STUDIES IN THE GENUS DIGITALIS

PART VI. VARIATIONS IN GLYCOSIDAL CONTENT OF BRITISH CLONES OF *Digitalis purpurea*

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Received April 28, 1960

Seeds of wild *Digitalis purpurea* from 150 different areas in 20 counties of Great Britain have been collected; weight of 100 seeds was 3·5–**8·6**–11·6 mg. Each batch of seed, regarded as a clone, was grown under uniform conditions and leaf collected from both first year and second year plants. Glycosidal content of each leaf sample, drawn from 8 plants within the clone, was estimated using 3,5-dinitrobenzoic acid and results expressed as u./g. by comparison with the Standard Preparation of Prepared Digitalis. Values for clones were: first year 9·8–**13·7**–18·9 u./g.; second year 3·1–**7·6**–11·4 u./g. Clone values for dried leaf yielded per plant were: first year 38–**79**–137 g.; second year 12–**23**–47 g. The weight of parent seed does not affect the yield of leaf or its activity (first year); nor is leaf activity affected by leaf yield (first year). A positive relation existed for first and second year leaf activity values for each clone and suggested genetical control of activity.

VARIATIONS in potency of different samples of dried leaves of *Digitalis purpurea* estimated by biological assay have been reported by various workers. Wokes[1] examined eight commercial samples of English leaves and found variations of 64–148 per cent from average. Watson and James[2] collected leaf and seed samples from 16 different plants in England and Wales and concluded that the potency variation 5·5–**12·4**–21·2 u./g. was not related to environmental factors of soil or altitude. The first and second year plants from these collected seeds were examined for genetical factors controlling potency[3,4]. Mather and Dyer[5] examined six strains of plants from wild and cultivated parents and concluded that heritable differences in activity and in yield of leaf existed between strains within the species. Barnard and Finnemore[6] selected one variety of seed because of the high potency of its progeny. More recently van Os and collaborators[7] have studied the heredity of proportionality between different glycosides in the total glycosidal complex for different lines of *D. purpurea*, using chemical methods of estimation.

Investigations of the natural variation within *D. purpurea* and of the possible existence of genetically controlled strains of high therapeutic potencies have been limited by the biological assays involved. The modern use of colorimetric methods of estimation of digitalis glycosides makes possible a much larger survey. The present work was undertaken to investigate the range of activity found in British samples of *D. purpurea* using a chemical method of estimation and with a view to subsequent examination of the heritability of high or low activities.

Collections

Each seed sample was collected from a few fully matured capsules on one inflorescence axis of a single plant. At the same time an objective assessment of the plant (A+ to C) was recorded together with its exact location and its environment. A distance of not less than one mile separated each plant from which collection was made and thus it was hoped that each sample of seeds might represent a separate clone of plants. Collections of 150 samples were made in September, 1950, from 20 counties in England, Wales and Scotland. These counties and the numbers of samples therefrom are recorded in the first two columns of Table I. Some predominance of samples from North Wales was arranged

TABLE I
GEOGRAPHICAL SOURCES OF SEEDS

County	Number of seed samples collected	Range of activity of progeny (first year leaf) u./g.*
Buckinghamshire	1	14·5
Cheshire	3	12·0–13·1–13·9
Cornwall	7	10·0–12·4–15·0
Cumberland	7	13·8–15·1–16·6
Derbyshire	4	12·3–13·9–15·6
Devonshire	1	13·6
Co. Durham	13	12·4–14·6–17·9
Kent	5	12·4–13·0–14·4
Shropshire	2	12·9–14·4–15·8
Staffordshire	10	11·5–13·9–15·6
Sussex	2	11·5–13·3–15·0
Westmorland	6	13·2–14·3–14·8
Anglesey	3	11·7–12·8–14·9
Caenarvonshire	32	9·8–13·1–18·6
Denbighshire	21	11·9–14·3–18·9
Merionethshire	1	13·3
Aberdeenshire	19	11·5–13·9–16·3
Dumfriesshire	11	11·3–13·2–15·4
Lanarkshire	1	11·3
Stirlingshire	1	*No germination*
Total	150	

* Total glycosides were estimated by the 3,5-dinitrobenzoic acid process and the results expressed as units per gram by comparison with the Standard Preparation of Prepared Digitalis.

because of the findings of Watson and James[2]. No leaf samples were collected from any plants since at the time of seed maturity the leaves are in an advanced state of senescence.

Weight of 100 Seeds

The weight of 100 seeds is an accepted diagnostic character[8,9] but its relation with yield or activity of the subsequent plant has not been explored, although some preliminary work was reported by Miller[10]. The present experiment offered such a possibility. The 150 seed samples were allowed to become air dried by spreading in thin layers in the laboratory for some days. Dirty samples were shaken over No. 30 and No. 60 sieves, the former retained portions of capsule wall, placenta, etc., the latter retained the seeds but passed fine dust and, in occasional samples, unfertilised ovules. Seeds on the No. 60 sieve were finally winnowed to free from small portions of capsule wall. There was no evidence of fractionation of seeds by this process of cleaning; clean samples

were not sieved. Preliminary trials suggested that about 1,000 seeds were suitable for counting and weighing. Projection in a photographic enlarger was used to aid the counting; a quarter-plate of perspex or of glass was scratched with a suitable grid of 12 rectangles on the lower side; on the upper surface about 500 seeds were scattered, were picked over with forceps to remove any foreign matter and were mounted in the enlarger. The seed images were thrown on white paper at a suitable magnification and these were readily counted by marking each image. A second slide of about 500 seeds was then counted, the two lots of seeds were mixed and weighed accurately; from these results the weight of 100 seeds was calculated. Replicate results were: sample 38—9·5, 9·4, 9·5, 9·3, 9·2 mg.; sample 54—6·9, 6·8, 6·9, 6·8, 6·8 mg.; sample 55—10·3, 10·1, 10·1, 10·2, 10·1 mg.: it was thus concluded that the method gave dependable results. These values for the 150 samples of seeds are set out in Table II; the two samples of seeds in the lowest weight range did not germinate when subsequently sown and hence the seed of minimal weight which germinates

TABLE II

WEIGHTS OF 100 SEEDS

CLONES 1–150

Weight range mg.	Number of samples	Weight range mg.	Number of samples
3·0–3·9	2	8·0– 8·9	42
4·0–4·9	2	9·0– 9·9	33
5·0–5·9	6	10·0–10·9	17
6·0–6·9	7	11·0–11·9	10
7·0–7·9	31	Total ..	.. 150

Range of weights 3·5 (4·3)–8·6–11·6 mg. per 100 seeds

(4·3) is also shown in the summary of range of weights. The mean weight of 100 seeds collected from the 23 plants rated as A+ was 9·5 mg., for the 19 plants rated as C it was 7·9 mg.; thus suggesting a positive correlation of the robustness of the parent and the weight of its individual seeds.

CULTIVATION, PREPARATION AND ESTIMATION

All clones of seeds were raised in the Museum Experimental Gardens, Mayfield, near Ashbourne, Derbyshire. Sowings were made in pans of John Innes' compost in a heated greenhouse in late February, 1951, germination occurred in 13–20 days and final percentage germinations ranged 28–88–100 per cent. Young seedlings were pricked off as soon as possible into growing-on compost and, when sufficiently matured, were hardened off in a cool greenhouse, cold frame and finally out of doors. Planting out was done May 31–June 1 on to a well prepared bed with the following analytical report "pH 6·61, lime requirement nil, available phosphate 10 p.p.m., available potash 6 p.p.m."; the bed received a dressing of potassium sulphate 1 oz. per sq. yd. shortly before planting out. Ten plants of each strain were placed 18 in. apart in a row; rows were 30 in. apart. The land received normal horticultural tending during the growing period. Harvesting of first year leaf was during

October 9–25, that is approximately 130 days after planting out, and in the afternoon, as far as possible after sunlight. From two plants in each row all leaves, except any decayed outer leaves and the young crown buds, were gathered and weighed to give a measure of total leaf yield per plant as a clone value. Aliquot portions of this material and similar amounts from six further plants in the row were then taken as the clone sample for drying; about 150 g. of dried leaf being produced. The remaining two plants at the end of each row were not harvested. Twenty clone samples were weighed before and after drying to determine the moisture contents, the mean value being used to calculate dry weight per plant for each clone. Drying was carried out in a drying room maintained at 55–65° and with extraction fan; by placing the leaves in shallow, hessian-bottomed

TABLE III

RANGE OF LEAF ACTIVITIES: FIRST AND SECOND YEAR CROPS

CLONES 1–150

First year crop		Second year crop	
Activity range u./g.	Number of clones	Activity range u./g.	Number of clones
18·0–18·9	2	11·0–11·9	3
17·0–17·9	1	10·0–10·9	8
16·0–16·9	5	9·0– 9·9	12
15·0–15·9	21	8·0– 8·9	30
14·0–14·9	38	7·0– 7·9	49
13·0–13·9	36	6·0– 6·9	29
12·0–12·9	25	5·0– 5·9	12
11·0–11·9	16	4·0– 4·9	4
10·0–10·9	3	3·0– 3·9	1
9·0– 9·9	1	No germination	2
No germination	2		
Total	150	Total	150
Activity range: 9·8–13·7–18·9 u./g.		3·1–7·6–11·4 u./g.	

trays and turning the contents night and morning drying was completed in 24–30 hours. Samples were then milled to No. 44 powder, transferred to wide-mouthed bottles and allowed to stand in the drying room for a further 48 hours before putting on the screw caps and transferring to the laboratory for analysis. Moisture contents at 105° of 10 such samples were found to be 3·8–4·5–5·3 per cent.

The second year crop of leaf was harvested June 24–July 17, 1952, from the same eight plants (or as many as had survived) in each row; the leaves being gathered from the flowering axes when the lower half of the inflorescence was in full flower but when the upper part of the inflorescence was still in bud. Weight of leaf per plant was determined and drying was done as for the first year crop. The two plants at the end of each row were used for seed; one or more flowering axes of each plant were bagged before the flowers opened, some were left undisturbed and produced little amount of seed, others were carefully opened at intervals and pollination stimulated, after which the bags were replaced. In this way inbred seed for future breeding experiments was collected from every clone in September, 1952.

Objective descriptions of both first and second year plants of each clone were recorded; variation in leaf shape, stem colour shape and hairiness, also differences in inflorescence shape were noted. Rates of development differed between clones but these were least apparent at time of harvest when the slow growing clones had caught up with the others. Leaf weight does, however, show marked differences between clones and is recorded in Table IV for both first and second year plants.

All clone samples were estimated for total glycosidal content by means of the 3, 5-dinitrobenzoic acid process described in Parts I and III of this present series of papers[11,12]. Standard Preparation of Prepared Digitalis was also estimated by the same process at the commencement and at the conclusion of the series of estimations each year and from the results the equivalent activity in u./g. was calculated for each clone. Results for both first year and second year leaves are in Table III.

Discussion

The total glycosidal content shows wide variation amongst the 148 clones about the mean for each year's crop. For first year leaf this is 72–138 per cent; for second year leaf it is 41–150 per cent. This wide range is in general agreement with the findings of other workers[1–5] although a somewhat wider range of activities is here reported from this broad survey of British clones. Activities of first year leaves were satisfactory to good (Table III) and only one sample gave figures of less than 10 u./g., the mean of 148 samples was somewhat higher than the Standard Preparation of Prepared Digitalis and there were several very high-activity clones. A range of these high, medium and low activity clones forms the basis of genetical studies to be reported later.

A comparison of the activities of first year leaf with the geographical origin of the parent seeds is set out in column three of Table I. The mean values for each county are about the same; as the number of samples increases the range of values also increases and thus the larger collections in North Wales include both the poorest (9·8 u./g.) and the two richest (18·9 and 18·6 u./g.) clones. There is no clear evidence that one county produces digitalis of higher activity than another and the evidence is rather of random distribution within natural variation in each county. Comparison of activity of first year leaf and the ecological habitat of the parent also showed no correlation.

Dry weight of leaf yielded per plant (Table IV) was medium to good for first year crop with a range between clones of 48–173 per cent about the mean of 79 g. A comparison of these values with county of origin of parent seed showed a random distribution of values within and between counties with mean values for each county of the same order.

The weight of 100 original seeds (Table II) and either first year leaf yield (Table IV) or leaf potency (Table III); also first year leaf yield and its activity (Tables III and IV) were examined for correlation. These coefficients are given in Table V and it will be seen that no correlation exists of seed weight and leaf yield of progeny; the value 0·131 for seed weight and leaf activity of progeny is probably not significant; and there

is no correlation of leaf yield and its activity. This last is of economic importance since the activity yield per acre is significant and breeding of strains high in both yield and in activity is desirable.

For second year crop the activity range of clones is given in Table III and the dry weight of leaf per plant in Table IV. For both criteria the values are much lower than for first year crop; the mean activity is 7·6 u./g. and only 11 clones have activities of 10 u./g. or over; hence the second year leaves are of inferior quality and are much less to be preferred

TABLE IV

RANGE OF LEAF YIELDS PER PLANT: FIRST AND SECOND YEAR CROPS

CLONES 1–100

First year crop		Second year crop	
Weight of dry leaf, range g.	Number of clones	Weight of dry leaf, range g.	Number of clones
130–139	4	40–49	1
120–129	2	30–39	13
110–119	2	20–29	57
100–109	8	10–19	29
90– 99	9	—	—
80– 89	21	—	—
70– 79	17	—	—
60– 69	21	—	—
50– 59	10	—	—
40– 49	5	—	—
30– 39	1	—	—
Total	100	Total	100
Dry weight range: 38–79–137 g.		12–23–47 g.	

than those of the first year crop. Such a finding is in agreement with those of some other workers[13–16] but conflicts with other publications. Since leaf yield is also low with a variation of 52–204 per cent about the mean of 23 g. per plant, the second year crop is of much less economic significance than is that of the first year.

There was no obvious correlation of leaf activities and objective descriptions of either first year or second year plants and it is not possible to forecast from plant appearance the amount of glycosides present in the

TABLE V

CORRELATION COEFFICIENTS

CLONES 1–100

Correlation	r
Seed weight: Leaf yield, first year	−0·034
Seed weight: Leaf activity, first year	0·131
Leaf yield, first year: Leaf activity, first year	0·045
Leaf activity, first year: Leaf activity, second year	0·335

leaves. Despite the low potencies of second year leaves there is a correlation of them and the activity of the first year crop from the same clone. This is shown in Table V and it suggests a measure of genetical control of glycosidal content in addition to seasonal and environmental influences.

Acknowledgements. The author wishes to express his thanks to a number of friends for the collection of some of the seed clones, also to Miss F. L. C. Blackwall for all the cultivation work. He is also indebted to the Agricultural Research Council for a grant towards the cost of the drug drying plant employed in the Museum Experimental Grounds.

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