

CHAPTER 4

EXPERIMENT ONE: A COMPARISON OF THE P EFFICIENCY OF TAMA AND SIROSA BASED ON THE NUMBER OF LEAVES PER PLANT

4.1

INTRODUCTION

Analysis of the results presented in Chapter 3 demonstrated a clear need to take into account the ontogenetic stage of plant species when measures of their P efficiency are being compared (see Section 3.4.1.4). Yet, in the past the P efficiency of plant species has not been directly related to the ontogeny of the species under study. Nevertheless, some attempts have been made to circumvent the problems associated with comparing the P efficiency of species, or, indeed, genotypes from the same species, with either dissimilar ontogenies or different rates of ontogenesis. Snaydon and Bradshaw (1960) and Spencer *et al.* (1980) used plant weight as a crude measure of the ontogenetic stage of plants. They showed that when plants were compared at the same weight, as well as at the same time, the relative P efficiency of the plants was dependent on the basis of comparison. Ozanne *et al.* (1969), on the other hand, made some comparisons of the P efficiency of species on a temporal basis and another comparison after all the species reached flowering. A disadvantage of this method was that the species had been flowering for different lengths of time when compared. Rather than compare species on a whole plant basis Hart and Jessop (1983) used the youngest mature leaves of both white clover and lotus as the basis for a comparison of their efficiency of P utilisation. Although this method used plant parts of similar physiological age it is not applicable to many measures of P efficiency. There would, therefore, appear to be a need to relate measures of the P efficiency of plant species to specific stages in the development of plants.

The recording of the number of leaves and the number of tillers per plant for the two grass species, Tama and

Sirosa, examined in experiment one makes it possible to re-analyse the data so that some measures of the P efficiency of the two grass species are related to stages in their vegetative development and can be compared to the measures of their P efficiency made on a temporal basis and presented in Chapter 3. The two grass species, rather than the two legume species, will be used to test the hypothesis that the relative P efficiency of plant species at any given time is influenced by the ontogenetic stage of the species at that time because the developmental stages of Tama and Sirosa are comparable whereas those of Hamua and Ladino contrast (see Section 3.2.2). Thus, it would first be necessary to establish the equivalence or otherwise of the growth stages of Hamua and Ladino before they could be compared at similar stages of development.

4.2

MATERIALS AND METHODS

4.2.1 INTRODUCTION

The data presented in this chapter are from experiment one so consequently the design of the experiment, the growth conditions, the measurement schedule, the analytical methods, and the statistical analysis were as described in Section 3.2. The only changes to the materials and methods, as described in Section 3.2, were the restriction to the data for the two grass species, Tama and Siroso, and the additional statistical analysis detailed below.

4.2.2 STATISTICAL ANALYSIS

4.2.2.1 Polynomial functions The data for the shoot fresh weight per plant, the total P uptake per plant, and the number of leaves per plant were fitted against P rate and time after sowing with the polynomial function described in Section 3.2.6.2. The regression coefficients and the coefficients of determination for the specific polynomial functions are presented in Appendix 3.2 for shoot fresh weight and Appendix 4.1 for total P uptake and leaf number.

The values for shoot fresh weight and total P uptake at any given leaf number were generated by first calculating the time after sowing, for a given P rate, that Tama and Siroso attained a specified number of leaves per plant. This calculated time after sowing was then substituted in the polynomial functions relating shoot fresh weight and total P uptake to time after sowing and P rate so that the values for shoot fresh weight and total P uptake at that time after sowing, and hence at the specified number of leaves per plant, could be generated.

Due to the indirect nature of these calculations the hypothesis proposed in Section 4.1 was only tested with measures of P efficiency based on shoot fresh weight and total P uptake per plant. Clearly, if the data produced for these measures of P efficiency supported the hypothesis then a wider range of measures of P efficiency should be tested in a more comprehensive study.

4.2.2.2 t-tests The significance of any difference between the values calculated for Tama and Siroso at any given number of leaves per plant and P rate was tested with a t-test, after the heterogeneity of the variances for Tama and Siroso was taken into account (V. Bofinger pers. comm.). The variance of each value ($Y_{P,T}$) for shoot fresh weight, or total P uptake, at a given P rate and a given time of sowing, and thereby a specified number of leaves per plant, was calculated with the expansion, presented below, of the general polynomial given in Section 3.2.6.2.

Variance ($Y_{P,T}$)

$$\begin{aligned}
 &= \text{Var}(B_0) + P^2 \text{Var}(B_1) + \dots + P^6 T^2 \text{Var}(B_9) \\
 &+ 2P \text{Cov}(B_0, B_1) + 2P^2 \text{Cov}(B_0, B_2) + \dots + 2P^3 T \text{Cov}(B_0, B_9) \\
 &+ 2P^3 \text{Cov}(B_1, B_2) + \dots + 2P^4 T \text{Cov}(B_1, B_9) \\
 &+ 2P^5 \text{Cov}(B_2, B_3) + \dots + 2P^5 T \text{Cov}(B_2, B_9) \\
 &+ 2P^3 T \text{Cov}(B_3, B_4) + \dots + 2P^6 T \text{Cov}(B_3, B_9) \\
 &+ \text{etc.} \dots \dots \dots \\
 &+ 2P^5 T^3 \text{Cov}(B_8, B_9)
 \end{aligned}$$

where: $P = \ln (\text{kgP} \cdot \text{ha}^{-1})$

$T =$ days after sowing (or by substitution
number of leaves per plant)

$B =$ regression coefficient

$\text{Var}(B) =$ variance of regression coefficient

$\text{Cov}(B, B) =$ covariance of regression coefficients

4.3

RESULTS

4.3.1 MEASURES OF VEGETATIVE DEVELOPMENT

The number of leaves per plant on Tama exceeded that on Sirosa except at 20 and 80 kg P.ha⁻¹ at the final harvest time (Table 4.1). This more advanced vegetative development of Tama compared with that of Sirosa, at each harvest time, was in accordance with the observations that the overall development, or ontogeny, of Tama including the time at which Tama switched to the reproductive phase, was ahead of that of Sirosa (see Table 4.1). Nevertheless, the number of tillers per plant on Tama was only greater than that on Sirosa at 21 days after sowing and at the P rates from 5 to 20 kg P.ha⁻¹ at 40 days after sowing (Table 4.2). At 68 days after sowing the number of tillers per plant was similar for both Tama and Sirosa (Table 4.2).

Therefore, number of leaves per plant gave a stronger delineation between the vegetative development of Tama and Sirosa than did number of tillers per plant (see Tables 4.1 and 4.2). The ratio of expanded leaves to unexpanded leaves was similar for both Tama and Sirosa at each harvest time, regardless of the P rate, suggesting that further subdivision of the number of leaves per plant would not provide a more sensitive measure of the vegetative development of the two species (Table 4.1). For the above reasons number of leaves per plant was used as the measure of vegetative development in the following comparisons of Tama and Sirosa (see Sections 4.3.2 and 4.3.3).

4.3.2 SHOOT FRESH WEIGHT RELATED TO NUMBER OF LEAVES PER PLANT

The shoot fresh weight per plant of Tama and Sirosa was usually similar when they were compared, at a given P rate, at a similar number of leaves per plant (Table 4.3). Moreover, Sirosa needed up to twenty-six days more than Tama, at a given P rate to attain a similar number of leaves per plant to that of Tama, but the shoot fresh weight per plant of Sirosa was then similar to that of Tama (Table 4.3).

Table 4.1 The number of leaves per plant and the ratio of unexpanded to expanded leaves for Tama and Siroso at three P rates from 21 to 68 days after sowing (DAS)

DAS	P rate (kg P.ha ⁻¹)	No. leaves plant ⁻¹		Expanded:unexpanded leaves	
		Tama	Siroso	Tama	Siroso
21	5	4.7c [#]	3.7c	0.39a ^z	0.39a
	20	8.3b	3.7c	0.39a	0.22b
	80	11.3a	4.0c	0.36ab	0.22b
40	5	9.3d	5.7e	0.48a	0.42a
	20	22.3a	10.7d	0.39a	0.39a
	80	21.3a	13.0c	0.41a	0.40a
68	5	19.0d	16.0e	0.36a	0.46a
	20	23.0c	24.0c	0.35a	0.33a
	80	29.0b	34.0a	0.45a	0.48a

[#] Means for number of leaves per plant within each time of harvest that are followed by the same letter are not significantly different according to Duncan's Multiple Range test ($p < 0.05$).

^z Means for the ratio of unexpanded to expanded leaves within each time of harvest were separated as above.

Table 4.2 The number of tillers per plant for Tama and Sirosa at three P rates from 21 to 68 days after sowing (DAS)

DAS	P rate (kg P.ha ⁻¹)	Species	
		Tama	Sirosa
No. tillers.plant ⁻¹			
21	5	0.3	#
	20	1.3	#
	80	2.0	#
40	5	2.0c ^z	0.3d
	20	5.0a	2.0c
	80	4.5ab	4.0b
68	5	3.0d	3.0d
	20	4.0c	4.0c
	80	7.0b	8.0a

There were no tillers on Sirosa plants at 21 DAS.

^z Means within each time of harvest that are followed by the same letter are not significantly different according to Duncan's Multiple Range test ($p < 0.05$).

Table 4.3 The shoot fresh weight per plant of Tama and Sirosa compared at a similar number of leaves per plant for a given P rate. The values for shoot fresh weight per plant and number of leaves per plant were calculated from polynomial functions by the method given in Section 4.2.2

P rate (kg P.ha ⁻¹)	DAS		No. leaves. plant ⁻¹		Shoot fresh weight		
	Tama	Sirosa	Tama	Sirosa	Tama	Sirosa	
					g.plant ⁻¹		
5	29	40	7	7	0.33	0.33	NS ^z
10	29	40	8	7	0.42	0.37	NS
20	24	40	7	8	0.40	0.47	NS
40	24	40	10	10	0.63	0.73	NS
80	29	40	14	14	1.05	1.44	*
5	48	68	15	15	1.07	1.06	NS
10	44	68	14	14	1.13	1.32	NS
20	42	68	16	16	1.43	1.94	*
40	55	68	25	25	3.16	3.43	NS
80	68	68	29 [#]	33	4.82	4.77	NS

^z Denotes whether the two preceeding means in that row were found significantly different by a t-test.
NS = not significantly different, * = significantly different at the 5% level. Also see Section 4.2.2.

[#] Maximum number of leaves per plant for Tama at 80 kg P.ha⁻¹ and the final harvest.

4.3.3 P UPTAKE PER PLANT RELATED TO NUMBER OF LEAVES PER PLANT

The P uptake per plant of Tama and Sirosa was usually similar when they were compared, at a given P rate, at a similar number of leaves per plant (Table 4.4). Nevertheless, the P uptake per plant of Sirosa was greater than that of Tama at 80 kg P.ha⁻¹, and on one occasion at 20 kg P.ha⁻¹ (Table 4.4). The greater P uptake per plant of Sirosa at 80 kg P.ha⁻¹ when there was thirty-three leaves and twenty-nine leaves per plant respectively on Sirosa and Tama was possibly the result of the greater leaf number of Sirosa. Although there was no apparent explanation for the other occasions that the P uptake per plant of Sirosa was greater than that of Tama such fluctuations could possibly have been artefacts resulting from the polynomial functions used to generate the data.

Table 4.4 The P uptake per plant, or plant P content, of Tama and Sirosa compared at a similar number of leaves per plant for a given P rate. The values for both P uptake and number of leaves per plant were calculated from polynomial functions by the method given in Section 4.2.2

P rate (kg P.ha ⁻¹)	No. leaves.plant ⁻¹		P uptake per plant	
	Tama	Sirosa	Tama	Sirosa
			$\mu\text{g P.plant}^{-1}$	
5	7	7	178	152 NS ^z
10	8	7	218	170 NS
20	7	8	218	226 NS
40	10	10	482	459 NS
80	14	14	1513	1821 *
5	15	15	455	553 NS
10	14	14	502	681 NS
20	16	16	685	997 *
40	25	25	1821	1922 NS
80	29 [#]	33	4989	5937 *

z # See Table 4.3

4.4

DISCUSSION

The shoot fresh weight per plant under all P conditions (5 to 80 kg P.ha⁻¹) and the shoot fresh weight per plant per unit of applied P of Tama and Sirosa was similar when the two species were compared at similar leaf numbers per plant (see Table 4.3). Also, the P uptake per plant, or plant P content, of Tama and Sirosa was similar when there was a similar number of leaves per plant on the two species (see Table 4.4). Therefore, the P efficiency of Tama and Sirosa as measured by shoot biomass production and capacity to absorb P under both low and high levels of P supply was similar when the two species were compared at a similar stage in their vegetative development. This conclusion contrasts with the results of the comparison of Tama and Sirosa on a temporal basis that showed both the shoot fresh weight and P uptake per plant of Tama were greater than that of Sirosa at 40 days after sowing (see Sections 3.3.2 and 3.3.3).

Although the results in Section 4.3 supported the hypothesis that the relative P efficiency of plant species at any given time is influenced by the ontogenetic stage of the species at that time there were some qualifications to that support. It would appear that in the specific case of Tama and Sirosa that the differences in the P efficiency of the two species on a temporal basis were entirely, rather than partly, the result of differences in the vegetative development of Tama and Sirosa at any given time (see Tables 4.1 and 4.2). A limitation of this study, however, was that only the vegetative development, and not the reproductive development, of Tama and Sirosa was quantitatively monitored. Furthermore, by 68 days after sowing Tama was in the reproductive phase whereas Sirosa was still in the vegetative phase. Thus, there is a need to compare the P efficiency of Tama and Sirosa when they are at similar reproductive stages, as well as when at similar vegetative stages, so that the relationship between the P efficiency of the two species and their ontogenesis can be fully examined. That is, a more complete study on the relative P efficiency

of Tama and Siroso on both a temporal and an ontogenetic basis is required.

Despite the limitations of this study it is apparent that ranking the P efficiency of plant species on the basis of their performance at a given time, or times, could produce misleading results if the rate of ontogenesis of the species is dissimilar. Yet, comparison of the P efficiency of plant species on a temporal basis has been the usual method reported in the literature (e.g. Keay et al. 1970; Blair and Cordero, 1978; Caradus, 1980).

CHAPTER 5

EXPERIMENT TWO: THE P EFFICIENCY OF TAMA AND SIROSA COMPARED ON A TEMPORAL AND AN ONTOGENETIC BASIS

5.1

INTRODUCTION

The shoot fresh weight per plant and the total P uptake, or P content, per plant were similar for Tama and Sirosa when there was the same number of leaves per plant (see Section 4.3). These results suggested that the superior P efficiency of Tama observed when Tama and Sirosa were compared on a temporal basis (see Section 3.4) was related to differences in the rate of development, or ontogenesis, of the two species.

Michelini (1958) and Bates (1971) have argued that it is necessary to consider the ontogenetic stage of plant genotypes when their physiological processes are being compared. Indeed, many of the physiological and metabolic processes involved in the P nutrition of plants have been shown to change as plants develop, or age, independently of the treatments being imposed. For example, the C_{min} for P uptake by roots has been shown to increase as plants age (Edwards and Barber, 1976), and the P concentration of plants has been found to decrease as plants age (Ozanne *et al.*, 1969; Bates, 1971). Yet, there appears to have been no attempts to relate the yield response to P of pasture genotypes to their ontogeny although the existence of such a relationship has been previously recognised (see Snaydon and Bradshaw, 1962; Keay *et al.*, 1970; Spencer *et al.*, 1980).

Therefore, an experiment was designed to test the hypothesis that the P efficiency of Tama and Sirosa was the same when they were at the same ontogenetic stage. The hypothesis was tested by comparing measures of the growth, the P physiology, and the P metabolism of Tama and of Sirosa on both a temporal and an ontogenetic basis at a low and a high level of P supply.

5.2

MATERIALS AND METHODS

5.2.1 DESIGN OF EXPERIMENT

The experiment was a factorial in a randomised complete block design. There were two species, two rates of applied P, seven destructive harvests and three blocks (see Table 5.1), plus an additional block that was used only for monitoring the reproductive status of the plants, for some photosynthetic measurements, and to calibrate the leaf area measurements.

Table 5.1 Treatments used in the randomised complete block design

Species	x	kg P.ha ⁻¹	x	Harvests (days after sowing)
Italian ryegrass		5		16
		20		29
Phalaris				42
				51
				63
				78
				112

5.2.2 SPECIES

The two species used were the grasses Italian ryegrass (Lolium multiflorum Lamk. cy. Tama), hereafter referred to as Tama, and phalaris (Phalaris aquatica L. cy. Sirosa), hereafter referred to as Sirosa. Tama is a temperate annual grass whereas Sirosa is a temperate perennial grass. Tama and Sirosa differ in their vernalization requirements. Sirosa requires a period of cold temperatures, 3 to 8°C over 2 to 4 weeks is optimum, as well as a switch from short to long days (10 hours) for it to initiate (McWilliam, 1968). On the other hand, Tama can initiate without a vernalization

period, but cold temperatures and short then long days will shorten its time to initiation (Cooper, 1960).

5.2.3 P AND OTHER FERTILIZER RATES

The two P rates of 5 kg P.ha⁻¹ and 20 kg P.ha⁻¹ were chosen, with reference to experiment 1, because they represented a P rate known to cause the development of P deficiency symptoms in Tama and Siroso, and a P rate known to be about the rate necessary for Tama and Siroso to attain their maximum growth.

The mineral nutrients added to the soil and their rates of application on a surface area basis are shown in Table 5.2. All nutrients were mixed thoroughly through the soil before sowing. The same nutrient rates were used for both the trays used to germinate the seedlings and the pots. NH₄NO₃ at 50 kg.ha⁻¹ (17.5 kg N.ha⁻¹) was applied to all pots at sowing and at 30, 51, 76, and 91 DAS.

5.2.4 MEASUREMENT SCHEDULE

The plants were harvested and measurements were made 16, 29, 42, 51, 63, 78, and 112 DAS. Each harvest took two days, with the second day being designated as the harvest day. On the first harvest day the following measurements were made: -

1. Photosynthetic rate of leaves.
2. Chlorophyll concentration of leaves.
3. Leaf area per plant.
4. Number of leaves and number of tillers per plant
5. Reproductive status of the plants.

On the second harvest day the following measurements were undertaken: -

1. Fresh weight of shoots and of roots.
2. A sub-sample of the shoots and of the roots was taken for later P fractionation.
3. A sub-sample was taken for later measurement of root length and of mycorrhizal infection.

Table 5.2 Mineral nutrients added to the soil used
in experiment 2

Nutrient	kg.ha ⁻¹	g.15 cm pot ⁻¹
Ca(H ₂ PO ₄) ₂ .H ₂ O	20.4*	0.036
Ca(H ₂ PO ₄) ₂ .H ₂ O	81.4*	0.144
NH ₄ NO ₃	50	0.088
K ₂ SO ₄	125	0.221
MgSO ₄ .7H ₂ O	70	0.124
CaSO ₄ .2H ₂ O	120	0.212
H ₃ BO ₃	4.4	0.008
ZnSO ₄ .7H ₂ O	7.8	0.014
CuSO ₄ .5H ₂ O	7.8	0.014
Na ₂ MoO ₄ .2H ₂ O	0.8	0.00053

* Ca(H₂PO₄)₂.H₂O rates are equivalent to 5 and 20 kg P.ha⁻¹ respectively

5.2.5 GROWTH CONDITIONS

The seeds were germinated in seedling trays (28 cm x 34 cm) in a glasshouse. Tama seedlings were transplanted into pots 8 days after sowing (DAS) whereas Sirosa seedlings were transplanted into pots 13 DAS. There were eight seedlings per pot. Both species were at the one folded leaf stage when transplanted. Free draining plastic pots (15 cm diameter x 15 cm height) with punctured plastic liners were used.

5.2.5.1 Soil The soil used is described in section 3.

5.2.5.2 Watering All pots were watered to field capacity at least once each day. The pots were watered to weight two or three times a week to check they were at field capacity.

5.2.5.3 Temperature The glasshouse temperature was regulated so that for the first 38 DAS the day temperature was 17 to 21°C and the night temperature was 2 to 11°C. From 39 DAS to 112 DAS the day temperature was 17 to 21°C and the night temperature was 14 to 16°C.

5.2.5.4 Daylength As the experiment began in April the daylength of about 8 hours per day was decreasing. From 43 DAS onwards supplementary artificial lighting was used to increase the daylength to 14 hours per day. The contribution of the artificial lighting to the photon flux density (400-700 nm) at the top of the plants was small, being about $100 \mu\text{E.m}^{-2}.\text{s}^{-1}$.

5.2.6 DETERMINATION OF THE DEVELOPMENTAL STAGE OF GRASSES

5.2.6.1 Evaluation of measures of plant age and development

It is often desirable to relate the physiological activities of a plant, such as P uptake, to the morphological age of a plant (Michelini, 1958). The relating of a physiological measure to a morphological, or ontogenetic, index as well as to time enables the effect of an experimental treatment imposed on a plant to be separated into two components. Namely, the effect of the treatment on the physiological activity being measured, and the effect of the treatment on the ontogeny of the plant.

The ontogeny of a plant can be affected by an experimental treatment in two ways. One, the treatment can alter the rate of ontogeny of the plant, and two the events that constitute the ontogeny of a plant can be changed so that some are omitted or re-ordered.

Alternatives to time used to monitor plant age and development can be divided into two groups: morphological indices and biomass indices. Examples of these indices are given below.

MORPHOLOGICAL INDICES

- Number of leaves per plant or per main stem
- Plastochron index (Michelini, 1958)
- Feekes growth stage scale (Large, 1954)
- Decimal code for growth stages (Zadoks et al., 1974)

BIOMASS INDICES

- Plant weight (Spencer and Hely, 1982)
- Relative growth rate
- Unit leaf rate (Barrow, 1978)

Within the morphological indices group those indices based solely on measures of leaf number (e.g. plastochron index) are only effective, particularly in relation to P nutrition research, while the plants are in the vegetative phase. The switch to the reproductive phase, initiation in grasses and cereals, is accompanied by changes in the P nutrition of plants (Milthorpe and Moorby, 1974). Thus, the comparison of two plants - one vegetative, the other reproductive - could lead to erroneous conclusions. The advantage of growth stage indices such as that of Zadoks et al. (1974) is they cover the complete ontogeny of plants, and so distinguish between plants in the vegetative and reproductive phases.

Although biomass indices have been used in P efficiency research (e.g. Spencer and Hely, 1982) they have several disadvantages. Plants of the same weight have not necessarily reached that weight via the same ontogeny (Hunt,

(1982). A similar disadvantage is that the relative growth rate and unit leaf rate of a plant can change from internal influences independent from those being imposed by experiment (Hunt, 1982; Beever and Woolhouse, 1975). Furthermore, a plant can have the same relative growth rate or unit leaf rate at two different times or two different growth stages. Also, as biomass indices are based on plant weight the development of mathematical relationships between physiological activities such as P concentration or leaf area ratio and a biomass index can result in functions that are mathematical aberrations (see Hunt, 1982).

Indices such as Zadoks growth stages are better measures of the ontogenetic stage of plants than morphological indices, based solely on vegetative characteristics; and biomass indices. Nevertheless, biomass indices possibly have a role in the comparison of species with incompatible ontogenies. For example, grass and legume species.

5.2.6.2 Measurements of plant ontogeny

Phenology The phenology of the plants was measured by recording the number of leaves and tillers per plant and the reproductive status of the plants. The number of leaves per plant and the number of leaves on the main stem of each plant was counted. Leaves were recorded as being fully unfolded (that is, a collar present) or as being folded. The number of observable tillers per plant was also counted. At each harvest the leaves and tillers on all plants were counted, including those plants designated for harvest later. This counting method was used to ensure all plants were developing at the same rate.

Reproductive status The plants in block four were used for the observations of the reproductive status of the plants that required destruction of the plant. All conclusions on the reproductive status of the plants were based on at least five plants for each treatment. After the start of head emergence evaluation of the reproductive status of the plants was based on all the plants present at each harvest. The reproductive status of the plants was noted at each

harvest and at lesser intervals when initiation (double ridge stage) was imminent. The reproductive status of the inflorescences was gauged according to the descriptions used by Kirby and Appleyard (1981).

Ontogeny The phenological data and the reproductive status of the plants were used to describe the ontogeny of both grass species by the following two methods: -

1. Total number of leaves per plant. All visible leaves on the main stem of each plant and on the tillers were counted at each harvest. This was the same as the method used in experiment 1.
2. Zadoks growth stage. The growth stage of the plants, at each harvest, was recorded according to a modified version of the decimal code for the growth stages of cereals proposed by Zadoks et al. (1974). For the sake of brevity this decimal code is hereafter called Zadoks growth stages. The modification to Zadoks growth stages is given in Table 5.3. The fuller definitions of Tottmann et al. (1979) were used for all growth stages.

The modified stages presented in Table 5.3 were regarded as reasonable equivalents of the stages used by Zadoks et al. (1974) with the advantage that they were more specific measures of the plants reproductive status.

5.2.7 MEASUREMENT TECHNIQUES

5.2.7.1 Biomass The fresh and dry weights of both the shoots and the roots of the plants were measured at each harvest. The soil was washed from the roots with tap-water (<1.0 ppm P) then the roots of the entire plant were kept submerged in tap-water to prevent wilting, until they were blotted dry immediately before being weighed. Dry weights were determined after drying the plant material at 80°C in a forced draught oven for 48 hours, and allowing for the sub-samples removed for other measurements.

5.2.7.2 Fresh weight versus dry weight Plant weights were expressed as fresh weights rather than as dry weights for the following reasons: -

Table 5.3 Comparison between the modified and the standard description of Zadoks growth stage 3 (also see Tottman et al., 1979)

Modified description		Standard description	
0	Germination	0	Germination
1	Seedling growth	1	Seedling growth
2	Tillering	2	Tillering
3	Shoot apex development	3	Stem elongation
30	-	30	Pseudostem (leaf sheath) erection
31	Double ridge stage (initiation)	31	First node detectable
32	-	32	2nd node detectable
33	Glume primordium stage	33	3rd node detectable
34	-	34	4th node detectable
35	Lemma primordium stage	35	5th node detectable
36	-	36	6th node detectable
37	Floret primordium stage	37	Flag leaf just visible
38	-	38	-
39	Terminal floret stage	39	Flag leaf ligule (just visible)
4	Booting	4	Booting
5	Inflorescence emergence	5	Inflorescence emergence
6	Anthesis	6	Anthesis
7	Milk development	7	Milk development
8	Dough development	8	Dough development
9	Ripening	9	Ripening

1. P concentrations expressed on a per unit fresh weight basis are more appropriate for physiological interpretation (Leigh et al., 1982). That is, the P concentration of tissue water in the plant is a better indication of the physiological activity of P in the plant than P concentration on a dry weight basis (Leigh et al., 1982; Asher and Loneragan, 1967).
2. Root fresh weight approximates root volume more closely than does root dry weight and thus P uptake per root fresh weight is a better estimate of P influx than P uptake per root dry weight.
3. The use of fresh weights allowed direct comparison of the results from experiment 2 with the results from experiment 1.

Tables 5.4 and 5.5 show that the dry weights of the plants, when expressed as a percentage of the fresh weights of the plants, of Tama and of Sirosa were similar at most harvests. At 63 and 78 DAS, however, the percentage dry weight was greater in both the shoots and the roots of Tama relative to the shoots and the roots of Sirosa (Tables 5.4, 5.5). This decreased tissue hydration of Tama relative to that of Sirosa was probably the result of the greater maturity of Tama at that time. Within each species there was no effect of P rate on tissue hydration at any given harvest (Tables 5.4, 5.5).

Table 5.4 The dry weight of the shoots as a percentage (%) of the fresh weight of the shoots of Tama and of Sirosa at two P rates over time

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
16	9.17bc ^z	9.97b	8.37c	13.22c
29	10.79a	11.23a	10.09a	10.77a
42	9.89b	12.57a	10.27b	9.59b
51	11.77b	11.80b	14.45a	11.92b
63	16.89a	11.78b	17.78a	16.99a
78	18.32a	14.62b	17.78a	18.19a
112	29.94a	29.01a	27.27b	30.0a
DMR 5%(min) [#] =1.50				
DMR 5%(max) [#] =1.71				

^z Values within a single row that are followed by the same letter are not significantly different at $p < 0.05$ using Duncan's Multiple Range test

[#] The minimum and the maximum values for Duncan's Multiple Range test ($P < 0.05$) within each column of values

Table 5.5 The dry weight of the roots as a percentage (%) of the fresh weight of the roots of Tama and of Sirosa at two P rates over time

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
16	12.30b ^z	16.30a	9.39b	15.60c
29	7.04a	6.67a	7.24a	6.07a
42	8.93a	7.00a	8.52a	5.99a
51	9.66a	9.13a	10.32a	8.74a
63	13.44b	9.37c	17.97a	10.22bc
78	15.36b	11.15c	19.80a	12.93bc
112	20.41b	21.01b	25.35a	20.06b
DMR 5%(min) [#]	=3.22	3.22	3.22	3.22
DMR 5%(max) [#]	=3.67	3.67	3.67	3.67

^z [#] See Table 5.4

5.2.7.3 Root length A sub-sample of the plants' root system (1 g fresh weight, when available) was stored in approximately 30 ml of FAA (13 ml formalin; 5 ml glacial acetic acid, 200 ml 50% ethanol) at each harvest. The length of the root sub-sample was later measured on a bizzometer (Adelie Enterprises). The bizzometer uses a television camera to measure the length of the root sub-sample as it is rotated through 360 degrees. The length measurements were based on a development of the Newman (1966) intercept method put forward by Evans (1970). The linear relationship between the fresh weight and the length of the sub-sample was used to calculate the length of the entire root system.

5.2.7.4 Leaf area The length and the width (at the widest point) of each leaf on three plants per pot was measured at each harvest. The length times width measurements were related to leaf area at each harvest by measuring the leaf area of the plants in block four with a planimeter.

5.2.7.5 Photosynthetic rate The photosynthetic rates of the oldest leaf and the youngest fully expanded leaf on the main stem of one plant per pot were measured for all treatments at each harvest. The maximum photosynthetic rate of the leaves was measured using a Clark-type O₂ electrode and the following optimum conditions (Kemp and Campbell, 1980). Quantum flux density was 2,000 $\mu\text{E}\cdot\text{m}^{-2}\text{ s}^{-1}$, the temperature was 25°C, and the solution the leaf slices were suspended in contained 15 mM KHCO₃ and 20 mM HEPES-NaOH (pH 7.6).

The chlorophyll concentration of the leaf slices used for the photosynthetic measurements was determined by the method of Jeffrey and Humphrey (1975).

5.2.7.6 P fractionation The P in the shoots and the roots was separated into three fractions (i.e. acid-soluble-P, P-lipid, and residue-P) as described previously (see section 3.2.5). For this experiment the sub-sample size was reduced to 0.5 g fresh weight, which was extracted in 10 ml MCFW

(see section 3.2.5). This reduction in sample size avoided the previous need to dilute the digested samples before analysing with the auto-analyser, without observed change in the sensitivity of the method.

5.2.7.7 Procedure for staining vesicular arbuscular mycorrhizas Roots were cleared and stained for vesicular arbuscular (VA) mycorrhizal fungi according to the procedure of Phillips and Hayman (1970). Roots stored in FAA were heated at 90°C for about one hour in 10% w/v KOH to clarify them, then were rinsed in water and acidified with 0.1N HCl. The roots were stained by simmering for 5 minutes in 0.05% w/v trypan blue in lactophenol. The excess stain was removed in clear lactophenol.

5.2.7.8 Measurement of the percentage of root infected The percentage of the root cortex infected with VA mycorrhizal fungi was estimated with the two methods recommended by Giovannetti and Mosse (1980). Visual estimates of the percentage infection were found to be as accurate as the grid intersect method (cf. Giovannetti and Mosse, 1980) and so visual estimates were used for the experimental results. For visual estimation of the infection root sub-samples were viewed at a magnification of 45 times under a stereoscopic microscope.

5.2.8 STATISTICAL ANALYSIS

5.2.8.1 Analysis of variance To normalize variance the data from most measurements were subjected to a natural log (ln) transformation before the analysis of variance.

The results presented in tables are the means of the original data, although the mean separations indicated were carried out on the ln transformed data with Duncan's Multiple Range Test (Little and Hills, 1978). The means of the original data are presented so they can be compared with values given in the literature and elsewhere in this thesis.

5.2.8.2 Fitting of functions Where appropriate functions were fitted to ln transformed data (see section 5.2.8.1) and were presented as such in the figures.

The functions fitted to the data were chosen empirically, that is for their goodness of statistical fit rather than to fit a pre-conceived model. Nevertheless, the functions used, logistics and polynomials, were biologically relevant to plant processes. Although a logistic was the function that best described the relationships between growth parameters, such as yield, P uptake, and P content, and time, and growth parameters and ontogeny for all four treatments there were occasional difficulties with the data for Tama grown at 5 kg P.ha⁻¹. These difficulties resulted from growth parameters such as the acid-soluble-P content of the roots of Tama, at 5 kg P.ha⁻¹, decreasing towards the end of the experiment rather than asymptoting as did the growth parameters for the other three treatments.

The logistic function used to describe the data took the following form (Ross, 1981).

$$Y = \frac{A}{(1 + b.e^{-k(t-t_0)})}$$

Y = ln (the predicted value of the dependent variable)

A = the final limiting value attained by Y

b = is related to the starting size of the system

k = the systems inherent rate of increase

t = is the independent variable related to Y

t₀ = the point at which the system started. This was designated as the value of t at the first harvest.

The logistic function was fitted to the ln transformed data with the BMDP 3R program (Dixon et al., 1983).

A difficulty with fitting the logistic function to measures of Y versus Zadoks growth stage was that the spacing between harvests was based on time intervals. Hence, although the values for Zadoks growth stage were, as is necessary, pre-determined they were not always at intervals conducive to minimising the variance of values of Y generated from the fitted function.

The adequacy of the fit of a logistic is measured by the residual mean square (RMS), but its significance cannot be assessed without some independent measure of the random error of the experiment (Ross, 1981). The independent measures used to assess the goodness of fit of the logistics were 1) a comparison with a polynomial with the same number of parameters (Bell, 1981), and 2) a comparison with the polynomial, found by stepwise regression, with the highest coefficient of determination (r^2) (Ross, 1981). The same polynomial function was the most suitable for comparison with the logistic in both categories. The function was: -

$$Y = A + B.T. + C.T.^{0.5}$$

A comparison of the logistic and the polynomial, fitted to some of the data sets, is given in Table 5.6. That the variance ratio was greater than unity in all cases demonstrated that the logistic fitted the data better than the polynomial (Table 5.6).

5.2.8.3 Confidence intervals The logistic functions fitted to the data for each treatment were compared at any given value of the independent variable by calculating the confidence interval for the predicted value at that point. The confidence interval (C.I.) was calculated as follows: -

$$C.I. = Y \pm t.s_y$$

where: Y is a single predicted value of Y for a specific independent variable (i.e. time or growth stage).

t is the tabulated value for t when $p=0.05$.

s_y is the standard deviation of the predicted value (Y). Thus, s_y takes into account the error of the regression coefficients, the error of the mean, and the variation of the replicated measurements of Y around the predicted mean.

Table 5.6 A comparison of the logistic function $Y = A/(1 + b.e^{-k(t-t_0)})$ and the polynomial function $Y = A + B.T. + C.T.^{0.5}$ fitted to the same data sets

Species	kg P.ha ⁻¹	Polynomial RMS	r ²	Logistic RMS	Variance ratio F _{18,18}
Total P uptake.plant ⁻¹					
Tama	5	0.42993	0.94	0.16525	2.602 [#]
Sirosa	5	0.58652	0.92	0.32737	1.792
Tama	20	0.37102	0.95	0.11423	3.248
Sirosa	20	0.43284	0.96	0.24512	1.766
Fresh weight.plant ⁻¹					
Tama	5	0.184169	0.99	0.02103	8.758
Sirosa	5	0.35445	0.98	0.05476	6.473
Tama	20	0.17778	0.99	0.01087	16.355
Sirosa	20	0.24464	0.99	0.01883	12.992
Leaf area.plant ⁻¹					
Tama	5	0.29721	0.96	0.07077	4.200
Sirosa	5	0.43870	0.96	0.09881	4.440
Tama	20	0.35262	0.93	0.10585	3.331
Sirosa	20	0.51398	0.95	0.13094	3.925

[#] F_{18,18}(5%) = 2.42

5.2.8.4 Polynomial functions All polynomials used were derived via stepwise regression. As a check on the veracity of the functions some values between the known values were calculated to ensure the function gave values that were biologically expected.

5.2.8.5 Growth analysis The functional approach was used for plant growth analysis (see Hunt, 1982). Logistic functions were fitted to plant weight versus time and leaf area versus time, and from these functions instantaneous values of relative growth rate and unit leaf rate were calculated after the method of Hughes and Freeman (1967).

5.3

RESULTS

5.3.1 THE BIOMASS ALLOCATION AND ONTOGENY OF TAMA AND SIROSA

5.3.1.1 Biomass allocation

Total plant fresh weight At each rate of applied P the fresh weight per plant of Tama was either greater than or not significantly different from that of Sirosa at each day onwards from the first harvest (Figure 5.1a). At 20 kg $P\cdot ha^{-1}$ the plant fresh weight of Tama was greater than that of Sirosa at each day from the first harvest (Figure 5.1a).

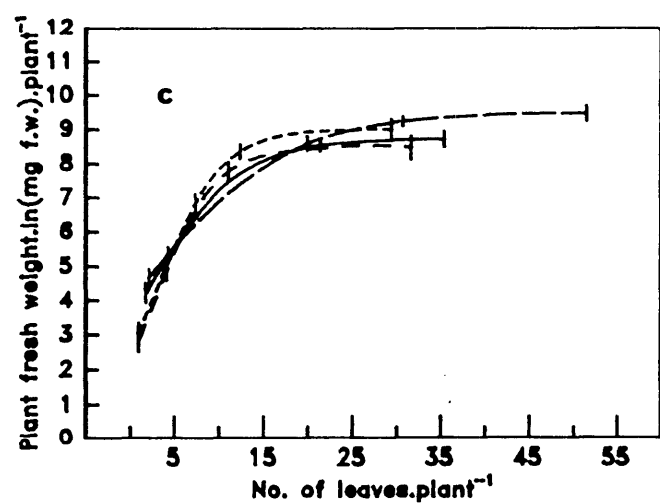
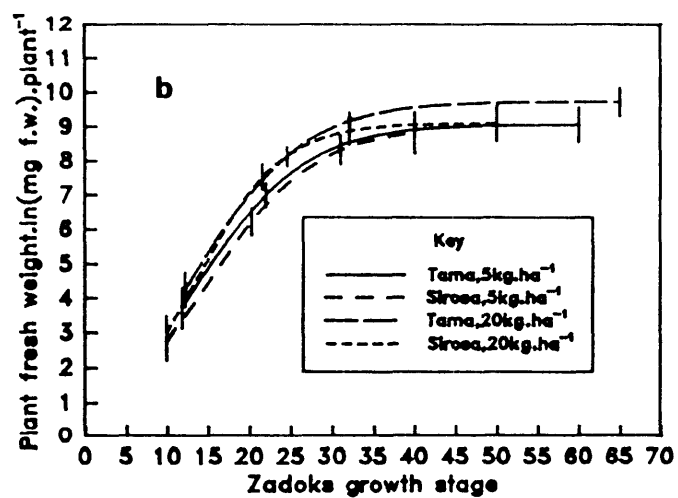
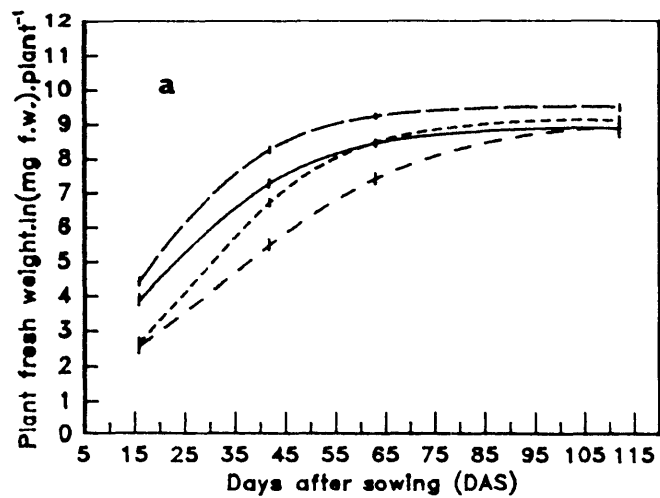
In contrast, when fresh weight per plant was related to the ontogeny of the grasses, using two different measures of ontogeny, there was no significant difference, overall, between Tama and Sirosa when compared at either of the two P rates (Figure 5.1b and c). That is, the total biomass production of Tama and Sirosa was the same when they were at the same ontogenetic stage, but on a temporal basis the biomass of Tama was produced at a faster rate due to the ability of Tama to develop faster than Sirosa.

The plant fresh weight of both species was greater at 20 kg $P\cdot ha^{-1}$ than at 5 kg $P\cdot ha^{-1}$ whether they were compared on a temporal or on an ontogenetic basis (Figure 5.1).

Shoot fresh weight When Tama and Sirosa were compared on a temporal basis their shoot fresh weights per plant were significantly different at both 5 and 20 kg $P\cdot ha^{-1}$ until about 100 DAS, after which they were similar (Figure 5.2a). In contrast, the shoot fresh weights per plant of Tama and Sirosa, when related to Zadoks growth stage, were similar when compared at either 5 or 20 kg $P\cdot ha^{-1}$ (Figure 5.2b).

A within species analysis of the response of the shoot fresh weight per plant of both Tama and Sirosa to increasing the P rate, from 5 to 20 kg $P\cdot ha^{-1}$, shows that the shoot fresh weight per plant increased on a temporal basis, but was not significantly different on an ontogenetic basis (Figure 5.2). That is, the greater shoot fresh weight per plant observed for both species at 20 kg $P\cdot ha^{-1}$ relative to

Figure 5.1 The relationship between plant fresh weight and (a) days after sowing, (b) Zadoks growth stage, and (c) number of leaves per plant for both Tama and Siroso at two rates of applied P. The fitted curves are described by the logistic function presented in Section 5.2. The coefficients for the function are given in Appendix 5.1. The vertical lines on the curves represent the 95% confidence interval at that point.



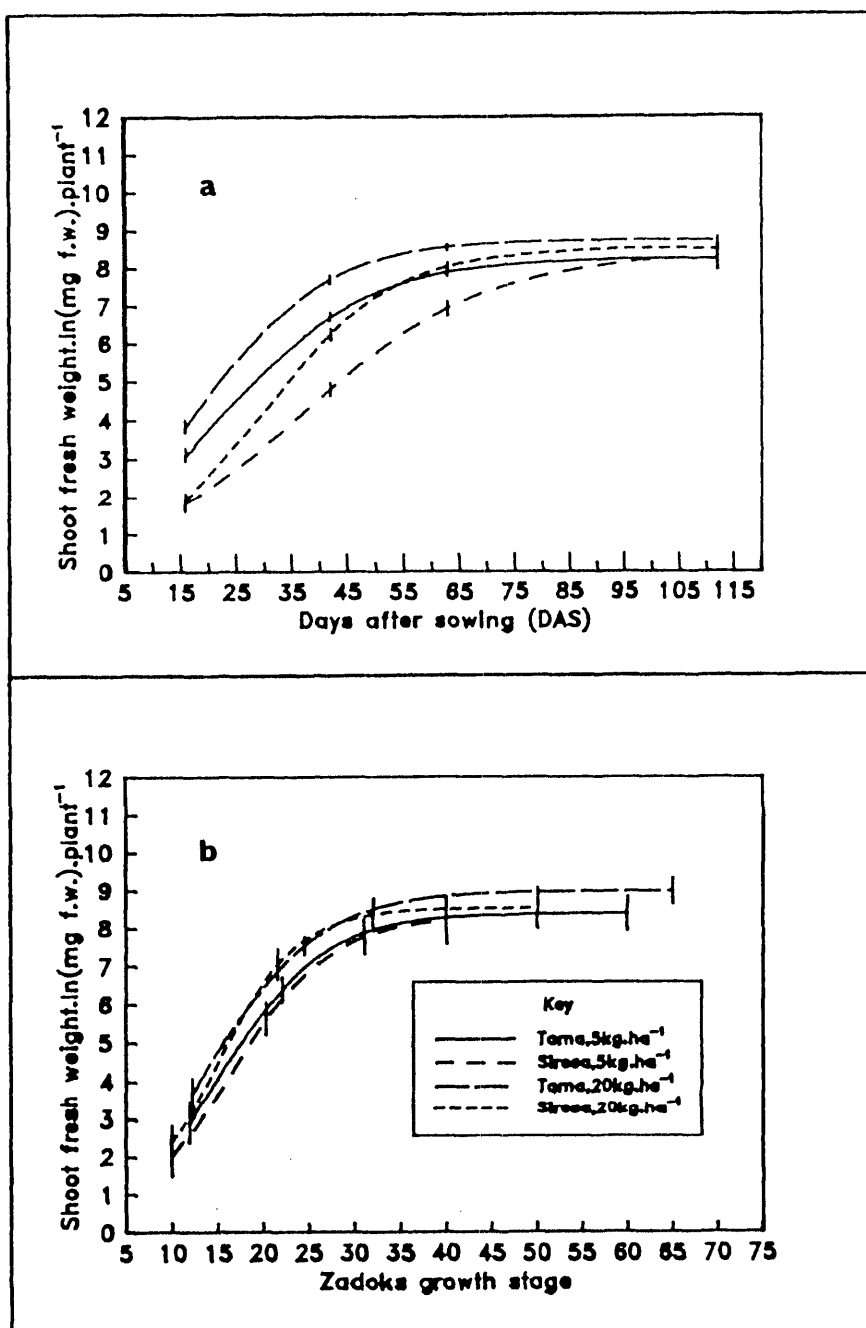


Figure 5.2 The relationship between shoot fresh weight per plant and (a) days after sowing, and (b) Zadoks growth stage for both Tama and Sirosa at two rates of applied P. See Figure 5.1 for further detail.

that at 5 kg P.ha⁻¹ at any given time after sowing largely resulted from the higher P rate advancing the ontogeny of the plants, and thereby the biomass of the plants, rather than from an independent effect on the biomass of the plants (Figure 5.2).

Root fresh weight The effect of P rate on root fresh weight per plant was similar to its effect on shoot fresh weight. On a temporal basis Tama roots outyielded Sirosa roots at both 5 and 20 kg P.ha⁻¹, whereas on an ontogenetic basis the root fresh weight per plant of the two species was not significantly different at either P rate (Figure 5.3). Therefore, once again the difference between the two grass species was in their rate of ontogenesis rather than in their inherent ability to produce root biomass at a given ontogenetic stage.

Shoot:root ratio The shoot:root ratio of Tama at 20 kg P.ha⁻¹ decreased by about 40% after 42 DAS, and thereafter was lower than the shoot:root ratio of Sirosa at 20 kg P.ha⁻¹ (Table 5.7). Moreover, at 5 kg P.ha⁻¹ Tama exhibited a significant reduction in shoot:root ratio of about 40% between 78 and 112 DAS (Table 5.7).

The shoot:root ratio of Tama at 20 kg P.ha⁻¹ was also significantly lower than that of Sirosa at 20 kg P.ha⁻¹ when they were compared at Zadoks growth stages greater than about 25. Interestingly, the sudden decrease in the shoot:root ratio that was observed for Tama at 20 kg P.ha⁻¹ was not observed at 5 kg P.ha⁻¹ until Tama was at a relatively more advanced Zadoks growth stage (Table 5.7, also see Figure 5.5).

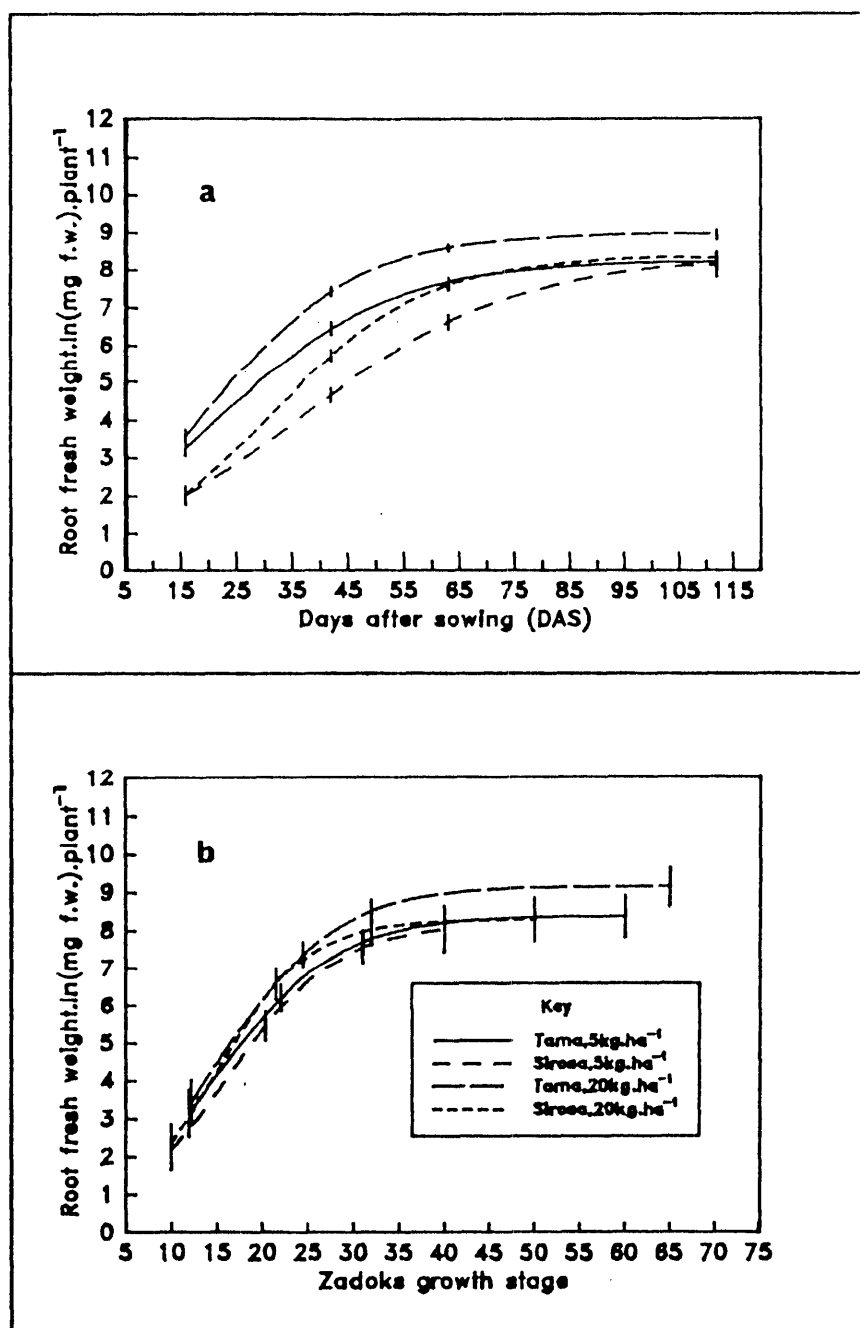


Figure 5.3 The relationship between root fresh weight per plant and (a) days after sowing, and (b) Zadoks growth stage for both Tama and Sirosa at two rates of applied P. See Figure 5.1 for further detail.

Table 5.7 The shoot:root ratios for Tama and Sirosa at two P rates measured from 16 to 112 days after sowing (DAS). The values in brackets are the Zadoks growth stages that were similar for both species within each P rate

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
16	0.82b ^z	0.66b	1.18a (12)	0.78b
29	1.27b	1.31b	1.68a (22)	1.51ab (13)
42	1.28b (22)	1.10b	1.41b	1.77a (22)
51	1.02b	1.17b	0.86b	1.52a
63	1.39a (31)	1.18ab (22)	0.91b (32)	1.29a
78	1.41a	1.50a (31)	0.95b	1.55a (32)
112	0.87b	1.14ab	0.92b	1.30a
DMR# 5% (min.) = 0.33				
DMR# 5% (max.) = 0.41				

z # See Table 5.4

The effect of increasing the P rate from 5 to 20 kg P.ha⁻¹ on the shoot:root ratio of each species was different. When the species were at Zadoks growth stage 22, that is early in their development, the shoot:root ratio of both Tama and Sirosa at 20 kg P.ha⁻¹ was greater than at 5 kg P.ha⁻¹ (cf. Table 5.7). Despite this early similarity in the responses of the two species after approximately Zadoks growth stage 25 the shoot:root ratio of Tama at 20 kg P.ha⁻¹ decreased to a ratio significantly lower than that of Tama at 5 kg P.ha⁻¹, whereas the shoot:root ratio of Sirosa at 20 kg P.ha⁻¹ remained significantly greater, or the same as, the ratio for Sirosa at 5 kg P.ha⁻¹ over all Zadoks growth stages (Table 5.7). Nevertheless, the shoot:root ratios of Tama at 5 and 20 kg P.ha⁻¹ were not significantly different when Tama reached its final Zadoks growth stages for those respective P rates (cf. Table 5.7 and Figure 5.5). Thus,

over the course of the experiment the root weight relative to the shoot weight of neither species exhibited a clearcut increase, that is a decreased shoot:root ratio, in response to the P-limiting conditions provided by the 5 kg P.ha⁻¹ rate.

5.3.1.2 Shoot growth and development

Vegetative development The number of leaves per plant was equal between species for the 5 kg P.ha⁻¹ treatment at 112 DAS but at all other P rates, and at all P rates for other harvest dates there were more leaves per plant on Tama than on Sirosa when they were compared at the same rate of applied P (Table 5.8). A within species comparison showed more leaves were emerged per plant on Tama at 20 kg P.ha⁻¹ than at 5 kg P.ha⁻¹ at most harvests (Table 5.8). In contrast, at most harvests, the number of leaves per plant on Sirosa was unaffected by the P rate (Table 5.8). That is, the phenotypic variation in response to P rate was less in Sirosa than in Tama.

The leaf number results were reflected in the number of tillers per plant data (Table 5.9). When there were more leaves per plant on Tama than on Sirosa there was usually also a greater number of tillers per plant on Tama (cf. Tables 5.8 and 5.9).

On the main stem the number of leaves emerged per plant for Tama and Sirosa was similar at most harvests (Table 5.10). A within species comparison at each harvest showed that on both Tama and Sirosa the number of leaves per main stem was unaffected by P rate (Table 5.10).

Overall, the rate of emergence of leaves and tillers was greater on Tama than on Sirosa when they were compared at each P rate and each harvest (see Tables 5.8 and 5.9). When there were more leaves per plant on Tama than on Sirosa it was the result of the greater number of tillers per plant on Tama than on Sirosa (Table 5.9) rather than the result of changes in the phenology of the main stem (Table 5.10).

Table 5.8 The number (No.) of leaves emerged per plant and the percentage (%) of those leaves that were unfolded for Tama and Sirosa at two P rates measured from 16 to 112 days after sowing

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
number of leaves per plant				
16	1.9a [#]	1.0b	2.2a	1.0b
29	4.4b	2.9c	7.9a	2.9c
42	10.5b	4.3d	20.0a	7.5c
51	17.0b	7.5d	24.0a	10.5c
63	21.5b	11.3c	30.8a	12.5c
78	26.5ab	19.3c	34.0a	24.3bc
112	35.5b	31.8b	51.5a	29.5b
% unfolded leaves				
16	52.5b	11.8c	64.2a	6.3c
29	54.9ab	50.4b	57.5ab	61.5a
42	52.1b	71.3a	49.3b	57.1b
51	58.2a	62.8a	65.7a	58.5a
63	64.4a	70.9a	68.2a	72.0a
78	71.6a	64.3a	74.1a	72.0a
112	82.2a	81.1a	82.4a	85.1a

[#] Means, within each row, are not significantly different (p<0.05) when followed by a common letter. Mean comparisons were made on transformed data.

Table 5.9 The number of tillers plus the main stem per plant for Tama and Siroso at two P rates measured from 16 to 112 days after sowing (DAS)

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Siroso	Tama	Siroso
16	1.0a ^z	1.0a	1.0a	1.0a
29	1.9ab	1.0b	2.6a	1.0b
42	3.0b	1.3c	5.5a	2.5bc
51	5.0ab	2.0c	6.0a	3.5bc
63	6.8a	3.0b	7.8a	3.5b
78	6.0b	4.5b	8.8a	5.8b
112	6.0b	5.3b	8.5a	5.3b
DMR [#] 5% (min.) = 1.6				
DMR [#] 5% (max.) = 1.8				

^z# See Table 5.4

Table 5.10 The number of leaves emerged on the main stem of each plant for Tama and Sirosa at two P rates measured from 16 to 112 days after sowing (DAS).

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
16	1.9a ^z	1.0a	2.2a	1.0a
29	2.2a	2.6a	3.3a	3.0a
42	4.5b	3.8b	6.0a	4.0b
51	5.0a	5.3a	4.8a	5.0a
63	4.8b	6.5a	5.8ab	6.3a
78	4.8c	6.3ab	5.5bc	7.0a
112	7.5a	8.3a	7.8a	8.3a
DMR [#] 5% (min.) = 1.3				
DMR [#] 5% (max.) = 1.5				

^z # See Table 5.4

The percentage of leaves per plant that were unfolded was similar for both species at the two P rates except at 16 DAS (Table 5.8).

At 112 DAS the 47% of yellow or brown leaves, that is senesced leaves, in the total number of leaves for Tama at 5 kg P.ha⁻¹ was significantly greater than the 29% for Sirosa ($p < 0.05$). There was no significant difference between the 46% and the 35% senesced leaves on Tama and Sirosa respectively, at 20 kg P.ha⁻¹ (unpresented data).

Leaf area The leaf area per plant of Tama was greater than that of Sirosa at any given time between 16 and 75 DAS at 5 kg P.ha⁻¹ and 16 and 55 DAS at 20 kg P.ha⁻¹ (Figure 5.4). In contrast, at both 5 and 20 kg P.ha⁻¹ the leaf area per plant of Tama and Sirosa was not significantly different when they were at corresponding Zadoks growth stages (Figure 5.4). This greater leaf area of Tama, relative to that of

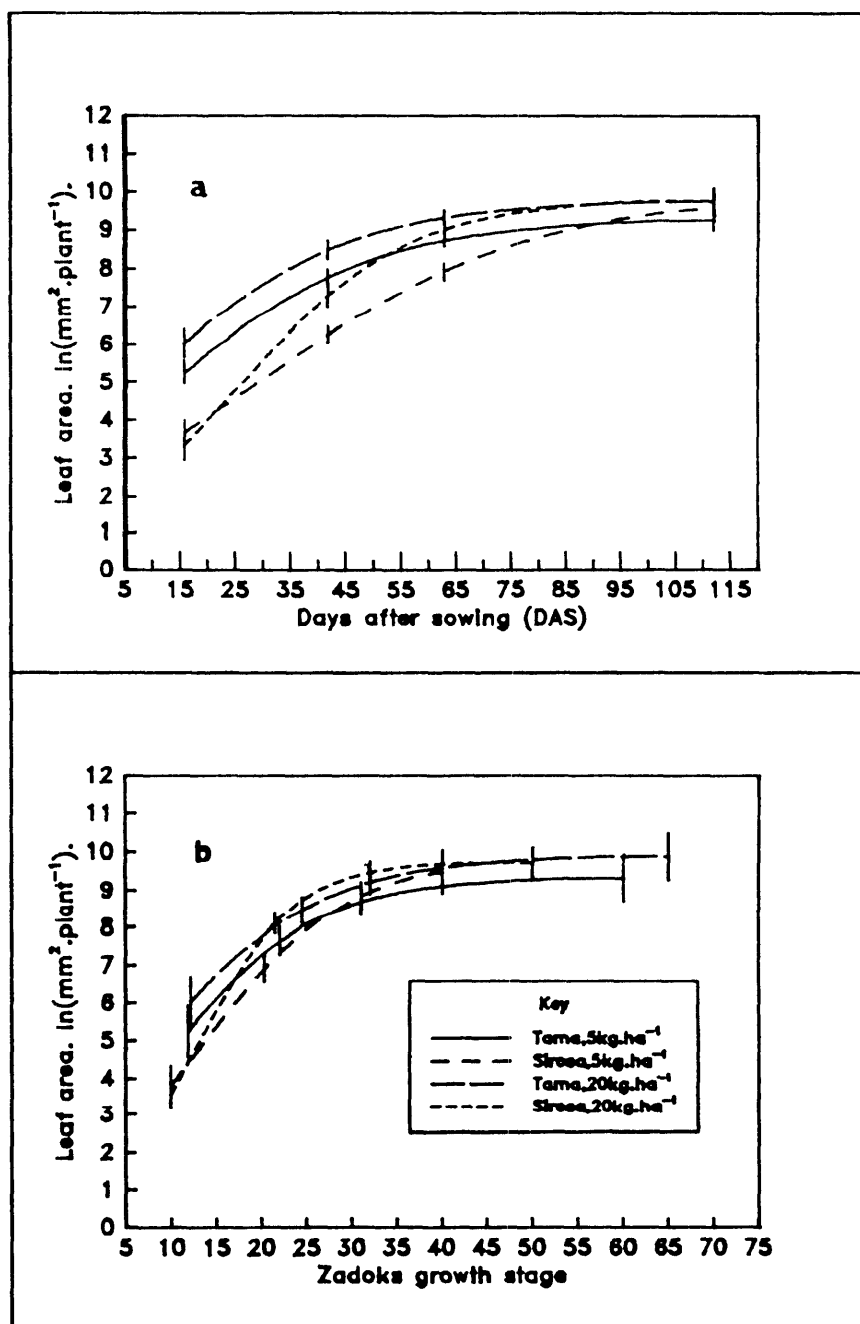


Figure 5.4 The relationship between leaf area per plant and (a) days after sowing, and (b) Zadoks growth stage for both Tama and Sirosa at two rates of applied P. See Figure 5.1 for further detail.

Sirosa, in the first eight to ten weeks after sowing reflected not only the faster growth rate of Tama but showed that in this period the potential for photosynthate production at any given time was greater in Tama than in Sirosa.

Reproductive development The outstanding effect of P rate on reproductive development was the failure of Sirosa, at 5 kg P.ha⁻¹, to develop its inflorescence beyond the double ridge stage (Table 5.11). Although the inflorescences in some Sirosa plants growing at 5 kg P.ha⁻¹ were found moving up the stem they were still at the double ridge stage. Sirosa, at 5 kg P.ha⁻¹ maintained for six weeks after the completion of the experiment also failed to produce heads. Sirosa at the 20 kg P.ha⁻¹ developed normally, but more slowly than Tama, with approximately one head fully emerged per pot by 112 DAS. Such variation between plants in their reproductive status was typical for Sirosa during the latter half of the experiment.

Tama at both 5 and 20 kg P.ha⁻¹ developed from initiation to anthesis during the course of the experiment (Table 5.11). The reproductive development of Tama at 5 kg P.ha⁻¹ was from 2 to 7 days slower than that of Tama at 20 kg P.ha⁻¹ (Table 5.11).

Table 5.11 The days after sowing (DAS) at which Tama and Sirosa reached various reproductive stages on the main stem of the plant

Reproductive Stage	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
Initiation (double ridge)	63	78	61	76
Glume primordium	76	*	69	93
50% heads emerged	102	*	97	*
Anthesis started	112	*	106	*
Anthesis - 50% of heads	*	*	112	*

* Failed to reach this development stage by 112 DAS

Ontogenetic development The ontogeny of Tama and Sirosa, as measured by Zadoks growth stage, at both P rates is shown in Figure 5.5. At both P rates the ontogeny of Tama was more advanced than that of Sirosa (Figure 5.5). The ontogeny of Tama at 20 kg P.ha⁻¹ was usually one to three days ahead of that of Tama at 5 kg P.ha⁻¹, whereas the ontogeny of Sirosa at 5 kg P.ha⁻¹ was similar to that of Sirosa at 20 kg P.ha⁻¹ except after 78 DAS (Figure 5.5). After 78 DAS the Zadoks growth stage of Sirosa at 5 kg P.ha⁻¹ was lower than that at 20 kg P.ha⁻¹ (Figure 5.5) as a result of Sirosa failing to further its reproductive development after initiation (Table 5.11).

5.3.1.3 Root growth and development

Root length The effect of P rate on the root length per plant was largely similar to its effect on root fresh weight per plant (cf. Table 5.12 and Figure 5.3). Again, at each harvest the root length per plant of Tama was greater than the root length per plant of Sirosa when they were compared at either P rate (Table 5.12). Also, as was observed with other measures of biomass allocation, the root lengths per plant of Tama and of Sirosa were similar when compared at the same Zadoks growth stages at either P rate (cf. Table 5.12 and Appendix 5.3).

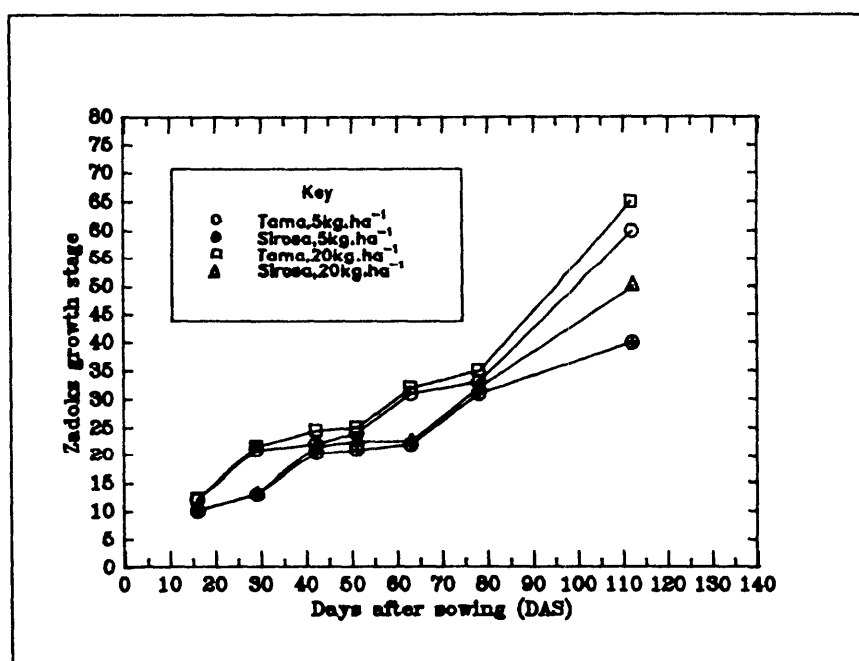


Figure 5.5 The Zadoks growth stage of Tama and of Sirosa at seven harvest times from 16 to 112 days after sowing, and at two rates of applied P.

Table 5.12 The root length per plant of Tama and Sirosa at two rates of applied P measured from 16 to 112 days after sowing (DAS)

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
	(m.plant ⁻¹)			
16	0.25a [#]	0.04b	0.37a	0.05b
29	2.27b	0.22d	4.60a	0.42c
42	7.99b	1.29d	15.05a	2.24c
51	18.47b	2.71d	42.64a	7.19c
63	38.59b	12.16c	62.12a	29.18b
78	48.45b	23.06c	94.60a	42.31b
112	118.25ab	47.92c	168.63a	77.39b

See Table 5.8

Up to 78 DAS the root length per unit fresh weight of Tama was substantially unchanged as was that of Sirosa throughout the course of the experiment (Table 5.13). There was, however, a difference between Tama and Sirosa in the way their root size changed from 78 to 112 DAS. In contrast to the little or no change in the root fresh weight per plant in this period (Figure 5.3) there was a large increase in root length per plant between 78 and 112 DAS, particularly by Tama (Table 5.12). This rapid proliferation in the root length of Tama relative to that of Sirosa was mainly the result of Tama, at both P rates, increasing its root length per unit root fresh weight between 78 and 112 DAS (Table 5.13). Apart from the change in Tama between 78 and 112 DAS there was no effect of either P rate or species on root length per unit root fresh weight (Table 5.13).

Root extension rate The mean root extension rates of Tama and of Sirosa were apparently sensitive to the switch by both species from the vegetative to the reproductive phase. For all four treatments the mean root extension rate increased with time up to the time of initiation (Table 5.14). At initiation the mean root extension rates of the four treatments were either decreasing, with a subsequent increase during later harvest intervals, or were starting to plateau (Table 5.14). The times that the plants in the four treatments were observed to initiate were 63 and 61 DAS for Tama at 5 and 20 kg P.ha⁻¹ respectively and 78 and 76 DAS for Sirosa at 5 and 20 kg P.ha⁻¹ respectively (see Table 5.11).

Table 5.13 The length per unit fresh weight of the roots of Tama and of Sirosa at two P rates measured from 16 to 112 days after sowing (DAS)

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
	(m.g fwt ⁻¹)			
16	9.1a [#]	4.5a	9.9a	6.3a
29	5.8a	9.1b	14.5ab	9.0b
42	3.1a	13.1a	9.1a	7.2a
51	1.7a	8.1a	11.0a	7.8a
63	9.2a	13.1ab	12.6b	13.3ab
78	5.0a	18.1a	14.7a	14.8a
112	39.8a	13.4c	22.6b	18.5bc

See Table 5.8

Table 5.14 The root extension rate of Tama and of Sirosa at two P rates measured from 16 to 112 days after sowing (DAS). The root extension rates were calculated by taking the mean daily root extension rate for each harvest interval

DAS	5 kg P.ha ⁻¹				20 kg P.ha ⁻¹			
	Tama		Sirosa		Tama		Sirosa	
(mm root.day ⁻¹)								
0- 16	y ^A	16a ^B	x	2b	x	23a	y	3b
16- 29	x	155a	w	14b	w	326a	x	29b
29- 42	xw	440a	v	82b	v	804a	w	140b
42- 51	uv	1165b	v	158c	u	3066a	v	550b
51- 63	uv	1677a	u	787a	v	1623a [#]	u	1832a
63- 78	vw	616b [#]	u	727b	u	2165a	uv	875b [#]
78-112	u	2080a	u	731b [#]	u	2177a	uv	1032a

[#] Harvest interval in which initiation occurred

A Means within each column preceded by the same letter are not significantly different (p<0.05)

B Means within each row followed by the same letter are not significantly different (p<0.05)

Infection of roots with VA mycorrhizal fungi At

20 kg P.ha⁻¹ the percentage of the root cortex infected with vesicular arbuscular mycorrhizal fungi in Tama was less than half that in Sirosa (Table 5.15). In contrast, the percentage infections of Tama roots and of Sirosa roots, at 5 kg P.ha⁻¹, were similar (Table 5.15). That is, the percentage infection with VA mycorrhizal fungi of Tama roots was greatly reduced when the P rate was increased from 5 to 20 kg P.ha⁻¹ whereas the percentage infection of Sirosa roots was usually unresponsive to P rate (Table 5.15). This low percentage of infection in Tama roots at 20 kg P.ha⁻¹

suggested that mycorrhizas did not play a significant role in P absorption by these roots, but did so in the P absorption by the roots of the plants in the other three treatments.

Table 5.15 The percentage of the root cortex infected with vesicular arbuscular mycorrhizal fungi in the roots of Tama and of Sirosa at two P rates measured from 16 to 112 days after sowing (DAS)

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
16	5.0a [#]	5.0a	5.0a	5.0a
29	19.5a	29.5a	5.0b	28.0a
42	69.5a	76.0a	18.0c	49.0b
51	66.0b	81.0a	20.5d	41.5c
63	73.0a	79.0a	21.0b	68.5a
78	91.0a	91.0a	30.0c	70.0b
112	78.0a	80.0a	19.5b	70.0a

[#] See Table 5.8

5.3.2 GROWTH ANALYSIS OF TAMA AND OF SIROSA

5.3.2.1 Temporal growth analysis At 5 kg P.ha⁻¹ the unit leaf rate of Tama was greater than that of Sirosa until 44 DAS, after which the unit leaf rate of Sirosa exceeded that of Tama (Figure 5.6). At 20 kg P.ha⁻¹ the unit leaf rate of Sirosa was greater than that of Tama until 36 DAS (Figure 5.6). There was little difference between the unit leaf rate of Sirosa and of Tama after 36 DAS (Figure 5.6).

That after 56 DAS the unit leaf rate of Sirosa at 5 kg P.ha⁻¹ was greater than the unit leaf rate of the other three treatments was further evidence that the ontogeny of Sirosa was markedly slowed at the low P rate, and as a result was in the vegetative phase longer (Figure 5.6).

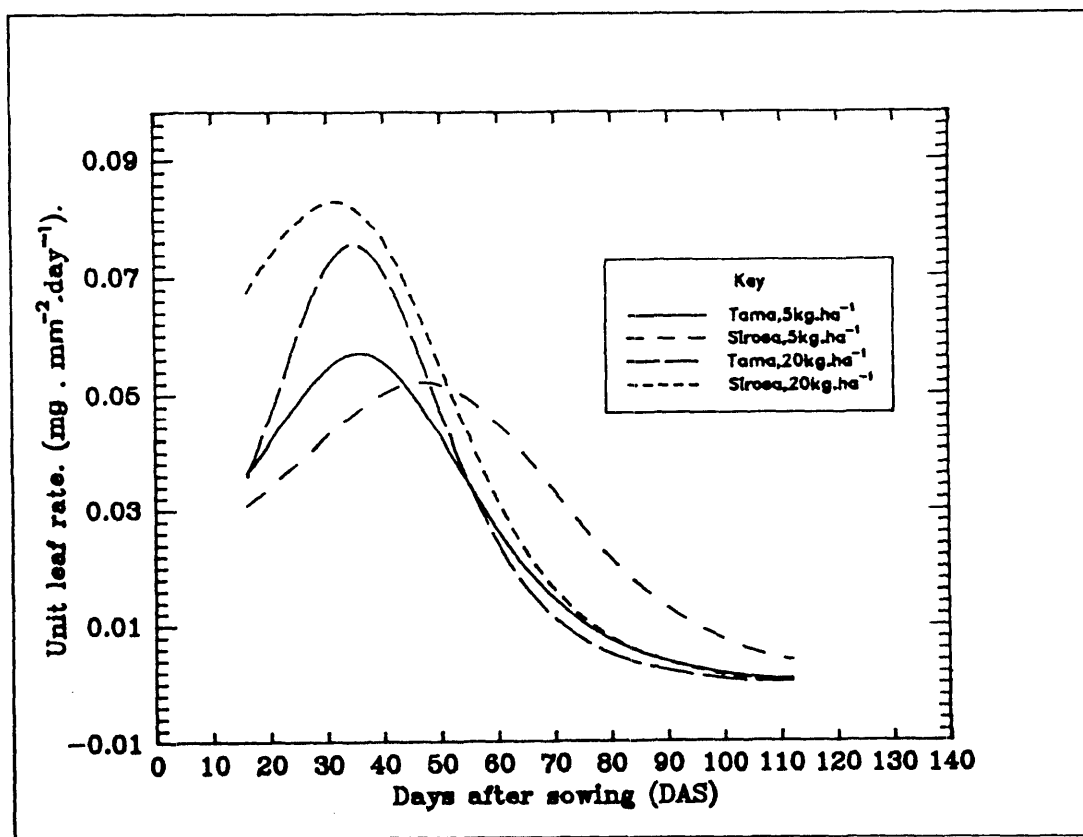


Figure 5.6 The instantaneous unit leaf rate (ULR) of Tama and of Sirosa from 16 to 112 days after sowing, and at two rates of applied P.

Examination of Table 5.16 shows that the above differences in the unit leaf rates of the treatments were the result of corresponding differences in both the relative growth rate and the leaf area ratio of the treatments.

5.3.2.2 Ontogenetic growth analysis Usually the unit leaf rates of Tama and Sirosa, compared at the same P rate, were similar at any given Zadoks growth stage (Appendix 5.2). The only exception was at 20 kg P.ha⁻¹ when the unit leaf rate of Sirosa was greater than that of Tama between Zadoks growth stages 12 and 20 (Appendix 5.2).

5.3.2.3 Photosynthetic rate Overall, there was no consistent significant difference between either Tama and Sirosa or between 5 and 20 kg P.ha⁻¹ in the net photosynthetic rate per unit fresh weight, whether it was measured on the oldest or the youngest fully expanded leaf on the main stem, on either a temporal or an ontogenetic basis (Table 5.17; cf. Appendix 5.3). Nevertheless, at 20 kg P.ha⁻¹ the net photosynthetic rate of the oldest fully expanded leaf of Sirosa at Zadoks growth stages 10 and 13 (16 and 29 DAS) was greater than the net photosynthetic rate of the equivalent leaf of Tama at Zadoks growth stage 12 (16 DAS) (Table 5.17). This superior net photosynthetic rate of the oldest fully expanded leaf of Sirosa, at 20 kg P.ha⁻¹, relative to that of Tama, at 20 kg P.ha⁻¹, when there was one or two unfolded leaves per plant was in agreement with the unit leaf rate results (see Appendix 5.2, Table 5.17).

The chlorophyll concentrations of the oldest and youngest leaves on the main stems of the plants were similar for Tama and Sirosa within each P rate (Tables 5.18). A within species comparison showed there was usually no effect of P rate on chlorophyll concentration (Table 5.18).

Table 5.16 The instantaneous relative growth rate (RGR) and the leaf area ratio (LAR) for Tama and Sirosa measured from 16 to 112 days after sowing (DAS). The values were generated from the equations fitted to total plant fresh weight (see Figure 5.1) and to leaf area (see Figure 5.4)

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
RGR (day ⁻¹)				
16	0.15	0.10	0.18	0.14
29	0.14	0.12	0.16	0.17
42	0.09	0.11	0.09	0.13
51	0.06	0.10	0.05	0.09
63	0.03	0.07	0.02	0.05
78	0.01	0.04	0.01	0.02
112	0.00	0.01	0.00	0.00
LAR (mm ² .mg ⁻¹)				
16	4.01	3.03	5.04	2.02
29	2.52	2.72	2.17	2.01
42	1.65	2.15	1.25	1.78
51	1.41	1.85	1.09	1.69
63	1.30	1.64	1.06	1.68
78	1.30	1.62	1.12	1.75
112	1.38	1.90	1.23	1.84

Table 5.17 The net photosynthetic rate (P_N) per unit fresh weight of the oldest and the youngest fully expanded leaves on the main stem of Tama and of Sirosa at two P rates measured from 16 to 112 days after sowing (DAS)

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
P_N (nmoles O ₂ .g fwt ⁻¹ .s ⁻¹) oldest leaf				
16	48a #	71a	29b	63a
29	59a	70a	61a	50a
42	50ab	64ab	47b	71a
51	34a	56a	35a	49a
63	31ab	47a	17c	30b
78	29ab	39a	18c	22bc
112	32a	27a	27a	23a
youngest leaf				
29	33a	36a	35a	43a
42	47a	40a	45a	51a
51	44a	35a	39a	45a
63	38a	41a	33a	43a
78	32a	43a	37a	35a
112	54a	28b	52a	36b

See Table 5.8

Table 5.18 The chlorophyll concentration of the oldest and youngest fully expanded leaves on the main stem of Tama and of Sirosa at two P rates measured from 16 to 112 days after sowing (DAS)

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
(mg chlorophyll.g.fwt ⁻¹) oldest leaf				
16	1.94a [#]	1.67ab	1.77ab	1.32b
29	2.78a	3.06a	2.51a	2.80a
42	2.11b	3.09a	2.18b	2.60ab
51	2.54a	2.62a	1.92b	2.63a
63	1.86a	1.65a	1.35a	1.53a
78	2.03a	2.00a	1.29b	1.09b
112	1.15a	0.93a	0.71a	0.91a
youngest leaf				
29	1.74a	2.05a	1.75a	2.03a
42	1.85a	1.53a	1.82a	1.65a
51	2.31a	1.70b	1.79ab	2.21ab
63	1.80a	1.57a	1.58a	1.84a
78	1.23c	2.09a	1.82ab	1.27bc
112	2.32a	1.25b	1.78b	1.20b

[#] See Table 5.8

5.3.3 P UPTAKE AND P DISTRIBUTION BY TAMA AND BY SIROSA

5.3.3.1 P uptake or total P content per plant The P uptake per plant, or total P content per plant, of Tama was greater than that of Sirosa at both 5 and 20 kg P.ha⁻¹ when they were compared at any given time, except between 80 and 112 DAS when at 5 kg P.ha⁻¹ the P uptake per plant of Tama was the same as that of Sirosa (Figure 5.7a). On the other hand, when the P uptakes per plant of Tama and Sirosa were compared at any given stage during their ontogeny they were the same whether the P rate was 5 or 20 kg P.ha⁻¹ (Figure 5.7b). That there was no difference between the P uptake per plant of Tama and Sirosa at any given ontogenetic stage was further corroborated when the P uptake per plant of each species was related to the number of leaves per plant (Figure 5.7c).

Nevertheless, as was observed with plant yield (see Section 5.4.1.1) and related measurements, the P uptake per plant of Tama at 20 kg P.ha⁻¹ was greater, but not significantly so, than the P uptake per plant of Sirosa at 20 kg P.ha⁻¹, after approximately Zadoks growth stage 30 (Figure 5.7).

5.3.3.2 P uptake per unit of root When the P uptakes per unit root length of Tama and of Sirosa were compared on a temporal basis the P uptake per unit root length of Sirosa was greater, overall, than that of Tama at 5 kg P.ha⁻¹, but there was no difference between the two species at 20 kg P.ha⁻¹ (Table 5.19). However, this difference between Tama and Sirosa on a temporal basis at 5 kg P.ha⁻¹ was, at least, partly the result of Tama being at a more advanced stage in its ontogeny than Sirosa was at any given time (see Figure 5.5).

Comparison of the P uptake per unit root length of Tama with that of Sirosa, at 5 kg P.ha⁻¹, at harvest times where the Zadoks growth stages of the two species were alike (see Appendix 5.3) demonstrates that on this basis the P uptakes of Tama and of Sirosa were similar (Table 5.19). At 20 kg P.ha⁻¹ the P uptake per root length of Tama was no

Figure 5.7 The relationship between P uptake per plant, or P content per plant, and (a) days after sowing, (b) Zadoks growth stage, and (c) number of leaves per plant for both Tama and Sirosa at two rates of applied P. See Figure 5.1 for further details.

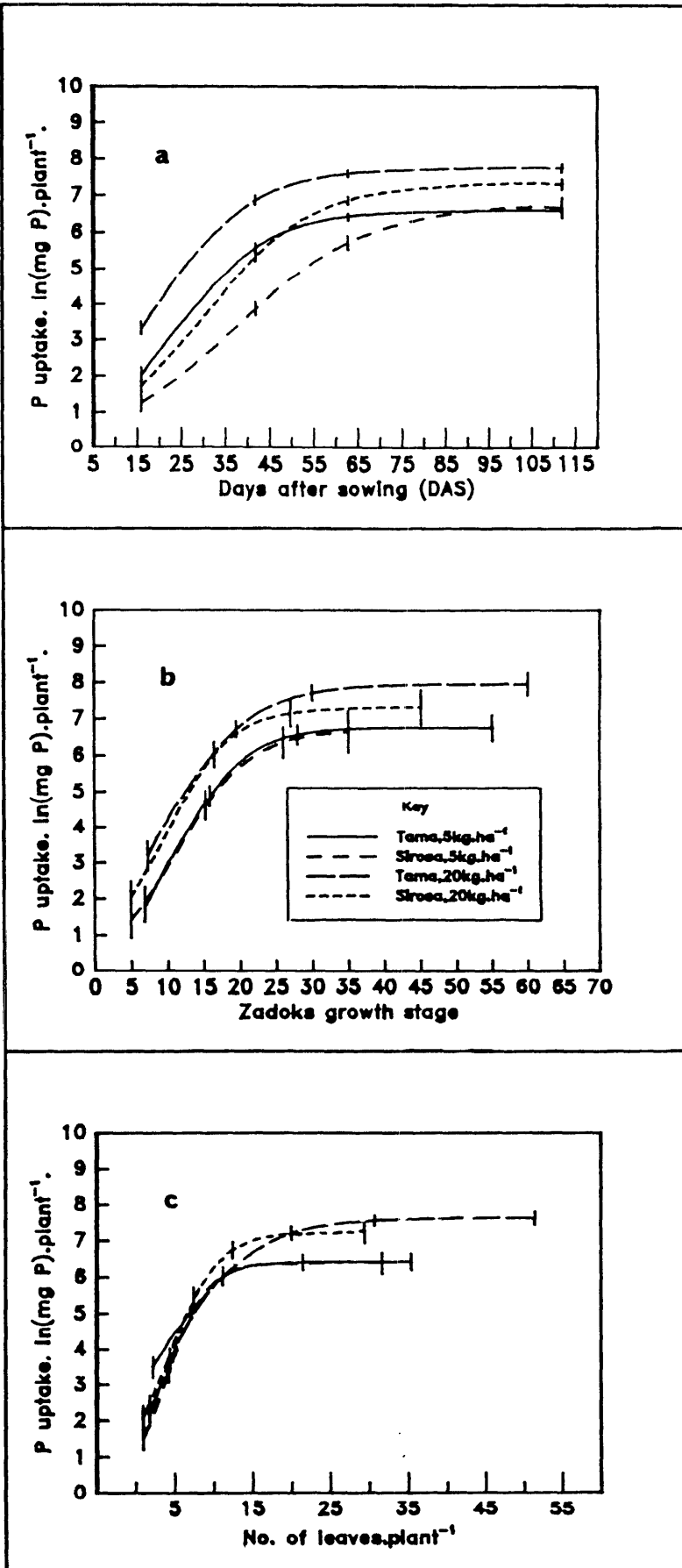


Table 5.19 The P uptake per unit root length and the P uptake per unit root fresh weight of Tama and of Sirosa at two P rates measured from 16 to 112 days after sowing (DAS)

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
P uptake.root length ⁻¹ (µg P.m ⁻¹)				
16	29.6b [#]	104.0a	75.6a	103.3a
29	28.4c	52.8b	71.1ab	95.4a
42	32.3b	32.9b	78.5a	100.4a
51	25.2c	53.6ab	33.1bc	65.1a
63	17.3b	31.7a	32.4a	33.1a
78	19.6b	20.4b	22.4ab	33.3a
112	5.3b	18.4a	15.3a	20.2a
P uptake.root fresh weight ⁻¹ (µg P.g ⁻¹)				
16	268c	462b	725a	637ab
29	449b	477b	803a	834a
42	397b	428b	619a	713a
51	280c	431ab	339bc	489a
63	321a	408a	407a	431a
78	291b	353b	321b	487a
112	191b	242b	335a	369a

See Table 5.8

different from that of Sirosa when they were compared on an ontogenetic basis (Table 5.19, see Appendix 5.3).

Alternatively, when the P uptakes per unit root fresh weight of Tama and of Sirosa were compared there was no difference, overall, between the species at either of the two P rates regardless of whether the comparison was on a temporal or an ontogenetic basis (Table 5.19, see Appendix 5.3).

5.3.3.3 P content of roots per plant Figure 5.8 shows that the P content of the roots (on a per plant basis) of Tama and of Sirosa was influenced by time and by the ontogeny of the plants in a similar fashion to that noted for P content per plant (see Section 5.4.3.1). Again, as with plant P content, within each P rate the root P contents of both species were similar when they were compared at the same Zadoks growth stage (Figure 5.8).

At 5 kg P.ha⁻¹ the root P concentration of Tama ranged from a maximum of 144 to a minimum of 84 µg P.g fresh weight⁻¹ whereas the respective range for Sirosa at 5 kg P.ha⁻¹ was from 260 to 104 µg P.g fresh weight⁻¹. At 20 kg P.ha⁻¹ Tama exhibited a range in the P concentration of its roots from 187 to 111 µg P.g fresh weight⁻¹ compared with the range for Sirosa of 311 to 113 µg P.g fresh weight⁻¹.

5.3.3.4 P content of shoots per plant The response of the shoot P content of Tama and of Sirosa paralleled the response of plant P content and root P content (Figure 5.9). That is, shoot P content of both species was greater at 20 than at 5 kg P.ha⁻¹, and the difference between Tama and Sirosa at each P rate on a temporal basis was again shown to be related to their different rates of ontogeny (Figure 5.9).

The P concentration in the shoots of Tama ranged from a maximum of 240 to a minimum of 118 µg P.g fresh weight⁻¹ at 5 kg P.ha⁻¹ and from 461 to 175 µg P.g fresh weight⁻¹ at 20 kg P.ha⁻¹ during the course of the experiment. The range in the shoot P concentrations for Sirosa at 5 kg P.ha⁻¹ was

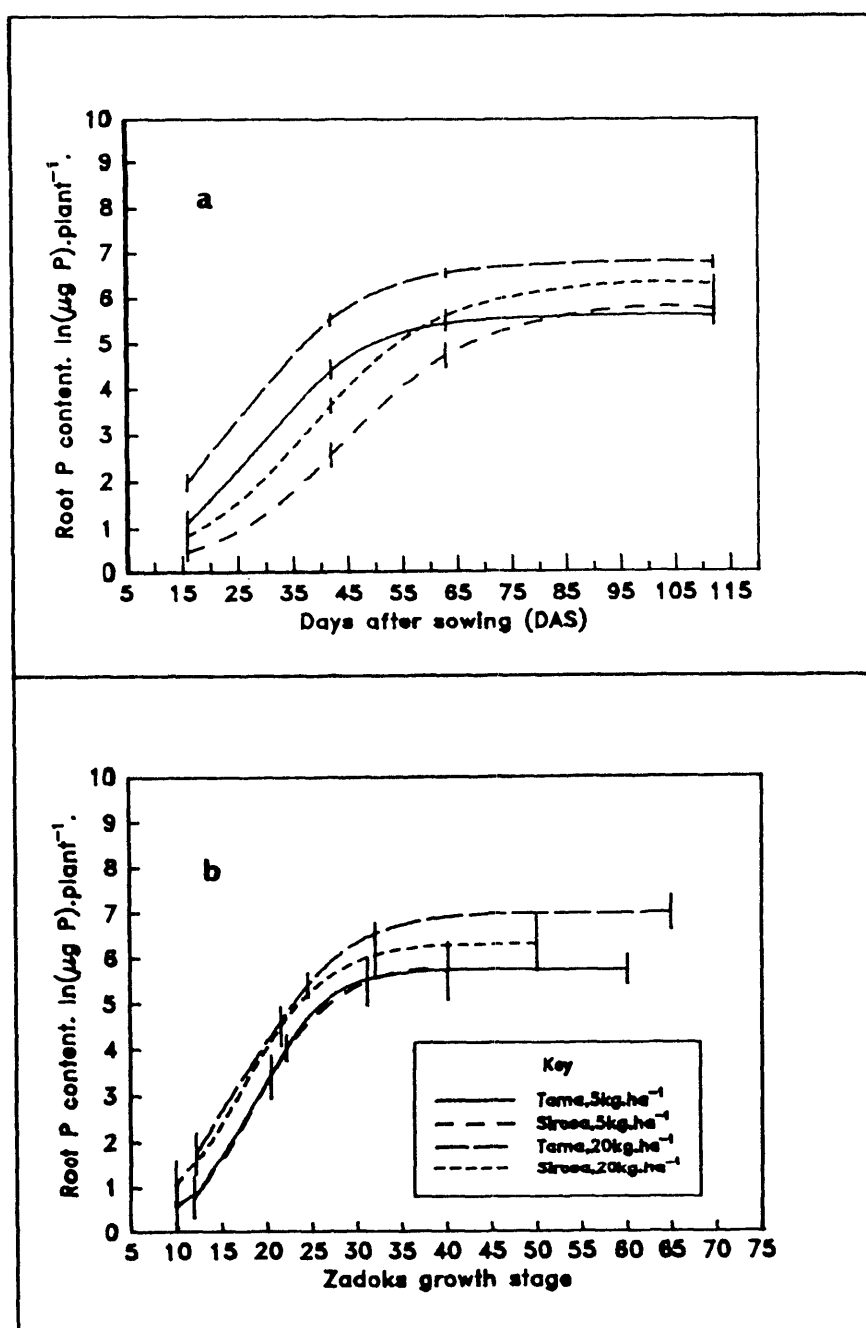


Figure 5.8 The relationship between root P content per plant and (a) days after sowing, and (b) Zadoks growth stage for both Tama and Sirosa at two rates of applied P. See Figure 5.1 for further details.

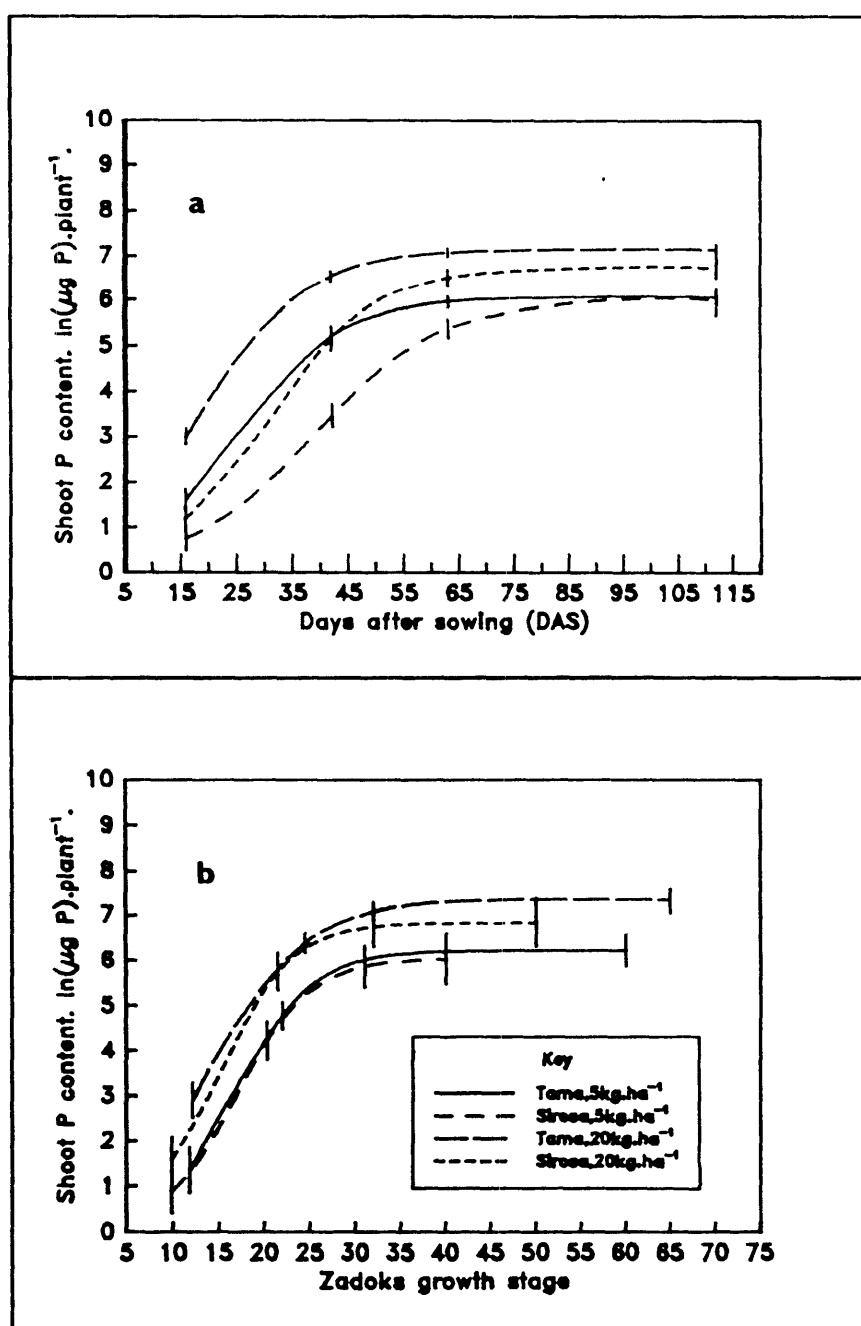


Figure 5.9 The relationship between shoot P content per plant and (a) days after sowing, and (b) Zadoks growth stage for both Tama and Sirosa at two rates of applied P. See Figure 5.1 for further details.

from 306 to 117 $\mu\text{g P.g fresh weight}^{-1}$ while at 20 kg P.ha $^{-1}$ it was from 437 to 179 $\mu\text{gP.g fresh weight}^{-1}$.

5.3.3.5 Net transport of P to the shoots On both a temporal and an ontogenetic basis the percentage net transport of P to the shoots of both Tama and Sirosa was similar, with greater than 50% of the P absorbed being transported to the shoots of both species (Table 5.20, see also Appendix 5.3).

An exception to this similarity occurred at 16 DAS when the percentage net transport of P to the shoots of Tama was greater than that of Sirosa, but this difference was no doubt related to differences in the ontogeny of the two species at that time (Table 5.20, see also Figure 5.5). Overall, the percentage net transport of P to the shoots of both species was similar at both P rates (Table 5.20). Therefore, relative to each other the shoot P content of neither species was limited by a capacity to transport P from the roots to the shoots.

Table 5.20 The percentage (%) net transport of P to the shoots of Tama and of Sirosa at two P rates measured from 16 to 112 days after sowing (DAS)

DAS	5 kg P.ha $^{-1}$		20 kg P.ha $^{-1}$	
	Tama	Sirosa	Tama	Sirosa
16	63.8a [#]	43.7b	74.1a	49.6b
29	67.4b	70.9ab	79.3a	79.2a
42	69.0b	73.5b	73.9b	83.9a
51	64.9a	66.9a	67.3a	73.1a
63	63.6a	61.8a	63.3a	65.5a
78	67.6a	63.5a	63.3a	70.8a
112	55.1a	55.0a	59.7a	63.2a

[#] See Table 5.8

5.3.3.6 P fractions content of roots At 5 kg P.ha⁻¹ the acid-soluble-P content of the roots of Tama decreased between 63 (Zadoks growth stage 31) and 112 DAS (Zadoks growth stage 60) whereas over the same time period (Zadoks growth stages 22 to 40) the acid-soluble-P content of the roots of Sirosa increased (Tables 5.21, 5.23; see also Figure 5.5). As a result of this decreased acid-soluble P content of the roots of Tama, at 5 kg P.ha⁻¹, the acid-soluble P concentration of its roots at 112 DAS was 1 µg P.g root fresh weight⁻¹ compared with 9 µg P.g root fresh weight⁻¹ for Sirosa at 5 kg P.ha⁻¹, and with 14 and 15 µg P.g root fresh weight⁻¹ for Tama and Sirosa respectively at 20 kg P.ha⁻¹.

At 20 kg P.ha⁻¹ the acid-soluble P contents of the roots of Tama and of Sirosa were similar up to about Zadoks growth stage 30 (Table 5.23). After Zadoks growth stage 30 the acid-soluble-P content, at 20 kg P.ha⁻¹, of the roots of Tama was about twice that of the roots of Sirosa (Table 5.23). Nevertheless, as reported for the total P content of roots, the acid-soluble P content of the roots of Tama was greater than that of the roots of Sirosa when they were compared on a temporal basis (Table 5.21).

Both the P-lipid and the residue-P contents of the roots of Tama and of Sirosa behaved similarly to the total P content of the roots when they were related to either days after sowing or ontogeny (cf. Tables 5.21, 5.23; and Figure 5.8). The usually greater contents of P-lipid and of residue-P in the roots of Tama relative to the roots of Sirosa at any given time were clearly related to the temporal differences in the ontogeny of the two species (cf. Tables 5.21 and 5.23).

5.3.3.7 P fractions content of shoots The content of all three P fractions, acid-soluble-P, P-lipid, and residue-P, was usually greater in the shoots of Tama than in the shoots of Sirosa at any given time or P rate (Table 5.22). The exceptions to this overall trend were the lower acid-soluble-P content of Tama shoots relative to that of Sirosa shoots at 5 kg P.ha⁻¹ and 112 DAS, and the similar residue-P contents of Tama and Sirosa at 112 DAS at both P rates (Table 5.22).

On an ontogenetic basis there was no difference between Tama and Sirosa at 5 kg P.ha⁻¹ in the way in which the three P fractions in the shoots responded to the plants' development (Table 5.24). At 20 kg P.ha⁻¹, however, the content of the P fractions in the shoots for Tama and Sirosa was similar at the early growth stages but differed after about Zadoks growth stage 30 (Table 5.24). At these later growth stages both the acid-soluble-P and the P-lipid contents of the shoots of Tama were greater than those in the shoots of Sirosa (Table 5.24). Nevertheless, the residue-P content of the shoots of both species was not different at any given growth stage (Table 5.24). As the residue-P fraction provided about two-thirds of the total P content of the shoots for both species it tended to dampen the effect of the variation between Tama and Sirosa in the acid-soluble-P content and P-lipid content of their shoots (Table 5.24) when the total P content of the shoots of the two species was compared (see Figure 5.9).

Table 5.21 The P content of the roots of Tama and of the roots of Sirosa present as acid-soluble-P, as P-lipid, and as residue-P, on a per plant basis, at two P rates over time

DAS	5 kg P.ha ⁻¹				20 kg P.ha ⁻¹			
	Tama		Sirosa		Tama		Sirosa	
μg P as acid-soluble-P								
16	y ^A	<1b ^B	x	<1b	x	1a	y	1a
29	x	2b	x	<1c	w	9a	y	2b
42	wv	6b	x	<1c	v	42a	x	5b
51	vu	10c	w	5d	u	78a	w	22b
63	u	12c	v	12c	u	119a	wv	32b
78	wvu	9d	v	14c	u	88a	vu	36b
112	w	4c	u	37b	u	112a	u	60b
μg P as P-lipid								
16		<1b		<1b		1a		<1b
29		4b		<1c		10a		2c
42		13b		2d		59a		5c
51		27b		8c		108a		21b
63		31c		23c		138a		55b
78		30c		27c		127a		69b
112		35c		39c		147a		62b
μg P as residue-P								
16		2b		1b		5a		1bc
29		14b		2d		33a		5c
42		56b		9d		169a		25c
51		117b		33d		250a		74c
63		189b		112c		469a		235b
78		216c		120d		515a		303b
112		292c		319c		810a		432b

A B See Table 5.14

Table 5.22 The P content of the shoots of Tama and of the shoots of Sirosa present as acid-soluble-P, as P-lipid, and as residue-P, on a per plant basis, at two P rates over time

DAS	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
µg P as acid-soluble-P				
16	1b ^B	1b	6a	1b
29	6b	2c	40a	4b
42	15c	4d	213a	30b
51	36b	10c	313a	43b
63	46c	32c	394a	92b
78	49c	34c	284a	139b
112	43c	94b	390a	144b
µg P as P-lipid				
16	1b	<1b	3a	1b
29	5b	1c	23a	4b
42	18b	4c	95a	22b
51	38b	14c	111a	36b
63	55b	34c	147a	60b
78	69b	31c	202a	126a
112	37c	30c	180a	68b
µg P as residue-P				
16	4b	1c	12a	1c
29	32b	6d	136a	21c
42	135b	23c	458a	127b
51	215b	66c	483a	243b
63	302c	167d	730a	461b
78	401b	229c	792a	715a
112	319b	385b	1085a	756a

B See Table 5.8

Table 5.23 The P content of the roots of Tama and of the roots of Sirosa present as acid-soluble-P, as P-lipid, and as residue-P, on a per plant basis, at two P rates over a range of Zadoks growth stages. The contents were derived by detransforming values generated from the logistic functions developed to describe the relationship between the P content of each P fraction in the roots and Zadoks growth stage (see Appendix 5.1)

Zadoks growth stage	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
µg P as acid-soluble-P				
12	<1	<1	<1	1
15	<1	<1	11	3
20	3	1	11	10
30	7	19	76	33
40	8	31	118	51
50	8	-	129	59
µg P as P-lipid				
12	<1	<1	1	1
15	2	1	3	3
20	7	5	18	12
30	27	24	96	46
40	36	40	159	67
50	38	-	179	74
µg P as residue-P				
12	2	2	4	3
15	6	6	11	9
20	28	23	51	46
30	153	134	319	241
40	273	296	663	402
50	324	-	855	460

Table 5.24 The P content of the shoots of Tama and of the shoots of Sirosa present as acid-soluble-P, as P-lipid, and as residue-P on a per plant basis, at two P rates over a range of Zadoks growth stages. The contents were derived by detransforming values generated from the logistic functions developed to describe the relationship between the P content of each P fraction in the shoots and Zadoks growth stage (see Appendix 5.1)

Zadoks growth stage	5 kg P.ha ⁻¹		20 kg P.ha ⁻¹	
	Tama	Sirosa	Tama	Sirosa
µg P as acid-soluble-P				
12	1	1	3	2
15	2	2	13	7
20	9	8	64	33
30	37	38	265	111
40	51	71	376	145
50	54	-	406	153
µg P as P-lipid				
12	1	1	3	2
15	2	2	8	7
20	10	9	31	31
30	39	26	121	70
40	53	31	177	77
50	57	-	195	78
µg P as residue-P				
12	3	3	1-	7
15	12	10	34	34
20	61	50	150	189
30	263	229	630	560
40	379	342	957	654
50	411	-	1068	668

5.4

DISCUSSION

5.4.1 THE RELATIONSHIP BETWEEN P EFFICIENCY AND ONTOGENY

On a variety of measures of P efficiency Tama was similar to Sirosa on an ontogenetic basis but superior on a temporal basis. Clearly, it is necessary when evaluating the comparative P efficiency of genotypes to relate measures of P efficiency of pasture plant genotypes to the ontogenetic stage at the time of measurement. This makes it possible to distinguish between ontogenetically based and physiologically based differences in the P efficiency of plant genotypes.

5.4.1.1 Biomass allocation and growth rate The biomass allocation of Tama and of Sirosa was similar on an ontogenetic basis (e.g. Figure 5.1). Yet, on a temporal basis the shoot and root yields of Tama were greater than those of Sirosa at both 5 and 20 kg P.ha⁻¹ (Figures 5.2, 5.3). Thus, if an agronomic definition of P efficiency, such as shoot yield per unit of P applied (Blair and Cordero, 1978), was used then Tama would be deemed the more P efficient of the two species at any given time because although it produced the same shoot yield as Sirosa when at the same Zadoks growth stage it developed faster.

The greater yields of Tama relative to those of Sirosa at any given time differed from the findings of Caradus (1980) for perennial ryegrass and phalaris. He found that although the root yield of perennial ryegrass was greater than that of phalaris at both low and high rates of applied P the relative positions of the two species were reversed for shoot yield.

Caradus (1980) reported mean shoot:root ratios of 1.3 and 2.0 for perennial ryegrass and phalaris respectively, whereas the values found in this study for Tama and Sirosa were lower at 0.9 and 1.2 respectively. Nevertheless, the variation in shoot:root ratio found in introduced pasture grasses is demonstrated by the values of 2.0 reported for annual ryegrass (Ozanne et al., 1969) and of 0.8 reported

for Chewings fescue (Festuca rubra) (Caradus, 1980). In neither Tama or Sirosa was the shoot:root ratio decreased in response to the lower P rate of 5 kg P.ha⁻¹ (Table 5.7). This lack of plasticity in the shoot:root ratios of Tama and Sirosa in response to P deficient conditions was similar to that reported for annual ryegrass (Ozanne et al., 1969) and mulga grass (Thyridolepis mitchelliana (Nees) S.T. Blake), but in contrast to that reported for a number of other pasture species (Christie and Moorby, 1975; Clarkson and Hanson, 1980).

The greater yield of Tama relative to that of Sirosa, at any given time, was related to it being at a later stage of its ontogeny than Sirosa (Figure 5.5), rather than directly related to any inherent difference in the unit leaf rate (ULR) or relative growth rate (RGR) of the shoots, or the root extension rate of the roots of the two species (Figure 5.6, Tables 5.14, 5.16).

The unit leaf rate and relative growth rate of Tama and Sirosa were similar when they were compared at the same Zadoks growth stage. These results were further supported by the photosynthetic rate data (Table 5.17). The unit leaf rates of Tama and Sirosa ranged from 0.00 to 0.08 g fresh weight.m⁻².day⁻¹ which was in broad agreement with the unit leaf rate of 0.01 g fresh weight.m⁻².day⁻¹ for Italian ryegrass obtained by Heath and Gregory (1938) and the range of rates of 0.04 to 0.07 g fresh weight.m⁻².day⁻¹ for buffel, Mitchell, and mulga grass reported by Christie and Moorby (1975). Similarly, the relative growth rates of Tama and Sirosa, which ranged from 0.001 to 0.185 day⁻¹, corresponded to those that were observed by other workers with Biddiscombe et al. (1969) finding the relative growth rate of annual ryegrass was in the range 0.018 to 0.143 day⁻¹.

Although differences in ULR and RGR between genotypes have sometimes been found to be the major cause of differences in the P efficiency of genotypes this was not the case here (e.g. Rorison, 1968; Christie and Moorby, 1975; Barrow, 1978; Chapin et al., 1982). The similarity in

the ULR and RGR of Tama and Sirosa was probably at least partly due to their being introduced pasture grasses selected for seedling vigour and high yields as opposed to experiments where the genotypes being compared were native or natural genotypes, for example Chionochloa species (Chapin et al., 1982), known to differ in growth rate and yield under low P conditions.

There are shortcomings with the use of ULR or RGR to compare the P efficiency of genotypes at a given P level or with relating ULR to indices of P efficiency such as P concentration (see Barrow, 1978). These shortcomings include the variation of ULR and RGR with plant ontogeny independently of the treatment being imposed, and the potential of plants to have the same ULR and RGR at two different times and ontogenetic stages (see Figure 5.6, Table 5.16; Hunt, 1982).

The root extension rate of Tama was similar to that of Sirosa at each Zadoks growth stage and at the later harvest times but was greater than that of Sirosa at the earlier harvest times (Table 5.14). The root extension rates of Tama and Sirosa, which ranged from 2 to 3066 mm.day⁻¹ depending on the P rate and the ontogenetic stage of the species, were comparable to the root extension rates recorded by Evans (1970) for perennial ryegrass grown in pots of sand, which ranged from 4 to 600 mm.day⁻¹.

The root extension rate of perennial ryegrass was observed by Evans (1970) to drop sharply when nodal roots first grew and then to subsequently increase. Beever and Woolhouse (1975) found the root growth of Perilla frutescens L. Britt. was similarly depressed at flowering and noted this phenomenon was frequently reported for other species. Moreover, the root extension rate of both Tama and Sirosa was demonstrated to decrease at initiation, when the plants switched to the reproductive phase, and to later increase again (Table 5.14). In a similar vein Colvill and Marshall (1984) found the production and survival of tillers on perennial ryegrass was largely determined by competition for assimilate at flowering.

One difference between the root systems of Tama and Sirosa was the degree to which they were infected by VA mycorrhizal fungi. At 20 kg P.ha⁻¹ the infection of the roots of Tama by VA mycorrhizal fungi was apparently inhibited by the plant (see Abbott and Robson, 1982) with the level of fungi present being unlikely to be effectively involved in P uptake (Giovannettii and Mosse, 1980, Table 5.15). Sirosa at 20 kg p.ha⁻¹ and both Tama and Sirosa at 5 kg P.ha⁻¹ were highly infected with VA mycorrhizal fungi which would have been effectively involved in the P uptake by the plants under these three treatments (Table 5.15; see Giovannetti and Mosse, 1980).

5.4.1.2 P uptake, content and distribution When the total P uptake (Figure 5.7), the P uptake per unit of root (Table 5.19, the shoot P content (Figure 5.9), and the root P content (Figure 5.8) of Tama and Sirosa were compared at each P rate the values for Tama were commonly different on a Sirosa on an ontogenetic basis, but were greater on a temporal basis. Within each species the values for all the above measures of P uptake and P content were greater at 20 kg P.ha⁻¹ than at 5 kg P.ha⁻¹. These results support the observations of Keay et al. (1970) and Hart and Jessop (1982) that the P uptake and P content of Mediterranean pasture species, and white clover and stylosanthes respectively were influenced both by the level of P supply and the physiological age of the plant material sampled.

The P uptake rate per unit of root for Tama and Sirosa was similar to that reported for related grass species grown under similar conditions. For example, Caradus (1980) found the P uptakes per unit root length of perennial ryegrass and phalaris were 28 and 48 $\mu\text{g P.m}^{-1}$ respectively compared with the range for Tama of 5 to 79 $\mu\text{g P.m}^{-1}$ and the range for Sirosa of 18 to 104 $\mu\text{g P.m}^{-1}$ (Table 5.19). Similarly, the P uptake per unit fresh weight of Tama and Sirosa ranged between 190 and 835 $\mu\text{g P.g f.w.}^{-1}$ (Table 5.19) compared with a range of 240 to 1900 $\mu\text{g P.g f.w.}^{-1}$ for annual ryegrass (Biddiscombe et al., 1969).

The P uptake rate per unit of root of a variety of crop and pasture species was shown to decline as plants age by Jungk (1974), Christie and Moorby (1975), Edwards and Barber (1976) and Chapin and Bielecki (1982). Also, the P uptake rate per unit of root for Tama, Siroso, Hamua, and Ladino was found to decrease over the course of the experiments (Figure 3.4, Table 5.19). Furthermore, Edwards and Barber (1976) demonstrated that the C_{min} for soybean roots was increased as the plants aged.

The total P uptake per plant, or plant P content, for Tama was greater than that of Siroso at each P rate and harvest time, except for 5 kg P.ha⁻¹ at the final harvest (Figure 5.7). Yet, the P uptake per unit root of Tama was either significantly lower or similar to that of Siroso at any given time (Table 5.19).

A poor relationship between P uptake rate and total P uptake, was also found by Chapin (1982) when comparing genotypes of Hordeum, Chionochloa, and Zea , and by Caradus (1983) in a comparison of eight genotypes of white clover. It would appear that the greater P uptake of Tama relative to that of Siroso, on a temporal basis, was mainly due to the greater root length and greater shoot demand for P of Tama, resulting from the faster rate of ontogenesis of Tama, as was demonstrated for a number of pasture grasses and legumes by Hart et al. (1981), Chapin (1982), and Caradus (1983).

The P concentration of most plant genotypes decreases as the plants age and as the level of P supply is lowered (Ozanne et al., 1969; Bates, 1971; Hart and Jessop, 1982). A confounding influence on this age and P supply related decrease in plant P concentration is that the level of P supply frequently affects the rate of growth and the rate of ontogenesis of plant genotypes (Bates, 1971; see also Figures 5.5, 5.6). It is, therefore, essential to define the ontogenetic stage plants are at when P concentration is determined (Bates, 1971). As the P contents and the fresh weights of Tama and Siroso were similar at each Zadoks growth stage the P concentrations of the plants were also similar (see Section 5.3.3).

No major differences between Tama and Sirosa in the biochemical patterns of P use were apparent from the ways in which P was allocated to the three P fractions (see Tables 5.21, 5.22, 5.23, 5.24). Nevertheless, there were some discrepancies between the two species. The acid-soluble-P content and concentration of Tama at 5 kg P.ha⁻¹ were approximately 25% and 10% of the values for Sirosa when both species were at Zadoks growth stage 40. Yet, although the flag leaf sheath was extending in both species at Zadoks growth stage 40 the P demand for inflorescence development would have differed between them. This was because inflorescence development was arrested at the double ridge stage in Sirosa whereas Tama produced mature inflorescences, at least partly, apparently through remobilisation of acid-soluble-P (see Table 5.11). Also, the acid-soluble-P content of the roots, and of the shoots, of Tama was greater than that of Sirosa after about Zadoks growth stage 30, although the concentration and the proportion of total P allocated to acid-soluble-P were similar for both species (see Tables 5.21, 5.22, 5.23, 5.24; Figures 5.2, 5.3). Chapin (1982) also concluded that there were no major differences in the biochemical patterns of P use of the species he compared within the Hardeum, Chionochloa, and Zea genera.

As found by other workers (e.g. Bielecki, 1973; Chapin et al., 1982; Hart and Jessop, 1983) the acid-soluble-P content and concentration of both Tama and Sirosa was the P fraction most sensitive to changes either in the ontogeny of the plants or in the level of P supply (Tables 5.21, 5.22, 5.23, 5.24). Although the variable nature of the content of the acid-soluble-P fraction in Tama and Sirosa was in agreement with its role as the major P storage pool the results also supported the suggestion of Chapin and Bielecki (1982) that the P-lipid and residue-P fractions act partly as P storage pools (see Tables 5.23, 5.24).

5.4.2 THE EFFECT OF P SUPPLY ON ONTOGENY

5.4.2.1 Rate of ontogenesis The rate of ontogenesis of plant genotypes is commonly found to increase in response to higher levels of P supply (Bates, 1971). The ontogenesis of Tama at 20 kg P.ha⁻¹ was from one to seven days more advanced than at 5 kg P.ha⁻¹, depending on the ontogenetic stage at which the comparison was made, but the rate of

rate (Figure 5.5, Table 5.11). In contrast, Williams (1946) found that the number of tillers per plant on phalaris was increased when the P rate was increased. Chapin et al. (1982) demonstrated that as the level of P supply to two species of Chionochloa was increased the number of tillers per plant also increased whereas the number of leaves per tiller was unaffected. Similarly, the number of leaves on the main, or apical, shoot of both Tama and Siroso was unaffected by the rate of P applied (Table 5.10, see also Williams, 1946). Although the number of tillers per plant on Tama was greater at 20 kg P.ha⁻¹ than at 5 kg P.ha⁻¹ the number of tillers per plant on Siroso was the same at both rates of P (Table 5.9). These results are in general agreement with the conclusion by Chapin (1980) that the number of leaves on the apical shoot was less affected than the number of tillers per plant on forage grasses at low levels of P supply.

5.4.2.2 Pattern of ontogenesis Michelini (1958) observed that a limitation to the use of indices of plant ontogenesis was the possibility that the treatments used could change the established pattern of ontogenesis in the plant genotypes under study. Such a change in the ontogenesis of plant genotypes could make it necessary to re-establish the relationship between the ontogenetic index being used and the genotype, but would have the benefit of providing a sensitive measure of the reaction of a genotype to various treatments (Michelini, 1958). Siroso developed at the same rate at both 5 and 20 kg P.ha⁻¹ until the plants reached the double ridge stage at 78 DAS (Figure 5.5, Table 5.11). The inflorescences in the Siroso plants at 5 kg P.ha⁻¹ failed to develop beyond the double ridge stage whereas the reproductive phase of the Siroso plants at 20 kg P.ha⁻¹ continued after the double ridge stage (Table 5.11). That is, after the double ridge stage (Zadoks growth stage 31 or 78 DAS) Siroso grown at 5 kg P.ha⁻¹ followed a pattern of ontogenesis qualitatively different from that of Siroso at 20 kg P.ha⁻¹ or, indeed, Tama at either 5 or 20 kg P.ha⁻¹ (Table 5.11). This difference in the reaction to P

deficiency, at 5 kg P.ha⁻¹, of Tama (an annual) and Sirosa (a perennial) supports the conclusion of Chapin (1980) that annual grasses respond to P deficiency by leaf senescence (see Section 5.3.1.2) and transporting P from leaves to inflorescences, whereas perennial grasses respond to P deficiency by reduced growth and reduced allocation to reproduction.

5.4.3 EVALUATION OF ZADOKS GROWTH STAGE AS A MEASURE OF ONTOGENY

The decimal code of Zadoks growth stages is better suited to use in statistical analysis and computer programs than related ontogenetic scales such as that of Feekes (see Large, 1954; Zadoks *et al.*, 1974). Zadoks growth stages are also a superior method of monitoring the ontogeny of many grass species than indices based solely on vegetative characteristics, the plastochron index (Michelini, 1958) for example, or on plant weight (see Section 5.2.6.1). Nevertheless, there are some shortcomings in the Zadoks growth stages as applied in this study and there is a need for similar scales to be developed for pasture legumes.

One shortcoming of Zadoks growth stages in this study was the relatively small proportion of the scale given over to describing the vegetative phase of grass species. For pasture grass species, where vegetative yield rather than reproductive yield is the prime concern, there is a need for greater numerical differentiation between species during the vegetative phase. Perhaps the number of stages describing the reproductive phase of plants could be rationalised and the vegetative stages expanded to include characteristics such as the number of leaves on the tillers as well as characteristics already included such as the number of leaves on the main stem and the number of tillers per plant (see Tottman *et al.*, 1979).

Adherence to Zadoks growth stages, as described by Zadoks *et al.* (1974) or Tottman *et al.* (1979), to describe the ontogeny of Sirosa and Tama in experiment two could have

resulted in Sirosa grown at 5 kg P.ha⁻¹ being mistakenly regarded as developing normally in its reproductive phase (see Table 5.3 and Section 5.3.1.2). Although destructive harvests showed that the reproductive development of Sirosa at 5 kg P.ha⁻¹ was arrested at the double ridge stage the main stem still elongated and developed nodes (see Section 5.3.1.2). Therefore, when Zadoks growth stages are being used for research applications it is apparently necessary to support the non-destructive evaluation of the ontogeny of plants with some direct observations of the developing inflorescence.

In addition to the need to refine the Zadoks growth stages for pasture grass species there is a need to develop ontogenetic indices for pasture legumes. Although there would be difficulties associated with comparing pasture legume species with contrasting growth habits, for example red and white clover (see Ryle *et al.*, 1981), the comparison of genotypes within a species would present fewer problems. Kalu and Fick (1981), for instance, developed a decimal code system to describe the ontogenesis of lucerne (Medicago sativa L.).

5.4.4 CRITIQUE OF OTHER METHODS OF EVALUATING THE P EFFICIENCY OF SPECIES

Two alternatives techniques to evaluating the P efficiency of plant genotypes on an ontogenetic, or a temporal, basis that have been regularly used are: -

1. diagrams relating the yield of genotypes at a high level of P supply to the yield of the genotypes at a low, or deficient, level of P supply (Caradus and Dunlop, 1978; Caradus, 1980), and
2. the comparison of the concentration of P in plant parts of the same physiological age from plant genotypes sampled at the same time (Bates, 1971).

The hypothesis behind the use of high P versus low P diagrams has been that plant genotypes that yield relatively

well at both low and high levels of P supply use P efficiently by means other than a slow relative growth rate, and are also capable of responding to an increase in P supply (Caradus, 1980). The failure of this method to consider the ontogenetic condition of the genotypes being evaluated results in the rankings of those genotypes being related to only one harvest time. Figure 5.10 shows the yields of Tama and Sirosa re-presented as a high P versus low P diagram. This Figure 5.10 demonstrates that the relative positions of Tama and Sirosa changed with respect to each other as well as moving to the upper right of the diagram over time.

The use of plant parts at a standardised physiological age, such as the youngest fully expanded leaf (YFL) in subterranean clover (Bates, 1971; Jones et al., 1972), and white clover and lotus (Hart and Jessop, 1983), has been one technique developed to avoid the difficulties associated with comparing the P concentrations of plants at different ontogenetic stages. Although this technique is limited in that P concentration is only one measure of the P efficiency of a genotype it is, nevertheless, a useful method for comparing plant genotypes harvested at the same time (see also Bates, 1971). A disadvantage of this technique is that it does not take into account that plant parts of the same physiological age can be found on plant genotypes at disparate ontogenetic stages and thereby at different stages of P nutrition (Bates, 1971), or that treatments such as repeated defoliation can alter the P concentration found in plant parts of a given physiological age (Jones et al., 1972).

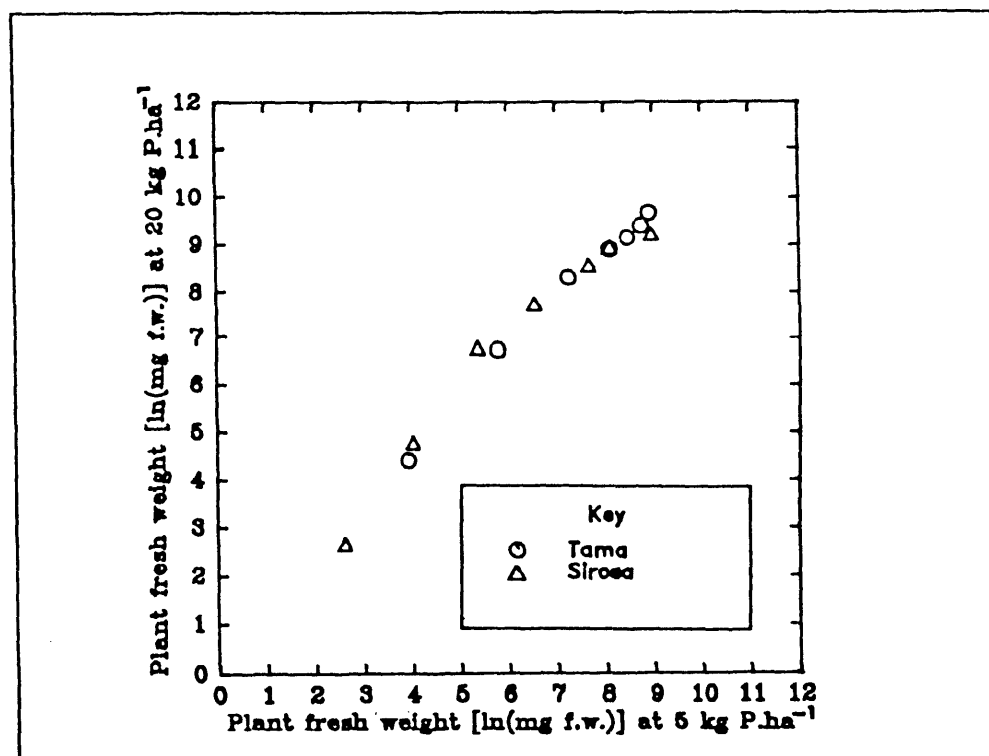


Figure 5.10 The plant fresh weight of Tama and of Sirosa at a high and a low rate of applied P. The values for the first harvest (16 DAS) are in the lower left of the figure, the values for the final harvest (112 DAS) are in the upper right of the figure.

CHAPTER 6

SUMMARIES AND CONCLUSIONS

6.1

INTRODUCTION

In the experiments reported in Chapters 3, 4, and 5 a wide range of measures of P efficiency were used on four registered cultivars of temperate pasture species. Both the comparative P efficiency of the four species, Tama, Siroso, Hamua, and Ladino, and the usefulness of the measures of P efficiency were evaluated. The results of the experiments both supported and expanded the conclusions that were drawn from a review of the literature and presented in the Introduction (see Chapter 1).

6.2 GRASSES VERSUS LEGUMES AND MEASURES OF P EFFICIENCY

The comparison between the two grass species, Tama and Siroso, and the two legume species, Hamua and Ladino, used in this study supported the general conclusions drawn from the literature (see Chapter 1). Namely, the two grass species attained their maximum yield at a lower level of P supply than the two legume species, and the P concentration of the shoots and of the roots of the two grass species was lower than that of the two legume species (Sections 3.3.2 and 3.3.4). Also, this study gave support to the conclusion from the literature that pasture grass species tend to be more P efficient than pasture legume species when the level of P supply is limiting to plant growth (Section 3.4, Trumble and Fraser, 1932; Biddiscombe *et al.*, 1969; Caradus, 1980). Therefore, to increase the P efficiency of a mixed sward it would appear that the emphasis needs to be placed on improving the P efficiency of the legume component of the sward.

The areas of P efficiency in which legume species require the greatest improvement relative to grass species encompass both the acquisition and the utilisation of P by plants. The ability of grass species to attain their

maximum yield at lower levels of P supply than legume species appears to result from the larger and finer root system of grass species, the lower minimum concentration of P in the soil solution from which grass species can absorb P, and the lower P concentration of the roots, and in some cases the shoots, and thus lower demand for P per unit of plant growth, of grass species relative to legume species (Section 3.4, Caradus, 1980; Mouat, 1983; Barrow, 1975b). The generally shorter and thicker roots of legume species relative to the roots of grass species, appear to place legume species at a disadvantage when foraging for P particularly when in competition with roots from grass species (Section 3.4; Evans, 1977; Caradus, 1980). Rate of P uptake per unit of root is usually greater for roots of legume species than for grass species, and moreover, tends to be greater in grass species with short, thick roots (e.g. *Sirosa phalaris*) than in grass species with long, thin roots (e.g. *Tama Italian ryegrass*) (Sections 3.3.3.2, 5.3.1.3 and 5.3.3.2; Caradus, 1980). Furthermore, the demand of a plant for P as governed by the total biomass, growth rate, and P concentration of the plant appears to interact with the size of the root system of the plant and the level of P supply available to the roots in determining the P uptake per unit root (Section 3.4.1.3, Clarkson and Hanson, 1980). That is, P uptake per unit root is neither a stable property of the roots of each species, nor is it the limiting step in the acquisition of P by plants (Chapin et al., 1982; Caradus, 1983). Conversely, for legume species to increase their efficiency of P acquisition would require an increase in the size, or biomass, of their root system or a change in their root morphology (Chapin et al., 1982).

Although the minimum P concentration in the soil solution required for absorption by plants (C_{min}) was not measured in the experiments reported in this thesis there is evidence that C_{min} varies between plant species, and that C_{min} tends to be higher for legume species than for grass species (Mouat, 1983; Bielecki, 1976). Moreover, this general conclusion was supported by Hamua and Ladino, the

legume species examined in this thesis, attaining their maximum yield at a higher rate of applied P than that required by Tama and Siroso, the two grass species (Section 3.4.1.2). A higher C_{min} would further disadvantage the roots of legume species competing against the roots of grass species. Nevertheless, there are difficulties associated with the use of C_{min} as a measure of P efficiency. Species with contrasting P efficiencies have sometimes been shown to have similar values for C_{min} (Chisholm, 1979); the value of C_{min} is difficult to calculate particularly in a manner that approximates P uptake by roots from the soil solution (Mouat, 1983); and the value of C_{min} for a given species changes as the plants develop, thus necessitating species to be compared at similar growth, or physiological, stages (Edwards and Barber, 1976; also see Section 6.3).

The scope for decreasing the root P concentration of pasture legume species relative to that of pasture grass species would appear to be more limited than that for shoot P concentration. This limitation results from the higher root P concentration of legume species, relative to that of grass species, being largely the product of the high P concentration in the root nodules formed by Rhizobium bacteria (Section 3.4.1.3, Hart et al., 1981; Robson et al., 1981).

Nevertheless, differences between the P concentration of plant species, both grasses and legumes, have often been reported (see Section 2.3.2.4). This greater efficiency of P utilisation by some species has been related to their greater capacity to use the P_i absorbed by the plant in metabolic and structural processes (i.e. P-lipid and residue-P), and to re-mobilise P from within the plant (Chisholm, 1979; 1981; Hart and Jessop, 1982; Hart and Jessop, 1983). Yet the differences between Tama and Siroso, when examined in this thesis, in the concentration of the three P fractions (acid-soluble-P, P-lipid, and residue-P) in their shoots and roots, at a given P rate, were largely the result of differences in the rate of ontogenesis of the two species rather than inherent differences in the

P metabolism of the two species (Sections 5.3.3.6, 5.3.3.7). The acid-soluble-P content of the roots of Tama at 5 kg P.ha⁻¹ was, however, lower than that of Sirosa (see Section 6.3).

Therefore, before measures of P efficiency can be used as selection criteria to evaluate pasture species it is apparent that any relationship between the ontogeny of the plant species and the measures of P efficiency needs to be taken into account. As a case study this relationship between ontogeny and measures of P efficiency was examined in Tama and Sirosa.

6.3 THE IMPORTANCE OF ONTOGENY IN THE DEVELOPMENT OF SELECTION CRITERIA FOR P EFFICIENT PLANTS

The differences in the P efficiency of Tama and Sirosa were related to differences in the ontogenesis rather than the P physiology or P metabolism of the two species. That is, Tama and Sirosa reached similar yields and P contents at the same ontogenetic stage, measured as either Zadoks growth stage or as the number of leaves per plant, but Tama attained each ontogenetic stage before Sirosa and, therefore, yielded more than Sirosa at any given time (Figures 5.1, 5.2, 5.3, 5.8, 5.9, 5.10). Moreover, under the P deficiency conditions prevailing at 5 kg P.ha⁻¹ the ontogeny of Tama was unaffected whereas Sirosa failed to produce a viable inflorescence (Table 5.11, also see Section 5.3.1.2). There were also differences, however, in the P physiology and P metabolism of the two species such as the lower percentage of infection by mycorrhizal fungi in Tama at 20 kg P.ha⁻¹ (Table 5.15), and the lower acid-soluble-P content in the roots of Tama, at a given Zadoks growth stage, relative to that of Sirosa (Table 5.23). Yet, overall these differences between Tama and Sirosa did not amount to any differences between the species on broader measures of P efficiency such as yield per unit of applied P, or more specific measures such as total P uptake, uptake per unit of root, and P content or concentration of roots and shoots at a given ontogenetic stage (see Section 5.3).

The difference in the P efficiency of Tama and Sirosa at any given time resulted from the different rates of ontogenesis of Tama and Sirosa, particularly under P stress conditions, and the differences in the way the course of ontogeny of Tama and Sirosa was affected by P stress. The importance of ontogenesis when evaluating the P efficiency of Tama and Sirosa suggests that selection criteria for P efficiency amongst high yielding or improved pasture grass genotypes need to be based on the rate of ontogenesis of the genotypes and the capacity of the genotypes to satisfactorily complete their ontogeny at low levels of P supply, rather than on criteria based on differences in the P physiology or P metabolism of the genotypes. There is certainly a need to test the generality of this hypothesis on a wider range of pasture grass species.

An analogy can be drawn between the influence of ontogeny on the P efficiency of improved pasture grass genotypes hypothesised above and studies on the P efficiency of plant genotypes adapted to low P environments (Chapin, 1982; Chapin and Bielecki, 1982; Chapin *et al.*, 1982). Chapin and his co-workers concluded that slow growth rate was the main adaptation that led to some plant species being able to survive low P conditions and not high P uptake capacity or more efficient biochemical patterns of P use. That is, they found that the P physiology and P metabolism of plant genotypes adapted to low P environments were fundamentally the same as those of plant genotypes adapted to high P environments with any dissimilarities being directly related to the different rate of growth of the two groups of genotypes.

There have been reports of plant genotypes that exhibit specific adaptations in their P acquisition or P utilisation that make them relatively more P efficient. Examples of these specific adaptations reported for some genotypes include the root exudates produced by lupins (Gardner and Parberry, 1982); the relatively low acid-soluble-P concentration in the leaves of lotus at both low and high

levels of P supply (Hart and Jessop, 1983); the imbalance in the ratio of cations and anions absorbed by buckwheat that enables the roots to acidify the rhizosphere (van Ray and van Diestm 1979); and the variation in root length, and thus total P uptake, between white clover genotypes (Caradus, 1983). There is a need to further evaluate these genotypes so that it can be determined how specific to a genotype or an environment such adaptive mechanisms are, how heritable they are, and whether they do indeed improve the overall P efficiency of a plant or whether they are different means to the same end. The endpoint being a measure of P efficiency such as yield per unit of applied P.

Studies on plant genotypes with apparently greater P efficiency have generally involved comparisons with other genotypes that have disparate ontogenies, or different rates of ontogenesis (e.g. Keay et al., 1969; Caradus, 1980; Chapin, 1982). There is clearly a need with these plants to evaluate them on an ontogenetic basis so the influence of ontogenesis on P efficiency can be distinguished from that of P physiology and that of P metabolism. Finally, plant genotypes have demonstrated differences in their methods of P acquisition and P utilisation, but too often the significance of these differences in the P physiology or the P metabolism of genotypes has not been fully evaluated. The evaluation of plant genotypes requires accounting for the differences in P efficiency between genotypes that result solely from the genotypes being at different ontogenetic stages, when compared at a given time. Plant genotypes compared at the same time but at different ontogenetic stages can have differences in their P physiology and P metabolism at that time that are directly related to their being at different ontogenetic stages and not to their P efficiency as such.

6.4

CONCLUSIONS

The biomass production from pastures when the level of supply of P is limiting to plant growth could be increased by improving the efficiency with which pasture species, but

particularly legume species, acquire and use P. The improvement of the P efficiency of pasture species requires the development of measures of P efficiency that can be used as selection criteria. Although a specific measure of P efficiency has not been developed and tested measures that integrate the capacity of the whole plant to acquire or use P such as the size of the root system or the level of P supply required for maximum shoot growth have, at present at least, less weaknesses than measures such as P uptake per unit of root. Nevertheless, the experiments reported in this thesis demonstrated that it is essential to take into account the ontogenetic stage of species when evaluating their P efficiency. That is, the usefulness of many comparisons of the P efficiency of plant species has in the past been clouded by the failure to differentiate between those species that use the P available in the soil more efficiently due to superior mechanisms for acquiring or of utilising P, and those species that, because, for example, greater seedling vigour or a shorter time to maturity, are at a more advanced ontogenetic stage, at a given time, than other species and, for that reason alone, appear more P efficient.