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NOTES AND COMMENTS

THE RELATIONSHIP BETWEEN ALTITUDE AND CYANOGENESIS IN WHITE CLOVER (*TRIFOLIUM REPENS*, L.)

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Summary

A total of 694 plants of *Trifolium repens* were collected in 17 samples to investigate the effect of altitude on the frequency of cyanogenic plants; the area chosen for this purpose was North Wales, with altitudes ranging from 100 to 1600 feet. A significant decrease in frequency of *AcLi* plants was observed, lower values obtained in high altitudes; the calculated regression coefficient was $-0{\cdot}01938$ (in angular measure), with $P < 0{\cdot}001$.

1. Introduction

It is a well-known fact that *Trifolium repens* is polymorphic for the production of HCN, with two unlinked loci responsible for the phenomenon: *Ac* which produces cyanogenic glucosides, linamarin and lotaustralin, and *Li*, which produces the enzyme linamarase. The mechanism that maintains such polymorphism has raised some discussion, since both abiotic and biotic selective agents seem to be involved. According to Daday (1954*a*, *b*), temperature is the most important factor, the cyanogenic form being at disadvantage in winter or in high mountains. Foulds and Grime (1972) have detected selection against the glucosidic form in wet soils, using an experimental approach.

On the other hand, evidence for the action of biotic factors comes from differential eating by snails and slugs, as well as small mammals, the acyanogenic forms being preferred (see Whitman, 1973, and Angseesing, 1974; for similar findings in *Lotus corniculatus*, see Jones, 1966, and Crawford-Sidebotham, 1972; negative findings were reported by Bishop and Korn, 1969). Jones (1973) provides a full discussion of these and related topics. The same author (Jones, 1970) advanced the interesting hypothesis that cyclical selection could be involved, acyanogenic forms being at an advantage in winter and cyanogenic plants being less predated in the summer as well as possessing some general physiological advantage.

The investigation reported here was conducted in order to show whether small variations in altitude in a restricted area were accompanied by changes in phenotypic frequencies.

2. Materials and methods

The area chosen for this study was North Wales. A total of 17 samples (ranging from 25 to 64 individual plants) were collected in unmanaged areas. In order to avoid collecting the same clone twice individual plants were taken from sites at least 10 feet apart. No attempt was made to follow along a transect.

At least four leaves of each plant were placed in a glass tube and brought to the laboratory, where they remained stored (in a cold room at 4°C) for about 24 hours. Two leaves were macerated and tested for cyanogenesis using sodium picrate papers, according to the usual procedures (Pusey, 1963). Those plants that failed to give a positive reaction after 2 hours were tested again by using the remaining leaves with others of known phenotype (*Acli* and *acLi*), previously identified and growing in a greenhouse. Such a procedure enabled us to recognise the four phenotypes.

3. Results

Frequencies of the four phenotypes for each sample are presented in table 1; a graphical representation of the frequency of cyanogenic plants can be seen in fig. 1. Up to 850 feet no significant trend with altitude was

TABLE 1

Variation of phenotypic frequencies associated with altitude in white clover

Altitude (ft)	Phenotypic frequencies *AcLi*	*Acli*	*acLi*	*acli*	Sample size
100	0·69	0·23	0·02	0·06	49
100	0·32	0·56	0·04	0·08	25
200	0·55	0·28	0·05	0·12	40
200	0·28	0·49	0·08	0·14	49
450	0·51	0·30	0·08	0·11	47
500	0·30	0·50	0·02	0·18	40
500	0·57	0·38	0·00	0·05	42
600	0·67	0·20	0·03	0·10	30
750	0·63	0·29	0·02	0·06	51
850	0·31	0·38	0·05	0·26	64
900	0·27	0·27	0·10	0·36	30
900	0·09	0·53	0·05	0·33	45
900	0·42	0·37	0·05	0·16	38
1050	0·09	0·28	0·20	0·43	35
1200	0·19	0·55	0·10	0·16	31
1200	0·22	0·60	0·05	0·13	40
1600	0·11	0·47	0·00	0·42	38

observed (Fisher's exact test has been used where the numbers were too small to calculate a chi-square). However, at 850 feet and higher altitudes there is a drop in the proportion of *AcLi* plants. Another interesting fact is that plants carrying *Ac* are more frequent than those homozygous for *ac*; plants carrying *Li*, on the other hand, are in no such excess.

Regression analysis was applied to the variation in the frequency of cyanogenic plants in relation to altitude; the calculated regression coefficient was $-0{\cdot}01938$ in angular measure, corresponding to a decrease of about 11 per cent for each 1000 feet ($P<0{\cdot}001$).

4. Discussion

Although samples came from areas with presumably different soil conditions (*p*H, mineral composition, moisture), the effect of increasing

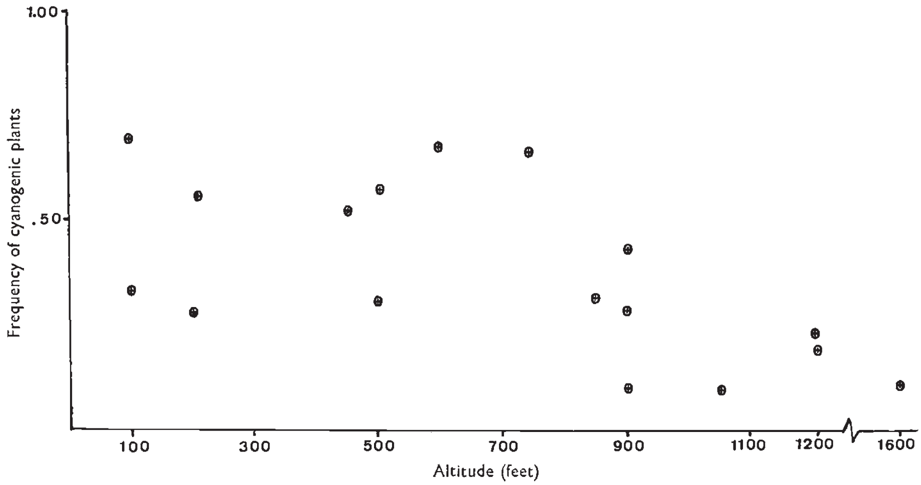

FIG. 1.—Variation in the frequency of cyanogenic plants with altitude.

altitudes seems likely to play an important role in the fall of *AcLi* frequency. Fig. 1 also suggests a non-linear relationship between the frequency of cyanogenic plants and altitude; though this point is not clearly established.

An interesting fact is the low frequency of *acLi* plants in all the 17 samples (in two of them zero frequencies were obtained, which could be due to sampling error). A similar finding is reported by Daday (1954*b*).

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