PHOSPHORUS REQUIREMENTS IN LEGUMES: CALIBRATION OF A RAPID AND SIMPLE TISSUE TEST

A.Morón
Soils Department, INIA La Estanzuela, Colonia, Uruguay

ABSTRACT
The objective of this research was to adjust and calibrate a phosphorus (P) tissue test to diagnose in a simple and rapid manner the nutritional status of commonly used legumes in Uruguay. Field experiments conducted during 1993-94 with four legumes, showed a significant yield response to increasing P fertilizer rates. A colorimetric sap analysis was used to determine P status in legumes. A strong relationship was found between P sap analysis and relative yields. A critical level of 90-100 µg P/ml in sap to attain 90% of the maximum yield was obtained.

KEYWORDS
sap analysis, phosphorus, legumes, tissue test, rapid test

INTRODUCTION
A vast majority of Uruguayan soils are phosphorus (P) deficient. Inclusion of perennial pastures with legumes has the requirement of increasing phosphorus availability in soils. Available P in soils has been determined through soil analysis (Bray I, cation exchange resins). The more important limitation is inadequacy of these soil P tests for some soils (for example, granitic soils).

The objective of this research was to adjust and calibrate a P tissue analysis method to diagnose the P status of forage legumes most commonly used under Uruguayan conditions.

MATERIALS AND METHODS
Field experiments: The research was conducted at La Estanzuela Experimental Station located in Uruguay (34° S, 57° W). The soil type was a typic, messic, Argiudoll. Characteristics of the A horizon (0-25 cm) were: texture, loam-clay-silt soil; pH (H_2O), 5.7; % of organic carbon, 3.7 and available P Bray I, 4 µg P/g. Four experiments were established in fall of 1993. In each one of the experiments a different legume was planted: *Lotus corniculatus* cv. E. Ganador (birdsfoot trefoil), *Trifolium repens* cv. E. Zapicán (white clover), Trifolium Pratense cv. E.116 (red clover) and Medicago sativa cv. Creoula (alfalfa). Conventional tillage was used in all treatments. The experimental design was a randomized complete block design with four replications. Plot size was 5 by 5 m. Treatments imposed were: four rates of P (0, 40, 80 and 160 kg of P_2O_5/ha) as simple superphosphate (23 % P_2O_5). Fertilizer was broadcasted and disk incorporated. In February 1994, the same source and rates were topdressed.

Forage yields were evaluated in 1993-94 leaving a stubble residue of 4 cm. Cut forage was removed from the plots. In each one of the plots a forage sample was taken to determine P content in sap. Forage samples were dried at 70° C for 12 hours in a forced-air oyen.

Sap analysis: The determination is based on the reaction between inorganic P present in sap and ammonium molybdate. Phosphomolybdate resulting from this reaction is then reduced with metallic tin (Sn). This last reaction determines the formation of a blue color compound. The intensity of this color is directly proportional to P concentration present in the sap. A color chart with five different color intensities was developed; each one corresponding to the following P concentrations: 10, 30, 60, 90 y 200 µg P/ml.

In alfalfa, red clover and birdsfoot trefoil the upper 10 cm of the main stems were selected from 10 to 15 plants. Leaves, ramifications and flowers were discarded for this analysis. In white clover, during vegetative phase, the upper 10 cm of 10 to 15 petioles were selected, discarding leaves. During the reproductive phase, the upper 10 cm of the floral peduncles were selected, discarding the flowers. Whatman 1 Filter papers 19 by 10 cm were folded in four. The sample was placed inside as tight as possible. Nylon was placed outside the filter. A pincer was used to compress the tissue sample until the filter was impregnated with sap. The plant parts were then discarded and the following steps were made at the opposite side of the filter. One drop of an acid solution of ammonium molybdate was placed in four different sites of the filter. At each site, the drop was located approximately 5 mm apart from the edge wetted by the sap. The filter was then air-dried for 15 seconds and then it was rubbed smoothly with tin rod at the sap-ammonium molybdate interface. The filter was then air-dried for 30 seconds. A visual reading was then compared with the color intensity chart. A filter paper was placed behind the filter with sap before readings were taken. The visual readings were taken at a blue meniscus generally developed inside the stains. This step was followed in order to avoid interferences due to the green color given by chlorophyll (Bouma and Dowling, 1982). When visual readings were between two contiguous chart color intensities, an intermediate value was used. Reported values were the mean of two independent readings. A quadratic-response-and plateau model was used to describe the relationship between P sap values and relative yields. The term relative yield denotes yield expressed as a percentage of the treatment with maximum yield in each experiment and forage evaluation.

RESULTS AND DISCUSSION
All four legumes showed a significant yield response to increasing rates P. Eight forage evaluations were made in white clover, seven in red clover and birdsfoot trefoil and six in alfalfa.

The relationship between P sap analysis and relative yields for all legumes and evaluations is shown in figure 1. Three different categories can be established: 1) deficient zone with values ≤ to 30 µg P/ml; 2) intermediate with values between 30 and 90 µg P/ml, and 3) sufficient with values ≤ to 90 µg P/ml. A critical level of 90-100 µg P/ml, to attain 90% of the maximum yield was defined.

REFERENCES
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