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FIELD OBSERVATIONS ON THE CYANOGENESIS POLYMORPHISM IN
TRIFOLIUM REPENS

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SUMMARY

We surveyed a population of *Trifolium repens* L. polymorphic for cyanogenesis to determine whether either of two biotic factors were concerned as selective forces affecting the balance between the cyanogenic and acyanogenic forms.

We found no difference between the distributions of cyanogenesis scores for uninfected leaves and for leaves infected by the fungus *Cymadothea trifolii* Wolf. Acyanogenic plants, however, were more heavily eaten than cyanogenic ones, the principle herbivores being the slugs *Arion ater* L. and *Agriolimax reticulatus* Muller. Selective eating of isolated plants in a herbaceous border was due both to more acyanogenic leaves being touched and to more being eaten per a cyanogenic leaf. For plants embedded in the lawn selective eating was due mainly to the latter cause.

Possible explanation for the differences in degree of selective eating between lawn plants and border plants and between this survey and choice experiments are discussed.

1. INTRODUCTION

MOST populations of *Trifolium repens* L. in Europe are polymorphic for cyanogenesis, with the leaves of cyanogenic plants releasing HCN gas when they

are damaged. The cyanogenic form is selected against by low temperatures (Daday, 1954; Jones, 1972) and soil water stress (Foulds and Grime, 1972), and it is believed that this form is maintained in the population by balanced selection. The balancing selective force has usually been assumed to be selective eating of acyanogenic plants by herbivores. The evidence for this consisted of passing references to selective eating (Corkill, 1952; Daday, 1955), together with experimental evidence (Jones, 1962, 1966) that herbivores select against the acyanogenic form of *Lotus corniculatus* L., another plant polymorphic for cyanogenesis. More recently Bishop and Korn (1969) failed to find selective eating by molluscs in experimental work on the polymorphism in *T. repens*. They concluded that some other factor must maintain the cyanogenic form in polymorphic populations and suggested the possibility of protection against micro-organism infection. Other experimental work (Crawford-Sidebotham, 1972; Angseesing 1973) achieved directly opposite results and suggested that Bishop and Korn were premature in dismissing selective eating as a factor maintaining the polymorphism. Results in the latter work (Angseesing, 1973) also showed how experimental conditions could cause significant variations in selective feeding by slugs. To be absolutely sure that laboratory conditions had not induced an artificial selectivity, we decided to carry out the field survey described below.

2. METHODS

The survey was carried out in the months of August, September and October 1972 on our own clover-rich lawn in Cheltenham. Leaves were picked at random from the lawn and were scored as follows. Firstly a leaf was typed according to whether or not it was infected with the black blotch fungus *Cymadothea trifolii* Wolf. This fungus affected nearly 50 per cent. of all leaves scored towards the end of September, but was not often seen in August and October. Secondly the leaf was typed according to the quantity of each leaflet which had been consumed by herbivores, each leaflet being scored separately. Five categories were used: W. (whole *i.e.* untouched), S (slightly nibbled—less than 10 per cent. of the leaflet eaten), N (nibbled—between 10 and 25 per cent. eaten), H (heavily eaten)—25 to 75 per cent. eaten) and T (almost totally eaten—more than 75 per cent. gone). For example, if a leaf had two leaflets nibbled and one heavily eaten it would be recorded as $H\ 2N$. The leaf was then placed in a numbered tube, bruised in toluene, and sealed in with a picrate paper. The degree of cyanogenesis was estimated by the colour of the picrate paper 24 hours later. A scale running from 0-5 was used. The scale was initially subjective, with test comparisons being made against a standard set of paper strips. Two decisions had therefore to be made about each leaf—the degree of eating and the degree of cyanogenesis—and borderline classification was rather subjective. However bias was avoided by one of us scoring the leaves for eating and the other scoring for cyanogenesis without knowing the eating scores.

There remained the possibility that very heavily eaten leaves would give a lower score than whole leaves from the same cyanogenic plant would have given. To overcome this possibility a number of whole plant analyses were carried out. Over a score of clover plants had been allowed to grow in our herbaceous borders. During October each plant was pulled up and three leaves, all whole, were separately tested for cyanogenesis. The mean

of these scores was taken as the cyanogenesis score for the plant. Every leaf on the plant was then scored for eating.

There was the similar possibility that the presence of *Cymadothea trifolii* had affected the cyanogenesis scores of infected leaves. To test whether this had occurred an uninfected leaf from the same plant was scored at the same time as each of 30 infected leaves.

3. RESULTS

(i) *Fungus distribution*

In table 1*a* it may be seen that there is no difference in the distributions of cyanogenesis scores for uninfected leaves and for those infected by the black blotch fungus, *Cymadothea trifolii*.

The comparison of 30 infected leaves with uninfected leaves from the same plants provides no evidence for any influence of *C. trifolii* infection on cyanogenesis score. 25 pairs of leaves, including 22 acyanogenic pairs, had identical scores. The remainder had scores that were merely one or two points higher for one leaf or the other (table 1*b*).

TABLE 1*a*

Percentage distributions of cyanogenesis scores for uninfected and black blotch (Cymadothea trifolii) infected leaves of Trifolium repens. Plants with a score of zero are acyanogenic

	Cyanogenesis score: N	0	1	2	3	4	5
Uninfected	364	62·6	15·7	6·0	7·7	3·6	3·8
Infected	107	62·6	16·8	6·5	6·5	5·6	1·9

TABLE 1*b*

Comparison of cyanogenic scores of infected leaves with those of uninfected leaves from the same plant. In addition to the scores below there were 22 pairs of leaves where both leaves were acyanogenic

Sample	1	2	3	4	5	6	7	8
Infected	5	2	2	3	3	2	3	5
Uninfected	3	3	1	3	3	2	1	3

(ii) *Lawn survey*

The first objective was to see if there was any difference between cyanogenic and acyanogenic leaves in the ratios of untouched leaves (all three leaflets untouched) to eaten leaves (at least one leaflet slightly nibbled). The untouched proportion was only slightly higher among the cyanogenic leaves and the difference was not significant (table 2*a*). A closer examination was then made of the eaten leaves. A second contingency test was made on the cyanogenic/acyanogenic partition among nibbled leaves (no leaflet more than 25 per cent eaten) and heavily eaten leaves (at least one leaflet more than 25 per cent eaten). This time the difference was significant and showed that cyanogenic leaves tend to be nibbled rather than heavily eaten (table 2*b*). Although only one-third of the leaves were acyanogenic, over one-half of the slightly nibbled scores were cyanogenic.

166 cyanogenic and 305 acyanogenic leaves were sampled in the survey. 57 cyanogenic and 130 acyanogenic leaflets were consumed, representing 11·5 and 14·2 per cent of the samples respectively. This gives an eating differential of 1·23 acyanogenic leaves per cyanogenic leaf.

TABLE 2

Contingency χ^2 for the partition of eating scores between cyanogenic and acyanogenic plants in the survey and in the whole plant analysis

(*a*) Whole versus eaten leaves in the lawn survey

	Acyanogenic	Cyanogenic
Whole	87	54
Eaten	218	112

$$\chi_1^2 = 0·82 \ (P > 0·05).$$

(*b*) Nibbled (*S* and *N*) versus heavily eaten (*H* and *T*) leaves in the lawn survey

Nibbled	128	80
Heavily eaten	90	32

$$\chi_1^2 = 5·13 \ (P < 0·05).$$

(*c*) Whole versus eaten leaves in the whole plant analysis

Whole	51	100
Eaten	294	252

$$\chi_1^2 = 19·06 \ (P < 0·001).$$

(*d*) Nibbled versus heavily eaten leaves in the whole plant analysis

Nibbled	176	191
Heavily eaten	118	61

$$\chi_1^2 = 15·63 \ (P < 0·001).$$

(iii) *Whole plant analysis*

The differences between whole and eaten and between nibbled and heavily eaten leaves were much greater in this survey, and both proved highly significant in the contingency test (table 2*c* and 2*d*). The distribution of leaflets scored is set out in table 3. Cyanogenic scores are clearly higher for whole and slightly nibbled leaflets but lower for all other categories. Although

TABLE 3

Percentage distribution of eating scores for cyanogenic and acyanogenic leaflets in the whole plant analysis

Eating category:	*W*	*S*	*N*	*H*	*T*	Total leaves
Cyanogenic	58·0	3·7	30·4	5·2	2·8	1056
Acyanogenic	46·0	1·7	36·4	11·9	3·9	1065

W = untouched leaflets; *S* = less than 10 per cent. eaten; *N* = 10-25 per cent eaten; *H* = 25-75 per cent eaten; *T* = more than 75 per cent eaten.

the leaflet totals for the two types of clover are similar, there were 202·25 acyanogenic and 136·75 cyanogenic leaflets eaten. This means that herbivores were eating about 40·5 per cent. cyanogenic leaves in the *Trifolium repens* part of their diet.

4. Discussion

Selective feeding on acyanogenic clover has previously been shown in choice experiments using two slug species, *Arion ater* L. and *Agriolimax reticulatus* Muller (Angseesing, 1973). These two species were chosen for experimental work because they are very common on our lawn and have actually been observed feeding off *T. repens* on a number of occasions. Indeed, a systematic examination of the lawn during the time of the clover survey showed that these two species accounted for over 90 per cent. of all slug sightings (J. Angseesing, unpublished). As large herbivores were excluded it seems reasonable to conclude that most of the eating observed was due to our slug population and to these two species in particular.

This survey, then, has confirmed selective eating as a factor controlling the cyanogenesis polymorphism in the field. A previous survey with a similar aim has been conducted by Whitman (1973), who looked at a number of populations grazed by unidentified, and probably various, herbivores. Whitman examined about the same number of leaves as ourselves but obtained a significant association in only one instance; this was when he combined all his observations into two categories: heavily eaten as against slightly eaten plus untouched. There are several possible reasons why his results are less conclusive than ours: (i) we were fortunate in working with a population containing nearly 50 per cent. cyanogenic plants whereas Whitman's total sample included only 10 per cent., making for a more effective contingency χ^2 in our case; (ii) Whitman compounded observations from a number of localities which had differing degrees of grazing; this must have resulted in a loss of information unless each locality contained exactly the same frequency of cyanogenic plants; (iii) most of the grazing in our survey was due to two species which have been shown to prefer acyanogenic clover in choice experiments; Whitman's herbivores were unidentified and may have included cyanide-tolerant species (see Jones, 1973); (iv) there are almost certainly differences in selective eating between the clover morphs in different plant communities; an example of this is the difference between the two situations we have examined.

The large and significant differences between the nibbled:heavily eaten ratios of cyanogenic and acyanogenic leaves may be an indication that slugs cannot differentiate between these until they have tasted them. In support of this is the lack of a significant difference between whole and eaten leaves in the lawn survey. Furthermore, although less than half of the leaves that were scored were cyanogenic, over two-thirds of the slightly nibbled category were cyanogenic. On the other hand, there is the highly significant contingency χ^2 for the untouched versus eaten comparison in the whole plant analysis. The two surveys were carried out, however, in different situations and it is possible that the taste-and-reject hypothesis applies in one situation only. If the slugs can learn to avoid a particular isolated plant, repeated tastes of cyanogenic plants would be unnecessary and a higher proportion of whole leaves would be expected on cyanogenic plants. This would not apply to the lawn if the merging of clover plants with each other and with grasses was sufficient to prevent easy identification of particular plants by slugs. In favour of slugs being able to identify individual plants is Angseesing's (1973) data that changing the plants offered to slugs confined in choice chambers depressed the quantity of food consumed for a day or two.

The degree of discrimination observed differs between the two field surveys and between the field surveys and laboratory choice experiments. After adjusting to a 50:50 cyanogenic:acyanogenic population 45 and 40 per cent. respectively of the leaflets consumed in the lawn and the border were cyanogenic as compared with 29 per cent. (*Arion ater*) and 33 per cent. (*Agriolimax reticulatus*) in the laboratory (Angseesing, 1973). There are several possible reasons for this. Firstly slugs may be able to identify plants more easily in a lunchbox than in a border or lawn. Then the lawn survey almost certainly undersampled totally eaten leaves whereas bare petioles were often scored in the border and were scored with absolute certainty in experimental work. There is a further explanation, very likely the most important source of difference between field and laboratory work. This is that more than 50 per cent. of the cyanogenic leaves scored in the field survey were only weakly cyanogenic whereas the choice experiments used the strongest plants available (Angseesing, 1973).

Although all the cyanogenic leaves taken have been given a numerical score, the results presented have been concerned only with selection between acyanogenic and cyanogenic plants. There are difficulties in comparing cyanogenic plants with each other as cyanogenic scores vary with leaf size, leaf position (Hughes, 1968), season of the year and time of day (de Waal, 1942). Further data is being accumulated to ascertain the relationship between selective eating and degree of cyanogenesis.

Jones (1973) has reminded us of the possible importance of molluscs as grazers, and slug densities of up to 277 animals per m². have been recorded (see Crawford-Sidebotham, 1970). This paper bears this out. Overall 13 and 17 per cent. respectively of the total leaf area of autumn lawn and border samples of *Trifolium repens* had been eaten by slugs.

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MAINTENANCE OF MALE STERILITY IN PLANT POPULATIONS
II. HETEROTIC MODELS

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SUMMARY

Gynodioecious populations contain male-sterile and hermaphrodite individuals, and gynodioecy has been interpreted as an outbreeding system.

Male-steriles require a mechanism for their maintenance in gynodioecious populations.

A theoretical study showed that for given values of heterozygote advantage, equilibrium proportions of male-steriles depended upon genetic control of the male sterility. These proportions were greatest where control was digenic with complementary gene action; lower where control was through a monogenic recessive; lower still where control was digenic with recessive suppressor gene action; and least when control was through duplicate genes.

With digenic inheritance, linkage between the sex-control genes was generally associated with reduced proportions of male-steriles.

1. INTRODUCTION

MANY species of flowering plants comprise, in addition to hermaphrodites, considerable proportions of male-sterile individuals. Such species are called gynodioecious, and gynodioecy has been interpreted as an outbreeding mechanism (Mather, 1940). Because of their sterility with respect to hermaphrodites, male-steriles would be lost from a population containing both forms, unless they had some mechanism for their maintenance. In natural populations of gynodioecious plants male-steriles may be maintained by their greater fruit set compared to hermaphrodites (Burrows, 1960) or by the lethality or partial lethality of some hermaphrodite genotypes (Lewis and Crowe, 1956). Theoretical studies of the effects of increased seed production by the male-steriles on their maintenance in gynodioecious populations were made by Lewis (1941), and by Ross and Shaw (1971). These authors found that male-steriles were only maintained if they produced a minimum of somewhat over twice as many seeds as hermaphrodites. Heterozygote advantage for fitness might also constitute a mechanism for maintenance of male-steriles. For example, with monogenic recessive male sterility and heterozygote advantage, male-steriles could be maintained because of recombination among heterozygous hermaphrodites. Jain (1961) studied

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