STUDIES IN THE GENUS DIGITALIS

PART VII. VARIATIONS IN GLYCOSIDAL CONTENT WITHIN CLONES OF *Digitalis purpurea*

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Received April 28, 1960

First year leaves of *Digitalis purpurea*, collected in July or August, contain smaller amounts of glycosides than those collected in September, October or early November when the values are relatively constant, with a coefficient of variation of about 7 per cent. Second year leaves contain uniform amounts of glycosides until the plant is in full flower, coefficient of variation about 8 per cent, after which glycosidal content decreases. Weight of leaf per plant increases throughout the first year of growth; it is less for second year plants and reaches a maximum in the early stages of flowering. Defloration of second year plants changes the accumulation pattern of glycosides to that of first year plants. Coefficient of variation in glycosidal content within groups of up to 10 plants in one clone was 9·4 per cent for first year plants (265 plants from 13 clones, grown on four sites, three growing seasons) and was 13·3 per cent for second year plants. Between groups of 10 plants coefficients of variation were 8·5 per cent (first year) and 11·5 per cent (second year). Season of growth and site where grown both influence the glycosidal content of plants; the latter is probably due to climate rather than to nutrition. In comparative experiments on different sites the influence of the site may be eliminated by expressing all results as percentages of the corresponding mean site values; this has been done for 45 comparisons on each of four sites when the coefficients of variation were first year plants 10·5 per cent, second year plants 12·0 per cent. It is concluded that these figures are a measure of the coefficient of variation of total glycosidal content in a clone of *Digitalis purpurea* leaves.

THE total glycosidal contents of 150 different clones of *Digitalis purpurea* leaves were reported in Part VI of this series[1] and some of these clones have been taken for further selection and breeding experiments. To assess the validity or otherwise of any such breeding experiments it is necessary to study in detail the natural variation which may occur within single clones.

Glycosidal content may be influenced by external factors such as nutrition, environment and climate, all of which are closely interrelated and which we must seek to control when examining the possible inheritance of a factor for high or low glycosidal content. Wasicky[2] considered that the method of cultivation influenced the proportions of the different glycosides present in the glycosidal mixture and he suggested that plants should be cultivated under standard conditions. In Part IV of this series[3] we have studied the influence of fertilisers upon leaf yield and upon glycosidal content of those leaves and the remarkable constancy of glycosidal content under all treatments excepting that with lime was found to be most significant. Thus no further study of nutritional factors seems desirable but the complex interaction of climate and nutrition to be found at different sites of cultivation needs to be investigated. Accordingly

much of the following work was carried out at four different sites in England. Also the experiments were repeated for several years to determine the influence of season upon glycosidal content. Many of the determinations have been made upon both first and second year plants.

The amount of glycosides present in leaves from a plant at different ages has not been fully investigated. Tattje[4] found a maximum glycosidal content in first year plants in July, 140–150 days after sowing; this was followed by a decrease and a subsequent second but low maximum in October. He also found that first year leaves were richer in total glycoside than were second year leaves[5]. Yamamoto and others[6] found a maximum in first year leaves in the month of August, followed by a gradual decrease and then an increase in second year leaves up to flowering in May. Klepsaite[7] found a maximum in September and a decline in October in first year leaves, whilst for second year leaves the glycosidal content was greater before blooming than afterwards. In studies of *Digitalis lanata* Court and Allemann[8] found that in second year leaves the glycosidal content fell rapidly as flower stems developed. Since all previous work in this series of papers has been based upon leaves of first year plants collected in late September, and of second year plants harvested when the upper flowers were opening, it was decided to make a detailed study of the total glycosidal content of leaves gathered throughout the life history of this biennial plant.

Apart from the age of a plant and also the possible inheritance of glycosidal content, to be investigated subsequently, the possibility of natural variation of glycosidal content within a clone must be envisaged and due to internal factors of which we have no knowledge. No previous work upon plant by plant variation of glycosidal content could be found and thus a large scale experiment was set up to investigate this fundamental fact within a number of clones of *D. purpurea.*

Experimental

The seed clones used were those reported in Part VI[1]. Horticultural details were the same as those set out in that paper and in Part IV[3] and all young plants were reared at site B at Mayfield near Ashbourne, Derbyshire. For the work carried out on other sites the young plants were chosen at random from this nursery and were rapidly transferred to the planting out sites. These were site M, the Farm Institute, Morley, Derbyshire; site S, Department of Horticulture, University of Nottingham, Sutton Bonington; site W, the gardens of Allen and Hanbury, Ltd., Ware. Harvesting and rapid drying were carried out as described previously[1,3]. Total glycosides were estimated by the 3,5-dinitrobenzoic acid process described previously[8] and results were expressed as "units" per gram, by comparison with the Standard Preparation of Prepared Digitalis.

Variations in Leaf Yield and Activity with Age of Plant

Leaves were collected twice a month from the time of planting out (July) until the first year plants died back because of frosts in November.

Collections were recommenced the following spring from rosette leaves and then from axial leaves as the inflorescence developed; samples were taken until the plants were completely senescent. The state of maturity of the inflorescence was noted and is recorded in the second part of Table I. The experiment was made at site B and was repeated in three successive

TABLE I

VARIATIONS IN LEAF YIELD AND ACTIVITY WITH AGE OF PLANT
(BI-MONTHLY HARVESTS)

Season	1951–52		1952–53		1953–54	
Clone	A0004		B0033		A1033	
Planted out	9.6.1951		10.6.1952		1.7.1953	
Date of harvest	Dry weight of leaf per plant g.	Activity u./g.	Dry weight of leaf per plant g.	Activity u./g.	Activity u./g.	Notes
First Year Crop						
July 1	1·6	10·4	—	—	7·7	Activity values in years 1951–52 and 1952–53 are based upon harvests of 4 plants on each occasion. In 1953–54 a group of 34 plants was reared and each assay is based upon a sample of 68 leaves, 2 leaves being taken from each plant.
July 16	3·8	9·7	3·5	9·1	—	
August 1	10·8	11·9	—	—	6·8	
August 16	31·0	10·5	7·5	10·5	9·6	
September 1	58·0	12·3	16·3	14·8	9·4	
September 16	46·5	11·1	25·5	12·9	8·3	
October 1	66·5	12·1	18·5	14·2	—	
October 16	66·0	13·2	27·5	13·8	8·9	
November 1	82·5	14·4	54·8	13·8	—	
November 16	84·0	12·7	51·0	14·7	—	
Second year crop						
March 16	18·0	9·4	—	—	5·0	
April 1	9·5	10·1	15·0	8·5	—	
April 16	12·0	8·8	15·0	7·9	—	
May 1	13·0	8·2	20·0	7·3	—	
May 16	25·0	8·3	30·5	8·9	6·1	Inflorescence axis showing
June 1	40·0	7·6	57·0	7·6	7·3	Inflorescence developing
June 16	27·0	8·5	—	—	6·8	Upper flowers opening
July 1	31·0	8·8	47·0	9·2	—	In full flower
July 16	14·0	7·7	40·0	7·9	6·6	In full flower
August 1	—	—	30·0	6·4	6·9	Some fruit well developed
August 16	10·0	7·7	—	—	—	Some fruit mature
September 1	9·0	6·5	—	—	—	Fruit mature, plant senescent

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

pairs of growing seasons 1951–52, 1952–53 and 1953–54. In the two first experiments all the leaves, apart from dead outer ones and young buds, from a block of four plants were taken for each bi-monthly harvest and hence the weight of dry leaf per plant at that date was also recorded (columns 2 and 4 of Table I). The plants were then left until the following year and were again harvested in the same succession as in the first year. This design of experiment does not take account of any variation within the garden upon which some 16 blocks of four plants were raised. Thus, for the experiment in 1953–54 a block of 34 plants was reared and at each bi-monthly harvest two typical leaves were taken from each plant. Under these conditions the yield of dry leaf per plant at each harvest could not be determined, but the total variation between plants was represented in each sample.

The results are set out in Table I from which it is seen that the weight of leaf yielded by a first year plant increases steadily with the age of the plant up to the month of November. The glycosidal content of first year leaves is lowest for young plants (July and August) but retains a relatively constant, high value for plants harvested during the months of September, October and the first half of November. The three sets of results show some variations in values during this September to November period, but the variations are not constant and the results are evidence of one uniform high value rather than of a pair of peak values during this period. It is thus satisfactory to harvest first year plants at any time during the period September to mid-November and the value for glycosidal content will then have a coefficient of variation, v, of about 6·8 per cent. (Table I, first year crop, September to November values; column 3 mean = 12·6, s = 1·1, $v = 1{\cdot}1 \times 100/12{\cdot}6 = 8{\cdot}7$ per cent; similarly column 5 $v = 5{\cdot}0$, column 6 $v = 6{\cdot}7$.) For second year plants the weight of leaf per plant increases with age until the time of flowering (June) after which it decreases markedly. The maximum weight yield of leaf does not exceed that of the first year plants, and generally it is less. The amount of glycosides present in these leaves remains relatively constant until the time of full flowering after which there is a decline. Thus it is satisfactory to harvest second year leaves during the period of inflorescence development (approximately mid-May to mid-July) and the value for glycosidal content will have a coefficient of variation of about 7·7 per cent.

Defloration

If young flowering axes are removed as they arise from second year plants, the basal rosette of leaves continues to develop throughout the growing season and such plants may live for several years by repetition of this treatment[9]. As a parallel to the bi-monthly harvests reported above some groups of second year plants of clone A0004 were freed from young inflorescence axes as they developed in 1952 and leaf samples were taken at monthly intervals. The same groups of plants were similarly treated in 1953 when they were in their third year of growth; the defloration of second year plants of clone A1033 was also carried out in 1953. Assays are given in Table II and it will be seen that the pattern of glycosidal

content at monthly intervals resembles that of first year rather than of second year plants; thus there is no falling off in values after July and in two of the series there is an increase in values in August or September. It may thus be concluded that the internal factor of plant maturity in terms of flowering and fruiting exerts a marked influence on the total glycosidal content of its leaves.

Variations in Leaf Activity within a Clone

Individual plants. Ten individual plants were reared in a row from each clonal batch of seed, leaf samples were collected from first year plants in late September or from second year plants during the period of flowering. Each individual sample was estimated for total glycosides. The investigation was made on site B during 1953, 1954 and 1956: in 1955 the investigation was made on the four sites B, M, S and W and in 1956 these plants

TABLE II

VARIATIONS IN LEAF ACTIVITY WITH AGE OF DEFLORATED PLANTS

(c.f. Table I)

u./g.

Season	1952	1953	1953
Clone	A0004		A1033
Crop	Second year	Third year	Second year
April 16	—	—	8·4
May 16	—	—	8·9
June 1	7·6	9·5	—
July 1	—	8·5	8·0
August 1	8·5	10·6	8·1
September 1	10·6	—	8·2

on sites B and M were continued as a second year crop. Individual figures for estimations of the 10 plants within each row were recorded and their coefficients of variation, *v*, were calculated. Typical results for five clones are shown in Table III, Part A. Totals of 265 first year plants from 13 clones and 124 second year plants from 10 clones were estimated and Table IV expresses in summarised form the means and standard deviations for each group of plants; where the number of plants in each group differs from 10, the actual figure is shown in brackets.

It will be noted that for first year plants on site B in three years the values of *s* vary between 0·58 and 1·34 and on four sites in one year *s* varies between 0·52 and 1·88. The calculated coefficients of variation of activity are for site B (three years) 4·5–8·8–11·9, and for four sites in one year 5·3–13·7. The mean value for all sites in the four years is 9·4 per cent. The values of *s* for first year crop in 1955 on four sites (Table IV, part B) suggest that the clones A2042 and A2060 are less variable than the other three clones: the coefficients of variation on the four sites are: clone A2007 11·0 per cent, A2012 11·3 per cent, A2034 10·3 per cent, A2042 9·0 per cent and A2060 7·2 per cent.

For second year plants on site B the values of s vary between 0·40 and 1·86 and for 1956 crop on the two sites the range of s is 0·40 to 1·45. The coefficients of variation of mean activity values on site B for second year plants grown in three seasons are 4·5–**14·3**–27·8. The mean for all second year plants on both sites is **13·3** per cent: thus the glycosidal content of second year plants shows a greater variation than for first year plants. Results for second year plants also confirm the conclusion drawn from first year assays that there is less variability within clones A2042 and A2060 than within the other three clones grown in seasons 1955–56.

TABLE III

VARIATIONS IN LEAF ACTIVITY: FIRST YEAR CROP, 1955

SITE B

u./g.

A. Individual plants in a row (c.f. Table IV)

Clone	Plant number										Mean	Standard deviation s
	1	2	3	4	5	6	7	8	9	10		
A2007 ..	10·0	9·1	11·6	12·4	12·6	11·4	13·3	11·4	13·0	12·6	11·7	1·34
A2012 ..	11·9	11·9	11·6	13·6	14·9	13·5	12·3	11·3	11·7	13·9	12·7	1·22
A2034 ..	11·0	12·0	9·6	12·1	13·4	12·9	12·9	12·6	14·0	13·7	12·4	1·33
A2042 ..	11·4	10·0	9·2	10·3	10·0	9·7	9·2	11·0	9·3	11·4	10·2	0·86
A2060 ..	10·3	10·1	9·3	9·6	10·0	10·3	10·4	9·2	8·9	8·0	9·6	0·77

B. Individual rows each of 10 plants (c.f. Table V)

Clone	Row Number					Mean	Standard deviation s
	1	2	3	4	5		
A2007	11·7	13·7	13·6	13·4	13·4	13·2	0·83
A2012	12·7	13·6	13·9	14·6	12·4	13·4	0·90
A2034	12·4	13·9	14·6	13·4	13·3	13·5	0·81
A2042	10·2	12·4	12·4	11·9	11·7	11·7	0·90
A2060	9·6	10·1	9·4	10·7	10·1	10·9	0·51

Groups of plants. It has been the normal custom in this series of papers to make estimations of glycosides on a leaf sample drawn representatively from a group of plants of the clone, the number in such a group being up to 10. Such values should have a lower standard deviation than assays based on individual plants. The individual plant experiments, reported above, were also designed to examine this variation between groups of plants within the clone. For each clone five rows each of 10 plants were reared. The individual plants in one row were harvested for the investigation reported above (and the mean values used in this work); representative handfuls of leaves from each of the 10 plants in row 2 were mixed and dried to form the sample for that row and samples for rows 3, 4 and 5 were similarly drawn. Plants from five clones of seeds were investigated in this way upon the four sites during 1955, and on site

TABLE IV

VARIATIONS IN LEAF ACTIVITY: INDIVIDUAL PLANTS (10 PER ROW)
MEAN VALUES AND STANDARD DEVIATIONS WITHIN ROWS

(c.f. Table IIIA)

A. SITE B

First year crop				Second year crop			
Year	Clone	Activity u./g.	*s*	Year	Clone	Activity u./g.	*s*
1953	A1005	13·6 (5)	1·29	1953	B0011	9·5	1·30
	A1102	13·0	1·22		B0047	7·9	1·59
1956	B2005	13·0	0·58	1954	A1012	5·5	0·75
	B2012	11·8	1·22		A1109	6·8	1·10
	B2035	12·4	1·03		A1113	6·7	1·86
	B2060	10·1	0·62				
	B2100	10·2	0·60				
	B2150	8·1	0·96				

B. FOUR SITES. 1955–56 CROPS

Clone	A2007		A2012		A2034		A2042		A2060	
Site	Activity	*s*	Activity	*s*	Activity	*s*	Activity	*s*	Activity	*s*
First year crop, 1955										
B ..	11·7	1·34	12·7	1·22	12·4	1·33	10·2	0·86	9·6	0·77
M ..	9·7	1·09	12·4	1·31	14·6	1·05	11·5	0·75	10·4	0·85
S ..	12·4	1·21	13·1	1·45	15·7	1·70	11·1	0·99	9·8	0·52
W ..	16·2	1·84	13·1	1·79	15·3	1·88	13·2	1·57	—	—
Second year crop, 1956										
B ..	8·4	1·45	10·0 (6)	0·82	10·8 (9)	1·32	6·7 (7)	0·53	6·6 (7)	0·40
M ..	5·8 (2)	1·27	6·8 (6)	0·77	7·8 (8)	0·53	7·1 (9)	0·65	7·5	0·51

Note: Figures in brackets are numbers of plants estimated where other than 10.

B the 25 rows were continued to the second year crop in 1956. Representative results for rows, the mean value and its standard deviation for each clone are set out in Table III, part B. A summary of these mean values and of their standard deviations is given in Table V.

For first year plants on the four sites values of *s* about the mean values per row within a clone vary between 0·23 and 2·25. Coefficients of variation of activity are 2·3–**8·5**–17·3 for first year plants; the corresponding values for second year plants are 8·0–**11·5**–15·6 per cent; it is thus confirmed that the mean standard deviation, *s*, and the coefficient of variation, *v*, for groups of 10 plants are less than for individual plants in both first and second year crops. When values of *s* for individual plants are compared with those for rows in the 19 instances of the 1955 first

year crop, the former is greater than the latter in 13 cases; whilst 15 values of v are greater for individuals than for rows. For the four aberrant instances, three of which were on site M, it must be supposed that the external factors influencing glycosidal content were not uniform between rows: it should also be noted that the actual activity values on site M were very low in 1955 and it is probable that such seasonal edaphic factors producing these low values may also accentuate the natural variation within the clones. The low variability found within clones A2042 and A2060 in Table IV was not found in Table V.

TABLE V

VARIATIONS IN LEAF ACTIVITY: INDIVIDUAL ROWS (EACH OF 10 PLANTS) MEAN VALUES AND STANDARD DEVIATIONS BETWEEN FIVE ROWS

(c.f. Table IIIB)

FOUR SITES. 1955–56 CROPS

u./g.

Clone	A2007		A2012		A2034		A2042		A2060	
Site	Activity	s	Activity	s	Activity	s	Activity	s	Activity	s
First year crop, 1955										
B ..	13·2	0·83	13·4	0·90	13·5	0·81	11·7	0·90	10·9	0·51
M ..	9·3	0·35	10·3	1·49	12·3	1·55	11·1	0·64	8·1	1·40
S ..	11·9	0·82	11·8	1·17	13·8	1·23	11·3	1·85	10·0	0·23
W ..	16·1	0·97	16·3	2·25	16·7	1·05	13·3	0·79	—	—
Second year crop, 1956										
B ..	7·5	0·60	8·8	0·81	9·3	1·40	8·0	1·25	7·7	0·75

Variations in Leaf Activity with Site of Cultivation and Season of Growth

To investigate the influences of season and of site of cultivation upon the glycosidal content of individual clones of *D. purpurea*, seeds from 15 clones representative of high, medium and low glycosidal content were employed. Rows of 10 plants of each clone were grown on each of the four sites in 1952 and clone samples were collected for assay in late September. Plants were continued into second year in 1953 and representative clone samples taken when the plants were in flower. The investigations were repeated in 1953–54 and again in 1954–55. Results are given in Table VI; a number of second year plants failed to mature on one or more of the sites and such clones have been omitted from the lower half of the Table.

It must be stressed that Table VI does not attempt to illustrate the inheritance within a clone of a factor for glycosidal content upon each of the four sites of cultivation. All seed samples were taken from inbred clones growing at site B for inheritance studies and hence first year plants grown on sites M, S or W in 1953 or 1954 do not stem from those grown

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TABLE VI

VARIATIONS IN LEAF ACTIVITY: FOUR SITES; THREE SEASONS
CLONE SAMPLES FROM 10 PLANTS

u./g.

First year crop

Site ..	1952 B	1952 M	1952 S	1952 W	Site ..	1953 B	1953 M	1953 S	1953 W	Site ..	1954 B	1954 M	1954 S	1954 W
Clone ..					Clone					Clone				
B0002	13·3	12·7	12·0	10·7	A1002	11·7	9·2	14·2	10·7	B1002	8·6	8·2	9·9	11·3
B0005	14·3	13·6	15·2	13·9	A1005	12·4	9·5	10·8	13·8	B1005	9·6	13·3	13·6	12·3
B0007	13·5	13·4	12·4	13·6	A1007	9·8	8·1	12·5	13·1	B1007	10·4	9·5	12·7	13·7
B0012	10·4	11·9	10·8	12·3	A1012	9·6	8·0	15·8	8·9	B1012	9·3	10·3	10·1	12·8
B0026	10·3	10·9	15·0	14·8	A1026	10·7	—	14·6	10·5	B1026	10·1	10·8	13·3	13·6
B0042	11·0	10·1	14·5	11·7	A1042	8·3	6·3	11·5	9·1	B1042	11·4	12·3	—	13·3
B0046	10·6	11·0	10·4	11·3	A1046	8·3	5·9	12·4	11·3	B1046	11·7	11·4	10·3	—
B0060	9·1	11·6	12·1	10·7	A1060	8·2	7·6	13·4	10·4	B1060	9·9	12·9	11·2	10·3
B0062	10·2	14·1	14·4	12·3	A1062	11·0	7·5	14·7	13·5	B1062	12·1	9·8	12·2	12·6
B0075	12·3	13·1	12·5	14·5	A1075	8·8	6·2	15·2	10·5	B1075	11·2	11·1	11·6	14·2
B0093	11·1	11·8	11·5	13·2	A1093	12·1	8·5	16·0	13·2	B1093	11·5	12·4	12·0	14·8
B0094	11·3	10·4	12·5	12·5	A1094	10·7	10·7	14·4	9·9	B1094	11·2	12·7	11·2	11·9
B0100	11·1	10·5	9·5	7·9	A1100	7·8	6·6	8·1	5·5	B1100	9·7	9·1	9·7	11·5
B0102	13·2	10·1	14·1	—	A1102	13·1	9·5	15·0	10·9	B1102	13·8	12·5	11·8	15·2
B0150	—	8·9	9·1	11·0	A1150	7·9	8·5	10·2	11·7	B1150	10·1	9·7	9·7	14·0
Mean	11·6	11·6	12·4	12·2		10·0	8·0	13·3	10·9		10·7	11·1	11·4	13·0

Second year crop

	1953 B	1953 M	1953 S	1953 W		1954 B	1954 M	1954 S	1954 W		1955 B	1955 M	1955 S	1955 W
B0002	10·4	9·5	14·7	9·2	A1002	7·4	6·8	10·6	—	B1002	9·4	9·1	9·1	11·0
B0005	11·1	10·7	13·5	10·2	A1005	8·7	6·0	8·3	—	B1005	9·0	11·1	10·4	14·1
B0042	9·7	7·3	9·5	9·8	A1042	7·1	4·6	7·0	—	B1042	8·6	8·6	7·9	13·1
B0046	7·4	9·1	9·6	8·9	A1046	6·8	5·5	9·0	—	B1046	6·9	6·0	8·4	—
B0062	9·9	11·8	15·5	9·9	A1062	9·5	8·0	12·6	—	B1062	10·7	11·7	10·4	13·4
B0093	11·1	11·5	8·9	9·5	A1093	8·7	6·4	8·2	—	B1093	7·6	8·0	7·1	11·7
B0094	9·9	7·3	10·3	9·6	A1094	9·3	5·4	7·8	—	B1094	7·1	8·0	7·4	14·0
B0100	8·1	8·2	8·9	8·7	A1100	5·5	5·2	7·6	—	B1100	6·4	5·7	7·4	11·1
B0102	12·9	9·0	11·5	—	A1102	7·5	4·5	9·3	—	B1102	10·3	9·4	10·3	17·0
B0150	—	9·6	8·1	8·2	A1150	5·1	7·1	7·7	—	B1150	8·1	9·0	7·0	13·4
Mean	10·1	9·4	11·1	9·3		7·6	6·0	8·8			8·4	8·7	8·5	13·2

Mean site values: B = 9·9, M = 9·4, S = 11·2, W = 11·8

Yearly Means: First year crop: 1952 = 12·0, 1953 = 10·6, 1954 = 11·5
Second year crop: 1953 = 10·0, 1954 = 7·5, 1955 = 9·6

on the same sites in 1952. In consequence a strict comparison may only be looked for within a clone on four sites in the same year: although the last three figures of the clone reference numbers are those of the original seed collections reported in Part VI of this series[1] and hence there is a relationship in reading completely across this Table.

A consideration of the results in Table VI reveals that there is a clear influence of the site upon the glycosidal content of plants grown thereon, and the sites may be ranked for glycoside production by comparing the mean site values. Thus by using first year plant values in each of the three years, sites B and M are variously ranked 4th or 3rd and sites S and W 2nd or 1st, whilst second year plants give rather wider variations in ranking in the three years. Mean site values for both first and second year plants in the four years of the experiment are set out in Table VI and these show a descending ranking of the sites in the order W, S, B and M, the difference between the first two and the second two being marked. Since all plots consisted of adequately manured and well tilled garden plots it seems possible that the variations were due to external factors other than nutrition and in this context it should be noted that site S is further south than either B or M; whilst site W is still further south.

Table VI also indicates a marked influence of the season of growth upon the glycosidal content of the plants. Ignoring differences between clones and between sites it is seen that for first year crop the glycoside production is in the descending series 1952, 1954, 1953; the year 1953 producing low potencies. A similar ranking based upon second year crop gives 1953, 1955, 1954 with 1953 as the best and 1954 as the worst year. This apparent contradiction between assessments based on the first or second year crops is explicable on the basis that the season exerts an influence upon first year plants and this is carried on into the subsequent year of growth. Thus 1952 was a "good" year as evidenced by yearly means for first year crops, and those plants retained this "good" influence in their second year of growth in 1953 although this year was a "bad" year as indicated by first year crop results. Similarly this 1953 "bad" influence on first year plants persisted in them in 1954 as second year crop despite the fact that 1954 was a "fairly good" year as shown by the first year crop.

We may thus summarise the conclusions of this section in stating that plants of *D. purpurea*, grown from the same batch of seeds, may differ in glycosidal content if grown upon different sites in the same year; they may also differ in glycosidal content if grown upon the same site but in different years. These two external factors must be considered in assessing the internal factors of the clone for glycoside production.

Discussion

The experimental results reported above have been discussed in each of the sub-sections, since the conclusions arrived at in one investigation determined the approach to the succeeding one. Each may now be considered as part of the whole work.

Bi-monthly harvests (Table I) have shown that first year plants have reached a uniform level of glycoside content by the commencement of

September if grown under normal conditions of cultivation. Since the weight of leaf per plant increases continually throughout the growing season up to mid-November it follows that each plant produces an increasing amount of glycoside throughout this period. We do not know if this "amount" constitutes a food store laid down by the plant, or if the "percentage" constitutes a threshold value in some stage of the metabolic cycle of the plant. It is interesting to note the different mobilities of the primary and secondary digitalis glycosides and aglycones of the A and B series and to speculate upon the influence of these on translocation and storage patterns in the living plant, but much more evidence is required upon the subject. We do, however, conclude that leaves collected from September onwards contain a normal percentage of glycosides; whereas commercial harvesting should take place as late as possible when the crop weight is at its maximum. For second year plants the percentage of glycosides present in the leaves remains fairly constant until the time of full flowering, after which the content decreases. Since the weight of leaf per plant increases up to the time of early flowering it follows that in this period there is an increased amount of glycoside produced by each plant; whilst during the later stages of senescence the glycosides disappear. Hence, such second year plants should be harvested when in early flowering; although commercially the crop yield and glycosidal content are both lower than for first year plants. When second year plants are prevented from flowering by removal of flowering axes as they appear, such plants behave as first year plants in growth habit and in accumulation pattern of glycosides. This is of interest in that the internal factor leading to senescence of the plant and decrease in glycosidal content has been influenced by external treatment.

The external factor, or group of factors, covered by the word season, influence the percentage of glycosides produced in the same clone of plants and this is especially significant in the first year of plant growth (Table VI). It follows that inheritance studies which spread over a number of growing seasons must take note of this fact. It is probable that light, warmth and rainfall are all concerned in the influence of season but the meteorological records have not been analysed for the five years and four sites involved in this work.

Although previous work showed that glycosidal content could not be much influenced by either manurial treatments or by starvation[3], the site of cultivation influences this content (Table VI) and it has been suggested that this is an influence of climate rather than of nutrition. Its existence means that individual figures for amount of glycoside present are not directly comparable between different sites in any one year.

When the external factors of site and season are excluded from our considerations and when plants are harvested at the time of constant and maximum glycosidal content, there still remains to be measured the individual variation within a clone. Table IV records the standard deviations within groups of 10 plants of one clone and, although the experiment extended over four different sites and over four growing seasons with corresponding differences in mean values expressed in terms

of u./g. in each instance, the different values of s within each clone were about the same. To obviate these site : season variables the values of s were converted into coefficients of variation ($v = 100\ s/\bar{x}$) and these values of v were of still closer similarity within clones and also between clones. The mean value of v for 13 clones of first year plants grown on

TABLE VII

VARIATIONS IN LEAF ACTIVITY WITHIN A CLONE
MEAN ACTIVITIES (PER CENT), STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION

(c.f. Table VI)

Clone	Mean	s	v	Clone	Mean	s	v	Clone	Mean	s	v
First year crop											
	1952				1953				1954		
B0002	102·2	12·2	11·9	A1002	109·3	8·6	7·9	B1002	82·0	6·2	7·6
B0005	119·0	4·5	3·8	A1005	112·7	21·2	18·8	B1005	105·9	16·0	15·1
B0007	135·9	7·5	5·5	A1007	103·4	11·6	11·2	B1007	99·9	11·2	11·2
B0012	95·0	7·8	8·2	A1012	99·1	15·3	15·4	B1012	91·7	5·2	5·7
B0026	106·3	17·3	16·3	A1026	104·4	7·1	6·8	B1026	103·3	9·9	9·6
B0042	98·7	12·8	13·0	A1042	83·0	3·2	3·9	B1042	106·5	4·3	4·0
B0046	90·7	4·7	5·2	A1046	88·4	12·9	14·6	B1046	100·8	9·6	9·5
B0060	90·9	9·9	10·9	A1060	93·3	8·0	8·6	B1060	96·5	15·3	15·9
B0062	106·6	15·3	14·4	A1062	109·6	12·3	11·2	B1062	101·3	11·0	10·9
B0075	109·7	7·9	7·2	A1075	94·0	15·6	16·6	B1075	103·9	4·0	3·8
B0093	99·6	6·9	6·9	A1093	117·2	7·3	6·2	B1093	109·6	5·1	4·7
B0094	97·6	5·7	5·8	A1094	110·0	17·8	16·2	B1094	102·2	9·8	9·6
B0100	81·9	14·0	17·1	A1100	68·0	14·9	21·9	B1100	86·6	3·8	4·4
B0102	104·9	15·4	14·7	A1102	115·7	12·9	11·2	B1102	115·5	10·6	9·2
B0150	79·6	9·3	11·7	A1150	92·3	16·7	18·1	B1150	93·7	10·2	10·9
Second year crop											
	1953				1954				1955		
B0002	108·9	15·8	14·5	A1002	110·4	11·8	10·7	B1002	101·7	12·7	12·5
B0005	113·8	5·6	4·9	A1005	102·9	10·4	10·1	B1005	116·0	10·6	9·1
B0042	91·2	12·1	13·3	A1042	83·2	8·9	10·7	B1042	98·4	4·0	4·1
B0046	88·1	10·9	12·4	A1046	94·5	6·8	7·2	B1046	83·3	14·9	17·9
B0062	117·4	18·7	15·9	A1062	133·8	9·1	6·8	B1062	121·5	14·2	11·7
B0093	103·7	17·7	17·1	A1093	104·8	10·8	10·3	B1093	88·7	3·7	4·2
B0094	92·9	11·0	11·8	A1094	100·3	19·1	19·0	B1094	92·4	9·7	10·5
B0100	85·3	6·4	7·5	A1100	81·8	8·2	10·0	B1100	78·2	9·6	12·3
B0102	109·0	16·7	15·3	A1102	93·1	16·1	17·3	B1102	120·2	8·7	7·2
B0150	87·8	14·6	16·6	A1150	91·0	25·8	28·4	B1150	95·9	9·5	9·9

one to four sites in four years was 9·4 per cent. When groups of 10 plants were taken and five such groups examined the value of v was 8·5 per cent, based upon groups of four plants in two years and upon a group of 34 plants in the third year. For second year plants the corresponding values of v are: individual plants 13·3 per cent (from Table IV), groups of 10 plants 11·5 per cent (from Table V), groups of four or more plants 7·7 per cent (from Table I).

This individual variation within a clone may be abstracted by a further consideration of Table VI. The total mean site values for each of the four sites have been used to show the influence of site upon percentage of glycosides present in plants growing thereon; and this influence we believe to be climatic rather than nutritional. Proportional ranking of sites by their annual mean values differs from year to year even when only first year plants are considered. It seems reasonable, however, to expect that all plants of the same year are influenced in a similar manner by the site upon which they are growing and this is supported by the results of Tables IV and V. Such site variations can then be eliminated by converting all clone values into a percentage of the mean site value for that year and crop of plants. Table VI was recalculated on this basis and within each annual group each clonal percentage mean was calculated together with its standard deviation and hence the coefficient of variation of that clone. These are set out in Table VII. For the 45 first year clones the values of coefficient of variation, v, = 3·8–**10·5**–21·9 per cent; the figures for individual years are: 1952, 3·8–**10·2**–17·1; 1953, 3·9–**12·6**–21·9; 1954, 3·8–**8·8**–15·9; hence 1953, which was a poor growing season, showed a higher annual mean value of v and also the highest individual value compared with the other two years. For the 30 second year clones the values of v are: 1953, 4·9–**12·9**–17·1; 1954, 6·8–**13·1**–28·4; 1955, 4·1–**9·9**–17·9; three years, 4·1–**12·0**–28·4 per cent. The greater variability noted in the 1953 first year crop is continued in the 1954 second year crop. This mean value of v = 10·5 per cent for first year plants, based upon 45 groups of 10 plants each grown on four sites and corrected for site influence, is in good agreement with the value 8·5 per cent for groups of 10 plants grown in five rows on each of four sites the results being calculated within sites only (discussion on Table V). The higher value for these present calculations may be due only to the much larger group of 45 comparisons or there may still be some residual influence of the four sites causing a somewhat higher coefficient of variation of results within each clone. Similarly there is good agreement between values of v for second year crop; these are 12·0 per cent in this experiment and 11·5 per cent between rows (discussion on Table V). There is no marked correlation in values of v for related clonal groups of plants grown in different years, nor is the relationship between corresponding first and second year plants very pronounced, but the two clones 100 and 150, which are poorest in yield of glycosides in each season, also tend to have high values of v. It is reasonable to conclude that the normal coefficient of variation of glycosidal content to be expected within a clone of digitalis plants is about 10 per cent for first year crop and 12 per cent for second year crop.

Acknowledgements. The author wishes to express his warmest thanks for the provision of horticultural facilities to the Director, Farm Institute, Morley; the Professor of Horticulture, University of Nottingham; and to Allen and Hanbury, Ltd., Ware.

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After Dr. Rowson presented the papers there was a DISCUSSION. The following points were made.

The activity of the first year leaves was twice that of the second year plants and the leaf yield was three times as great. The leaves should be harvested as late as possible in the year before the frosts. The weight of leaves from the deflorated plants in the second year was about the same as the yield from the first-year plants.