to different soil constituents makes inorganic phosphorus immobile. If the concentration of P ions, or rather the

mechanism of sugar beet seems to be fixed on a very high release rate, which is not adjusted for deficiencies in external nutrient elements but rather follows the growth rate. If the pH buffering capacity of the soil is low, pH levels hazardous to the plant can easily occur whether nutrient availability is high or low.

A change in soil pH induced by roots in the rhizosphere cannot be expected to be a good indicator of the actual release of H^+ or OH^-/HCO_3^-, especially so in neutral and alkaline soils with a strong soil pH buffering capacity (Hinsinger, 1998). Schubert *et al.* (1990) showed, when comparing different soil types, that little or no decrease in pH was found for the soils that had a strong buffering capacity. In poorly buffered soils, a decrease in pH was observed. It is not only the soil constituents that determine whether a soil has a strong buffering capacity but also physical properties influencing water (infiltration) and gas (pore structure) flows. Extensive research is also needed in this area to determine dynamic pH buffering capacity.

Concentrations of nutrients, monitored in our experiments as conductivities, were found to promote growth rate best at low values, as found in soil solutions. In addition, a constant specific growth rate could be maintained without a constant concentration and could also be maintained in different concentrations. A constant growth rate, such as RGR, is a question of addition of nutrients with an increasing frequency or amplitude, or both, rather than a fixed concentration. Thus, the amount of nutrients needed by the plant increases with increasing size of the plant. The same situation can be translated into the supply rate and uptake rate of, for example, P in the soil. It is possible that low P in the soil solution can be continuously made available for uptake as an effect of root induced relative addition rate of protons releasing P, which is coupled to the relative growth rate and the increasing size of the plant.

Nutrient status of crops and soils is used by plant nutritionists to monitor and remedy nutrient deficiencies in the soil. However, it is not sufficient only to analyze the amount of nutrients per unit plant and the proportions of nutrient elements in the plants. It is also necessary to analyze growth rate. Amounts and proportions of nutrients do not mirror the uptake rate and its effect on the growth rate. As seen in **Fig. 8** the root:plant ratio can have the same value from maximum growth, i.e. growth under optimal conditions, to minimum. This means that optimal nutrient proportions can be taken up at any growth rate not in itself indicating limitations to the uptake rate of the nutrients.

Availability for uptake of the correct nutrient elements is a function of presence and accessibility, which is a question of elements being available in the right amounts at the right time - as a continuous increasing demand controlled by plant growth. A major challenge will be to take into account the spatial and temporal variability of the physical and chemical changes occurring in the rhizosphere in the soil induced in different ways by the plants. Plant properties involved in the dynamic interaction with the soil to promote uptake of nutrients have to be extensively investigated with relevant approaches and methods. The approach and methods we have used open up a new way of studying plant properties and their interaction with environmental conditions.

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