Herbage Studies. II.—*Variation in* Lotus corniculatus *and* Trifolium repens (*Cyanophoric Plants*).

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Lotus corniculatus.

In Part I of these studies, it is shown that *Lotus corniculatus* is a plant in which a glucoside containing cyanogen is frequently present together with the corresponding enzyme. During 1911 we were able to make observations practically over the whole of Europe, owing to the assistance we received from Dr. Eyre, which led us to the conclusion that the glucoside and enzyme were normal constituents of the plant in almost all districts, though occasionally, in close proximity to plants which were cyanophoric, others were met with in which little if any cyanide could be detected. In Scotland, in South Ayrshire, the plant was uniformly *acyanophoric*, except on the coast; nor could cyanide be detected in plants collected in Norway.

Lotus major, which is a sufficiently distinct variety to have been recognised by botanists as a separate species, was uniformly free from cyanide and also apparently from enzyme; but no regular distinction could be made between the various other forms which botanists look upon as merely varieties of the plant. *Lotus major*, it should be added, always affects damp situations and is a rank grower; it can be distinguished by the manner in which the large number of flowers in the umbel spread out from a common centre instead of at intervals from the flower stalk; the calyx teeth also tend to spread outwards, whereas in other forms they are almost uniformly strongly incurved. We have found the double form of *L. corniculatus* (var. *pleno*) to be strongly cyanophoric.

During 1912, we have again examined specimens of *L. corniculatus* from many localities in England and Wales and, as a rule, have found them to be strongly cyanophoric.* Out

* During 1912, in testing for cyanide, the Guignard picrate paper used was always prepared as required and we have substituted toluene for chloroform in order to avoid the possibility of any trace of acid being introduced. In the morning, before going into the field, strips of paper were impregnated with the alkaline picrate solution and the moist strips were at once placed in the tubes which were to be used in testing the plants. The tubes were then incubated in the pocket, in order to ascertain whether any hydrogen cyanide was retained in the cork. Recently prepared undried paper is usually more sensitive than paper which has been dried and then moistened just before it is to be used; the once dried paper rarely has the bright yellow appearance of freshly stained paper.

of about 30 specimens collected day by day by a cyclist during a fortnight's tour from London to Wales and back, in the earlier part of July, only two were found to be almost free from cyanide—one of these was obtained at Church Stretton, Somerset, the other from the foot of Cader Idris, Wales. Specimens sent to us by Mr. Pickering from Woolacombe, South Devon, and by Mr. Stapledon (*L. incanus*, Gray, *L. villosus*, B. and H.) from Westward Ho, North Devon, were strongly cyanophoric ; on the other hand, material sent to us from near the Lizard, Cornwall, gave no response to the test.

At the end of June, one of us found the plant growing very freely, in full bloom, on the retaining wall at the foot of the hill slope bordering the whole length of Rydal Water, Westmorland ; of seven specimens, presenting no difference in appearance, picked from this wall at fairly regular intervals, all but one were more or less strongly cyanophoric ; the exceptional specimen contained the faintest trace, if any, of cyanide.

During August, a very thorough study of the plant was undertaken by one of us, chiefly in the valley of the River Cree on the borders of Ayrshire, Kircudbrightshire and Wigtownshire, in the district where the previous year, at Whitsuntide, the specimens examined were all acyanophoric.

Again, in the places visited in the previous year, many specimens were found in which cyanide could not be detected ; here and there, however, along the river bank and in the adjoining fields, patches of the plant were met with now and then which were *faintly* cyanophoric.

On walking along the high road, across the moor, from Drumlamford, about five miles from Barrhill Station, to Newton Stuart, a distance of about 12 miles, a delicate stunted form of *Lotus corniculatus* was frequently found growing at the roadside among grass, *Lotus major* being plentiful in damp situations near Newton Stuart. Five specimens of the plant were secured in the course of 10 miles ; the first of these, obtained about three miles out, was strongly cyanophoric, the picrate paper being coloured brick red by the evening ; not a trace of hydrogen cyanide was observed in the case of the other four specimens. About two miles from Newton Stuart, where the River Cree comes into view, at the foot of a fairly steep hill, there was a profuse growth of *L. corniculatus* on the top of the retaining wall at the left hand side of the road ; several specimens were tested ; strange to say, none of them appeared to be cyanophoric.

Very faint indications of hydrogen cyanide were observed in some but not in all plants collected between Drumlamford and Barrhill Station. On walking from Pinwherry, the next station north of Barrhill, to the coast at Ballantrae, about eight miles, along the road passing through the valley of the Stincher, *Lotus major* was found to be abundant everywhere in the moist bank at the foot of the hill slope on the right. *L. corniculatus* was also met with here and there at the edge of the road ; specimens collected within the first, within the second and within the next two miles, all gave fair to strong indications of cyanide.

At Ballantrae, as in 1911, *L. corniculatus* was growing freely in very coarse sand and stones, on the upper level of the beach, in large clumps or compact tufts consisting of long straggling stems bearing small, delicate leaves but large seed-pods, the underground root growth being very strong ; cyanide was present in moderate amount in this plant. A short distance away, across the main road, at the foot of the hill, delicate plants with small seed pods were found growing in the grass beneath a wire fence bordering the rough roadway leading up hill. In 1911, nothing was detected in the plant in this situation but last year distinct indication of the presence of cyanide was obtained—perhaps only because the test applied was a more delicate one. In any case, the very different conditions prevailing on the shore and near at hand on the hillside had favoured the development of very different types of plant.

The contrast is equally striking when the plant growing on the beach at Ballantrae is

compared with that found on the East coast near Dundee and St. Andrews on either side of the Tay estuary. *L. corniculatus* is abundant in these localities on the outer margin of the sand dunes, where it grows in fine blown sand. The growth is chiefly underground and is much less coarse in character than that at Ballantrae; usually, the leaves alone, which are very small and delicate, appear above ground. Cyanide was found to be present in this plant, though in very much smaller amount than in the coarse growing Ballantrae form.

Taking the observations made *in the field* last year and the previous year into account, it appeared to be little short of established that, in addition to the common widely distributed cyanophoric form of *L. corniculatus*, a botanically indistinguishable form exists, different from *L. major*, which, like this latter, is acyanophoric.

Considerable quantities of plant collected in the Cree valley were brought to London for the purpose of determining the enzymic activity. Among these were various samples in which cyanide had not been detected; when these were incubated at 37°, with a few drops of toluene, the presence of cyanide in the plant became obvious within 24 hours, though the amount detected was very small in all cases. On testing *L. major* in the same way, *no trace* of cyanide was detected.

It may be added that traces of cyanide have been found in plants grown during 1912 from seed gathered the previous year in Norway from plants in which cyanide could not then be detected.

Whilst therefore it appears that *L. major* is uniformly acyanophoric and that the common forms of *L. corniculatus* are more or less strongly cyanophoric, a form of this latter species undoubtedly exists in which the power of producing the cyanophoric glucoside is all but suppressed.

Enzymic Activity of L. corniculatus *from Various Localities.*

The determination of the enzymic activity of plants differing in habit and from various localities is obviously of importance in view of the probability that the cyanophoric glucoside and the enzyme are in close correlation.

It should be noted that the activity of *L. major* towards linamarin is so slight as to be negligible (see Part I) and it may almost be assumed that this species is not only acyanophoric but also free from the specific enzyme met with in *L. corniculatus*.

The enzymic activity of all the specimens of *L. corniculatus* examined during 1911 in which the cyanophoric glucoside was present was high. Of the four specimens from Norway, in which cyanide was not detected, however, one was moderately active, one but slightly active and two active.

The results obtained on examining specimens collected last year are as follows:—

Enzymic Activity of *Lotus corniculatus*.

Source of specimen.	Percentage hydrolysed.	
	Linamarin.	Salicin.
Moscow,* from American clover seed	84·2	—
,, ,, West Russian clover seed	80·5	—
Ballantrae (August 27, 1912)	57·0	20·0
River Cree, Ivy Pool (August 29, 1912)	74·0	31·0
Lane from Dalnaw Farm (September 1, 1912)	40·5	—
Face of bank below Farm, near bridge (September 1, 1912)	2·6 3·0	3·0
Edge of ditto (September 1, 1912)	71·7	28·0
Barry Links, Taymouth (September 8, 1912)	3·0 3·0	3·2
Pilmour Links, St. Andrews (October 9, 1912)	49·5 45·2	21·2
Lotus tenuis	—	50·4

* We are indebted to Prof. Williams for these samples; they are referred to in the table given in Part I.

It will be seen that there is a marked difference between the plants collected on opposite sides of the River Tay, near Dundee and at St. Andrews, the one having but little activity, the other being moderately active. So far as we were able to judge, the two plants were identical in external appearance.

Still more remarkable is the difference between Nos. 8 and 9. Both were strong plants showing faint traces of cyanide: the one specimen was taken from a large tuft overhanging the very edge of the bank; the other from a large patch at most a yard or two distant from the first, growing below it where the bank began to slope away to the river.

It appears, therefore, to be established that whilst the "normal form" of *L. corniculatus* generally met with in the southern parts of Britain contains both a cyanophoric glucoside and the correlated enzyme, in Scotland and also in Norway a form prevails which is "rich" in enzyme but contains mere traces of the glucoside; and that a third form exists in which the amount of enzyme is also very small. The second form appears to be widely distributed though less common than the first: but the third has been met with only rarely (once at home and perhaps in Norway). We hope that we shall be able to obtain further information, during the present year, which will enable us to throw more light on the nature of the relationship between the different varieties.

Inasmuch as the glucoside is sometimes all but absent in cases in which an apparently "normal" amount of enzyme is present, it appears probable that the presence of these two "factors," which undoubtedly are to be regarded as correlated, is not sufficient and that some other factor or factors are concerned in the production of the glucoside if not of the enzyme. As traces of cyanide

are usually found, except in *L. major*, it is scarcely probable that the power to produce either cyanide or acetone is altogether lacking; it is more likely that the conditions of concentration may not be suitable and that the factor in question is one influencing concentration. In any case, taking into account the fact that plants possessed of very different characters have been found in close proximity, it is difficult to avoid the conclusion that the differences observed are less the consequence of the operation of special conditions of environment—though these may contribute—than of the presence or absence of definite factors.

Trifolium repens.

It was pointed out in the first part of these studies that our special object is the development of methods of appraising the value of the various constituents of the herbage of pasture lands. The study of *L. corniculatus* is of interest from this point of view, as the plant affords a striking case of variation under natural conditions and it is to be supposed that any light thrown on the causes of variation will be of assistance in dealing with the general problem of variation. But in other respects, the plant is not well suited to our purpose, as it ranks rather as a weed than as a fodder plant and is an altogether minor constituent of most pastures.

By far the most important leguminous plant in pastures is white clover, *Trifolium repens*. We have been the more attracted to this plant by the appearance of the account given by Hall and Russell of their exhaustive study of the remarkable conditions prevailing in Romney Marsh,* where pastures are found, side by side, one of which is good sheep-fattening land but unsuitable and unsafe for ewes with lambs, whilst the other has no fattening qualities and is used only in rearing lambs, which thrive on it. *Trifolium repens* is abundant in these pastures and is an important constituent of the herbage, particularly of the fattening fields and especially throughout the earlier part of the year.

Mirande has anticipated us in making known the presence of a cyanophoric glucoside in this plant.† In our experience, the wild plant, wherever tested, always contains cyanide; the amount is not large—from 0·0036 to 0·039 according to Mirande's determinations—and always below that present in the markedly cyanophoric forms of *L. corniculatus*.

It is noteworthy that seedsmen and agriculturists recognise two varieties of *Trifolium repens*—cultivated and wild white clover; and that of late years the latter has acquired some popularity as the more lasting variety. An

* 'Journ. Agric. Sci.,' vol. iv, p. 339.

† 'Comptes Rendus,' 1912, vol. 155, p. 651.

interesting account of Wild White Clover, by Dr. A. Gilchrist, Professor of Agriculture in the Armstrong College, Newcastle-on-Tyne, was published in the 'Journal of the Board of Agriculture' in 1909 (vol. XVI, No. 91). As an illustration of the difference between this form and the commonly cultivated variety, Prof. Gilchrist cites an experiment made at Cockle Park Farm, Northumberland, in which two quarter-acre plots of the poorest type of boulder clay soil, laid down to grass in April 1906, each received together with other seeds 4 lbs. of cultivated or commercial white-clover seed per acre and one of them (Plot II), in addition, 4 lbs. per acre of the wild white-clover seed; the hay produced on the two plots was as follows:—

	Weight of hay.	
	Plot I.	Plot II.
	cwt.	cwt.
1907	30½	35
1908	18¼	28¼
1909	15½	21¾
Average	21½	28½

To quote Prof. Gilchrist, "The aftermath has been grazed every year. White clover and practically all the clovers disappeared from Plot I after the first year but now some natural clover plants are spreading on this plot. Plot II has always had a thick and close sward of white clover and this continues to be so. It may be noted that on this cold clay soil meadow fescue seed has failed to produce plants. A striking result is that on Plot I the grasses have not been nearly so luxuriant as on Plot II. This was so even in the first year's hay crop and is undoubtedly due to the collection of nitrogen from the atmosphere by means of the nodules on the clover roots and to the stimulating effects of the nitrogen on the grasses."

We have not succeeded in finding cyanide in white clover raised from "cultivated" seed at any stage of growth. When the wild white seed is germinated, faint traces of cyanide can be detected even on the fourth or fifth day, as soon as the cotyledons begin to assume a green tinge, the response to the cyanide test being more distinct a day or so later when they are fully green though only just emerging from the seed husk.

Enzymic Activity of Trifolium repens.

The method adopted in estimating the enzymic activity was that described in Part XVII of our Studies on Enzyme Action involving the use of the dried leaf material.*

The following are the results obtained:—

Enzymic Activity of *Trifolium repens.*

	Linamarin.	Amygdalin.	Prunasin.	Salicin.
Rothamsted Park permanent grass plots—				
5^2	—	—	—	7·0
6	—	2·5	12·0	11·0
7	17·7	—	—	15·5
Romney Marsh—				
Westbroke	19·2	5·0	26·5	15·9
Blacklocks	19·2	—	—	14·7
Midley Paddock	—	—	—	18·6
Midley (poor)	—	—	—	13·9
Somerset—				
Non-scouring land (Nov. 1, 1912)	—	—	—	4·5
Scouring land (Nov. 9, 1912)	4·5	3·0	18·2	12·6
Non-scouring land (Nov. 9, 1912)	4·5	2·0	18·2	11·2
Armstrong College, County Demonstration Farm	13·2	2·2	17·0	11·2
Kent	(average of 4 samples)			12·7, 9·5, 12·6
Kitchen End { Lawn	—	—	—	11·8
Kitchen End { Pasture	—	—	—	7·5
Woburn	—	—	—	13·1
Cultivated white (Sutton's seed)	1·0	—	2·5	11·7

The striking fact brought out in this Table is that, whereas with one exception all the specimens examined are moderately active towards salicin, the "cultivated" variety alone is practically without action on linamarin and prunasin. This observation is of special interest in connexion with the question raised in Part XVII of our Studies on Enzyme Action.

Apparently we are in face of differences similar to those which have been discovered in *L. corniculatus.*

Should this discovery be confirmed, it is obvious that the object we have in view will have been in part attained, as we shall be in possession of varieties of a plant which is acknowledged to be one of the most valuable constituents of the herbage of pasture land; it will be all important to ascertain whether the chemical peculiarities already recognised, presented by the two types of the plant, are accompanied by others and are in any way to be correlated, directly or indirectly, with their value as food materials. Hitherto, no distinction has been drawn between them in this respect, the wild form

* 'Proc. Roy. Soc.,' B, vol. 85, p. 363.

having been favoured of late years but only on account of its more perennial character.

We have to thank Messrs. Temperley and Co. of Hexham and Newcastle-on-Tyne for placing seed of the Wild White variety of Clover at our disposal and also Prof. Gilchrist, Mr. C. T. Gimingham, Dr. Russell, Prof. Somerville and Dr. J. Voelcker for the trouble they have taken in assisting us to procure specimens.

The Trypanosomes found in the Blood of Wild Animals Living in the Sleeping-Sickness Area, Nyasaland.

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INTRODUCTION.

The chief object of this Commission in coming to Nyasaland was to inquire into the relation of the African fauna to the maintenance and spread of trypanosome disease.

The Commission arrived at their camp on Kasu Hill on January 12, 1912. As this was the rainy season the low country was covered with dense vegetation and much of it under water. Nothing could therefore be done in the study of the fauna until about the beginning of June, when the dry season was well established.

The camp at Kasu is situated on one of the hills (lat. 13° 40′ S., long. 34° 12′ E.) which rise on the western edge of the flat country adjoining Lake Nyasa. This low-lying lake-coast plain looks quite flat when viewed from the camp, and extends from the lake shore some 20 miles inland. The camp lies about 10 miles from the edge of this low country, and, therefore, some 30 miles from the lake. This plain is covered with thorn scrub, except near the lake, where there are large grassy plains, or "dambos," dotted over with palm trees. The thorn-scrub is the home of the tsetse-fly and also of numerous wild animals.

* Dr. Davey resigned his membership of the Commission in October, before the completion of the work here recorded.