NOTES AND COMMENTS

HERBIVORE FEEDING AND CYANOGENESIS IN *TRIFOLIUM REPENS* L.

RICHARD J. WHITMAN

Department of Entomology, Cornell University, Ithaca, New York 14850

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SUMMARY

Qualitative measures of herbivore feeding damage and cyanogenesis were obtained for 1000 *Trifolium repens* L. growing in unmanaged areas. An association between low herbivore feeding damage rating and cyanogenesis is indicated by the data. If only those plants given the highest feeding damage rating have significant reduction of vegetative growth and/or seed production, the data presented help to explain the maintenance of the polymorphism of cyanogenesis in *T. repens*.

1. INTRODUCTION

Trifolium repens L. is polymorphic for the presence or absence of cyanogenic β -glucosides as well as for the presence or absence of a β -glucosidase. Production of the glucosides and the β -glucosidase is determined by alleles at the unlinked loci *Ac* and *Li*, respectively. The polymorphism was discussed by Jones (1967).

Daday (1965) reported that acyanogenic *T. repens* is significantly more resistant to frost damage than is cyanogenic *T. repens*. Jones (1962) suggested that cyanogenic glucosides in plants act as a defensive mechanism against feeding by some herbivores. He reported that in the case of *Lotus corniculatus* L., acyanogenic or weakly cyanogenic plants were preferred over strongly cyanogenic plants by certain molluscs (Jones, 1962, 1966). Bishop and Korn (1969) did not find selective eating of cyanogenic and acyanogenic *T. repens*, using two species of molluscs. However, Crawford-Sidebotham (1972), using many more species of molluscs, found that cyanogenic plants of both *L. corniculatus* and *T. repens* are eaten less than acyanogenic plants. It has been suggested by Jones (1970) that in *T. repens*, selection is cyclical, involving frost resistance in winter and physiological fitness and herbivore feeding in summer.

The present investigation was designed to provide qualitative measures of herbivore feeding damage and cyanogenesis of individual *T. repens* growing in unmanaged areas.

2. MATERIALS AND METHODS

T. repens is an introduced species in North America but it is considered to be naturalised (U.S. Dept. Agric., 1953). For this study, 20 areas in which *T. repens* grows in a wild state were located in Tompkins County, New York State, U.S.A. These areas were roadsides, mature fields, and power transmission rights-of-way, none being near pastures or lawns where *T. repens* may

have been seeded. Fifty individual plants at each area were given a qualitative herbivore feeding damage rating as well as a cyanogenesis rating. One thousand plants were sampled in all.

To select a plant to be tested, the investigator moved through the area in one direction until an individual *T. repens* compound leaf was sighted. The stolon associated with this leaf was located and its growing tip found. Then, the terminal three, fully expanded compound leaves on the stolon were examined for herbivore feeding damage. Where no feeding damage was found a rating of 0 was assigned to the plant. Very slight to slight feeding damage (damage of less than 3 mm. diameter on one or a few leaflets) was assigned a rating of 1. Medium feeding damage (damage of greater than 3 mm. diameter on one or a few leaflets) was assigned a rating of 2. Heavy feeding damage (extensive to complete destruction of two or more leaflets out of nine) was assigned a rating of 3. Thus a plant rated 0 or 1 was relatively undamaged, a plant rated 2 had received modest damage, and a plant rated 3 had been extensively damaged. No attempt was made to determine which herbivores caused the observed feeding damage.

Upon determination of the feeding damage rating, the terminal fully expanded leaf was plucked for use in the cyanogenesis test. If this leaf was unsuitable due to feeding damage, the second leaf or another young leaf from the same plant was used. Hughes (1968) reported that the first and second expanded leaves on a stolon have the maximum β -glucosidase activity.

The test for cyanogenesis used was that of Corkill (1940), with the modifications of Jones (1966). The test was begun in the field as soon as the leaf was plucked, as cyanogenesis proceeds rapidly (Melville, Coop, Doak and Reifer, 1940). The tubes containing the cyanogenesis tests were incubated for 24 hours at 37° C. upon return to the laboratory. The test used gives a positive reaction only to those plants having both the β -glucosides and the β -glucosidase. These plants were identified as cyanogenic, all other plants being classified as acyanogenic.

The colour change of the picrate paper was scored as 0 to 6. Since collection of the leaves in a variety of habitats made use of a constant leaf size impossible, the score distribution of the cyanogenic plants will not be presented.

3. RESULTS

The results of the investigation are summarised as a contingency table in table 1. It was found that 109 of the 1000 *T. repens* plants tested were cyanogenic, and that these were distributed between the four herbivore feeding damage ratings. However, upon calculation of the expected values of the respective cells (Ferguson, 1966), it was found that there were more cyanogenic plants given a feeding damage rating of 0, 1 and 2 than expected, and fewer cyanogenic plants given a rating of 3 than expected. The results for the acyanogenic plants were the converse. Using the chi-square test of independence, it was found that $0.10 > P > 0.05$. Thus the data gathered were not significant at the 0.05 level but an association between low feeding damage rating and cyanogenesis is indicated.

Considerable variation was found in the 20 areas with respect to frequency of cyanogenic plants and amount of herbivore feeding damage. There was

TABLE 1
The polymorphism of cyanogenesis related to herbivore feeding damage rating

Classification	Feeding damage rating				Totals
	0	1	2	3	
Cyanogenic	27	19	34	29	109
Acyanogenic	167	134	244	346	891
Totals	194	153	278	375	1000

$$\chi^2_{(3)} = 6.558; 0.10 > P > 0.05.$$

no significant correlation between these two measures over the 20 areas studied. It is believed that each area should be sampled extensively, and at several times during the year, if a correlation coefficient between cyanogenesis and herbivore feeding damage is to be calculated. Some of the variation found in these two measures would be due to differences in factors such as animal density, alternate food plants available, and climatic conditions affecting feeding prior to the test day.

Considering that 1000 plants were tested in all, it is believed that 10.9 per cent. is a good estimate of the occurrence of cyanogenic *T. repens* plants in the unmanaged area of Tompkins County, New York.

4. DISCUSSION

The data indicated that there is an association between low herbivore feeding damage rating and cyanogenesis in *T. repens*. It is not expected that a plant resistant to herbivore feeding will be immune to such feeding. Jones (1970) has noted that individuals or species of herbivores might be partly or wholly resistant to HCN in the quantities evolved by *T. repens*.

The data, as analysed, are not significant. However, if the feeding damage ratings 0, 1 and 2 in table 1 are grouped, and compared to feeding damage rating 3, it is found that $0.02 > P > 0.01$. In light of the above, it would be valuable to obtain data as to what level of herbivore feeding (artificial or real) causes a significant reduction in vegetative growth and/or seed production. It may be that only herbivore feeding damage rated as 3 (extensive to complete destruction of two or more leaflets out of nine) causes a significant reduction in these two measures. If this should be found, then the differences reported here as to herbivore feeding damage ratings of cyanogenic and acyanogenic *T. repens* would help to explain the maintenance of the polymorphism.

Further research should be carried out in North America to determine the more important diurnal and nocturnal herbivores on *T. repens*. Field experiments designed to show the degree to which these herbivores exhibit selective eating would be valuable. Information is also needed as to what level of herbivore feeding damage (artificial or real) significantly reduces vegetative growth and seed production of *T. repens*. Coupled with the present data, this information would provide an estimate of one selective force.

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HAEMOGLOBIN PATTERNS OF SECOND-GENERATION HYBRIDS BETWEEN PLAICE AND FLOUNDER

K. SICK, O. FRYDENBERG* and J. T. NIELSEN*

*Institute of Genetics, University of Copenhagen, Øster Farimagsgade 2A,
DK-1353 Copenhagen K, Denmark*

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SUMMARY

Haemoglobin electrophoresis of F_2 hybrids between plaice and flounder revealed besides the parent and F_1 patterns only one new pattern, which is most likely due to trisomy or triploidy.

HYBRIDS between the two flatfish species, plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*), can be produced in aquaria and are also known to occur in Nature. We have previously described the electrophoretic patterns of haemoglobins from the parent species and from their first generation hybrid (Sick *et al.*, 1963). Both species have multiple haemoglobins; the patterns, as these are revealed by agar electrophoresis (Sick, 1965) are, however, clearly different. The pattern of the F_1 hybrid is very complex, and it does not resemble a simple superposition of the parent patterns. These patterns are shown as Nos. 1-3 in fig. 1.

* Present address: Institute of Genetics, University of Århus, Ny Munkegade, DK-8000 Århus C, Denmark.