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The Relation between Nitrogen Deficiency and Second Leaf Senescence in Wheat Plants

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Abstract. Life span of the second leaf of wheat (*Triticum aestivum* L., cv. Grana) plants was studied from day 8 to day 50 of plant age in a variant with nitrogen (+N) and in a variant in which plant senescence was induced by the omission of nitrogen from the nutrient solution (–N). Seed protein was the sole source of nitrogen for these plants. Specific leaf mass (SLM) in the –N variant, and specific leaf area (SLA), the mass of fresh leaf, soluble protein content and total nitrogen content in the +N variant peaked by day 22 of plant age (that is by day 19 of leaf age). Dry matter content, leaf length and leaf area, and SLM in the +N variant peaked by day 29 of plant age (that is by day 26 of leaf age). The ontogeny of the second leaf in the variant with enhanced senescence was shorter by at least 14 days. Plants from this variant showed typical symptoms of N deficiency, that is yellowing of leaves, tip burn, and lack of tillering. However, the growth and biochemical characters studied did not indicate an earlier onset of the senescence of the second leaf of −N plants. Both +N and −N variants reached their peaks (with the exception of an earlier peak by day 12 in case of total nitrogen content in the −N variant) on the same day of leaf age. Thus the first part of the leaf life span from leaf growth initiation to full expansion was of the same length in both the control and N-deficient plants. The stage of the proper senescence of the second leaf of –N plants was very short; the leaf completely died away within 7 days after senescence onset.

In cereal plants, above all the flag leaf and leaves inserted just below it have been studied from the viewpoint of nitrogen nutrition. This is caused by the fact that flag leaf and leaves preceding it contribute with their nitrogen and their photosynthates directly to the seed and in this way influence the final yield (SIMPSON and DALLING 1981, MAE and OHIRA 1982, BERGER *et al.* 1985, FLECK *et al.* 1986, DE MOLINO *et al.* 1986).

The vegetative phase has been studied to a lesser extent from these viewpoints, but there exist data on its significance for an adequate nitrogen supply in early growth stages. This situation later manifests itself favourably in the number of ears expressed per leaf area (MENGEL and KIRBY 1979, SIMPSON *et al.* 1982, MEI and THIMANN 1984). The second leaf of wheat plants which is characterized by

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a relatively long life span is a typical representative of the vegetative phase. Its ontogeny (with the exception of leaf area) can be followed from day 8 of plant age, when the leaf during sampling still must be loosened from the sheath of the first leaf. Its development on plant apex starts by day 4, and then can be followed to day 50 of plant age under conditions of adequate full nutrition, that is when plants are grown in nutrient solutions containing all the necessary macro- and micronutrients.

The aim of this paper was to obtain information on important stages of the ontogeny of an intact second leaf under conditions of full nutrition and to compare them with "induced senescence" obtained under conditions of nitrogen deficiency. Seed protein was the sole source of nitrogen for plants grown in nutrient solutions lacking nitrogen. To characterize aging, data on fresh matter, dry matter, leaf length and leaf area, specific leaf area (SLA), specific leaf mass (SLM), and net assimilation rate (NAR), obtained in growth analysis, were employed. In the sphere of nitrogen metabolism, total nitrogen content and total protein content were chosen as indices characterizing leaf ontogeny in this preliminary study which formed a basis for further enzymic and structural studies.

MATERIAL AND METHODS

Plant Cultivation

Winter wheat (*Triticum aestivum* L., cv. Grana) seeds were germinated for 3 days in distilled water. The seedlings obtained were transplanted into darkened cultivation vessels containing:

1) Modified Knop's nutrient solution (control variant, +N): $Ca(NO_3)_2$ 4.878 mM; KH_2PO_4 1.148 mM; KNO_3 1.978 mM; $MgSO_4$ 0.811 mM; KCl 1.341 mM; EDTA-Fe 5 p.p.m.

2) Nutrient solution lacking nitrogen (–N): $CaCl_2$ 5.551 mM; KH_2PO_4 1.148 mM; $MgSO_4$ 0.811 mM; KCl 3.353 mM; EDTA-Fe 5 p.p.m.

Both these nutrient solutions were derived and modified according to Knop's nutrient solution by DVOŘÁK (1960). Both solutions were supplemented with microelements: H_3BO_3 2.8×10^{-6} M, $MnCl_2$ 4.5×10^{-6} M, $ZnSO_4$ 3.7×10^{-7} M, $CuSO_4$ 1.6×10^{-7} M, $(NH_4)_6Mo_7O_{24}$ 5.5×10^{-8} M. Ammonium molybdate was omitted from the –N nutrient solution. Nutrient solutions were renewed once a week. Solution volume per plant was 60 ml in both variants. Plants were grown under controlled cultivation conditions: irradiance 200 μmol m^{-2} s^{-1}; temperature 20 ± 1 °C; relative air humidity 80 ± 2 %; photoperiod 16 h.

Leaves were sampled 9 h after the beginning of the photoperiod. Control plants were studied from day 8 of plant age, when they had a fully developed first leaf. During the first sampling, the second leaf had to be released from the sheath of the first leaf. Control plants were studied up to day 50 of plant age at which time 7 leaves developed on the main stalk and besides that on average approximately 8 tillers. Plants of the –N variant were also studied from day 8 (at the stage of the first leaf),

but only to day 36, at which time plants had three developed leaves but no tillers. After this period (day 50 in +N and day 36 in −N variants), the second leaves died

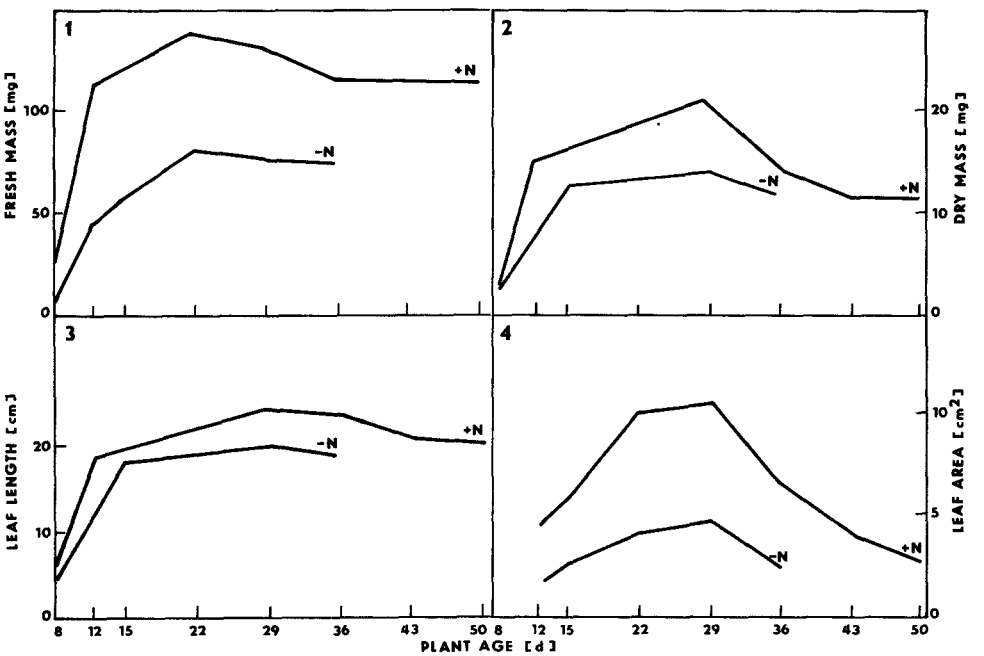
Growth and Biochemical Characters and Indices

Leaf area was measured by means of a Leaf Area Meter (LI–COR+ Lincoln, Nebraska, USA).

Parameters of growth analysis, that is specific leaf area (SLA; $\frac{A}{W_1}$; $m^2\,g^{-1}$), net assimilation rate (NAR; $E = \frac{1}{A} \cdot \frac{dW}{dt}$; $g\,m^{-2} \cdot d^{-1}$), and specific leaf mass (SLM ; $g\,m^{-2}$) were calculated according to ŠESTÁK *et al.* (1966). Soluble protein was determined according to LOWRY *et al.* (1951). Total reduced nitrogen was determined after HUMPHRIES (1956).

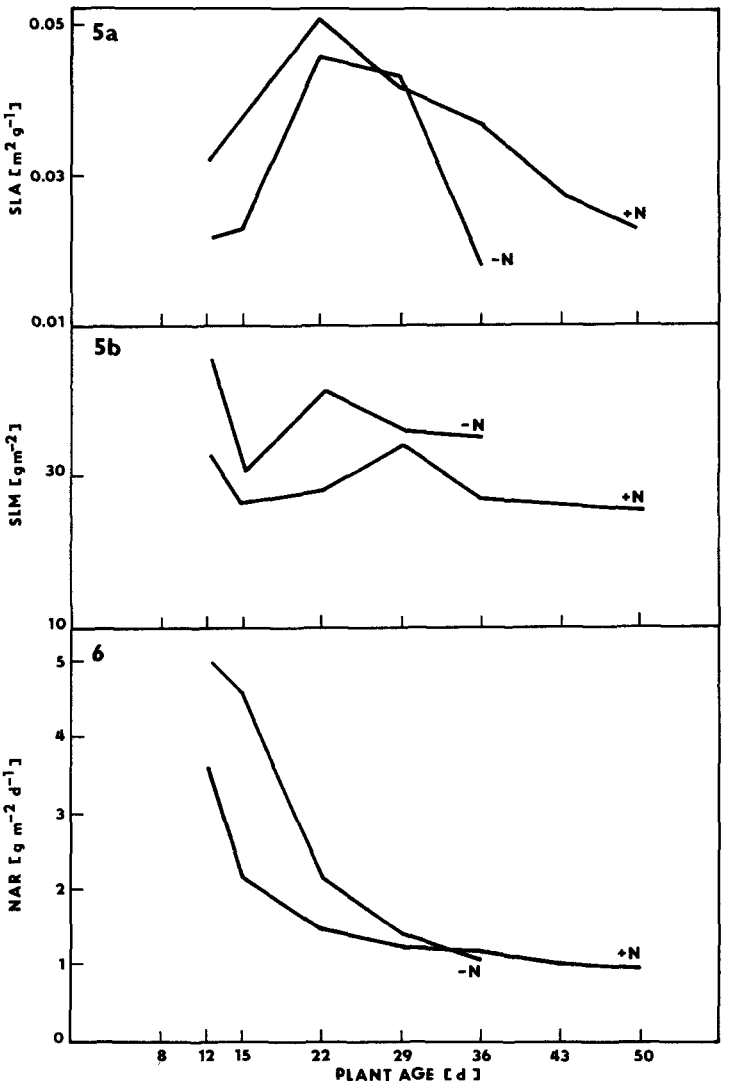
RESULTS AND DISCUSSION

Fresh matter of the second leaf of both plants grown in the +N nutrient solution and plants grown in the −N nutrient solution reached its peak by day 22 (Fig. 1). But



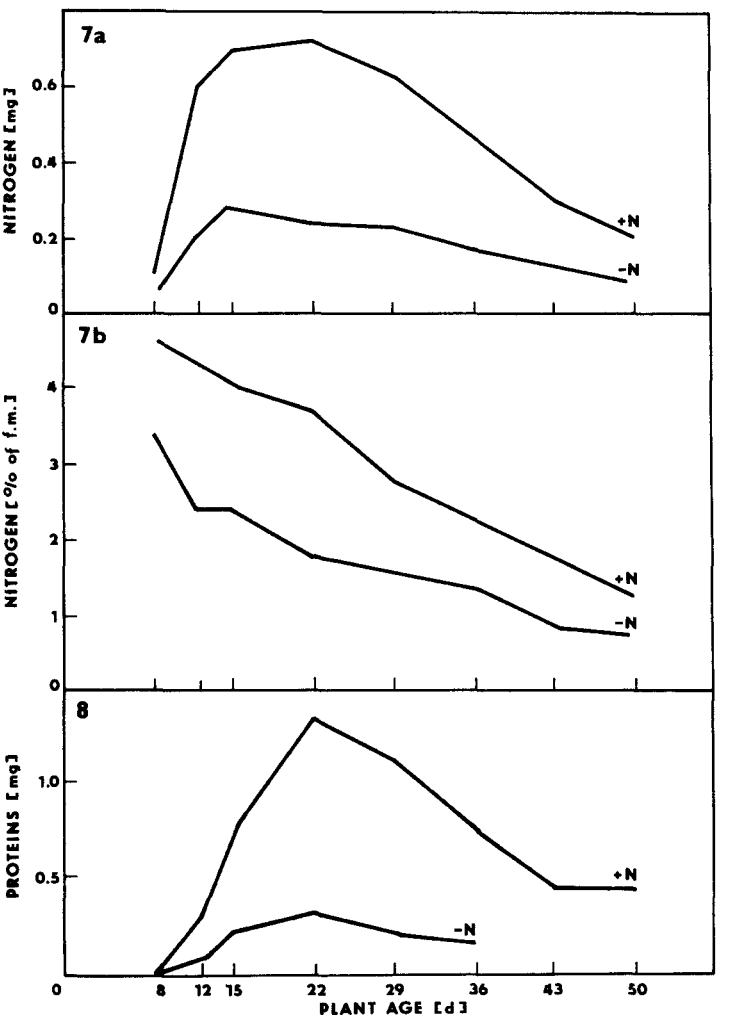
Figs. 1–4. Growth parameters [per leaf] of the second wheat leaf during its ontogeny. +N: full Knop's nutrient solution as a control variant; −N: Knop's nutrient solution without nitrogen as a variant of induced senescence. Leaf age = plant age − 3 days.

leaves of the −N variant reached only 57.50 % of the value recorded in the +N variant. Dry matter content of both variants reached its peak by day 29 (Fig. 2), at which time the second leaf of the −N plants reached 67 % of the value recorded with +N plants.Leaf length was also affected by nitrogen deficiency (Fig. 3). Leaf length peak was also recorded on day 29, when the second leaf of the −N plants represented the highest value in comparison with the control, that is 88 %. Leaf area was not measurable up to day 8. The highest expansion of leaf area was also recorded by day



Figs. 5 and 6. Specific leaf area (SLA, Fig. 5a), specific leaf mass (SLM, Fig. 5b) and net assimilation rate (NAR, Fig. 6) during ontogeny of the second wheat leaf.

29 of plant age (Fig. 4), that is by day 26 of leaf age. The leaf of the −N variant reached at this peak only 51 % of the value recorded in the control (+N variant). SLA of the second leaf reached its maximum by day 22 in both variants (Fig. 5a). SLM (Fig. 5b) showed higher values in the −N variant through the entire period. The second leaf of the −N plants showed higher NAR values when compared with the second leaf of the +N plants (Fig. 6). NAR decreased with leaf age in leaves of both variants. The peak in nitrogen content occurred in control (+N) plants by day 22, whereas by day 15 in −N plants (Fig. 7a). Nitrogen content in the second leaf of the



Figs. 7 and 8. Nitrogen (Fig. 7a, b) and protein (Fig. 8) contents [per leaf] during ontogeny of the second wheat leaf.

−N plants amounted only to 42 % of that recorded in control (+N plants). The percentage nitrogen content decreased fluently during leaf ontogenesis in both variants (Fig. 7b). The lowest values, when the second leaf of the −N plants was compared with the +N control plants, were recorded in case of soluble protein. At the time of the peak which occurred by day 22, soluble protein content of −N leaves amounted to only 24 % of the control (Fig. 8). A chart of the ontogeny of the second leaf of wheat plants with three typical phases is shown in Fig. 9. A conspicuous similarity between leaf senescence and N deficiency syndrome has been known for a long time. GREGORY (1937) described this phenomenon already in the thirties. In cereal crops, symptoms of nitrogen deficiency include chlorosis, narrowing of leaves, and tip burn. In our experiments, N deficiency was also accompanied by the inability of plants to form tillers. The life span of the second leaf was in this variant shorter by 14 days and more. Fresh mass of the second leaf reached its peak in both variants by day 22 (Fig. 1) which was not the result of dry matter accumulation, because dry matter content reached its peak by day 29 (Fig. 2). It appears that at least two factors, also reaching their peaks by day 22 (protein content, see Fig. 8, and a high water content – 87 %), contributed to the fresh matter peak. By contrast, dry matter content percentage was low at this sampling term (13 % and 16.5 %, respectively). In case of total nitrogen content, the second leaf of the −N plants was also analyzed at that time, when it already was completely yellow and dry (Fig. 7a, b). These analyses showed that nitrogen had been completely drained from the senescent leaf to newly developing structures of younger leaves. It is understandable that the lowest levels, when comparing the −N variant with the +N variant, were recorded in cases of protein and total nitrogen contents. However, SLM and NAR values were higher in the −N variant. SLM as a ratio of dry matter to leaf area shows characteristic dynamics. It first decreases with respect to a fast growth of leaf area, but later increases as a result of increasing leaf thickness, and then again decreases during the aging of the leaf (TICHÁ 1985). We did not study the photosynthetic behaviour of the

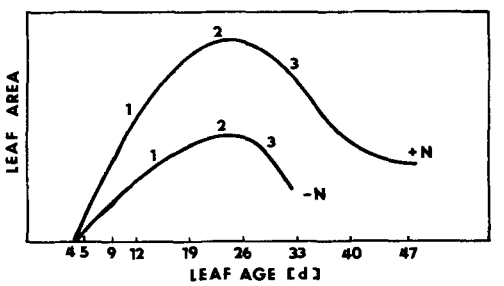


Fig. 9. Second leaf ontogeny. 1 – Leaf as a net carbon-importing structure untill the full development of photosynthetic activity. 2 – The photosynthetic phase; the leaf becames an asset to the carbon economy. 3 – The senescence phase follows the photosynthetic phase – a period of mobilization and export of carbon, nitrogen and minerals (STODDART and THOMAS 1982). All values have resulted in at least 3 experiments. In each experiment 10 leaves were always measured.

second leaf and for this reason we could not correlate SLM with leaf photosynthetic rate.

FLECK *et al.* (1986) associated high SLM with peaks in starch and sucrose accumulation. This phenomenon can be of special significance in the variant with nitrogen deficiency. Higher SLM can represent in this variant not only a higher leaf thickness, but also a higher capacity of the leaf to utilize triose phosphate in the direction of saccharide synthesis and accumulation under conditions of a low level of nitrogen incorporation into carbon precursors of amino acids, amides, and proteins, and in addition to that of a decreased growth rate. The higher NAR in −N plants can be explained similarly.

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REFERENCES

BERGER, M. G., WOO, K. C., WONG, S. Ch., FOCK, H. P.: Nitrogen metabolism in senescent flag leaves of wheat (*T. aestivum* L.) in the light. – Plant Physiol. **78** : 779–783, 1985.

DVOŘÁK, M.: Influence of mineral nutritives on respiration of *Cucurbita* plant roots. – Acta Univ. Carol.-biol. **1** : 65–86, 1960.

FLECK, I., FRANSI, A., FLORENSA, I., RAFALES, M.: Relationship between inorganic phosphate and carbohydrate content in flag leaves of *Triticum aestivum* L. cv. Kolibri. – J. Plant Physiol. **126** : 283–291, 1986.

FLECK, I., FRANSI, A., VIDAL, D.: Senescence patterns of several physiological and biochemical parameters of field grown flag leaves of *Triticum aestivum* L. cv Kolibri. – J. Plant Physiol. **123** : 327–338, 1986.

GREGORY, F. G.: Mineral nutrition of plants. – Annu. Rev. Biochem. **6** : 557–578, 1937.

HUMPHRIES, E. C.: Mineral components and ash analysis. – In: PAECH, K., TRACEY, M. V. (ed.): Moderne Methoden der Pflanzenanalyse. Vol. 1, Pp. 479–481. Springer-Verlag, Berlin 1956.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., RANDALL, R. J.: Protein measurements with Folin phenol reagent. – J. biol. Chem. **193** : 265–275, 1951.

MAE, T., OHIRA, K.: Relation between leaf age and nitrogen incorporation in the leaf of the rice plant (*Oryza sativa*). – Plant Cell Physiol. **23** : 1019–1024, 1982.

MEI, H. S., THIMANN, K. V.: The relation between nitrogen deficiency and leaf senescence. – Physiol. Plant. **62** : 157–161, 1984.

MENGEL, K., KIRBY, E. A.: Principles of Plant Nutrition. – International Potash Institute, Worblaufen – Bern 1979.

DE MOLINO, M. M., ULLOA, M., MARTINEZ-CARASCO, R., PÉREZ, P.: Characterization of grain yields and nitrogen level in relation to flag leaf senescence of wheat varieties. – Physiol. Plant. **66** : 503–508, 1986.

SIMPSON, F. J., LAMBERS, H., DALLING, M. J.: Translocation of nitrogen in a vegetative wheat plant. – Physiol. Plant. **56** : 11–17, 1982.

STODDART, J. C., THOMAS, H.: Leaf senescence. – In: BOULTER, D., PARTHIER, B. (ed.): Nucleic Acids and Proteins in Plants. I. Pp. 592–636, Springer-Verlag, Berlin-Heidelberg-New York 1982.

ŠESTÁK, Z., ČATSKÝ, J.: Metody Studia Fotosyntetické Produkce Rostlin. [Methods of Studying Photosynthetic Production of Plants]. – Publ. House Czechosl. Acad. Sci., Praha 1966.

ŠESTÁK, Z. (ed.): Photosynthesis during Leaf Development. – Academia, Praha 1985.

THOMAS, H., STODDART, J. L.: Leaf senescence. – Annu. Rev. Plant Physiol. **31** : 83–111, 1980.

TICHÁ, I.: Ontogeny of leaf morphology and anatomy. – In: ŠESTÁK, Z. (ed.): Photosynthesis during Leaf Development. Pp. 16–50. Academia, Praha; Dr. W. Junk Publ., Dordrecht – Boston – Lancaster 1985.