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Nitrate accumulation in spinach
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Summary

Leafy vegetables, like spinach, may contain high concentrations of nitrate. In the Netherlands, about 75% of mean daily intake of nitrate originates from the consumption of vegetables. Hazards to human health are associated with the reduction of nitrate to nitrite. Acute nitrite poisoning causes methemoglobinemia: nitrite binds to haemoglobin and reduces the O₂ transport. Chronic nitrite poisoning may be caused by carcinogens, which are formed from nitrite and secondary N-compounds.

Nitrate accumulation in vegetables is most pronounced in winter and results from the excessive use of fertilizer in order to increase yield. The legally permitted nitrate concentration in spinach is decreased regularly by the government. This provides difficulties for spinach growers, especially in winter, when spinach is grown in un-heated glasshouses. It is therefore of high importance to elicit the mechanism of nitrate accumulation, in order to advise the growers how to stay within the range of the legally permitted nitrate concentration.

Spinach, grown at low PFD and with ample nitrate, exhibited a diurnal rhythm in the nitrate concentration of the leaves. During the day, nitrate left the vacuoles to be metabolized in the cytoplasm at a rate eight times higher than that during the night. The nitrate concentration of the leaves decreased during the day, as nitrate reduction in the leaves exceeded the uptake and transport of nitrate to the leaves. In the vacuoles, nitrate is replaced by organic solutes. In the first h of the night, nitrate uptake increased suddenly. Nitrate is transported directly into the vacuoles of the leaves, and the nitrate concentration of the leaves increased. The remaining part of the night is characterized by an unchanged nitrate concentration, as both nitrate uptake by the roots and nitrate reduction in the leaves remained at a low stable level (chapters 2 and 7).

The high NO₃ concentration of the leaves in the morning, can be reduced by subjecting the plants to a single "low light" treatment on the night preceding harvest. In the "low light" night, nitrate uptake is not increased and the nitrate concentration of the leaves is not increased either (chapter 3). At the same time, nitrate reduction in the leaves is increased (chapters 3, 4 and 7). The most optimal "low light" PFD to diminish the nitrate accumulation in spinach appeared to be 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In this "low light", the nitrate concentration of the shoot was reduced by 25% in the morning. In the vacuoles of the leaves, nitrate is replaced by organic solutes, synthesized in the "low light". The decrease in the nitrate uptake by the roots during a "low light" night is not caused by decreased transport of carbohydrates to the roots (chapter 4).

Chapter 5 describes the role of nitrate in leaf elongation. By comparing IER in darkness and during a "low light" night, we were able to conclude that NO₃ is not a obligatory osmoticum for leaf elongation: NO₃ may be replaced by organic solutes, without affecting IER. We concluded that in spinach grown at low PFD, NO₃ is used as an osmoticum due to lack of enough available organic solutes.

Spinach with high nitrate concentrations is usually grown in un-heated glasshouses. These plants were compared to spinach grown in a nutrient solution in a growth chamber.

Despite the differences in growth rate, the plants could be fully compared at the time of the harvest. Furthermore, a single "low light" night decreased the nitrate concentration by 25% under all conditions investigated (chapter 6).

Using $^{15}\text{NO}_3$, we found that nitrate reduction is located largely in the leaves, during the day. In a "low light" night, more NO_3 was reduced in the leaves and transported to the roots than in darkness. Neither the increased organic nitrogen transport to the roots, nor the increased organic nitrogen concentration in the roots was correlated to the decreased NO_3 uptake in a "low light" night (chapter 7).

The results are summarized and combined in chapter 8. Concluding, nitrate accumulation in spinach, grown at low PFD, is not caused due to lack of regulation of nitrate uptake. In contrast, nitrate uptake is well regulated by the demand of the shoot for nitrate. This demand is determined by both the rate of nitrate reduction and by the availability of organic osmotica. The nature of the factor, transported from the shoot to the roots, that regulates nitrate uptake is not clear. Whatever the nature of the factor, it seems to regulate nitrate uptake by regulation of the loading of nitrate into the xylem. Furthermore, a single "low light" treatment on the night preceding harvest may provide a tool to diminish nitrate accumulation in spinach, grown in winter in un-heated glasshouses.