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## Growth of Subterranean Clover in a Range Soil as Affected by Microclimate and Phosphorus Availability. III. Comparative Growth of Subterranean and Rose Clovers at Cold Soil Temperatures<sup>1</sup>

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### ABSTRACT

The biological yield potential of winter annual range legumes is influenced by the timing of rainfall adequate to ensure germination. If rains are late, the establishment phase of growth must occur at cold soil and air temperatures. On low-P soils, this combination of abiotic factor constraints can limit symbiotic N fixation.

We conducted three controlled-environment experiments to compare the seedling growth of rose clover (*Trifolium hirtum* All., 'Hykon') and subterranean clover (*T. subterraneum* L., 'Mt. Barker', hereafter referred to as subclover) on a P-deficient soil (Sobrante-Las Posas association), simulating winter conditions of cold soils and low light. Comparative growth responses, especially intraplant dry matter partitioning and nodulation, were observed for several soil P availability levels.

A sunlit phytotron provided a common shoot environment of seasonal (fall/winter) ambient light intensity and photoperiod, with a programmed smooth diurnal temperature cycle of 20 C maximum and 5 C minimum. Three independently-controlled water baths maintained soil temperature experiments at a constant 5 C, a constant 10 C, and a diurnal 5 C to 10 C alternation. Phosphorus levels were 0, 45, 90, and 180 kg/ha equivalents. Growth intervals ( $5 \pm 0.5$  trifoliate-leaf stage), varied between 58 and 114 days.

Leaf area of subclover was greater than that of rose clover, and responded more to increasing P levels. Leaf areas tended to increase with higher soil temperatures. Nodule weight was positively correlated with leaf area; nodule weight:leaf area regressions suggested that nodule development was adequate at 5 C and that nodule development was primarily related to time rather than to morphological development of the shoot. Nodule weights were similar for the two species, and low at 0 P.

The results suggest that while the practical soil threshold temperature for overall growth is near 5 C, some intraplant growth processes may proceed, but only at adequate availability levels of mineral nutrients. These relationships may be particularly important where range annual legumes are being introduced as part of a more general range improvement program.

**Additional index words:** Annual range, Controlled environments, Root:shoot ratio, Nodulation, Leaf area, N fixation.

THE biological yield of forage legumes consists of a combination of dry matter yield, nutrients (both organic and inorganic) which are ingested by livestock, and symbiotically-fixed N, part of which is available for transfer to associated species in a mixed plant community.

In the specific case of California annual range legumes, biological yield potential frequently is constrained by the characteristics of the Mediterranean-type climate (1, 2, 7, 8). Moreover, in the complex mixed-species plant communities of these "grasslands", it has long been recognized that seasonal weather conditions result in substantial between-year variation in both dry matter yield and species balance (18, 19), and the expressions "good clover year" and "poor clover year" succinctly describe the outcome of various climatic, edaphic, and biotic factor effects.

Simple and unique explanations are seldom possible. A classic example of interacting abiotic factors is the profound effect of date of sufficient fall rain to ensure both germination and sustained seedling growth. Talbot and Biswell (18) found that late rains favored legumes while early rains favored competing plants such as *Bromus mollis* and *Erodium botrys*. Guerrero and Williams (6), however, concluded that late rains, with concomitant low temperatures, could instead lead to *Erodium* dominance.

Similarly, Gibson (3, 4) concluded that while nodule formation in winter annual legumes could occur between 7 and 33 C, the low-temperature threshold for effective symbiotic fixation occurred at 12 C. More recent work (5, 12) suggests that N fixation per se may not be adversely affected by cold temperature, but conversion of fixed N to new plant tissue through growth is. Root zone temperature is of particular interest because it strongly influences behavior of the shoot (16, 21) and because it is related to the seasonally lowering temperatures of fall and winter.

The purpose of this study was to compare the establishment-phase growth of rose clover (*Trifolium hirtum* All., 'Hykon') and subterranean clover (*T. subterraneum* 'Mt. Barker') in three experiments conducted in an environment with a common light and air temperature but with separate root zone temperatures maintained at late fall and winter cold soil levels. Particular attention was given to monitoring gross

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morphological characters which would assist in interpreting whole-plant responses of the symbiotic N fixation system to these environments and to several levels of phosphorus availability established for a soil known from previous work (11, 14) to be both P-deficient and P-fixing.

## MATERIALS AND METHODS

Two Mediterranean climate, winter growing, annual range legumes, rose clover and subterranean clover (hereafter referred to as subclover), were grown in a P-deficient, P-fixing range soil and subjected to four levels of P availability in three different root temperature experiments.

The experiments were conducted in the sunlit Zscheile rotating phytotron (22) during the period mid-November to mid-March. Being sunlit, light intensity and duration varied daily and with the seasons and represented a sample of fall and winter conditions. The ambient air temperatures for these experiments were 5 C for 12 hours, a 4-hour gradual warming to 20 C, with the peak temperature attained at 1200 hours and maintained for 4 hours, followed by a 4-hour gradual cooling to 5 C.

Within the phytotron, three experiments were conducted simultaneously, employing different soil temperatures. These were: Exp. 1) 5 C constant; Exp. 2) 10 constant; Exp. 3) 5 C for 12 hours and 10 C for 4 hours (1200-1600 hours each day) with 4 hours of gradual change between set temperatures. The soil temperature was maintained by submersing stainless-steel, soil pots (5 × 37.5 × 20 cm width, length, depth, respectively) in water baths equipped with individual compressors, circulating pumps, thermostats, and cooling and heating coils. To minimize the effect of solar radiant heating of exposed soil, round, white, styrofoam pellets (2 mm diam) were placed on the soil surface; the open surface of coolant water between the pots also was covered with styrofoam pellets (10 × 30 mm).

The three soil temperature experiments each were designed to test the effect of four levels of P on the two species in a completely-randomized, split plot design. The main plots were the P levels with the two species forming subplots. In the four P treatments of 0, 45, 90, and 180 kg/ha, P was applied as single superphosphate. The P treatments were replicated five times in each experiment as a pot containing both rose and subterranean clovers. A rigid, plexiglass insert divided the pot in half transversely, separating the soil volume and roots of the two species.

Soil for the experiment was a Sobrante-Los Posas association obtained from the Univ. of California Sierra Foothill Range Field Station, Browns Valley, Yuba County. These soils are members of the fine-loamy or fine mixed thermic family of mollic haploxeralfs. A rotary tiller was used to break the surface centimeter of soil and the surface plant litter and soil were raked off. The soil was then tilled to a depth of 10 cm, removed and screened through a 1 cm mesh screen. The soil was steam-sterilized to control damping off organisms. Because of the extremely low N content of the soil, 22 kg/ha of N (as ammonium nitrate) was added initially to all treatments.

Gypsum was applied to the two treatments with the lowest levels of P (0 and 45) at a rate of 112 kg/ha of S equivalent, to ensure that S was not limiting. The two treatments with the highest levels of P had more than 112 kg/ha of S, contributed by the single superphosphate, and no S was added.

The single superphosphate, ammonium nitrate, and gypsum were mixed with approximately 2,670 cc of soil and the mixture poured into the rectangular metal pots.

Seeds of the two species were sized (using only uniformly large seeds) and inoculated with fresh "WR" inoculant (Nitragin Co.) using gum arabic as a binder and pelleted with calcium carbonate. The seeds were placed on the soil surface in the pots and covered with 6 mm of washed and sterilized river sand. The seeds were germinated on 10 Nov. 1976, at a uniform soil and air temperature of 15 C. The temperature was lowered to experimental temperatures at approximately the 0.08 unifoliate stage (15) at which time the plants were thinned to 10 uniform plants per species in each pot. Three plants of each species in each pot were marked for weekly recording of morphological development. All pots were individually watered so as to maintain adequate soil moisture.

Plants in each treatment were sampled when the average morphological development of the five replications of the 45, 90, and 180 kg/ha P levels reached the  $5 \pm 0.5$  leaf stage. The entire plant was removed, and the roots carefully washed free of soil. Nodules were removed with tweezers, dried, and weighed. Leaf areas were determined using a Lambda portable area meter, Model LI-3000. Dry weights of the roots and shoots were obtained and shoot/root ratios calculated.

## RESULTS

Analysis of variance revealed more similarities than differences in response for the morphological parameters measured in the three experiments (Table 1). A noteworthy exception was the P × species interaction occurring for shoot weight, root weight and S:R ratio in the 5 C experiment but not in the 5 C to 10 C or 10 C experiments. Nodule weights responded to P levels but did not vary between species.

Rate of morphological development data are given in Fig. 1 and Table 2. Linear regressions fitted these data extremely well, as indicated by uniformly high correlation coefficients (Table 2). This parameter responded markedly to addition of P but did not respond within P levels. Two exceptions were significant differences between 45 and 180 kg/ha P, shown only by subclover and only in the 5 C and 5 C/10 C experiments. Within-experiment differences between species were found only for 0 P (all three experiments) and for 180 P in the 5 C/10 C and 10 C experiments. As expected, slopes of the regression line clusters for the three experiments increased with increasing temperatures.

Leaf area of subclover generally was greater than that of rose clover (Table 3). Increase in leaf area due to P level was more pronounced for subclover but only in the 5 C and 5 C/10 C experiments. Because the three experiments were independent, statistical comparisons were not made between temperature levels. However, overall means of P treatment values suggested that leaf area responded positively to soil temperature increase.

Nodule weight varied only slightly with P level. Very small amounts existed at 0 P, with only traces recorded for subclover at 5 C and for rose clover at 5 C and 5 C/10 C (Table 3). The only significant difference between P levels of 45 kg/ha or more was found between 45 and 180 kg/ha for subclover in the 10 C experiment.

Shoot weight generally increased with an increase in P level in both species (Table 3). The greatest response was found for rose clover in the 5 C experiment, where a five-fold difference between 0 P and 180 P was observed. This was one of the few instances where the magnitude of response of rose clover over the range of P levels exceeded that of subclover, although the comparative response pattern overall, as shown by least significant differences, clearly favored subclover. Recalling that most plants were sampled at a common stage of morphological development it is clear that, although rate of shoot development (leaf production) was slowed by cooler soil temperatures, substantially more dry matter accumulated per leaf unit at these cooler temperatures.

Root weight varied less than shoot weight (Table 3), with the only clear P response observed between 0 P and all other P levels, and even this rather gen-

**Table 1. Analysis of variance for leaf area, nodule weight, shoot and root weights and shoot:root ratio of subterranean and rose clovers grown in a range soil at four levels of P availability.**

Source	df	Mean squares for experiment 1 (5 C root temperature)				
		Leaf area	Nodule wt.	Shoot wt.	Root wt.	S:R ratio
Replicates	4	11.41	0.74	173.11	90.73	0.03
P levels	3	299.32**	27.21**	16,795.88**	3,978.13**	0.93**
Linear	1	681.01**	41.04**	39,061.45**	6,212.70**	2.46**
Quad.	1	184.90**	32.67**	9,573.57**	4,278.89**	0.28*
Cubic	1	32.05	7.91*	1,752.67	1,442.81*	0.04
Error A	12	7.90	1.54	456.09	257.56	0.05
Species	1	41.68**	1.98	44,462.22**	20,403.29**	0.10*
P × spp.	3	12.46	0.82	1,374.09**	296.77*	0.11*
Repl. × spp.	4	1.04	0.25	23.29	19.22	0.03
Error B	12	4.45	0.71	211.03	80.36	0.02
Mean squares for experiment 2 (5 C-10 C root temperature)						
Replicates	4	13.83	0.20	187.07	31.21	0.07
P levels	3	296.01**	10.63**	4,980.27**	586.51**	0.77**
Linear	1	444.33**	16.50**	4,546.45**	70.56	1.81**
Quad.	1	398.36**	13.52**	9,864.98**	1,642.45**	0.47**
Cubic	1	45.33**	1.88**	529.38	46.51	0.04
Error A	12	9.22	0.31	193.97	92.66	0.04
Species	1	111.32**	0.20	31,208.98**	11,468.38**	0.01
P × spp.	3	17.69	0.44	81.01	142.71	0.05
Repl. × spp.	4	4.61	0.57	189.01	76.06	0.03
Error B	12	5.43	0.46	221.45	52.83	0.02
Mean squares for experiment 3 (10 C root temperature)						
Replicates	4	5.59	0.10	250.41	711.75	0.11
P levels	3	186.14**	1.85**	3,562.70**	99.03	2.27**
Linear	1	357.62**	4.07**	6,174.42*	90.02	5.61**
Quad.	1	137.10**	0.43	1,419.46	14.28	0.71
Cubic	1	63.71*	1.05**	3,094.23**	192.78	0.48
Error A	12	9.58	0.10	332.60	92.26	0.08
Species	1	185.33**	0.01	32,438.72**	11,108.89**	0.47**
P × spp.	3	20.96	0.16	615.82	40.18	0.01
Repl. × spp.	4	1.80	0.04	124.78	22.80	0.08
Error B	12	9.83	0.07	304.73	39.86	0.04

\*,\*\* P < 0.05, 0.01, respectively.

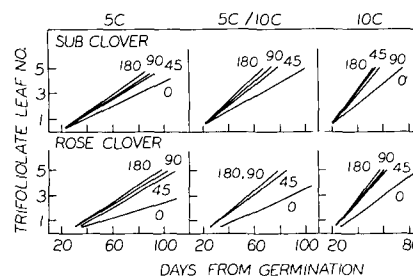
eral response was not observed in the 10 C experiment for either species.

Both species behaved similarly in shoot:root ratios (Table 3). Clearly, important shifts in intraplant partitioning began to occur only when P levels reached 90 (rose clover) or 180 (subclover) kg/ha.

Total plant weights (Table 3) from the 5 C experiment were of greatest interest. Subclover whole plant weights increased with increasing P level and merely reflected the sensitivity to increasing P level shown by other, intraplant, components such as leaf area. In contrast, a striking five fold difference between 0 P and 45 P levels was observed for rose clover, with the remaining P level comparisons nonsignificant.

Figure 2 graphically portrays P response patterns of the two species for shoot and root production. Standard errors clearly show the ease with which species differences can be separated and, more importantly, response similarity of the three P-added treatments. A notable exception is the shoot weight of subclover, where an almost linear relationship existed between 0 P and 90 P levels for all temperature experiments.

Figure 3 shows that leaf area was a more sensitive indicator of nutrient status in subclover than in rose clover, with, as for shoot weight, a near-linear response between 0 P and 90 P levels. Standard errors suggest little or no effect of the soil temperature treatments on leaf area of rose clover. The largest values for leaf area occurred in the 10 C experiment for subclover.



**Fig. 1. Morphological rate of development of subterranean and rose clovers as a function of P availability in three root temperature experiments conducted simultaneously. See Table 2 for regression equations and coefficient significance.**

Because leaf area obviously is an intraplant growth component of considerable diagnostic relevance to photosynthetic efficiency considerations, which, in turn, are directly linked to biological reduction of atmospheric N (e.g., 9, 21), it was of interest to compute the relationship of nodule weight and leaf area (Fig. 4). Linear equations fitted the data well, with correlation coefficients of 0.86 or larger. A somewhat better fit was shown for rose clover than for subclover. Regression coefficients between species within soil temperature experiments were significantly different ( $P < 0.05$ ) for only the 10 C level, although rose

**Table 2. Regression equations; significance of regression coefficients related to P level and species comparisons; and correlation coefficients for morphological rates of development shown in Fig. 1.**

P		Experiment			
		Exp. 1 (5 C root temperature)			
		Subclover			
0	$y = 0.047 \times -0.77$	$b = 0.047a \times \dagger$	$r = 0.995$		
45	$y = 0.062 \times -1.14$	$b = 0.062b \ x$	$r = 0.998$		
90	$y = 0.064 \times -1.14$	$b = 0.064bc \ x$	$r = 0.995$		
180	$y = 0.069 \times -1.25$	$b = 0.069c \ x$	$r = 0.992$		
		Rose clover			
0	$y = 0.033 \times -0.77$	$b = 0.033a \ y$	$r = 0.983$		
45	$y = 0.059 \times -1.40$	$b = 0.059b \ x$	$r = 0.991$		
90	$y = 0.062 \times -1.35$	$b = 0.062b \ x$	$r = 0.997$		
180	$y = 0.064 \times -1.37$	$b = 0.064b \ x$	$r = 0.996$		
		Exp. 2 (5-10 C root temperature)			
		Subclover			
0	$y = 0.055 \times -0.54$	$b = 0.055a \ x$	$r = 0.995$		
45	$y = 0.082 \times -1.28$	$b = 0.082b \ x$	$r = 0.998$		
90	$y = 0.088 \times -1.35$	$b = 0.088b \ x$	$r = 0.998$		
180	$y = 0.097 \times -1.53$	$b = 0.097c \ x$	$r = 0.998$		
		Rose clover			
0	$y = 0.044 \times -0.99$	$b = 0.044a \ y$	$r = 0.992$		
45	$y = 0.077 \times -1.54$	$b = 0.077b \ x$	$r = 0.997$		
90	$y = 0.082 \times -1.58$	$b = 0.082b \ x$	$r = 0.998$		
180	$y = 0.082 \times -1.59$	$b = 0.082b \ x$	$r = 0.997$		
		Exp. 3 (10 C root temperature)			
		Subclover			
0	$y = 0.079 \times -1.05$	$b = 0.079a \ x$	$r = 0.998$		
45	$y = 0.131 \times -2.14$	$b = 0.131b \ x$	$r = 0.998$		
90	$y = 0.125 \times -2.06$	$b = 0.125b \ y$	$r = 0.997$		
180	$y = 0.136 \times -2.18$	$b = 0.136b \ y$	$r = 0.999$		
		Rose clover			
0	$y = 0.072 \times -1.43$	$b = 0.072a \ y$	$r = 0.997$		
45	$y = 0.116 \times -2.19$	$b = 0.116b \ x$	$r = 0.996$		
90	$y = 0.118 \times -2.20$	$b = 0.118b \ x$	$r = 0.998$		
180	$y = 0.123 \times -2.18$	$b = 0.123b \ y$	$r = 0.999$		

\* b values with the same letter (abc) are not significantly different ( $P < 0.05$ ) within experiments (temperatures and species).

† Values with the same letter (xyz) are not significantly different ( $P < 0.05$ ) for P levels within temperatures and between species of the same temperature treatments (e.g., values for 0 kg/ha P were significantly different in Experiment 1 (5 C) but values for 45, 90, and 180 kg/ha P were not).

**Table 3. Leaf area, nodule weight, shoot, and root weights per plant; total plant weights; shoot:root ratio; and number of days to sampling (five-leaf stage) for subterranean and rose clovers grown in a range soil at four levels of P availability in three separate root temperature experiments.**

P	Leaf area				Nodule wt.				Shoot wt.				Root wt.				Shoot:root ratio				Total plant wt.				Days to sampling							
	cm				mg				mg				mg				mg				mg											
kg/ha	5 C	5 C, 10 C	10 C	Avg.	5 C	5 C, 10 C	10 C	Avg.	5 C	5 C, 10 C	10 C	Avg.	5 C	5 C, 10 C	10 C	Avg.	5 C	5 C, 10 C	10 C	Avg.	5 C	5 C, 10 C	10 C	Avg.	5 C	5 C, 10 C	10 C	Avg.				
	Subterranean clover																															
0	2.7	2.9	6.4	4.0	tr†	0.1	0.3	0.1	53	67	74	65	41	56	59	52	1.3	1.2	1.3	1.2	90†	120	130	110	114	114	87	105				
45	10.5	13.5	17.1	13.7	3.2	1.8	1.0	2.0	123	111	128	121	88	63	66	72	1.4	1.8	2.0	1.7	210	170	190	190	106	83	63	84				
90	13.7	15.9	16.8	15.5	3.8	2.3	0.9	2.3	147	123	109	126	92	69	55	75	1.6	1.8	2.0	1.8	240	190	160	200	106	73	59	79				
180	17.2	16.8	19.3	17.8	3.8	2.5	1.6	2.6	174	108	132	138	93	56	55	68	1.9	2.0	2.4	2.1	270	160	190	210	98	73	58	76				
L.S.D. (0.05)	3.1	2.7	3.0		1.5	0.8	0.4		26	14	23		6	9	NS		0.2	0.2	0.3		44	21	30									
X	11.0	12.3	14.9		2.7	1.9	0.9		124	103	111		78	61	59		1.5	1.7	1.9		200	160	170		106	86	67					
	Rose clover																															
0	1.7	2.1	6.3	3.4	tr	tr	0.4	0.1	13	17	38	23	11	13	25	16	1.2	1.4	1.5	1.4	20	30	60	40	114	114	87	105				
45	10.8	11.6	11.6	11.3	3.3	2.2	1.2	2.2	67	59	59	61	43	35	27	35	1.5	1.6	2.2	1.8	110	90	90	100	106	83	63	84				
90	11.2	12.2	11.6	11.7	2.8	2.1	1.0	2.0	71	61	55	62	37	33	25	32	2.0	1.8	2.3	2.0	110	90	80	90	106	73	59	79				
180	12.3	9.9	12.8	11.7	3.0	1.8	1.2	2.0	80	49	64	64	42	26	24	31	1.9	1.9	2.6	2.1	120	80	90	100	98	73	58	75				
L.S.D. (0.05)	3.5	4.5	4.5		1.1	0.9	0.4		17	23	22		11	13	NS		0.3	0.3	0.4		28	36	NS									
X	9.0	8.9	10.6		2.2	1.5	1.0		58	47	54		33	27	25		1.6	1.7	2.1		90	70	80		106	86	67					

† Trace amount.

‡ Values rounded to nearest 10.

clover consistently had higher values than did subclover. The largest regression coefficients were found in the 5 C experiment.

## DISCUSSION

A frequent source of frustration in field plot research with annual range legumes of the genus *Trifolium* growing under winter conditions (cold soil, low light) on P-deficient and/or P-fixing range soils is the inability to differentiate, by means of plant yield or botanical composition data, between levels of applied P which are expected to provide statistically significant and biologically important differences. Fundamental to this problem are four major considerations: 1) the unique set of requirements for effective nodulation and symbiotic N fixation of plants frequently being grown in N-poor soils, 2) the complex of relationships which delimits the concentration of P in the soil solution, its uptake by legumes, and its mobilization in plant growth, 3) genetic differences among available legumes, especially those related to relative maturity and to those characteristics which typify effective colonizers vs. those with greater expression of desirable agronomic traits (e.g., high yield potential, nutritional quality), and 4) the time-dynamic interaction of seasonal and diurnal variation in temperature and light with individual plant and plant community phenology.

Previous work (11) demonstrated that increasing P level in a stepwise manner over a range of 0 to 180 kg/ha (Sobrante-Las Posas soil series association) significantly increased the number of nodules per plant, percent crude protein of the shoot, and shoot weight. Further, an approximate 50% reduction in light intensity significantly reduced these P-related responses. In those experiments, conducted with a diurnal ambient air temperature cycle similar to that of the present study, soil temperature was not directly controlled and probably reached relatively high midday levels by absorption of solar radiation as is typical under field conditions where bright, sunny days occur (14). Nodule weight was considered by Nutman (10) to be a better morphological measure of N-fixing

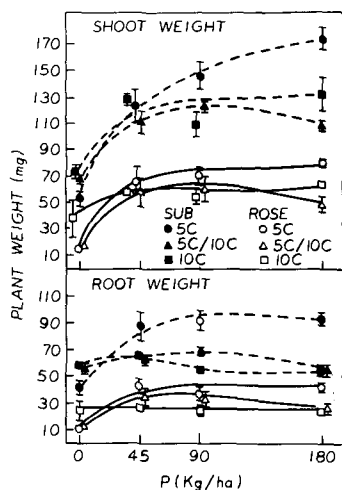


Fig. 2. Response of subterranean and rose clover shoot and root weights to P availability in three root temperature experiments.

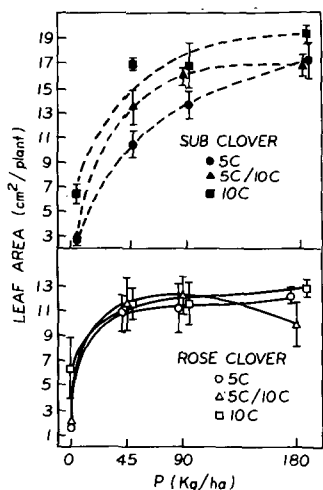


Fig. 3. Leaf area of subterranean and rose clovers as a function of P level in three root temperature experiments.

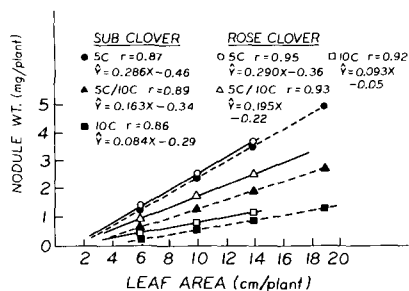


Fig. 4. The relationship of nodule weight to leaf area of subterranean and rose clovers with data combined for P levels, as influenced by root temperature.

capability in subclover than nodule number. More recently, Phillips et al. (13) have shown that for another cool-season annual, *Pisum sativum* L., plant N content was a near-linear function of plant nodule mass.

In the present study, response of nodule weight and other parameters (Table 3, Fig. 1, 2, 3) to increasing P level under controlled, cool soil temperatures generally was most pronounced between P levels of 0 and 45 kg/ha. Visual observations showed that most nodules were effective, with no important color differences between P treatments. Evidence for a soil temperature growth threshold near 5 C is found in the pattern of average regression coefficient increments for morphological development (Table 2, Fig. 1) between the three temperature levels, as follows:

P, kg/ha	Regression coefficient increment (2-species mean)	
	from 5 C to 5 C/10 C	from 5 C/10 C to 10 C
0	0.010	0.031
45	0.018	0.045
90	0.022	0.033
180	0.010	0.040

These computations suggest a non-linear response as temperature is increased, with a greater effect at low rather than at high temperatures.

A striking independence of nodule development and plant morphological development was observed (Table 3, Fig. 4). The largest nodule weights were found in the 5 C experiment, from plants which had developed most slowly, indicating that nodule development is somewhat independent of morphological development of the host plant, once nodule initiation has occurred. A further observation is relevant: while we did not attempt to statistically test responses across experiments, we found an interesting relationship when nodule weight at the various P levels was plotted as a function of time. Linear regressions fitted these data well, with positive regression coefficients with similar values for all three P levels, and a negative regression coefficient for the control. Therefore, it is of paramount importance that the seedling legume, if subjected to winter temperature and light conditions because of seasonally late rains, have adequate P available. In this case, although total winter plant growth is slow, P is assimilated in adequate amounts (14) despite cold temperatures and the plant achieves a more favorable status for further growth, interplant competition, and N fixation (20) when winter passes.

Speculation based on interspecific comparisons must be limited, since only one of many possible cultivars for each species was used in the experiments. However, the data do support the theory that rose clover, widely recognized as an effective colonizer and tolerant of site limitations such as low soil fertility, is more "conservative" in its growth expression than is subclover, which in turn is noted for desirable agronomic characteristics such as high yield potential and nutritional quality. In part, observed growth differences between species may simply have been due to seed weight differences, with the comparison valid

only prior to the log phase of growth. The P response data suggest that the Mt. Barker cultivar of subclover was more tolerant of cold soil than the Hykon cultivar of rose clover. Further, the subclover cultivar more readily translated improved nutrient status into significantly greater leaf area. This result, coupled with the close correlation of leaf area and nodule weight indicates that subclover has a greater capability for achieving levels of photosynthesis adequate to support the energy requirements of N fixation (9).

Properly managed in favorable environments, both of these species demonstrate a potential for high yield (17). The management option of fall irrigation coupled with fertilizer application (to take advantage of higher temperatures and higher nutrient levels) is of interest where value of the additional forage, produced at a time of year when forage availability normally is low, justifies cost of the additional resources. This strategy would be even more appealing if early, vigorous growth of the legumes could provide winter-available soil N for use by associated, non-legume species. Vaughn and Jones (20) suggest that peak N-fixing activity is associated with phenological development to the stage of macroscopic flowering, i.e., interspecies N transfer normally occurring in the spring or later. There is merit in further investigation of the opportunities for manipulating this germ-plasm-environment-resource input complex in order to provide fall-available legume-fixed N.

In summary, annual legumes of interest in the context of this paper probably have a low-temperature growth threshold near 5 C. Further discrimination of low temperature effects within the plant requires information on both soil and air ambient temperatures as well as their effects on growth components such as leaf area and N fixing capability. For many California, and other, P-deficient range soils it is essential to know the P-fixing capability, or to directly determine soil solution concentrations of P. Genetic variation of available species and cultivars is of considerable importance, particularly when results of physiological studies are to be interpreted at the ecosystem level and in the context of seasonal weather and climatic cycles. Finally, it is increasingly important that if nonfield studies are to be conducted, they should be done over suitable lengths of time in facilities which permit a reasonable simulation of important environmental variables.

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