# Nutrient deficiency symptoms and uptake relations in juvenile hazelnut (*Corylus avellana*) in response to macronutrient supply

W. Voogt[1,a], M. Butturini[2], W. Sun[2], E. Suarez Huerta[3] and L. Marcelis[2]

[1]Greenhouse Horticulture and Flower Bulbs, Wageningen University & Research, Wageningen, The Netherlands; [2]Horticulture and Product Physiology, Wageningen University, Wageningen, The Netherlands; [3]Agri Competence Centre, Hazelnut Company Division, Ferrero Trading Luxembourg, Senningerberg, Luxembourg.

***Abstract***

**The characterization of macronutrient (N, P, K, Ca, Mg, S) deficiencies in *Corylus avellana* is poorly documented, whereas early detection of nutrient shortages would help to prevent yield and quality reductions in hazelnut production. In a four-month greenhouse trial, nutrient deficiencies of macro-elements were induced by growing one-year-old 'Tonda di Giffoni' plants in 5-L containers with perlite with macronutrients individually applied at 0, 50 and 100% reduction in the supply concentration of a standard nutrient solution. Except for P, all nutrient disorders were visually induced in the zero treatments, after four (N and K) to six weeks (Ca, Mg, S). In the 50% treatments, deficiency symptoms appeared only for N, Ca, and S. The zero treatments of N, P, K, and S resulted in a significant reduction in biomass, whereas in the 50% treatments, growth was reduced significantly for N only. The nutrient contents in stem and foliage tissue demonstrated positive relationships with the nutrient concentrations applied at the root zone. Despite the clear response of biomass and tissue content to the P-supply, the lack of visible symptoms of P-deficiency was remarkable. The quantity of P in the initial plant was likely sufficient as a P-source, resulting in slight growth reduction but without initiating symptoms in this short growing cycle. Nevertheless, the average P contents in the final harvested leaves were extremely low (25 mmol $kg^{-1}$ DM) compared to standard values mentioned in literature (50 mmol $kg^{-1}$ DM).**

**Keywords:** nitrogen, potassium, calcium, magnesium, sulphur, phosphorous, fertilization, nutrient uptake, nutrient solution, tip burn

## INTRODUCTION

The macronutrient demand for hazelnut (*Corylus avellana* L.) has not been investigated as extensively as for other fruit crops. The majority of research data published about this topic is on nitrogen (N) and, to a lesser extent, on potassium (K) or phosphorous (P), but rarely on calcium (Ca), magnesium (Mg), or sulphur (S) (Tous et al., 1994; Li et al., 2019; Kizilkaya et al., 2022). With the modernization of hazelnut production, the economic value of a hazelnut orchard is high. Therefore, the potential risk of macro-nutrient deficiencies or nutrient imbalances, which could have long-term effects on productivity, should be avoided as there are quite some macro- (as well as micro-) nutrients involved, and the potential for imbalances is relatively high. To ensure long-term sustainable hazelnut cultivation and empower the hazelnut farming community, the knowledge level of mineral nutrition should be improved. Since nutrient deficiencies induce specific visual symptoms in leaves and shoots, early recognition of these symptoms will help to identify problems with hazelnut mineral nutrition. In addition, guide values for nutrient levels in plant tissue will be helpful to further investigate the nutrient status of a hazelnut orchard or specific trees. Nutrient deficiency symptoms in plants have some common features and have been described widely but can be quite distinct for plant species (Mengel and Kirkby, 2012). Özenç and Bender Özenç (2009) investigated the

[a]E-mail: Wim.voogt@wur.nl

specific symptoms in young hazelnut plants but did not publish pictures, and clear descriptions are lacking. Yet, a visual diagnostic decision support tool in the form of a field-guide with nutrient deficiency pictures is non-existent. Guide values for nutrient contents in leaf tissue have been published (Snare, 2008; Canali et al., 2005; Olsen, 2013) but these data are not very well established, and the ranges are quite different. To establish a library with pictures of nutrient deficiencies and accompanying data from tissue analysis, a trial was conducted to initiate nutrient deficiencies of all macro-nutrients (N, P, K, Ca, Mg, and S) in common hazelnut. Juvenile *C. avellana* plants were grown in a soilless system under greenhouse conditions to obtain fully controlled conditions to minimize confounding effects of biotic and abiotic stresses. The current experiment focuses on macro-elements, and to distinguish between mild and severe effects, 100 and 50% reductions of the element concentration from a standard nutrient solution were applied.

**MATERIALS AND METHODS**

Two-year-old 'Tonda di Giffoni' plants were purchased and raised in 0.5-L pots filled with a standard peat-based potting soil. Plants arrived on December 21, 2021, and were kept in a climate cell at 4°C to complete chilling unit accumulation. Before transplanting, shoots were pruned at 50 cm from the base of the stem, and potting soil was removed from the root balls of the dormant plants as much as possible. Plants were moved to a greenhouse on March 16, 2021, and the experiment was concluded on June 9, 2021. The plants were grown in 9.2-L (diameter 230 mm, height 225 mm) polyethylene (PE) pots filled with approximately 8 L of fine perlite substrate (Grain 1, 0.6-1.5 mm) (Pull b.v, Rhenen). The experiment was conducted in a 32 $m^2$ Venlo-type greenhouse compartment at Unifarm, Wageningen University and Research (52.0°N, 5.7°E), with fully automatic climate control of temperature, humidity, $CO_2$ level, and a sun-shading screen. The nutrient solution was supplied by drip irrigation, quantity adjusted to the plant development and weather conditions to achieve a drainage rate of approximately 70%, which was manually verified. Each treatment replicate (plot) had its individual closed nutrient solution system, consisting of a 300-L PVC container, a small pump, and drip irrigation; the nutrient solution was recirculated. The experiment was laid out in three replicates in a randomized block design (RBD) with three blocks, with three plants per plot. A basic nutrient solution was established using published data on mineral leaf contents of hazelnut for the mutual nutrient ratios (Tous et al., 1994; Miletic et al., 2001; Canali et al., 2005; Snare, 2008; Özenç and Bender Özenç, 2009; Olsen, 2013) which were then converted into a nutrient solution with a target EC of 1.3 mS $cm^{-1}$, with commonly-used procedures (Sonneveld and Voogt, 2009a, b). The resulting nutrient solution was used as a reference for the control treatment (Table 1). N, S, P, applied as $NO_3^-$ and $NH_4^+$, $SO_4^{2-}$, and $H_2PO_4^-$ respectively, K, Ca and Mg as $K^+$, $Ca^{2+}$ and $Mg^{2+}$, respectively. For the nutrient-deficient treatments, 100 and a 50% reduction of the concentration from the reference were applied, hereafter referred to as the 'Zero' and '50%' treatments, respectively. To avoid confounding EC effects, the reduction of the individual cations and anions was compensated by proportionally increasing the other anions or cations while keeping the same mutual ratios. An exception was made for $NH_4^+$, which was kept at 0.25 mmol $L^{-1}$ for all treatments, to avoid the associated pH effects in the root zone as much as possible (Sonneveld and Voogt, 2009a, b). To avoid too extreme $SO_4^{2-}$ and $H_2PO_4^-$ concentrations for the zero and 50% N treatments, chloride ($Cl^-$) was deliberately included for the anion corrections to compensate for the reductions in $NO_3^-$, $SO_4^{2-}$ and $H_2PO_4^-$ (Table 1). Individual fertilizer recipes were calculated for each treatment. Commercial straight fertilizers were used to prepare the solutions. Micronutrients were applied equally in all treatments, at concentrations of 24, 17, 6.2, 30, 1.3, and 0.4 µmol $L^{-1}$ for (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo), respectively. Initially, each box was filled with 230 L of the targeted nutrient solution. No additional water or fertilizers were added during the trial. The pH was kept between 5.8 and 6.2, and corrections were made when needed by adding aliquots of 1 mol $L^{-1}$ HCl solution or 2 mol $L^{-1}$ $K_2CO_3$.

Table 1. Composition of the nutrient solutions applied in the control and the targeted 50% and zero treatments of N, P, K, Ca, Mg, and S.

| Treatment | $NH_4$ | K | Ca | Mg | $NO_3$ | Cl | $SO_4$ | $H_2PO_4$ |
|---|---|---|---|---|---|---|---|---|
| | (mmol $L^{-1}$) | | | | | | | |
| Control | 1.36 | 3.25 | 3.61 | 1.56 | 12.92 | 0.00 | 0.45 | 1.13 |
| 50% N | 1.36 | 3.25 | 3.61 | 1.56 | 6.46 | 3.29 | 1.40 | 2.40 |
| 50% P | 1.36 | 3.25 | 3.61 | 1.56 | 12.92 | 0.57 | 0.45 | 0.57 |
| 50% K | 1.36 | 1.63 | 4.19 | 1.79 | 12.92 | 0.00 | 0.45 | 1.13 |
| 50% Ca | 1.36 | 5.10 | 1.81 | 2.44 | 12.92 | 0.00 | 0.45 | 1.13 |
| 50% Mg | 1.36 | 3.70 | 4.17 | 0.78 | 12.92 | 0.00 | 0.45 | 1.13 |
| 50% S | 1.36 | 3.25 | 3.61 | 1.56 | 12.92 | 0.45 | 0.23 | 1.13 |
| zero-N | 1.36 | 3.25 | 3.61 | 1.56 | 0.00 | 7.00 | 2.48 | 3.00 |
| zero-P | 1.36 | 3.25 | 3.61 | 1.56 | 12.92 | 1.13 | 0.45 | 0.00 |
| zero-K | 1.36 | 0.00 | 4.75 | 2.05 | 12.92 | 0.00 | 0.45 | 1.13 |
| zero-Ca | 1.36 | 6.89 | 0.00 | 3.35 | 12.92 | 0.00 | 0.45 | 1.13 |
| zero-Mg | 1.36 | 4.25 | 4.67 | 0.00 | 12.92 | 0.00 | 0.45 | 1.13 |
| zero-S | 1.36 | 3.25 | 3.61 | 1.56 | 12.92 | 0.90 | 0.00 | 1.13 |

All individual plants were visually examined weekly for appearing symptoms, which were recorded. Images were taken by the Robin PSI PlantScreenTM system (PSI, Czech Republic) through red-green-blue (RGB) imaging with a top-view of the plants to capture the nutrient deficiency symptoms on April 6, 21, and 29. Since the plant size exceeded the Robin system from early May onwards, a standard digital camera was used to take the images on May 4, 14, and June 7. Root images of each plant were taken at the end of the experiment on June 16. Ten juvenile plants were selected randomly for plant nutrient content determination at the start of the trial. At harvest, above-ground parts of all plants were divided into stems and leaves. Both fresh and dry weights of stems, leaves, and roots were measured after air-drying at 80°C for 72 h. Dry matter samples of stems and leaves were sent to a commercial lab for nutrient content analysis.

## RESULTS

Plants grown at the zero nutrient levels started to show symptoms after four weeks for N, and after six weeks for K, Ca, Mg, and S, after the initiation of the treatments (Figure 1).

### Deficiency symptoms

#### 1. Nitrogen.

At zero N, the plants started to develop discoloration of the oldest bottom leaves four weeks after the start, which turned from fresh green to pale/yellowish green progressively in two weeks. This was followed by discoloration of leaves higher up on the main stem. Eventually, the old leaves turned completely yellow. Younger leaves tended to remain green, but after two weeks started to develop yellow colouring, and towards the end of the experiment the most affected leaves started to drop. Secondarily, necrosis appeared on chlorotic leaves, started at the tip and margins, and progressed down the midrib towards the leaf base. In contrast, the primary, the secondary and some of the majority of smaller veins stayed green. Plant growth was significantly weaker, both in shoot length and leaf size, than the control and became visually apparent one week after the appearance of the leaf symptoms. Plants at 50% N showed much less intensive chlorotic symptoms and necrosis, which also started later, and growth was slightly less than the control.

#### 2. Phosphorus.

During the experimental period, no apparent symptoms of deficiency in the plants could be observed, neither at zero, nor at 50% P. Slight growth reduction at the zero treatment compared to the control was the only visible effect.

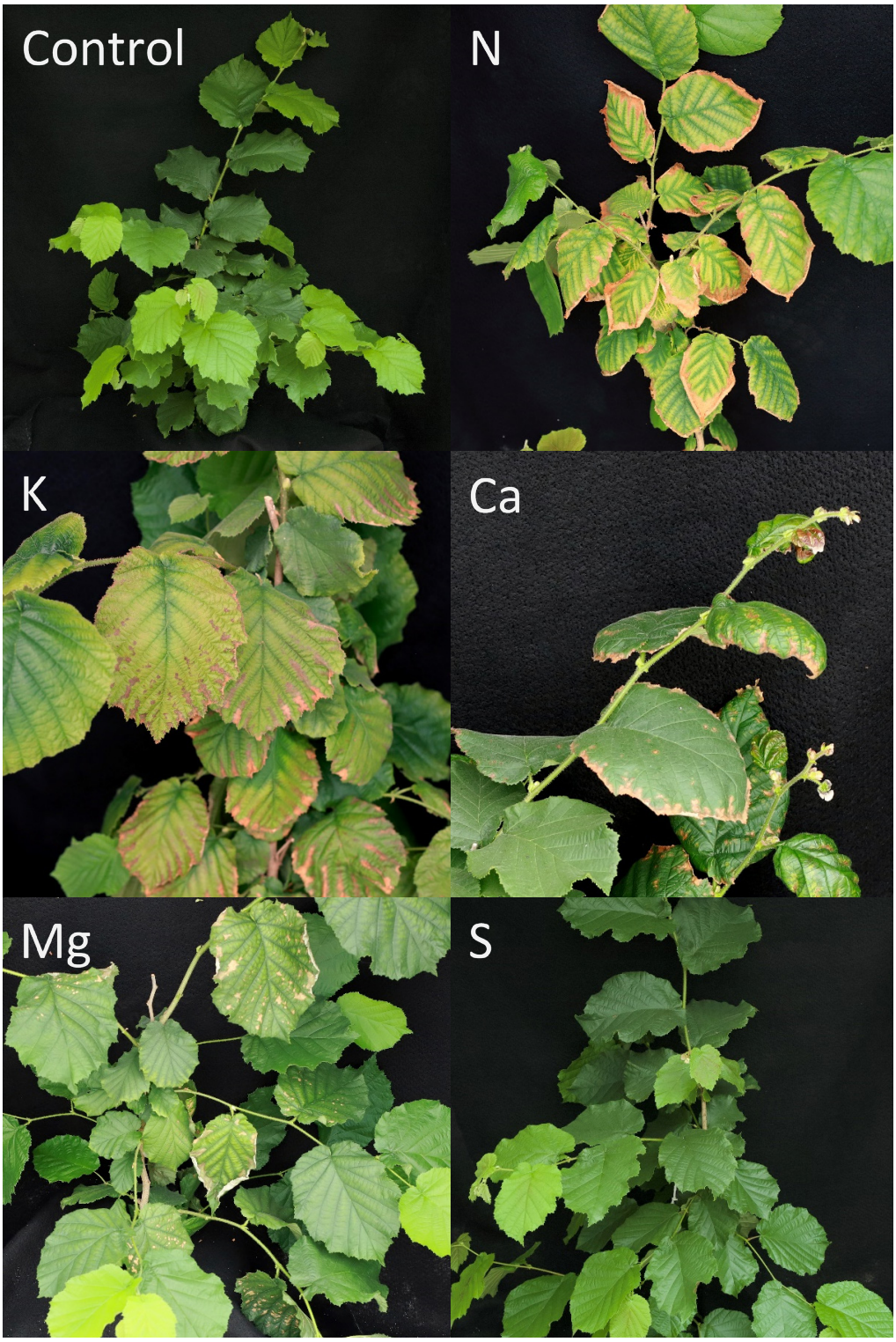


Figure 1. Typical symptoms of the induced nutrient deficiencies of N, K, Ca, Mg, and S in young hazelnut trees, compared to a control treatment. Pictures taken of plants and shoots approximately 12 weeks after the start of the trial.

**3. Potassium.**

The first symptoms appeared five weeks after the start in the zero K and started with small brown-red intravenous freckle-like necrotic spots in the oldest leaves, followed by

yellowish discoloration of the whole leaf. In a few weeks, the spots turned into intravenous necrotic stripes across the entire leaf. The overall growth of the plants was reduced compared to the control, both in shoot length and leaf size. Plants in the 50% K did not develop deficiency symptoms, and the growth was not visibly different from the control.

**4. Calcium.**

Symptoms appeared eight weeks after the start of the trial at zero Ca. The first visible symptoms appeared as necrotic lesions on the edges and tips of the very young leaves at the top of the growing shoots. Eventually, necrosis symptoms appeared along the complete leaf margin of the youngest leaves, resulting in the typical 'tip burn' symptoms. Gradually when the young leaves grew older, the necrotic edges hindered the expansion of the leaf margins compared to the inner part, which resulted in cupped leaves. All plants in the zero treatment showed more or less incidence of these symptoms and the growth of these plants was negatively affected compared to the control. Plants at 50% Ca showed these symptoms as well, but the incidence was less severe.

**5. Magnesium.**

Symptoms became visible in the zero treatment seven weeks after the start and appeared on old leaves first. Initially, it began as a yellowish discoloration between the veins, whereas the veins stayed green. Later, necrotic spots appeared, slowly expanding into larger necrotic patches. Toward the end of the experiment, the most symptomatic leaves tended to drop. All plants at zero-Mg developed these symptoms but did not show a reduction in growth. Plants at 50% Mg grew normally and did not develop Mg deficiency symptoms.

**6. Sulphur.**

Deficiency symptoms appeared on young leaves at zero S about seven weeks after the start of the trial. It started as a pale light-green discoloration in the youngest leaves of all shoots evenly spread over the whole leaf. In the course of time, the colour did not change much and stayed light grayish green, in contrast to control plants, where young leaves start light green also but turn to dark green in the course of time. The chlorosis appeared in all plants of zero S, and the deficiency caused a slight growth reduction. Plants at 50% S showed only minor discoloration in the young leaves of some of the shoots and did not affect growth.

**7. Other symptoms.**

Tipburn-like symptoms, equal to the tip burn that occurred in the Ca treatments, appeared in some shoots of some plants two months from the start and increased gradually throughout the experiment. The symptoms appeared in plants of various nutrient treatments and control plants. Another symptom was some purple colouring (anthocyanosis) appearing in the young leaves of some of the shoots, which gradually faded away when the leaves expanded. These symptoms appeared in many plants of zero P, though this phenomenon appeared also in many other treatments, including the control.

**Nutrient concentrations in water and plant**

The nutrient concentrations at the start and the end were all in agreement with the intended concentrations (data not shown), and the changes in concentrations during the trial were rather limited due to the sufficiently large water buffer. The analysis of shoots and stems clearly revealed the effects of the different nutrient levels in the treatments but with distinct differences between the nutrients (Table 2). The effects of the cations K, Ca, and Mg were clear and consistent. Compared to the control, the zero treatments of these nutrients induced a significant reduction in nutrient content, resulting in 76, 83 and 76% reduction for K, Ca, Mg, respectively. The 50% treatments did not halve the plant contents, though the reduction was still substantial at 22, 30, and 30%, respectively, for K, Ca, and Mg. The reductions in tissue contents were less evident for the anions N, P and S. For zero-P, the content in the shoot tissue was reduced by 64%, but only by 10% with 50%-P. For N and S, the zero treatments were reduced by only 23% (N) and 12% (S) and with the 50%- treatments the reduction was even

negligible for both nutrients. The nutrient contents in the stems were lower than in the shoots but reflected the different treatments in more or less the same way as for shoots (data not shown). The total dry weight of the foliage and stems of zero N and zero K were significantly lower than all other treatments (Table 3). Zero P and zero S were also significantly lower than the control for leaves, but not for stems. Zero Ca and zero Mg had lower leaf and stem weights than the control, but the difference was not significant. The 50% treatments did not reveal significant differences compared to the control, except for the stem at 50% N, which was significantly lower. The total water uptake was in accordance with the effects on biomass (data not shown): plants with zero N and K had a significantly lower water uptake than the control plants. The water uptake was not significantly lower than the control for all other zero treatments as well as the 50% N and K.

Table 2. Mineral contents (mmol $kg^{-1}$ dry matter) of leaves sampled at the end of the experiment, an average of all replicates.

| Treatment | K | Na | Ca | Mg | N-total | Cl | P | S-total |
|---|---|---|---|---|---|---|---|---|
| | (mmol $kg^{-1}$ dry matter) | | | | | | | |
| Control | 363 | 17.4 | 1218 | 436 | 1906 | 57 | 84 | 135 |
| 50% N | 434 | 6.5 | 1280 | 428 | 1903 | 63 | 143 | 318 |
| 50% P | 350 | 10.8 | 1288 | 440 | 2090 | 61 | 76 | 156 |
| 50% K | 281 | 4.3 | 1363 | 507 | 2013 | 62 | 110 | 117 |
| 50% Ca | 506 | 13.0 | 825 | 605 | 2023 | 58 | 91 | 143 |
| 50% Mg | 407 | 4.3 | 1385 | 305 | 1956 | 58 | 89 | 136 |
| 50% S | 366 | 6.5 | 1220 | 436 | 2076 | 62 | 98 | 159 |
| zero-N | 533 | 5.7 | 1406 | 515 | 1670 | 57 | 179 | 673 |
| zero-P | 343 | 4.3 | 1066 | 432 | 1860 | 59 | 30 | 206 |
| zero-K | 88 | 18.8 | 1820 | 810 | 1866 | 68 | 193 | 106 |
| zero-Ca | 743 | 8.7 | 212 | 596 | 1883 | 55 | 85 | 48 |
| zero-Mg | 485 | 17.4 | 1650 | 102 | 1950 | 59 | 106 | 126 |
| zero-S | 389 | 17.3 | 1308 | 420 | 1870 | 43 | 111 | 171 |

Table 3. Total dry weight g $plant^{-1}$ (mean ± SE) of leaves and stems at harvest of *C. avellana* plants under 13 nutrient treatments.

| Treatment | Leaf DW (g $plant^{-1}$) | | Stem DW (g $plant^{-1}$) | |
|---|---|---|---|---|
| | Mean | SE | Mean | SE |
| Control | $44.21^{ab}$ | 6.69 | $15.05^{a}$ | 2.54 |
| 50% N | $35.47^{bc}$ | 2.08 | $11.67^{b}$ | 1.22 |
| 50% P | $40.92^{b}$ | 3.80 | $14.63^{a}$ | 1.57 |
| 50% K | $44.1^{ab}$ | 3.03 | $15.27^{a}$ | 0.90 |
| 50% Ca | $36.47^{bc}$ | 5.18 | $14.1^{ba}$ | 1.66 |
| 50% Mg | $49.22^{a}$ | 4.25 | $15.63^{a}$ | 1.31 |
| 50% S | $42.3^{b}$ | 5.40 | $15.93^{a}$ | 2.43 |
| zero-N | $12.96^{d}$ | 1.12 | $7.71^{c}$ | 0.86 |
| zero-P | $30.14^{c}$ | 3.75 | $13.96^{ba}$ | 0.99 |
| zero-K | $17.44^{d}$ | 1.46 | $7.55^{c}$ | 0.79 |
| zero-Ca | $36.91^{bc}$ | 5.38 | $13.21^{ba}$ | 1.61 |
| zero-Mg | $40.28^{ab}$ | 6.55 | $12.43^{ba}$ | 2.24 |
| zero-S | $31.18^{c}$ | 2.83 | $13.16^{ba}$ | 0.95 |

Different letters indicate significantly different means within treatments ($P<0.05$; $n=3$).

## DISCUSSION AND CONCLUSIONS

The intended deficiencies and related symptoms of the macro-elements could be

established clearly, except for phosphorus. The symptoms described are overall in agreement with what has been described about nutritional disorders in the plant nutrition literature in general (Mengel and Kirkby, 2012; Winsor and Adams, 1987), and more specifically in woody plants (Aendekerk, 1996). For N, the progressive necrotic tissue appearing between the veins might be typical for hazelnut as usually necrosis, as a secondary symptom, is not common and is usually found in leaf margins only (Aendekerk, 1996). All other symptoms are not specific to *C. avellana.* Not surprisingly, the response to the reduction of N and K supply appeared more rapid and was more severe than for Ca, Mg, and S, due to the substantial difference in reduction of the absolute quantities for N and K relative to the concentration in the reference nutrient solution compared to the other elements. The limited response of plants to the reduced P supply was unexpected. Although the response in growth was significant compared to the control, no specific symptoms could be observed. This agrees with the findings of Özenç and Bender Özenç (2009), who also found no other symptoms than some reduced growth in their experiment. The contents in the plant also show a clear relationship with the applied concentration at the root zone and are very low at zero as well as at 50% P. A comparison of the biomass and the tissue analysis of the starting plant material and at the termination of the trial reveals that 23% of the total P at the end was already present in the starting plant (control treatment), whereas for the other elements it was only 3% (S), 6-8% (K, Ca, Mg) to 10% (N). So most likely the initial P content of the plant was high enough to provide it with sufficient P for the experimental period of 16 weeks. Nevertheless, for the zero-P treatments, the P content in the leaves were extremely low at the end of the test (25 mmol $kg^{-1}$ DM), which were much lower than the results from Özenç and Bender Özenç (2009), who reported 40 mmol $kg^{-1}$ DM at zero P. Other authors mentioned that normal P levels should be between 40 and 100 mmol $kg^{-1}$ DM (Canali et al., 2005; Miletic et al., 2001; Olsen, 2013). The leaf content of the control and treatments other than P agree with these standards. A few leaves with red-purple discoloration were found in immature leaves. This phenomenon (anthocyanosis) is an accompanying symptom usually found with P deficiency in many plant species (Mengel and Kirkby, 2012). However, in this trial, these symptoms appeared in several plants other than the reduced P treatments. It is, therefore, quite unlikely that this anthocyanosis is a typical characteristic of P-deficiency in hazelnut. In contrast to expectations, the zero-S produced clear symptoms. This likely has to do with the small contribution of the propagated plants to the total S intake. Only 3% of the total S intake was present in the plant at the start of the test. The tipburn, present in all zero-Ca and many 50% Ca plants, appeared in some plants of other treatments, including the control. It is therefore likely that it cannot be only explained by Ca-deficiency as lacking this element in the root zone. Tipburn-like symptoms are a well-known phenomenon in greenhouse crops (Sonneveld and Voogt, 2009a, b), and its appearance is connected with the prevailing greenhouse environmental conditions. These so-called 'physiological disorders' are commonly explained as being caused by weak cells due to specific Ca shortages in the cell walls. Although not all processes involved are fully understood yet, the over-arching cause is that it is initiated by rapid growth, high transpiration and therefore a deficient Ca-transport to young plant tissue (Olle and Bender, 2009). These weak cells easily collapse when the young leaves unroll and if then exposed to sudden high transpiration. High growth rates aggravate the problem, due to a higher dilution of Ca in the plant. After all, the symptoms are quite similar to ones other than the described Ca-deficiency symptoms, but in the latter, it is effectively caused by absolute Ca shortage in the root environment.

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