THE EFFECT OF PLANT AGE ON CRITICAL PHOSPHORUS CONCENTRATIONS IN TOWNSVILLE STYLO (*STYLOSANTHES HUMILIS* H.B.K.)

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ABSTRACT

A pot trial in which Townsville stylo (Stylosanthes humilis *H.B.K.*) *was grown at different rates of applied phosphorus was conducted at Brisbane, Queensland. Plants were harvested* 35, 50, 70 *and* 90 *days after germination and dissected into apical tissue (apex plus first fully expanded leaf), leaves and stem. Critical phosphorus concentrations were established for each tissue and the whole plant tops at each harvest.*

The critical phosphorus concentration (expressed on a dry weight basis) of the whole plant tops declined with plant age, whereas the critical concentrations of the apical tissue and leaves did not decline until after seed set occurred. The critical phosphorus concentration (fresh weight basis) of the whole plant tops remained constant throughout the duration of the experiment, as did the critical phosphorus concentration (dry weight basis) of the stem. However the wide confidence limits of the latter critical concentration invalidated its use for diagnostic purposes.

The critical phosphorus concentration (dry weight basis) of the apical tissue was found to be 0.26%, *while that of the whole plant tops was* 0.17% *at the immediate pre-flowering stage of growth.*

The agricultural implications of these findings are discussed.

INTRODUCTION

The decline in phosphorus concentration of whole plant tops of Townsville stylo (*Stylosanthes humilis*) with age has been well documented (Fisher 1970, Jones 1968, Robinson and Jones 1972) and a decline with age in the critical phosphorus concentration has also been reported for whole tops of maturing Townsville stylo and its companion "grass" by Jones (1968).

To eliminate the effect of plant age on the critical phosphorus concentration of whole plant tops, it is now common practice to sample plants at a definite physiological stage—Andrew and Robins (1969) sampled at "the immediate pre-flowering stage of growth"; Jones (1968) sampled at "full flowering"; and McNaught (1970) sampled legume when the pasture reached a "standard height". While critical concentrations for several elements have been established at these various stages of growth, Robinson and Jones (1972) state "a difference in the harvest time of a few days could cause a significant difference in the critical values obtained".

However, sampling of the whole plant top (which consists of a variety of tissues at different stages of development) does not comply with the basic principle behind the critical nutrient concept that "at the same nutrient level, nutrient concentrations are identical in leaves of the same physiological age, irrespective of the chronological age of the whole plant" (Smith 1962). As the apex (both shoot and lateral) is a readily identifiable tissue of constant physiological age, it was the aim of this experiment to determine the effects of plant age on the critical phosphorus concentrations of the apex and other component tissues of the whole plant top.

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MATERIALS AND METHODS

A pot trial was undertaken at Brisbane, Queensland, under glasshouse conditions, using the 0–15 cm horizon of a yellow podzolic soil from the Mt. Cotton area, Queensland. Extractable phosphorus measured in this horizon using Colwell's (1963) 0.5 M $NaHCO_3$ procedure was 3 ppm. Plastic pots (15 cm diameter) were lined with plastic bags and filled with 1800 g air dry soil. The treatments were seven phosphorus levels × four harvest times with three replications.

Rates of application of phosphorus were 0, 0.056 g, 0.112 g, 0.169 g, 0.225 g, 0.450 g and 0.900 g of Ca $(H_2PO_4)_2.H_2O$ per pot. These rates are approximately equivalent on an area basis to 0, 12.5, 25, 37.5, 50, 100 and 200 kg ha^{-1} of phosphorus and are designated P_0, $P_{12.5}$, P_{25}, $P_{37.5}$, P_{50}, P_{100} and P_{200} respectively. Sufficient calcium as calcium carbonate was applied to each phosphorus treatment to equalize the total amount of calcium being added to each pot. The varying additions of carbonate did not appreciably affect soil pH.

The basal fertilizer added to each pot was as follows: zinc sulphate, 0.010 g (*c.* 8.0 kg ha^{-1}); copper sulphate, 0.010 g (*c.* 8.0 kg ha^{-1}); ammonium molybdate, 1.25 mg (*c.* 1.0 kg ha^{-1}); magnesium sulphate, 0.095 g (*c.* 75 kg ha^{-1}); sodium borate, 2.5 mg (*c.* 2.0 kg ha^{-1}); and potassium sulphate, 0.382 g (*c.* 300 kg ha^{-1}).

The calcium carbonate was thoroughly mixed through the air dry soil and the basal fertilizer and $Ca(H_2PO_4)_2.H_2O$ then added in solution form. Sufficient water was added to bring the pots to 80 percent field capacity, and they were covered with plastic for 14 days to allow the fertilizer to equilibrate with the soil.

Scarified, inoculated seed of *Stylosanthes humilis* cv. Paterson was spread on each pot and the soil surface lightly cultivated. Ten days after germination, seedlings were thinned to six per pot and watered with a suspension of CB756 peat inoculum.

Pots were watered up to 80 percent field capacity once daily, and pot position was re-randomized weekly. Supplementary lighting was used to increase the photoperiod to 16 hours per day for the first 35 days of the experiment.

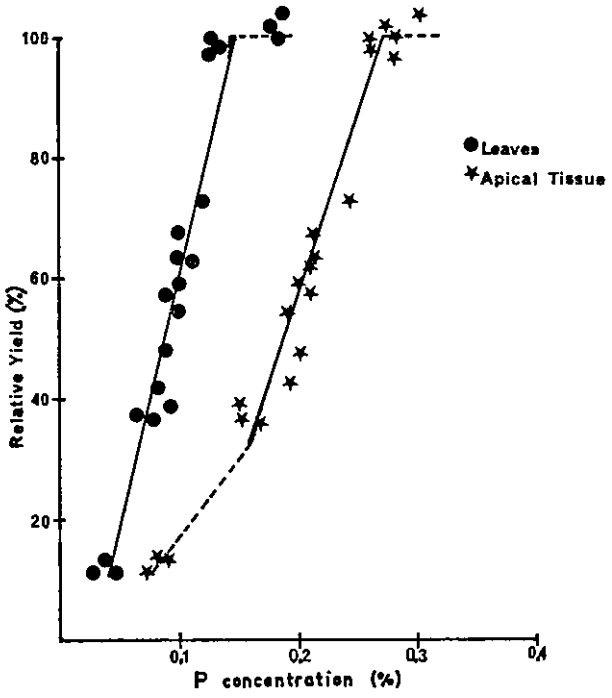
Plants were harvested 35, 50, 70 and 90 days after germination, and the fresh weight of the whole plant tops obtained immediately. Each plant was then dissected into apex plus first fully expanded leaf (including lateral apices), leaves and stem. Samples were dried at 70°C, weighed and ground. After Kjeldahl digestion, each replicate was analyzed for total nitrogen and phosphorus by an Auto Analyzer procedure (Roofayel, unpublished) based on the ammonia-phenate-hypochlorite reaction for N and sulphuric-molybdate-A.N.S.A. method for P.

The nitrogen concentrations in the whole plant tops at the 70 day harvest ranged from 2.27% to 3.03%—in agreement with the concentrations reported by Robinson and Jones (1972) for Townsville stylo plants of similar age.

The phosphorus concentrations of the whole plant tops were calculated using the dry weights and phosphorus concentrations of the component tissues.

A plot of relative whole plant top yield *versus* % P for each tissue at each harvest indicated that the response curve could be adequately represented by two intersecting straight lines—one where increasing relative yield was associated with increasing phosphorus concentration (the deficiency region) and the other where a constant relative yield of 100% was associated with increasing phosphorus concentration (the luxury consumption region) (Figure 1).

The linear regression equation representing the deficiency region was calculated using each replicate of the P_0 to P_{100} treatments inclusive as a point and taking phosphorus concentration as the independent variable. It was necessary to omit the P_0 points when deriving the linear regression equations for the apical tissue at the 50 day and 70 day harvests because of marked departure of these points from the linear trend of the $P_{12.5}$ to P_{100} points. The whole plant top yield corresponding to 100% relative yield was the mean of the yields of the P_{100} and P_{200} treatments.



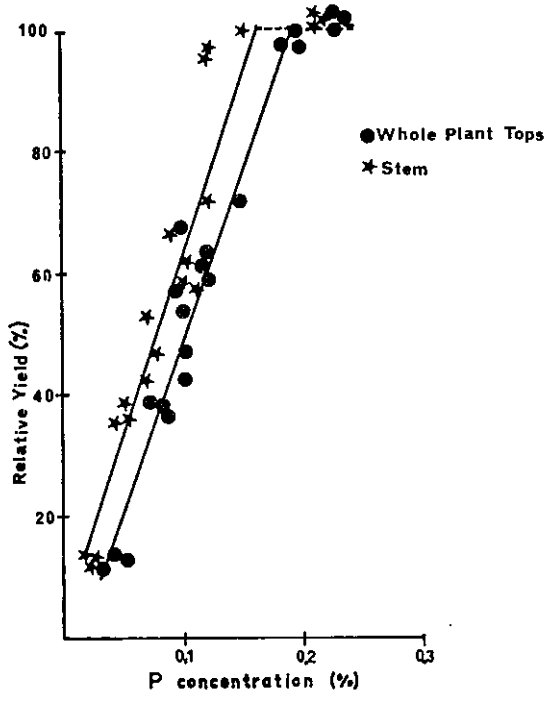


FIGURE 1

Relation between relative whole plant tops dry matter yield and phosphorus concentrations of whole plant tops and component tissues of Townsville stylo 50 days after germination.

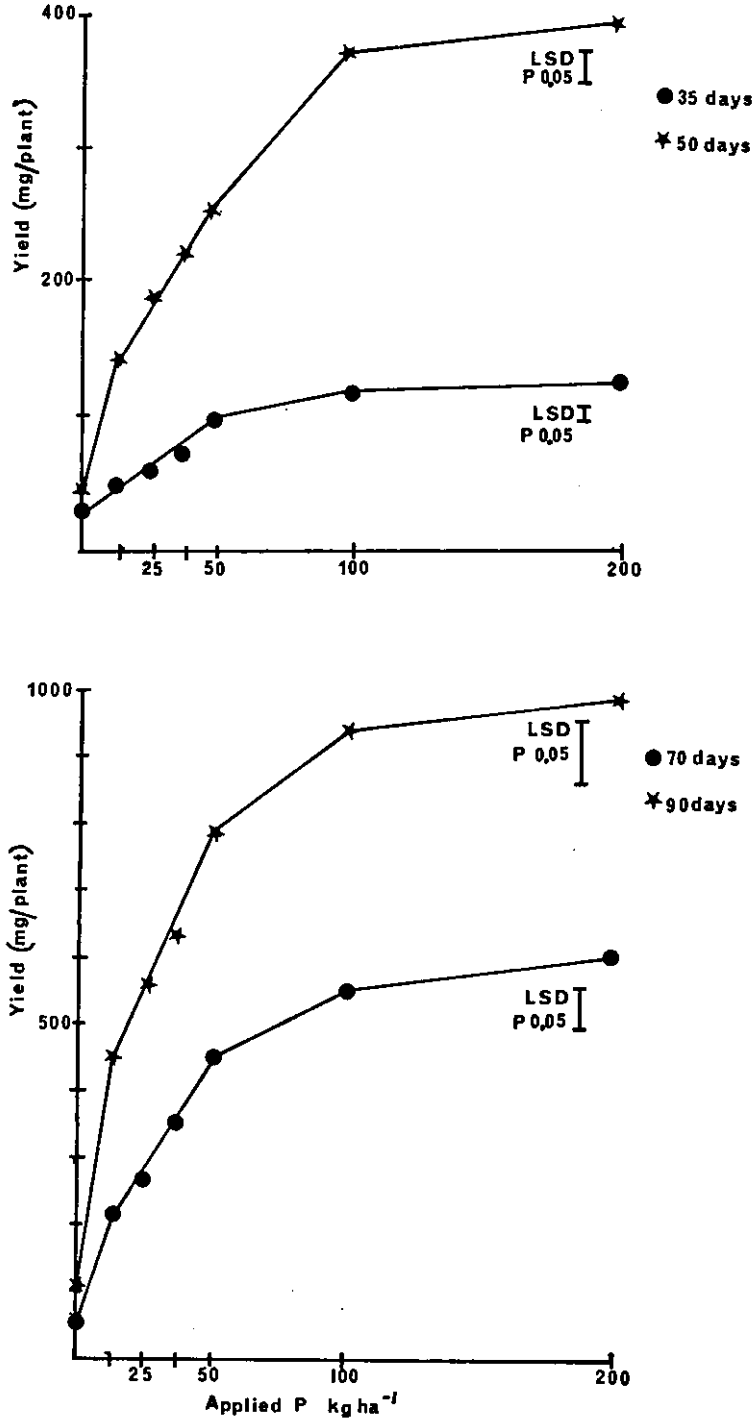


FIGURE 2
Relation between dry matter yield of whole plant tops of Townsville stylo and applied phosphorus.

As the critical nutrient concentration can be defined as the concentration in the plant part corresponding to 90% relative yield (Andrew and Robins 1969), it was calculated by interpolation from the linear regression equation.

RESULTS

Growth and dry matter yields

Maximum yield at all harvest dates was reached at the P_{100} level (Figure 2). Plants at the P_0 level were stunted with small, dark green leaves.

The first flowers appeared 63 days after germination on the plants growing at the P_{50}, P_{100} and P_{200} levels, while plants at the $P_{12.5}$, P_{25} and $P_{37.5}$ levels produced flowers a few days later. Plants at the P_0 level failed to flower.

The 35 and 50 day harvests corresponded to the vegetative stage of growth, the 70 day harvest to full flowering, and the 90 day harvest to seed drying and "haying off" which was indicated by leaf drop and plant wilting.

Phosphorus concentrations

Phosphorus concentrations (dry weight basis) in whole plant tops, stems and leaves decreased with plant age—the most rapid decline occurring between the 70 day and 90 day harvests (Table 1). Although there was no significant difference in the phosphorus concentrations at each phosphorus level in the leaves and stems between the 50 day and 70 day harvests, a decline in the concentrations did occur when they were related to relative yield at the respective harvests. The concentration in the apical tissue tended to remain constant until the 70 day harvest, but showed a decline at the 90 day harvest. However, the phosphorus concentration (fresh weight basis) of the whole plant tops remained constant over the entire 90 day period.

Critical phosphorus concentrations

The critical phosphorus concentration (dry weight basis) of the whole plant tops declined until "haying off" and seed drying occurred (70 to 90 day period), whereas the critical concentration expressed on a fresh weight basis remained constant (Table 2). The critical concentrations of the apical tissue and leaves remained constant until full flowering (70 day harvest) and declined thereafter. Any decline in the critical concentration of the stem with plant age was masked by the wide confidence limits attached to the critical concentration.

Total phosphorus distribution

Approximately 35% of the total phosphorus in the plant tops at the P_0 to P_{100} levels inclusive was located in the leaves and stem until full flowering (70 day harvest), but the percentage had decreased by the 90 day harvest (Figure 3). Plants at the P_{200} level tended to maintain 45% of the total phosphorus of the tops in the leaves and stem over all four harvests.

Dry matter content

Dry matter content of the whole plant tops increased significantly from 17% to 18% during the 50 to 70 day period, and from 18% to 23% during the 70 to 90 day period. Applied phosphorus rate had no significant effect on the dry matter content of the whole plant tops.

Dry matter distribution

The dry matter proportions of the different tissues are presented in Table 3. The percentages are the means of the $P_{12.5}$ to P_{200} treatments inclusive as neither the phosphorus rate main effect nor the phosphorus rate × harvest time interaction was significant at any harvest. Plants at the P_0 level had significantly higher percentages of stem and leaf at the 35 day, 50 day and 90 day harvests than plants growing at the other phosphorus levels.

TABLE 1

Effect of plant age on the phosphorus concentration (% P) expressed (a) on a dry weight basis and (b) on a fresh weight basis in tissues and whole plant tops of Townsville stylo

(a) Dry weight basis

Tissue	Harvest	P rate							Time Main Effect
		P_0	$P_{12.5}$	P_{25}	$P_{37.5}$	P_{50}	P_{100}	P_{200}	
									*
Apical Tissue	35 day	0.08	0.17	0.19	0.21	0.26	0.28	0.32	a
	50 day	0.08	0.16	0.20	0.21	0.22	0.27	0.28	a
	70 day	0.08	0.20	0.20	0.20	0.24	0.27	0.28	a
	90 day	0.08	0.16	0.19	0.19	0.19	0.24	0.32	b
Leaves	35 day	0.02	0.07	0.08	0.10	0.13	0.14	0.22	c
	50 day	0.03	0.07	0.09	0.09	0.10	0.13	0.18	c
	70 day	0.01	0.08	0.09	0.09	0.10	0.11	0.18	c
	90 day	0.01	0.06	0.06	0.06	0.07	0.07	0.11	d
Stems	35 day	0.02	0.06	0.09	0.10	0.10	0.14	0.23	e
	50 day	0.02	0.05	0.08	0.09	0.10	0.13	0.22	e
	70 day	0.01	0.04	0.08	0.08	0.09	0.12	0.21	e
	90 day	0.01	0.02	0.02	0.02	0.02	0.04	0.20	f
Whole Plant Tops	35 day	0.02	0.12	0.16	0.17	0.18	0.20	0.27	g
	50 day	0.03	0.09	0.10	0.12	0.13	0.20	0.23	h
	70 day	0.03	0.09	0.10	0.11	0.14	0.17	0.30	h
	90 day	0.01	0.08	0.10	0.11	0.11	0.14	0.24	i

(b) Fresh weight basis

Tissue	Harvest	P rate							Time Main Effect
		P_0	$P_{12.5}$	P_{25}	$P_{37.5}$	P_{50}	P_{100}	P_{200}	
									*
Whole Plant Tops	35 day	0.002	0.019	0.025	0.025	0.025	0.029	0.034	j
	50 day	0.007	0.019	0.023	0.025	0.028	0.034	0.046	j
	70 day	0.004	0.017	0.025	0.027	0.029	0.035	0.056	j
	90 day	0.003	0.019	0.021	0.023	0.025	0.033	0.054	j

*Harvests labelled with the same letter are not significantly different at $P<0.05$.

DISCUSSION

Critical phosphorus concentrations

This experiment has clearly established that the critical phosphorus concentration of the whole tops of Townsville stylo plants declined with increasing plant age when expressed on the conventional dry weight basis. However, when it was expressed on a fresh weight basis, the critical phosphorus concentration of whole plant tops remained constant. Furthermore, the critical phosphorus concentrations of the apical tissue and leaves, expressed on a dry weight basis, did not decline until "haying off" and seed drying commenced.

Several factors may be identified as contributing to the decline with age in the critical phosphorus concentration of whole tops expressed on a dry weight basis. Of particular importance are changes in the proportionate distribution of dry matter among the different tissues comprising the whole plant tops (Table 3), and changes with time in the dry matter content of the tops. Successive increases in the dry matter proportion of stem and, to a lesser degree, leaves up to the harvest at 70 days lowered the critical phosphorus concentration of the whole plant tops because these tissues have a much lower phosphorus concentration than the physiologically younger apical

TABLE 2

Linear regression equations of relative whole plant top dry matter yield (y) on phosphorus concentration (x) expressed on (a) a dry weight basis and (b) a fresh weight basis in tissues and whole plant tops of Townsville stylo. Critical phosphorus concentrations are interpolated from these equations at a relative dry matter yield of 90%

(a) Dry weight basis

Tissue	Harvest	Regression Equation	r^2	Critical P Conc. (%P) *
Apical Tissue	35 day	$y = 399.4x - 21.2$	0.83	0.28 ± 0.02
	50 day	$y = 576.0x - 56.7$	0.89	0.26 ± 0.01
	70 day	$y = 745.1x - 95.5$	0.69	0.25 ± 0.02
	90 day	$y = 523.4x - 32.3$	0.93	0.23 ± 0.01
Leaves	35 day	$y = 628.2x - 4.4$	0.75	0.15 ± 0.02
	50 day	$y = 742.1x - 13.9$	0.67	0.14 ± 0.02
	70 day	$y = 1686.7x - 97.8$	0.37	0.11 ± 0.05
	90 day	$y = 1479.7x - 23.8$	0.68	0.08 ± 0.02
Stem	35 day	$y = 480.8x + 16.3$	0.38	0.15 ± 0.06
	50 day	$y = 570.4x + 7.8$	0.89	0.14 ± 0.01
	70 day	$y = 672.4x + 6.9$	0.39	0.12 ± 0.06
	90 day	$y = 481.8x + 65.4$	0.36	0.05 ± 0.05
Whole Plant Tops	35 day	$y = 699.6x - 53.1$	0.78	0.20 ± 0.01
	50 day	$y = 587.5x - 9.9$	0.92	0.17 ± 0.01
	70 day	$y = 672.9x - 11.8$	0.74	0.15 ± 0.01
	90 day	$y = 656.3x + 2.0$	0.72	0.13 ± 0.02

(b) Fresh weight basis

Tissue	Harvest	Regression Equation	r^2	Critical P Conc. (%P). *
Whole Plant Tops	35 day	$y = 3626.1x - 33.3$	0.71	0.034 ± 0.003
	50 day	$y = 3071.7x - 5.2$	0.87	0.031 ± 0.002
	70 day	$y = 2650.5x - 2.8$	0.49	0.035 ± 0.014
	90 day	$y = 2452.3x + 9.1$	0.71	0.033 ± 0.005

* 95% confidence limits

TABLE 3

The relative contribution of different plant components to the total dry matter yield of Townsville stylo

Tissue	Harvest				LSD P=0.05
	35 day	50 day	70 day	90 day	
Apical Tissue	46	44	32	49	2
Leaves	23	20	26	18	2
Stem	31	35	42	33	1

tissue (Table 1). A similar increase in the proportion of stem to other tissues during lateral vegetative growth of Townsville stylo was reported by Fisher (1970) and Robinson and Jones (1972). The period between the 70 day and 90 day harvests was characterized by a significant increase in the proportion of apical tissue due to the formation of seed at all applied phosphorus levels except P_0. This strong increase in the proportion of apical tissue of relatively higher phosphorus concentration between 70 and 90 days offset the sharp increase in dry matter content of the whole plant tops which occurred during this period and the critical phosphorus concentration did not decline significantly.

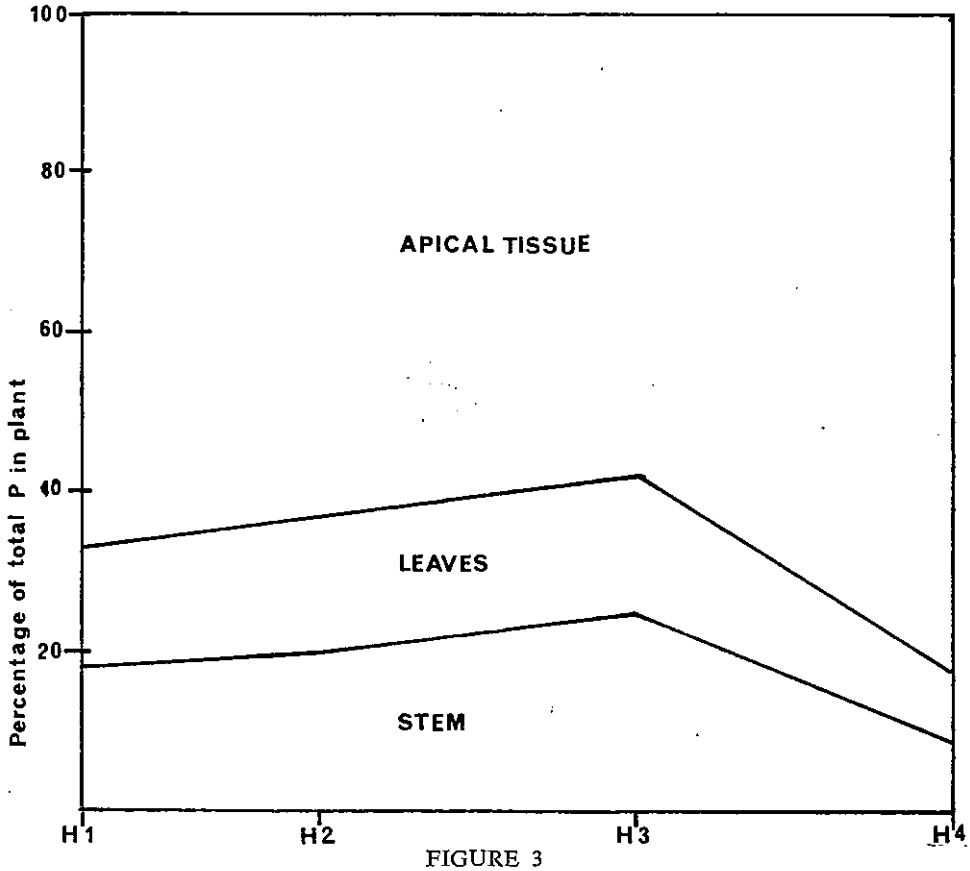


FIGURE 3

Percentage of total phosphorus in the whole plant tops of Townsville stylo present in various tissues 35(H1), 50(H2), 70(H3) and 90 days (H4) after germination at a phosphorus application rate of 50 kg P per ha.

A decline in critical phosphorus concentration in the leaves occurred during "haying off" and seed drying (70 to 90 day period) (Table 2). Two major factors appear to be operative. The phosphorus concentration in a particular tissue expressed on a dry weight basis would be expected to decrease if either an increase in dry matter content or a decline in the total amount of phosphorus occurred. The dry matter content of the whole plant tops increased significantly during the 50 to 90 day period, presumably due to the drying out of leaf and stem tissues as they aged, and to seed drying during the 70 to 90 day period. At the 90 day harvest, a strong decrease was also observed in the proportion of the total phosphorus in the whole plant tops which was present in the stems and leaves (Figure 3), presumably the result of translocation of phosphorus from these tissues to the apical tissue as seed developed. Such a translocation of phosphorus in Townsville stylo has been reported by Robinson and Jones (1972). The decline in critical phosphorus concentration in the leaves can therefore be attributed to the increase in dry matter content of this tissue at the 90 day harvest and the translocation of phosphorus to the apical tissue which is revealed by data for the 90 day harvest.

The apical tissue, which continued to be produced following flowering, maintained a constant critical phosphorus concentration up to and including the 70 day harvest (Table 2). The observed decrease in both the phosphorus concentration in

the apical tissue and the critical phosphorus concentration of this tissue at the 90 day harvest is attributed to the decrease in moisture content associated with seed drying between the 70 and 90 day harvests.

Agricultural implications

The findings reported herein have important practical applications in the assessment of the nutrient status of Townsville stylo pastures. Since the critical phosphorus concentration (fresh weight basis) of the whole plant tops and the critical phosphorus concentrations (dry weight basis) of the apical tissue and leaves were found to be independent of the chronological age of Townsville stylo, at least until "haying off", any of these could be used to assess the phosphorus status of Townsville stylo pastures. This finding represents a considerable advance over the currently used procedures where whole plant tops must be sampled at definite stages of development before comparisons with published values of critical concentrations can be made. Seed analysis for diagnostic purposes, as suggested by Jones (1968), requires study, as this may allow the determination of phosphorus status after "haying off" has occurred.

As the critical phosphorus concentration, at various stages of growth, of the leaves generally has wider confidence limits than that of the apical tissue, the latter would be preferable as a diagnostic value for distinguishing phosphorus deficiency from sufficiency.

Determination of the phosphorus concentration in the apical tissue has many practical advantages over that of determining the fresh weight phosphorus concentration in the whole plant tops. Fresh weight analysis requires that there is a minimum of elapsed time between sampling and weighing of the material, as even slight losses of moisture will affect results. For this reason fresh material must be placed in airtight containers which are kept cool until weighing is possible. On the other hand, sampling of the apical tissue does not require standardization of time of sampling, nor is it necessary to minimize moisture losses. It is therefore better suited to the routine determination of the nutrient status of field samples.

It is suggested that a critical phosphorus concentration of 0.26% in the apical tissue of Townsville stylo is applicable in southern Queensland. The critical phosphorus concentration of 0.17% in the whole plant tops of the same plants at the immediate pre-flowering stage of growth is in agreement with the critical concentration derived by Andrew and Robins (1969) from pot and field trials in this region.

However, results from a pot trial conducted at Darwin, N.T., using a similar soil, indicated a critical phosphorus concentration in the apical tissue of 0.15%, and in the whole plant tops of the same Townsville stylo plants at the immediate pre-flowering stage of growth of 0.11% (Moody 1977). Phosphorus uptakes in the pot trials at Darwin and Brisbane were similar for plants of the same age at a given level of applied phosphorus, but dry matter yields were markedly lower at Brisbane. Such large differences in the critical phosphorus concentrations at the two locations highlight the dangers of using critical nutrient concentrations determined in one environment for assessing the nutrient status of the same species, and in this case the same cultivar, in a different environment. The effect of the environment on critical phosphorus concentrations in whole tops and particularly the more generally useful apical tissue of Townsville stylo clearly requires further study.

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