THE PHYSIOLOGICAL RESPONSE TO MOISTURE SUPPLY OF CYANOGENIC AND ACYANOGENIC PHENOTYPES OF *TRIFOLIUM REPENS* L. AND *LOTUS CORNICULATUS* L.

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SUMMARY

When grown in moist or droughted conditions the cyanogenic and acyanogenic phenotypes of *L. corniculatus* and *T. repens* germinated with equal frequency, and produced similar vegetative yields and possessed equal sexual vigour.

There was no significant difference in mortality between the phenotypes of *L. corniculatus* when severely desiccated but there was a delay in stomatal closure by the cyanogenic form. The leaf tissue of the cyanogenic phenotype of *T. repens* was less adept at preventing water loss when subjected to negative water potentials but there was no difference between the rate of transpiration of the phenotypes of this species. This was confirmed by the equal number of fatalities of the phenotypes when severely desiccated.

Selection by drought against the cyanogenic phenotype of the two species appears to be due either to linkage of the genes responsible for cyanogenesis with genes causing fitness responses to environmental factors, or to the fact that these alleles are acting as modifiers of the fitness genes, rather than to the release of HCN from damaged tissue of cyanogenic individuals.

1. INTRODUCTION

CYANOGENESIS in *T. repens* is controlled by the interaction of two genes. The presence or absence of cyanogenic glucoside, lotaustralin, and enzyme, linamarase, are each governed by a pair of genes *Ac*, *ac* and *Li*, *li*, and their presence is dominant over their absence (Corkill, 1942; Atwood and Sullivan, 1943). Cyanogenic plants contain both *Ac* and *Li*, while acyanogenic plants may contain either one or neither of the dominant genes *Ac* and *Li*. The control of cyanogenesis in *L. corniculatus* is similar except that there are two pairs of each of the alleles *Ac* and *Li*. On damage to the tissue of the cyanogenic phenotype detectable amounts of HCN are released.

The maintenance of polymorphism of cyanogenesis in *T. repens* and *L. corniculatus* has been attributed to three selective forces. Many authors (Corkill, 1952; Jones, 1962, 1966; Crawford-Sidebottom, 1972; Angseesing, 1974) have suggested that selective eating by animals may be partly responsible. Daday (1954*a*, *b*; 1965) argued that temperature was a major factor in maintaining the polymorphism in *T. repens* and that low winter temperatures were unfavourable to the cyanogenic phenotype (*AcLi*).

Soil moisture as a selecting agent was demonstrated by Foulds and Grime (1972*a*) when measuring the phenotype frequency in natural populations of *T. repens* and *L. corniculatus* growing in moist and droughted habitats mainly in Derbyshire. They found that glucoside forms of *T. repens* were significantly less frequent on droughted soils than moist soils. There was, however, no difference in the frequency of the phenotypes with glucosidase present, supporting the view of Daday (1965) that the locus concerned with

cyanogenic glucoside production may be genetically linked to genes concerned with fitness responses to environmental factors. In contrast to results for *T. repens*, those for *L. corniculatus* gave no evidence of an effect of the glucoside gene (*Ac*) on survival at the sites subjected to droughting, but fewer glucosidase (*Li*) plants were found in the droughted populations. Thus two closely related species have responded to the same selection in different ways, *i.e.* a low frequency of cyanogenic plants being achieved by a reduction in frequency of *Ac* in *T. repens* while in *L. corniculatus* there has been a reduction in frequency of *Li*. Jones (1973) confirmed this drought effect on populations of *L. corniculatus* growing in large droughted areas (over 20 m²) though small areas (less than 4 m²) appear to be unaffected.

Foulds and Grime (1972*b*) confirmed these population studies for *T. repens* by showing that the glucosidic form was killed more easily by soil moisture stress than was the aglucosidic form. However, a summation of the fatalities of the three acyanogenic phenotypes show that 23 per cent were killed and an almost equal number of the cyanogenic form died (32 per cent). Under sublethal moisture stresses the vegetative yield of *Ac* and *ac* phenotypes were not significantly different, though in moist conditions *ac* phenotypes obtained higher yields than the *Ac* plants. This suggested a possible linkage between the *Ac* gene and genes affecting vegetative vigour. Cyanogenic plants displayed a low sexual vigour in all treatments and the effect of moisture stress was to inhibit flowering completely under conditions in which flowers were produced in all three acyanogenic forms. Confirmation of this result was needed.

In an attempt to elucidate the physiological factors responsible for this apparent disadvantage of the cyanogenic form in droughted conditions the following experiments were devised.

2. MATERIALS AND METHODS

The plants used in the experiments were grown from seed obtained from Derbyshire and the cyanogenic and acyanogenic phenotypes were distinguished using the picric acid paper technique (Daday, 1954*a*).

(i) *Growth, reproduction and mortality*

Seeds were cultivated in garden soil and transplanted into garden plots measuring one square metre. The depth of soil in the plots was varied by inserting 500 gauge polythene sheeting at a depth of 5 cm, cut larger than the actual experimented area and turned up at the edges to confine rooting to the plot and to prevent water from running from one plot to another. In the control treatment no plastic was present and the soil, a fertile sandy loam, exceeded a metre in depth. Eighty-one plots were arranged in random numbers along rows (16 controls and 65 treatments). A 10-cm space was left between rows. Each of the treatment plots was covered with a wire cage over which clear polythene was placed; this could be retained or removed so that the amount of water received by each plot could be controlled.

On 27th April 1974 into each control plot, one 6-week-old seedling, either cyanogenic or acyanogenic, of *T. repens* and *L. corniculatus* was transplanted. Into 10 treatment plots two 6-week-old seedlings of *T. repens* were

placed and a similar number of *L. corniculatus* replicates were transplanted into 10 more plots. Forty-five plots contained two *T. repens* and two *L. corniculatus* replicates. Wherever possible the two *T. repens* and two *L. corniculatus* consisted of one cyanogenic and one acyanogenic phenotype.

Each plot was watered initially with 500 cm³ of Crone-Bryan's solution (Fred, Baldwin and McCoy, 1932), then daily with tap water until the 11th May 1974. By this date the plants which survived were fully established. Subsequently, water was added only when the majority of plants in the 5-cm treatment wilted slightly, and the plots were covered over during periods of intense rain. During long periods of drought the control plots were also watered liberally. All plants were harvested on the 19th September 1974, except for those replicates from 20 treatment plots, selected at random, which were left without water for a further 2 weeks until they appeared stressed. None of these severely droughted plants were harvested.

In order to determine the most severe conditions of moisture stress to which the plants were subjected, soil samples were taken in the afternoon after a period of 10 days without water. At this stage some of the plants showed signs of wilting. The samples were taken from the entire 5-cm depth of soil and the water content was determined by oven-drying at 100°C for 24 hours. The pH of the soil in each treatment was determined at the beginning and end of the experiment. Measurements included the number of fatalities, dry matter production (root and shoot were weighed separately), radial expansion of the shoot, number of plants in flower, number of flowers per plant, number of florets per flower, number of seeds per floret and an estimated number of seeds per plant.

(ii) *Leaf response to desiccation*

Hygen's (1951) quick weighing method for the analysis of transpiration of detached leaves was used for the comparison of the relations between leaf water status and stomatal closure.

Newly opened trefoils of the three species were cut close to the leaflets and floated on water in the dark at 20°C for 12 hours, after which they were exposed for 60 minutes at a light intensity of 2800 lux to allow the stomates to open.

Trefoils which were rapidly surface-dried with filter paper and the cut ends sealed with petroleum jelly were weighed (saturated weight, SW) and placed on a line in a transparent container (temperature 22.5°C: relative humidity *c.* 50 per cent). Several trefoils of each plant were treated in this manner and were then reweighed every 5 minutes (fresh weight, FW) for 60 minutes, after which time the dry weight (DW) was determined.

Leaf water status at any point during a drying cycle was then measured as relative water content (RWC) where:

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{SW} - \text{DW}} \times 100 \text{ (Okali, 1971)}$$

In this way the change in leaf water status with time could be plotted; the curve obtained consists of an initial linear phase when stomates are open and rate of water loss steady and rapid, followed by a curvilinear phase during stomatal closure and containing finally another linear phase when stomates are closed and water loss is cuticular with a progressively

declining rate. The point of intersection of the two extrapolated linear portions of the curve is taken as indicating the relative water content at stomatal closure (Okali, 1971).

The basic data for the determination of points of stomatal closure were used to calculate rates of transpiration. Maximum rates were first calculated in $\text{mg dm}^{-2}\text{min}^{-1}$ from the weight loss during the first 10 minutes after recording saturated weight and leaf areas. The latter were determined by placing the trefoils on photographic paper, the relationship between weight and areas of which had previously been calculated.

Total transpiration rate was also calculated as the rate of loss of relative water content (which depends on the rate of water loss per minute derived from water lost during the first 10 minutes after recording saturated weight). Maximum cuticular rate of transpiration was similarly determined for the first 10 minutes after the estimated point of stomatal closure. Stomatal rate of transpiration was then obtained by difference.

(iii) *Water content of desiccated leaves*

The ability of leaf discs to retain water against applied negative water potentials were measured by using Okali's (1971) modified gravimetric vapour equilibrium technique of Slatyer (1958). Leaf discs 5 mm in diameter were punched from recently opened trefoils of plants grown in garden plots. All discs were saturated by placing them inside a lunchbox on nylon mesh resting on a water-saturated sponge. After 2 hours five saturated discs were surface-dried between filter paper and weighed to obtain saturated weight (SW) before transferring to micro-desiccators (polythene screw-capped containers) in which the discs were supported on a platform of nylon mesh. The mesh was supported above sponge saturated with NaCl solution, the concentration of which was varied in order to give a range of seven water potentials (0 to -120 bars). Each treatment was repeated three times. The micro-desiccators were maintained in a water bath ($25 \pm 1^\circ\text{C}$) for 48 hours after which the discs were reweighed in order to give the equilibrated weight (EW). The discs were next oven-dried overnight at 100°C to obtain the dry weight (DW). Water retained by the leaf tissue at each water potential was then calculated as:

$$\frac{\text{EW} - \text{DW}}{\text{SW} - \text{DW}} \times 100$$

(iv) *Seed germination*

In the summer of 1974 cyanogenic and acyanogenic phenotypes of *L. corniculatus* and *T. repens* were isolated, and seeds collected from them and stored at 3°C until required. They were scarified and placed in groups of 100 on indented blotting paper. The blotting paper was saturated with deionised water or mannitol solutions of 0.5, 1.0, 2.0, 4.0, 8.0 and 12 bars osmotic pressure. The seeds were germinated at the optimum temperature of 20°C for 12 days. Large numbers of seed from each site collected were germinated in seed boxes and when the plants were large enough each plant was classified as cyanogenic or acyanogenic by means of the "picric acid paper" test.

3. RESULTS

(i) *Growth, reproduction and mortality*

Measurements confirmed that there was little variation in soil pH either between treatment or over the whole course of the experiment (table 1). The mean maximum daily temperature during the period of the experiment was 18.6°C. The total rainfall experienced was 263 mm. The soil moisture content of the 5-cm plots (throughout the entire depth), measured during a period when there was no rain for 10 days, was found to be 5 per cent per g dry soil.

Observations at the end of the experiment suggested that there was probably no root competition between the replicates in the control plots and no excessive overlapping of the root systems took place in the 5-cm plots.

There was no significant difference in dry matter yield (table 2) between the phenotypes of *L. corniculatus* and *T. repens* in either the moist or droughted regimes. A similar pattern emerges when the radial expansion of the shoot is considered (table 2). With the exception of the cyanogenic form of *L.*

TABLE 1

Soil depth	Soil pH	
	Soil pH	
	Initial (27.5.74)	Final (19.9.74)
Control	7.0-7.2	6.9-7.2
5 cm	7.0-7.1	7.0-7.1

Values show the range over ten measurements.

corniculatus, grown in droughted soil, which possesses a greater root/shoot ratio than the acyanogenic form, there was no significant difference between the phenotypes of either of the two species, whether grown in moist or droughted soil with respect to both the root/shoot ratio and root length. Except for the root length of the acyanogenic form (table 3) of *L. corniculatus* the final length of the root and the ratio of the root to the shoot were greater in both phenotypes of *L. corniculatus* than those of *T. repens* in droughted conditions.

The flower production of the cyanogenic phenotypes of both species was difficult to compare because many replicates failed to produce flowers, particularly in the drought regime. The plants which did flower produced between 3 and 235 per plant in the case of *L. corniculatus* and between 1 and 163 in the case of *T. repens*. Table 4 shows that in moist conditions all the plants, cyanogenic and acyanogenic, of *L. corniculatus* formed flowers whereas only 15.4 per cent of the acyanogenic phenotypes flowered in drought conditions. Although the number of flowers per plant was considerably larger in moist soil than the droughted soil there was no significant difference between the cyanogenic and acyanogenic forms in either moisture regime. Only 80 per cent of the cyanogenic and acyanogenic phenotypes of *T. repens* formed flowers in moist productive conditions but when grown in droughted conditions a high percentage of plants still formed flowers. Half of the

acyanogenic forms flowered and 35.7 per cent of the cyanogenic phenotype. The cyanogenic and acyanogenic phenotypes of *T. repens* were similar to those of *L. corniculatus*, there being no significant difference between the number of flowers per plant of the two forms in either water regime.

In *L. corniculatus* the number of florets per flower or the number of seeds per floret of the cyanogenic phenotype was not significantly different from those of the acyanogenic forms in either of the moisture regimes (table 5).

TABLE 2

Vegetative yield for cyanogenic and acyanogenic phenotypes of L. corniculatus and T. repens grown in moist and droughted soils

Species (Water regime)	Phenotypes	No. of replicates	Dry matter production (g)	Plant diameter (cm)
<i>L. corniculatus</i>				
Moist	Cyanogenic (A)	9	55.5	54.8
	Acyanogenic (B)	7	50.0	46.4
Droughted	Cyanogenic (C)	26	2.3	14.7
	Acyanogenic (D)	41	1.3	10.7
<i>T. repens</i>				
Moist	Cyanogenic (E)	5	92.0	62.5
	Acyanogenic (F)	10	152.5	68.5
Droughted	Cyanogenic (G)	14	2.8	20.9
	Acyanogenic (H)	33	2.4	18.0
<i>F</i> values				
Between phenotypes			3.34 n.s.	1.94 n.s.
Between soil moisture			256.76***	638.89***
Between species			43.52***	42.49***
M.S. error			630.94	61.47
Scheffé comparisons of the means				
$\bar{X}_A - \bar{X}_C$			***	***
$\bar{X}_B - \bar{X}_D$			***	***
$\bar{X}_E - \bar{X}_G$			***	***
$\bar{X}_F - \bar{X}_H$			***	***
$\bar{X}_A - \bar{X}_F$			***	n.s.
$\bar{X}_B - \bar{X}_E$			n.s.	***
$\bar{X}_B - \bar{X}_F$			***	***
$\bar{X}_C - \bar{X}_G$			n.s.	*
$\bar{X}_D - \bar{X}_G$			n.s.	***
$\bar{X}_D - \bar{X}_H$			n.s.	***

n.s.—not significant; *—significant at the 0.05 level; **—significant at the 0.01 level; ***—significant at the 0.001 level.

Where all the parameters are insignificant the Scheffé comparisons have been omitted.

A similar pattern emerges when the results for *T. repens* were considered except that the cyanogenic had fewer florets than the acyanogenic phenotypes in moist conditions. Sexual reproduction, expressed as the number of seeds produced per plant, in the cyanogenic phenotype of *L. corniculatus* and *T. repens* is given in table 5, which shows that there was a drastic reduction in seed production when the plants were grown in droughted soil. However, the cyanogenic phenotype of both species were identical with the acyanogenic phenotypes in both moist and droughted soils in their ability to form seeds.

Of the *L. corniculatus* and *T. repens* plants that were severely desiccated at the end of the period of growth (table 6) there was no significant difference in the number of cyanogenic and acyanogenic phenotypes that died. The phenotypes of *T. repens* were more severely affected (half the plants being killed) than those of *L. corniculatus*.

TABLE 3

The root/shoot dry weight ratio and root length (mm) for cyanogenic and acyanogenic phenotypes of L. corniculatus and T. repens grown in moist and droughted soils

Species (Water regime)	Phenotype	Root/shoot ratio	Root length (mm)
<i>L. corniculatus</i>			
Moist	Cyanogenic (A)	0.22	492
	Acyanogenic (B)	0.23	708
Droughted	Cyanogenic (C)	1.21	507
	Acyanogenic (D)	0.81	404
<i>T. repens</i>			
Moist	Cyanogenic (E)	0.09	312
	Acyanogenic (F)	0.11	345
Droughted	Cyanogenic (G)	0.45	305
	Acyanogenic (H)	0.40	260
<i>F</i> values			
Between phenotypes		0.5 n.s.	1.13 n.s.
Between soil moisture		41.84***	16.08***
Between species		5.26*	87.54***
M.S.error		0.11	12564.91
Scheffé comparisons of the means			
$\bar{X}_A - \bar{X}_C$		***	n.s.
$\bar{X}_B - \bar{X}_D$		*	***
$\bar{X}_B - \bar{X}_E$		n.s.	***
$\bar{X}_B - \bar{X}_F$		n.s.	***
$\bar{X}_C - \bar{X}_G$		***	***
$\bar{X}_C - \bar{X}_H$		***	***
$\bar{X}_D - \bar{X}_H$		***	***
$\bar{X}_A - \bar{X}_D$		**	n.s.
$\bar{X}_B - \bar{X}_C$		***	*
$\bar{X}_C - \bar{X}_D$		**	n.s.

Number of measurements is the same as in Table 2.

Where all the parameters are insignificant the Scheffé comparisons have been omitted.

(ii) Leaf response to desiccation

The transpiration rate expressed in terms of leaf area of the cyanogenic phenotype of *L. corniculatus* and *T. repens* was not significantly different from any of the acyanogenic forms or an average of the three acyanogenic phenotypes (table 7). There was also no difference between cyanogenic and acyanogenic forms with respect to total, stomatal and cuticular transpiration (when expressed as loss of relative water content).

The relative water content of the leaves of the four phenotypes of *L. corniculatus* at which stomatal closure occurred is given in table 8 and shows

that the *AcLi* phenotype closes its stomates at a significantly lower relative water content than the *acLi* phenotype but not the other two acyanogenic forms. The *AcLi* phenotype also possessed a significantly poorer control of water loss than the three acyanogenic phenotypes when compared as a group. There was no significant difference between the phenotypes in their ability to avoid desiccation. The four cyanogenic phenotypes of *T. repens* were not significantly different in either the relative water content at which they close their stomates or in their ability to avoid desiccation.

TABLE 4

Percentage of plants flowering and number of flowers per plant (of those plants flowering) for cyanogenic and acyanogenic phenotypes of L. corniculatus and T. repens grown in moist and droughted soils

Species (Water regime)	Phenotype	Percentage of plants flowering	No. of flowers per plant
<i>L. corniculatus</i>			
Moist	Cyanogenic	100.0 (9)	80.1 (9)
	Acyanogenic	100.0 (7)	69.9 (7)
Droughted	Cyanogenic	15.4 (26)	2.3 (4)
	Acyanogenic	12.2 (41)	2.6 (8)
<i>T. repens</i>			
Moist	Cyanogenic	80.0 (5)	54.8 (4)
	Acyanogenic	80.0 (10)	22.3 (8)
Droughted	Cyanogenic	35.7 (14)	6.8 (5)
	Acyanogenic	51.5 (33)	2.5 (17)
<i>F</i> values			
Between phenotypes			1.23 n.s.
Between soil moisture			31.53***
Between species			2.64 n.s.
M.S. _{error}			1327.51

Figures in brackets are the number of measurements.

Relevant Scheffé comparisons of the means were insignificant.

It is clear from the comparison of the cyanogenic phenotype of the two species that a low rate of water loss in the phenotypes of *L. corniculatus* was due to the closure of stomates while the leaves still possessed a high relative water content.

(iii) *Water content of desiccated leaves*

There was no significant difference between any of the cyanogenic phenotypes of *L. corniculatus* in the ability of their leaves to retain water against negative water potentials (fig. 1). This was not the case with leaves of the *T. repens* phenotypes (fig. 2), the acyanogenic form, *acLi*, being by far the most adept, having significantly higher values at all moisture stresses except -20 bars. At all but the lower stresses the cyanogenic phenotype (*AcLi*) was less capable of retaining water, than the other three phenotypes considered together, and all but the *AcLi* phenotype when compared individually.

TABLE 5

Florets per flower, seeds per floret and estimated number of seeds per plant for cyanogenic and acyanogenic phenotypes of L. corniculatus and T. repens grown in moist and droughted soils

Species (Water regime)	Phenotypes	Florets per flower	Seeds per floret	Estimated no. of seeds per plant ^a
<i>L. corniculatus</i>				
Moist	Cyanogenic (A)	3.4 (165)	10.7 (175)	2975 (9)
	Acyanogenic (B)	2.4 (93)	11.3 (127)	1642 (7)
Droughted	Cyanogenic (C)	2.5 (30)	8.9 (50)	8 (26)
	Acyanogenic (D)	2.7 (20)	9.1 (50)	7 (41)
<i>T. repens</i>				
Moist	Cyanogenic (E)	67.1 (21)	2.3 (50)	8271 (5)
	Acyanogenic (F)	88.1 (35)	2.1 (30)	3361 (10)
Droughted	Cyanogenic (G)	59.6 (8)	2.4 (10)	459 (14)
	Acyanogenic (H)	69.6 (5)	2.3 (10)	240 (33)
<i>F</i> values				
Between phenotypes		41.21***	0.04 n.s.	7.36**
Between soil moisture		31.79***	3.51 n.s.	42.53***
Between species		3337.24***	111.30***	5.22**
M.S. error		44.40	31.74	7900326.61

Scheffé comparisons of the means

$\bar{X}_E - \bar{X}_F$	***	n.s.	n.s.
$\bar{X}_A - \bar{X}_C$	n.s.	n.s.	***
$\bar{X}_B - \bar{X}_D$	n.s.	n.s.	***
$\bar{X}_E - \bar{X}_G$	n.s.	n.s.	***
$\bar{X}_F - \bar{X}_H$	***	n.s.	***

^a Means were calculated from all individuals.

Figures in brackets are the number of measurements.

Where all the parameters are insignificant the Scheffé comparisons have been omitted.

TABLE 6

Total number of fatalities of cyanogenic and acyanogenic phenotypes of L. corniculatus and T. repens grown in droughted soil and severely desiccated

Species (Phenotypes)	Total no. of replicates	Alive	Dead	Fatalities (%)
<i>L. corniculatus</i>				
Cyanogenic	21	15	6	28.6
Acyanogenic	17	15	2	11.8
χ^2 ^a	2.77 n.s.			
<i>T. repens</i>				
Cyanogenic	10	5	5	50.0
Acyanogenic	15	8	7	46.7
χ^2 ^a	0.33 n.s.			

^a χ^2 values are for 2×2 contingency tables.

(iv) *Seed germination*

The batches of seed of both species of legume were classified into seeds with less than 30 per cent cyanogenic individuals, equal numbers of cyanogenic and acyanogenic forms and those with more than 60 per cent cyanogenic plants. There was no significant difference between the number of seeds that germinated at any of the osmotic moisture stresses of either *L. corniculatus* (fig. 3) or *T. repens* (fig. 4) whether the seeds contained a high or low percentage of the cyanogenic phenotype.

TABLE 7

Rates of transpiration of four cyanogenic phenotypes of L. corniculatus and T. repens calculated in terms of leaf area (mg/dm²/min) and as rate of loss of relative water content (stomatal values obtained by difference)

Species (Phenotypes)	No. of observations	Water loss (mg/dm ² /min)	Loss of relative water content/min		
			Total	Stomatal	Cuticular
<i>L. corniculatus</i>					
<i>AcLi</i> (A)	24	9.87	0.84	0.66	0.18
<i>Acli</i> (B)	10	5.64	0.42	0.28	0.15
<i>acLi</i> (C)	12	6.60	0.54	0.38	0.16
<i>acli</i> (D)	15	8.58	0.66	0.47	0.19
(<i>Acli</i> , <i>acLi</i> , <i>acli</i>)		6.94	0.55	0.38	0.17
<i>T. repens</i>					
<i>AcLi</i> (E)	20	10.25	1.34	1.16	0.19
<i>Acli</i> (F)	9	11.60	1.60	1.39	0.21
<i>acLi</i> (G)	12	10.28	1.46	1.26	0.20
<i>acli</i> (H)	15	10.75	1.37	1.17	0.21
(<i>Acli</i> , <i>acLi</i> , <i>acli</i>)		10.73	1.48	1.27	0.21
<i>F</i> values					
Between phenotypes		1.71 n.s.	0.58 n.s.	0.55 n.s.	0.49 n.s.
Between species		20.75***	231.24***	154.17***	5.07*
M.S.error		9.70	0.08	0.09	0.006

All relevant Scheffé comparisons of the means between phenotypes were not significant.

4. DISCUSSION

The experiments described in this paper were designed mainly to demonstrate whether or not the cyanogenic phenotype of *L. corniculatus* and *T. repens* is disadvantageous under droughted conditions. Foulds and Grime (1972*b*) could find no difference in vegetative yield or the number of fatalities of the cyanogenic phenotype in comparison with the acyanogenic phenotypes of *T. repens*. The results of this study confirm these findings in that of the total number of mature plants of *T. repens* that were severely desiccated, 50 per cent of both the cyanogenic and acyanogenic forms died. There was also no significant difference in vegetative growth between the phenotypes in moist or droughted soils. The reproductive vigour of the cyanogenic phenotype is not as low as the results of Foulds and Grime (1972*b*) suggested

because 80 per cent of the replicates flowered in the moist soils and 35 per cent in the droughted conditions. It should be noted, however, that although the number of flowers per plant and the seed production of the phenotypes was not different, in droughted conditions, a greater number of the acyanogenic replicates flowered (50 per cent), thus the cyanogenic phenotype is at a disadvantage in this respect.

The transpiration data confirm the above results as there is no difference between the phenotypes with respect to the rate of transpiration, the water

TABLE 8

Mean relative water content of four cyanogenic phenotypes of L. corniculatus and T. repens at points of stomatal closure and desiccation avoidance expressed as mean relative water content of leaves after 1 hour drying under standard conditions

Species (Phenotype)	No. of observations	Relative water content per cent	
		Stomatal closure	Desiccation avoidance
<i>L. corniculatus</i>			
<i>AcLi</i> (A)	24	83.00	78.73
<i>Acli</i> (B)	10	90.00	87.00
<i>acLi</i> (C)	12	91.87	86.79
<i>acli</i> (D)	15	90.62	85.23
(<i>Acli</i> , <i>acLi</i> , <i>acli</i>)		90.83	86.34
<i>T. repens</i>			
<i>AcLi</i> (E)	30	75.70	72.05
<i>Acli</i> (F)	9	74.86	69.66
<i>acLi</i> (G)	12	77.46	72.64
<i>acli</i> (H)	15	76.20	71.62
(<i>Acli</i> , <i>acLi</i> , <i>acli</i>)		76.17	71.31
<i>F</i> values			
Between phenotypes		3.64*	2.51 n.s.
Between species		122.65***	126.54***
M.S. _{error}		35.64	35.20
Scheffé comparisons of the means			
$X_A - X_C$		*	—
$X_A - X_{1/3(B+C+D)}$		**	—
$X_E - X_{1/3(F+G+H)}$		n.s.	—

Insignificant Scheffé comparisons have been mostly omitted.

content at which the stomates are closed or the ability of the leaves to avoid desiccation. However, when the leaves were subjected to negative water potentials the cyanogenic phenotype proved to be less adept at preventing water loss than the three acyanogenic phenotypes considered together.

The cyanogenic phenotype appears not to be at a disadvantage at the early stage of growth because there was no difference in the germination of cyanogenic or acyanogenic seeds when they were subjected to osmotic moisture stress, though it would be interesting to see the effect of severe desiccation on young seedlings.

The suggestion by Foulds and Grime (1972*b*) that the low frequency of the cyanogenic phenotype in natural populations of *L. corniculatus* was due

FIG. 1.—The relation between relative water content (per cent) and water potential (bars) of leaves of the four cyanogenic phenotypes of *L. corniculatus* grown in moist conditions. *AcLi*—solid circle; *Acli*—solid square; *acLi*—solid triangle; *acli*—open circle. The vertical line represents the 95 per cent confidence interval.

to reduction of the *Li* allele may be only a partial explanation of the reason for the disadvantage of the cyanogenic form in droughted habitats. During moisture stress the cyanogenic phenotype (*AcLi*) delays stomatal closure but this did not cause a higher rate of stomatal transpiration or tissue death.

FIG. 2.—The relation between relative water content (per cent) and water potential (bars) of leaves of the four cyanogenic phenotypes of *T. repens* grown in moist conditions. *AcLi*—solid circle; *Acli*—solid square; *acLi*—solid triangle; *acLi*—open circle. The vertical line represents the 95 per cent confidence interval.

Stomatal control was the only physiological process found to be associated with the cyanogenic phenotype because there was no difference between this form and the acyanogenic forms with respect to vegetative growth, flowering, seed production and germination when the plants were subjected to moisture stress.

FIG. 3.—The effect of osmotic moisture stress on the germination of cyanogenic and acyanogenic seeds of *L. corniculatus* grown at 20°C for 12 days in the dark. Less than 30 per cent cyanogenic seeds—circle; 50 per cent cyanogenic seeds—square; more than 60 per cent cyanogenic seeds—triangle. The vertical line represents the 95 per cent confidence interval.

Therefore selection against the cyanogenic phenotype of *T. repens* by drought may occur owing to genetic linkage of the locus concerned with glucoside production with genes responsible for fitness responses to environmental factors, as originally suggested by Daday (1956), or to the fact that this allele acts as a modifier of the fitness genes.

The greater susceptibility of the cyanogenic phenotype of *L. corniculatus* to desiccation is more difficult to explain as the possession of both the *Ac*

FIG. 4.—The effect of osmotic moisture stress on the germination of cyanogenic and acyanogenic seeds of *T. repens* grown at 20°C for 12 days in the dark. Less than 30 per cent cyanogenic seeds—circle; 50 per cent cyanogenic seeds—square; more than 60 per cent cyanogenic seeds—triangle. The vertical line represents the 95 per cent confidence interval.

and *Li* genes seem to be necessary before the factor becomes fatal. However, as with the case of *T. repens*, there is no evidence that it is the release of HCN from the leaves of the cyanogenic phenotype when droughted that is responsible for the death of the plant, but rather it is a matter of gene linkage or modification.

When the phenotypes of both legumes were frosted or droughted there was a reduction in the rate of photosynthesis, but not respiration (Foulds and

Young, 1977), but no difference between cyanogenic and acyanogenic phenotypes could be distinguished.

The complete elimination from droughted areas of the cyanogenic phenotype of *L. corniculatus* (Jones, 1962; Crawford-Sidebottom, 1972) and *T. repens* (Corkill, 1952; Angseesing, 1974) may be prevented by selective predation by animals which avoid cyanogenic plants.

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5. REFERENCES

- ANGSEESING, J. P. A. 1974. Selective eating of the acyanogenic form of *Trifolium repens* L. *Heredity*, 32, 423-430.
- ATWOOD, S. S., AND SULLIVAN, J. T. 1943. Inheritance of a cyanogenetic glucoside and its hydrolysing enzyme in *Trifolium repens*. *J. Hered.*, 34, 311-320.
- CORKILL, L. 1942. Cyanogenesis in white clover (*Trifolium repens* L.). V. The inheritance of cyanogenesis. *N.Z. J. Sci. Tech.*, 23, 178B-193B.
- CORKILL, L. 1952. Cyanogenesis in white clover (*Trifolium repens* L.). VI. Experiments with high-glucoside and glucoside-free strains. *N.Z. J. Sci. Tech.*, 34, 1A-16A.
- CRAWFORD-SIDEBOTTOM, T. J. 1972. The role of slugs and snails in the maintenance of the cyanogenesis polymorphisms of *Lotus corniculatus* and *Trifolium repens*. *Heredity*, 28, 405-411.
- DADAY, H. 1954a. Gene frequencies in wild populations of *Trifolium repens* L. I. Distribution by latitude. *Heredity*, 8, 61-78.
- DADAY, H. 1954b. Gene frequencies in wild populations of *Trifolium repens* L. II. Distribution by altitude. *Heredity*, 8, 377-384.
- DADAY, H. 1965. Gene frequencies in wild populations of *Trifolium repens* L. IV. Mechanism of natural selection. *Heredity*, 20, 355-365.
- FOULDS, W., AND GRIME, J. P. 1972a. The influence of soil moisture on the frequency of cyanogenic plants in populations of *Trifolium repens* and *Lotus corniculatus*. *Heredity*, 28, 143-146.
- FOULDS, W., AND GRIME, J. P. 1972b. The response of cyanogenic and acyanogenic phenotypes of *Trifolium repens* to moisture supply. *Heredity*, 28, 181-187.
- FOULDS, W., AND YOUNG, L. 1977. Effect of frosting, moisture stress and potassium cyanide on the metabolism of cyanogenic and acyanogenic phenotypes of *Lotus corniculatus* L. and *Trifolium repens* L. *Heredity*, 38, 19-24.
- FRED, E. B., BALDWIN, I. L., AND MCCOY, L. 1932. *Root Nodule Bacteria and Leguminous Plants*. University of Wisconsin Press, Madison.
- HYGEN, G. 1951. Studies in plant transpiration. I. *Physiologia Pl.*, 4, 57-183.
- JONES, D. A. 1962. Selective eating of the acyanogenic form of the plant *Lotus corniculatus* L. by various animals. *Nature*, 193, 1109.
- JONES, D. A. 1966. On the polymorphism of cyanogenesis in *Lotus corniculatus* L. I. Selection by animals. *Canad. J. Genet. Cytol.*, 8, 556-567.
- JONES, D. A. 1973. Co-evolution and cyanogenesis. In *Taxonomy and Ecology*, ed. V. H. Heywood, pp. 213-242. Academic Press.
- OKALI, D. V. V. 1971. Tissue water relations of some woody species of the Accra plains, Ghana. *J. Ecol.*, 59, 89-101.
- SLATYER, R. O. 1958. The measurement of diffusion pressure deficit in plants by a method of vapour equilibrium. *Anat. J. Biol. Sci.*, 11, 349-365.