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## Do phosphorus and nitrogen contents in young corn leaves represent contents in shoots?

Ingeborg Frøsig Pedersen, Peter Sørensen, Bent T. Christensen, and Gitte Holton Rubæk

**Abstract:** For corn grown on two light-textured soils, leaf P and N concentrations at the six-leaf stage represented their respective concentrations in shoots. At the seven/eight-leaf stage, the P concentration was higher in leaves than in shoots. Biomass at the seven/eight-leaf stage is linked to the N to P ratio in leaves and shoots at the six-leaf stage.

**Key words:** development stage, phosphorus and nitrogen concentration, shoot, youngest fully developed leaf, *Zea mays*.

**Résumé :** La concentration foliaire de P et de N au stade de la sixième feuille concorde avec celle relevée dans la pousse chez le maïs cultivé sur deux sols de texture légère. Au stade de la septième ou huitième feuille, la concentration de P était plus élevée dans les feuilles que dans la pousse. Mesurée au stade de la septième/huitième feuille, la quantité de biomasse est liée au ratio N/P qu'on observe dans les feuilles au stade de la sixième feuille. [Traduit par la Rédaction]

**Mots-clés :** stade de développement, concentration de phosphore et d'azote, pousse, plus jeune feuille entièrement développée, *Zea mays*.

Sufficient phosphorus (P) content in corn (*Zea mays* L.) shoots from seeding to the six-leaf stage is essential for optimum yields at harvest (Barry and Miller 1989). However, evaluation of short-term P and nitrogen (N) availability in soils with unevenly distributed nutrients is complex and may require plant analyses. Previous studies linking P and N availability in soil to corn growth have focused on concentrations in shoots or in leaves (e.g., Mallarino 1996; Plénet and Lemaire 2000; Bélanger et al. 2011). Analyses of nutrient concentrations in the youngest fully developed leaf (YFDL) are more feasible than harvesting shoots to evaluate crop N (e.g., Chapman and Barreto 1997) and P status (e.g., Frydenvang et al. 2015). Previous studies relating leaf to shoot concentrations considered plants beyond the V8 to V10 development stage (e.g., Ziadi et al. 2009; Bélanger et al. 2011) and it remains unclear if P and N concentrations in the YFDL represent concentrations in shoots during earlier growth stages, where optimal nutrient availability is critical for obtaining maximal final yields. Our objectives were to test if (i) P concentration in the YFDL was related to that in the shoot during the early growth of corn and (ii) P and

N concentration or N to P ratio in the YFDL and shoots could serve as indicators of subsequent shoot dry matter (DM) yield.

We sampled leaves and shoots of corn grown on two soils with a wide range of P uptake potentials, capitalizing on a previous experiment by Pedersen et al. (2017). Briefly, corn grew in pots in climate-controlled chambers. The soils were coarse sand (7% clay + silt) and sandy loam (20% clay + silt) with initial Olsen-P contents of 48 and 40 mg P kg<sup>-1</sup>, respectively. The coarse sand classifies as Orthic Haplohumod and the sandy loam as Typic Hapludalf (USDA Soil Taxonomy System). Each pot had 6.9 kg dry soil with a bulk density of 1.3 g cm<sup>-3</sup>. Soil water content was adjusted to 60% of field capacity before corn planting. The different P uptake potentials were established in the previous experiment by combinations of acidified cattle slurry [untreated slurry (pH6.5), moderately acidified slurry (pH5.5), or strongly acidified slurry (pH3.8)] and slurry application technique and treatments with and without mineral P (Na<sub>2</sub>HPO<sub>4</sub>). Each treatment had three replicates for each soil type. The number of pots totaled 99 of each soil type,

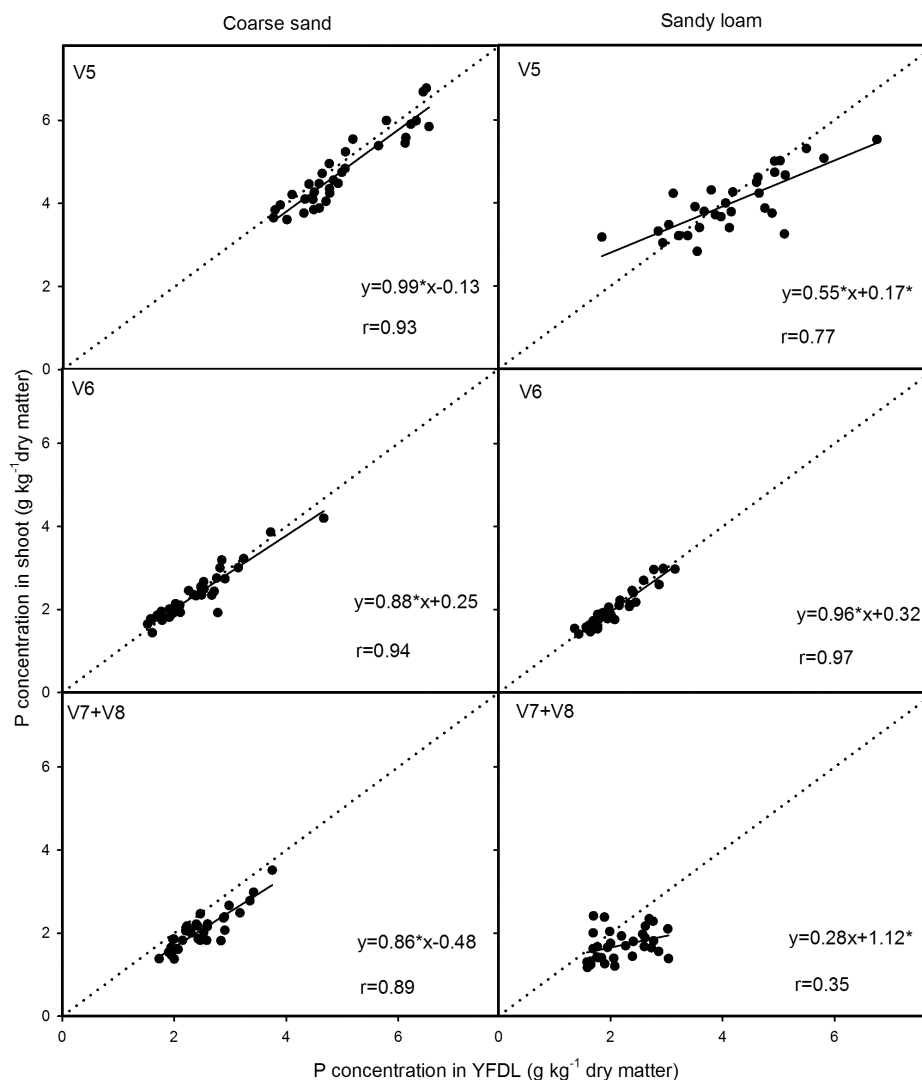
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I.F. Pedersen, P. Sørensen, B.T. Christensen, and G.H. Rubæk. Department of Agroecology, Aarhus University, AU Foulum, Blichers Allé 20, PO box 50, 8830 Tjele, Denmark.

**Corresponding author:** Ingeborg Frøsig Pedersen (email: ifp@agro.au.dk).

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**Fig. 1.** Relationship between P concentration in the youngest fully developed leaves (YFDL) and in shoots at the five-, six-, and seven/eight-leaf stage (V5, V6, and V7+V8, respectively) of corn grown on coarse sand and sandy loam. The dotted line indicates  $y = x$  while the solid line represents the linear regression across the development stages. Asterisks (\*) indicate significant slopes and intercepts ( $p < 0.05$ ).



sufficient for harvest at three growth stages. The P application rate corresponded to  $20 \text{ kg P ha}^{-1}$  for all treatments. Each pot received  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  corresponding to  $40$  and  $19 \text{ kg N ha}^{-1}$ , respectively, and sufficient rates of macro and micronutrients [potassium (K), sulfur (S), manganese (Mn), zinc (Zn), boron (B), copper (Cu), cobalt (Co), and molybdenum (Mo)].

Three corn seeds (*Zea mays* L. 'Adept') were planted per pot. Non-germinated seeds were replanted with spare maize seedlings grown in separate pots. Climate conditions simulated late spring/early summer conditions, with a mean daily temperature of  $15^\circ\text{C}$  with an amplitude from  $11^\circ\text{C}$  to  $19^\circ\text{C}$  during the first 10 d and then a mean temperature increase of  $0.1^\circ\text{C day}^{-1}$ . The relative mean air humidity was 75% and the photoperiod was 16 h, with light intensities ranging from 170 to

$1060 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The pots were irrigated with demineralized water to a water content of 60% of field capacity during the first 35 d of growth and then to 70% until harvest.

Plant development was recorded 45, 60, and 75 d after planting and corresponded to the five (V5), six (V6), and seven/eight (V7+V8) leaf stage, respectively. The YFDL from each plant (in total three leaves per pot) were sampled when the leaf collar was fully visible. Subsequently, all three plants were cut 1 cm above the soil surface. The three leaves from each pot were pooled, as were the three whole plants from each pot. Leaves and shoots were oven-dried at  $60^\circ\text{C}$  to a constant mass (min. 48 h) for determination of DM content. The dried plant samples were ball-milled prior to analysis.

The P concentration in plant tissues was determined by digesting 300 mg DM in 3 mL  $\text{H}_2\text{O}_2$  ( $9.7 \text{ mol L}^{-1}$ ) and 6 mL  $\text{HNO}_3$  ( $14.3 \text{ mol L}^{-1}$ ) using Teflon-coated vessels and a pressurized microwave oven (Anton Paar GmbH, Graz, Austria), with the P concentration in the diluted digest being determined by ICP-OES (Thermo Fisher Scientific, Waltham, MA). Plant tissue N concentration at V6 was determined with a PDZ Europa ANCA-GSL elemental analyser (Sercon Ltd., Cheshire, UK). The N and P concentrations are expressed on a DM basis.

The shoot biomass included the biomass removed in the corresponding YFDL. Statistical analyses were computed with the R-Project software package version 3.2.3 with data normality verified by Shapiro–Wilk statistics. We tested the relationship between P and N concentrations in leaves and in shoots, between N and P concentrations, and between N to P ratios and corn biomass by simple linear regression and Pearson's correlation coefficient ( $r$ ). Differences in the P concentration between the YFDL and the corresponding shoot at V7+V8 was examined by a paired  $t$  test. Significance was declared at the  $p \leq 0.05$  level of probability.

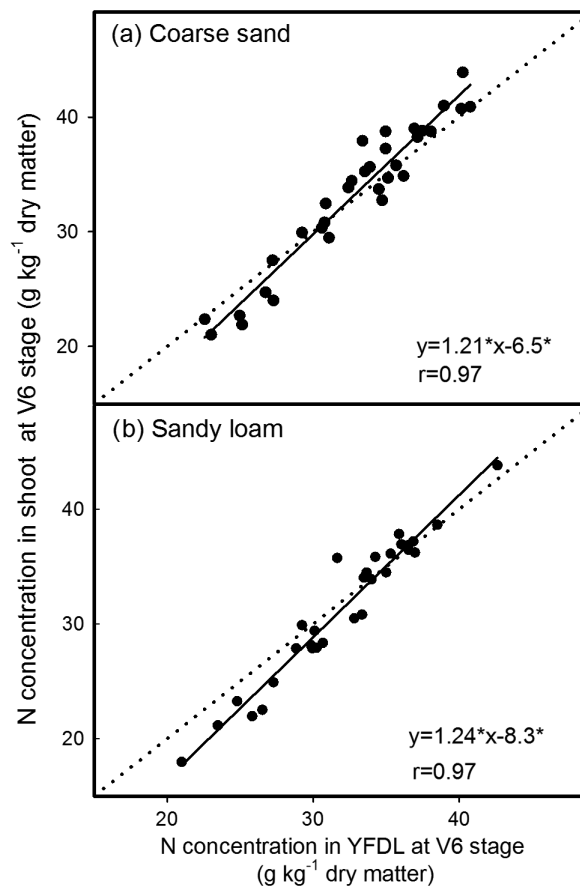
The P concentrations in the YFDL and shoots ranged from 1.4 to 6.8 and 1.2 to 6.9  $\text{g P kg}^{-1}$  DM, respectively. This interval represents concentrations well below and above the critical concentration of 3.4  $\text{g P kg}^{-1}$  DM reported by [Mallarino \(1996\)](#) for corn shoots at V5 to V6. The P concentrations in plant tissues decreased from V5 to V7+V8 ([Fig. 1](#)). This ascribes to increasing proportions of structural and storage tissues low in P ([Grant et al. 2001](#)).

For corn grown on coarse sand, the linear relationship between P concentration in the YFDL and in the shoot was significant at all three development stages. The relationship between P concentration in the YFDL and in the shoot at V5 and V6 was close to the  $y = x$  regression line ([Fig. 1](#)).

For corn grown on sandy loam, the linear relationship between P concentration in the YFDL and in the shoot was also significant at V6 and close to the  $y = x$  regression line. The low degree of correspondence between P concentrations in the YFDL and the shoot at V5 on the sandy loam compared with the coarse sand ([Fig. 1](#)) suggests that soil properties other than P may affect the link between P uptake potentials and the distribution of P between the shoots and the YFDL. However, our experimental setup does not allow for identification of specific mechanisms.

At V7+V8, the average P concentration was 0.43 and 0.48  $\text{g kg}^{-1}$  DM higher in the YFDL than in the shoots on the coarse sand and the sandy loam, respectively (paired  $t$  test,  $p < 0.05$ ). The higher P concentration in the YFDL compared with that in the shoot suggests P remobilization from senescing leaves to young leaves and may indicate a shift after V6 from uptake-dominated P supply to remobilization-dominated P supply, a process of particular importance in P deficient plants ([Veneklaas et al. 2012](#)). For plants older than V6, P concentration in

**Fig. 2.** Relationship between N concentration in the youngest fully developed leaves (YFDL) and in shoots at the six-leaf stage (V6) of corn grown on (a) coarse sand and (b) sandy loam. The dotted line indicates  $y = x$  while the solid line represents the linear regression for each soil type. Asterisks (\*) indicate significant slopes and intercepts ( $p < 0.05$ ).



the YFDL can therefore remain adequate, even for corn restricted by P deficiency. Therefore using P concentration in the YFDL as a diagnosing tool after V6 may ignore growth limitations due to shortage of plant-available P.

A close relationship was also observed for N concentration in the shoots and YFDL on both soils at V6 ([Fig. 2](#)). The N concentration ranged from 18 to 44  $\text{g N kg}^{-1}$  DM, again representing N concentrations below and above the concentration (34  $\text{mg N kg}^{-1}$ ) that is considered critical for growth of corn plants younger than V9 ([Plénet and Lemaire 2000](#)).

We related P and N concentrations at V6 to the shoot biomass at V7+V8 to test if these nutrient concentrations at V6 could serve as an early-season indicator for subsequent biomass production. We found a significant relationship between the N to P ratio at V6 and DM yield recorded at V7+V8 ( $r = -0.44$ ,  $p < 0.05$  and  $r = -0.47$ ,  $p < 0.05$  in the shoot and YFDL, respectively). The N to P ratio in the shoots and leaves at V6 ranged from 9 to 23. Soil texture did not affect this relationship. The P and N

concentrations were not significantly related to shoot biomass at V7+V8 except for a weak correlation to shoot N concentration ( $r = -0.31$ ,  $p < 0.05$ ). This underlines that not only the concentrations of individual nutrients but also the nutrient balance in plant tissues is important for early growth of corn. If the contents of N or P are limiting due to reduced uptake and not compensated for by redistributions within the plant, this is reflected in the N to P ratio (Veneklaas et al. 2012). The similar relationship between the N to P ratio in the shoots and YFDL at V6 and the subsequent DM yield of shoots at V7+V8 suggests that analyses of P and N concentrations in the YFDL at this growth stage may be of significant diagnostic value.

We found that the stage of plant development is decisive when P concentrations in the YFDL are used as a proxy of concentrations in shoots of young corn plants. Analyses of P and N concentration in the YFDL at V6 accurately reflected the status of these two elements in corn grown on light textured soils in a pot study. This may allow for improved nutrient management in subsequent corn crops, if validated in the field.

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