ALKALOID BIOGENESIS IN TETRAPLOID STRAMONIUM

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Investigations on the biosynthesis of alkaloids in plants have directed attention towards the site in which the synthetic processes take place. In the examination of this problem plants belonging to the Solanaceæ, and particularly those which produce tropane alkaloids, have been extensively employed, and a great deal of information has been obtained by the use of intergeneric grafts. A large number of different graft combinations have been employed and the results have been fully reviewed[1,2].

These experiments have directed attention towards the root as the locus of synthesis. Thus, when grafts are prepared in which *D. stramonium* or *D. tatula* are grown as scions on non-alkaloid-producing stocks, no alkaloid can be detected in any part of the plant; conversely when *D. stramonium* or *D. tatula* are used as the stock and non-alkaloid-producing plants used as the scions alkaloids are present in all parts of the plant in amounts comparable with those found in normal plants of *Datura*. Such experiments have been carried out by a number of workers[3–9], and their results have been confirmed by our own results. Heike[10] grafted *D. stramonium* on to *Nicotiana rustica* and found nicotine in the scion but no tropane alkaloids, as indicated by no mydriatic action. Some conflicting evidence has, however, been reported; Hegnauer[11] found small amounts of alkaloid in all parts of the plant when *D. tatula* was grafted on tomato; similar results are reported by Mothes and Romeike[12] using *D. stramonium* on tomato. A possible explanation of these results may be that different grafting methods were employed, and that the small amounts of alkaloid reported by these workers were produced in the scions while growing on their own roots before being severed from them and connected to the root of the stock.

The weight of evidence, therefore, does appear to suggest that in *D. stramonium* and *D. tatula* the root is the site of alkaloid synthesis; further evidence in support of this hypothesis is given by the exudation of tropane alkaloids from roots of *D. stramonium* grown *in vitro* after the aerial parts have been removed[7].

Investigations on the alkaloid content of species of *Datura* have shown that a large number of factors may influence the amount of total alkaloid present in the plant. Among these factors the influence of polyploidy on the alkaloid content in species of *Atropa*, *Datura* and *Hyoscyamus* was investigated by one of us (J. M. R.)[13] and it was shown that in tetraploid plants considerable increases in alkaloid content are found in the aerial parts when compared with diploid controls. This fact has been confirmed for *D. stramonium* and *D. tatula* by later workers[14,15]. When this work was commenced in October 1950, no information was available

on the influence of polyploidy on the alkaloid content of the roots of plants of any of these genera; however, Steinegger[16] has recently (December 1952) published a value, viz. 0·127 per cent., for the alkaloid content of the tetraploid root of *Datura stramonium*.

In view of the evidence suggesting the root as the locus of synthesis in *D. stramonium* and *D. tatula* it seemed of interest to investigate the relationship between the alkaloid content in the root and in the aerial parts in diploid and tetraploid plants of these species, and to compare the results of analyses of alkaloid content of root and aerial parts between individual diploid and tetraploid plants, and to confirm the conclusions by making graft experiments. It was hoped that in this way further information could be obtained on the locus of synthesis of the alkaloids and that this could be related to the increased alkaloid content of the aerial parts of tetraploid plants.

Experimental

The experiments were designed along two main lines:

(*a*) To compare the amount of total alkaloid present in the roots and in the aerial parts of diploid and tetraploid plants of *Datura stramonium* and *D. tatula* for individual plants grown under identical conditions.

(*b*) To produce series of reciprocal grafts between diploid and tetraploid plants of Stramonium 2ns/4ns, 4ns/2ns; also to produce grafts between diploid and tetraploid plants of the two species and diploid and tetraploid tomato plants as follows:—

1. Diploid tomato scion on diploid *D. stramonium* (or *D. tatula*) stock (2nt/2ns).
2. Diploid tomato scion on tetraploid *D. stramonium* (or *D. tatula*) stock (2nt/4ns).
3. Tetraploid tomato scion on diploid *D. stramonium* (or *D. tatula*) stock (4nt/2ns).
4. Tetraploid tomato scion on tetraploid *D. stramonium* (or *D. tatula*) stock (4nt/4ns).

thus to compare the amount of total alkaloid present in the roots and in the aerial parts (i.e., stocks and scions) of each graft with the results obtained in (*a*). In addition, a number of grafts of diploid stramonium scions on diploid tomato stocks were prepared (2ns/2nt).

On harvesting the grafts of diploid tomato scion on diploid stramonium root in 1952 it was found that two of the tomato scions had rooted at the graft union, so that two active root systems were present. These were collected and analysed separately.

In the production of the tetraploid plants of the *Datura* species 3 plants were obtained which on examination were found to be branch chimeras, i.e., tetraploid plants with diploid branches. These plants were allowed to grow to maturity, when the tetraploid and diploid branches were collected and analysed separately.

5 samples of *Datura stramonium* seed and 3 samples of *Datura tatula* seed used in these experiments were obtained from different geographical

sources. Different samples are indicated by the letters A, B, C, D, E for the *D. stramonium* seed, and Q, R, S, for the *D. tatula* seed. One sample of tomato seed, var. Sunrise, was used throughout. For all the samples the mature diploid plants conformed to the type descriptions of the species.

Production of tetraploids. In 1951 and 1952 tetraploidy was induced in seeds of both species of *Datura* by soaking in 0·8 per cent. aqueous solution of colchicine for 4 days, the seeds having previously been soaked in water for about 12 hours. In the treatment of the tomato seeds the same concentration of colchicine solution was used, but the seeds were allowed to remain in the solution until germination, after which they were removed from the solution and sown in the usual manner; it was found that a greater number of tetraploids could be obtained by this method.

After colchicine treatment and subsequent germination of the seeds, seedlings were selected which showed swelling in the region of the hypocotyl; in most cases these were later found to be tetraploid.

In addition to the use of colchicine-treated seed, in 1952 the filial generation (F_1), was grown from seed set by tetraploids produced in 1951. Similarly, seed was collected from the diploid plants in 1951 and used for some of the 1952 sowings. Tetraploidy was proved in the maturing plants by measurements of stomatal size, and confirmed in the majority of the plants by measurements of the pollen grain diameter. Previous workers[17] have shown that increase in the size of the stomata and of the pollen grains when compared with diploid control plants are good criteria of tetraploidy. Similar methods were used to confirm the tetraploid nature of tetraploid scions when grown on diploid stocks. In the reciprocal grafts, however, the aerial parts of the tetraploid stocks were cut off before sufficiently mature for measurement of stomatal sizes to be possible; for these grafts, therefore, chromosome counts were performed on root tip preparations obtained from the stock roots a short time before harvesting of the graft, using the aceto-lacmoid squash technique.

All the tetraploid plants grew well and apart from an occasional stunted plant, were as vigorous as the corresponding diploid plants. The average weight of dried material obtained per plant was approximately equal to that obtained from the diploid controls.

Method of Grafting. The grafts were carried out on young seedlings just past the cotyledon stage, i.e., with the first pair of leaves apparent but not fully expanded. The method known as side grafting was employed. The seedling to be used as the scion was cut off about 1 to 2 cm. below the growing point, and the stem shaped to form a wedge. An oblique cut penetrating to about the centre was made in the hypocotyl of the seedling to be used as the stock at a point about 1 cm. below the cotyledons. The scion shoot was inserted in this cut and the two stems firmly bound together with wet bast using 2 or 3 separate ties. The area was wrapped round loosely with more wet bast and left for 1 to 2 weeks. At the end of this period the aerial shoot of the stock was cut off just above the graft union, and new bast ties were applied as necessary to prevent constrictions in the expanding stem. After about a month

complete union had in most cases been effected, and no further ties were necessary.

In both growing seasons the majority of the grafted plants grew well, although a number of the tetraploid scions remained somewhat stunted and produced very few flowers.

TABLE I

Datura stramonium 2n AND 4n

STOMATAL SIZES AND ALKALOIDAL CONTENTS (per cent. dry weight)

Plant sample	Diploid control			Tetraploid		
		Alkaloidal content			Alkaloidal content	
	Stomatal size μ	Aerial parts	Root	Stomatal size μ	Aerial parts	Root
1951.						
DSC 1	38×27	0·34	0·13	49×35	0·49	0·20
2	38×23	0·32	0·15	56×35	0·40	0·32
3	33×22	0·38	0·18	47×35	0·27	0·29
4	37×24	0·34	0·13	49×32	0·39	0·17
5	35×24	0·18	0·06	48×32	0·45	0·16
6	36×28	0·13	0·12	52×35	0·42	0·20
	—	—	—	55×36	0·32	0·39
	—	—	—	50×32	0·46	0·18
DTR 1	37×25	0·47	0·18	49×37	0·76	0·24
2	35×24	—	—	45×31	0·66	0·18
1952.						
DSBI 1	29×22	0·14	0·05	41×28	0·25	0·11
(F_1) 2	37×24	0·12	0·04	45×35	0·38	0·20
3	28×21	0·20	0·06	43×30	0·30	0·13
4	34×23	0·16	0·08	41×27	0·33	0·14
5	—	—	—	43×28	0·23	0·13
6	—	—	—	46×28	0·27	0·18
7	—	—	—	44×28	0·28	0·09
8	—	—	—	49×33	0·23	0·15
9	—	—	—	51×32	0·23	0·12
10	—	—	—	49×29	0·25	0·09
DTR 1	34×23	0·32	0·14	40×31	0·59	0·14
(F_1) 2	35×24	0·29	0·16	48×31	0·45	0·15
3	33×22	0·35	0·11	48×32	0·55	0·18
4	31×22	0·35	0·14	49×32	0·38	0·19
5	33×22	0·33	0·15	47×34	0·65	0·26
6	35×23	0·30	0·11	49×32	0·55	0·22
1952–53.						
DSB 1	37×25	0·14	0·12	45×28	0·17	0·16
2	34×23	0·16	0·08	47×30	0·21	0·10
3	33×22	0·15	0·09	43×29	0·20	0·21
4	32×22	0·15	0·09	41×28	0·20	0·08
5	—	—	—	46×29	0·18	0·08

Cultural details. Colchicine-treated and untreated seeds of all the samples of *D. stramonium*, *D. tatula* and tomato were sown in March–April in John-Innes seed compost in a cool greenhouse. The seedlings were pricked off into boxes and planted out in the open in May–June. During the 1951 season the grafted plants were not planted out in the open but were potted on in John-Innes potting compost and kept in a cool greenhouse; some ungrafted tetraploid and diploid plants from the same seed sample were also kept in the greenhouse throughout to act as controls. Owing to the somewhat inconsistent results obtained from the analyses of

the tetraploid plants grown throughout in the greenhouse (see Table II) it was decided in the 1952 season to transplant all the plants into the open, the grafted plants being planted out as soon as the union was complete and bast ties were no longer necessary.

In both seasons 1951 and 1952 the complete set of experiments was carried out at two sites, one in London and the other in Derbyshire; at both sites the beds to which the plants were transplanted had received only the normal routine manuring treatment. Tetraploid and diploid samples, together with the grafts, were grown side by side in the same beds.

TABLE II

Datura stramonium 2n AND 4n
RANGE OF INDIVIDUAL PLANT ESTIMATIONS (cf. Table I)
ALKALOIDAL CONTENTS
(Per Cent. Dry Weight)

Plants	Aerial parts		Roots	
	Control	4n	Control	4n
1951.				
DSA plot ..	0·23–0·34–0·47	0·22–0·40–0·68*	0·10–0·14–0·17	0·10–0·14–0·19
DSA greenhouse	0·10–0·17–0·25	0·14–0·24–0·30*	0·06–0·11–0·17	0·08–0·09–0·11
DSB	0·26–0·31–0·34	0·19–0·30–0·45	0·11–0·14–0·20	0·12–0·15–0·21
DSC plot ..	0·13–0·28–0·38	0·26–0·39–0·49*	0·06–0·13–0·18	0·16–0·22–0·39*
DSC greenhouse	0·19–0·23–0·31	0·18–0·22–0·29	0·07–0·10–0·13	0·05–0·10–0·13
DSE plot ..	0·19–0·26–0·33	0·23–0·27–0·36	0·07–0·08–0·10	0·11–0·16–0·24*
DSE greenhouse	0·27–0·29–0·33	0·76*	0·09–0·12–0·15	0·20*
DTR	0·47	0·65–0·70–0·76*	0·18	0·18–0·21–0·24*
1952.				
DSB	0·13–0·17–0·20	0·09–0·22–0·37*	0·03–0·06–0·08	0·04–0·08–0·11
DSB I (F_1) ..	0·12–0·15–0·20	0·17–0·27–0·38*	0·04–0·06–0·08	0·09–0·13–0·20*
DSB II (F_1) ..	—	0·22–0·28–0·36*	—	0·10–0·12–0·13*
DSE	—	0·18–0·19–0·20	—	0·08
DTQ	0·34–0·39–0·41	0·12–0·20–0·26	0·11–0·13–0·16	0·11–0·22–0·32*
DTR (F_1) ..	0·29–0·32–0·35	0·38–0·53–0·65*	0·11–0·13–0·16	0·14–0·19–0·26*
DTS (F_1) ..	0·16–0·23–0·32	0·31–0·32–0·33*	0·08–0·11–0·14	0·20–0·21–0·22*
1952–53.				
DSB	0·14–0·15–0·16	0·17–0·19–0·21*	0·08–0·10–0·12	0·10–0·16–0·21*
DTR (F_1) ..	0·19–0·22–0·24	0·63*	0·09–0·10–0·11	0·14*

* Indicates significant increase compared with 2n control.

The 1952–53 experiment was commenced late in the season of 1952 and consequently the plants could not be transplanted to the open. All the plants were kept in a cool-warm greenhouse for the whole period of their growth, and artificial illumination was supplied, giving a daily light period of 10 hours. This experiment was carried out in London only.

All plants were harvested when fully grown and still in flower. The whole plant was dug up and separated into root and aerial parts; the more mature fruits and the thicker parts of the stems were discarded. In harvesting the grafted plants the stock and scion were separated at the graft union and the small portion of hypocotyl remaining at the top of the root was discarded. The tomato on stramonium grafts in which rooting of the tomato scion had occurred from the graft union were dug up and the scion roots carefully separated from those of the stock; similarly the tetraploid and diploid branches of the branch chimeras were carefully

separated from one another. All the root samples were washed free from soil, and together with the aerial parts, dried in a current of air at 55° C. Immediately after drying, each sample was reduced to a moderately coarse powder and stored in a well-closed container from which moisture was excluded by silica-gel. Roots and aerial parts of individual plants were kept separate throughout.

Method of estimation. The colorimetric method of Allport and Wilson[18] with slight modifications, was employed throughout this work to determine the amount of total solanaceous alkaloids present in the samples. A Unicam quartz spectrophotometer was used to measure the colour intensity; 1 cm. cells were used throughout. Preliminary experiments showed that the optimum wavelength for measurement of the colour was 555 mμ. Using a sample of pure atropine alkaloid, the relation between colour intensity and concentration of solanaceous alkaloid was obtained for a series of concentrations ranging from 0·2 to 1·6 mg. per cent. The graph of these results was a straight line (Fig. 1), showing that between these concentrations the reaction obeys the Beer-Lambert Law, K (1 mg. per cent. solanaceous alkaloid) = 0·652.

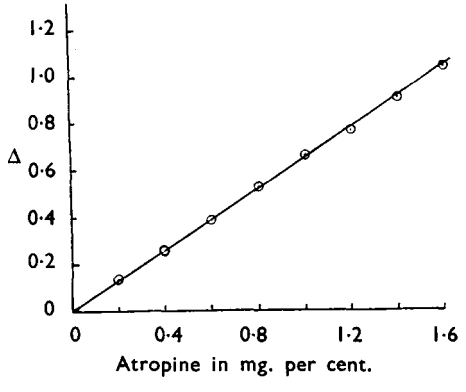

FIG. 1.

In the analyses of the plant samples certain modifications in the quantities of reagents used were necessary to allow for the wide variations in the alkaloid content. These variations from the standard method were shown, by estimation of known mixtures of atropine alkaloid with an inert substance (powdered grass) and by comparison with results obtained by the Pharmacopœial method of assay, not to affect the accuracy of the results.

RESULTS

The results are set out in Tables I to VII. Under the heading "plant sample" the initials DS are used to indicate *Datura stramonium* and DT to indicate *Datura tatula;* the third letter in each case represents the sample of seed used. The symbol (F_1) indicates plants raised from seed obtained from diploid and tetraploid plants grown in the previous year; hence when the symbol (F_1) is not given the tetraploid plants were obtained by direct colchicine treatment of the seeds. The samples DSBI (F_1) and DSBII (F_1) were grown in 1952 from seed collected in 1951 from 2 diploid and 2 tetraploid plants of DSB.

Table I gives examples of stomatal sizes and total alkaloid content of aerial parts and roots of individual diploid and tetraploid plants grown in 3 different seasons. Similar results have been summarised in Table II;

in the 1951 crop comparative analyses are given for teploitraploid and did plants from the same samples transplanted to the open (plot) and kept in the greenhouse. Table III gives the analyses of the 3 plants which had

TABLE III

4n DATURAS WITH 2n BRANCHES
ALKALOIDAL CONTENT
(Per Cent. Dry Weight)

	Aerial parts 2n branch	Aerial parts 4n branch	Roots (4n)
1951 DSC ..	0·24	0·34	0·18
1951 DSD ..	0·33	0·46	0·11
1952 DTQ ..	0·17	0·26	0·12

TABLE IV

Datura stramonium GRAFTS I
Scion = 2n plants. Stock = 4n plants
ALKALOIDAL CONTENT
(Per Cent. Dry Weight)

Plant sample	Aerial parts	Roots
1951.		
DSC Control 2n plants ..	0·19–0·23–0·31	0·07–0·10–0·13
Graft 1	0·22	0·06
" 2	0·19	0·07
" 3	0·17	0·13
DSE Control 2n plants ..	0·27–0·29–0·33	0·09–0·12–0·15
Graft 1	0·36*	0·11
" 2	0·43*	0·18*
1952.		
DSA Control 2n plants ..	0·30–0·48–0·59	0·05–0·12–0·16
Graft 1	0·19	0·19*
DSBI Control 2n plants ..	0·12–0·15–0·20	0·04–0·06–0·08
(F_1) Graft 1	0·24*	0·04
" 2	0·23*	0·05
" 3	0·18	0·06
DTS Control 2n plants ..	0·16–0·23–0·32	0·08–0·11–0·14
(F_1) Graft 1	0·39*	0·13
" 2	0·29*	0·13
" 3	0·36*	0·12
" 4	0·32*	0·17*
1952–53.		
DSB Control 2n plants ..	0·14–0·15–0·16	0·08–0·10–0·12
Graft 1	0·42*	0·16*
" 2	0·19*	0·11
DTR Control 2n plants ..	0·19–0·22–0·24	0·09–0·10–0·11
(F_1) Graft 1	0·41*	0·13*

* Indicates significant increase compared with 2n control.

diploid and tetraploid branches. The results of the graft experiments are set out in Tables IV to VII. The grafts on tomato stocks are not included as in every instance no trace of solanaceous alkaloid was found in either the tomato stock or the *Datura* scion. Analyses of the tomato roots from the 2 control grafts (diploid tomato scions on diploid stramonium stocks)

which had rooted at the graft union showed a small amount of solanaceous alkaloids (about 0·02 per cent. dry weight) to be present in each sample.

Discussion of Results

The results given in Tables I and II, which are obtained from analyses of more than 200 individual plants, grown in 3 successive years, support previous work showing that in general there is an increase in alkaloid content in the aerial parts of tetraploid plants of *Datura stramonium* and

TABLE V

Datura stramonium GRAFTS II

Scion = 4n plants. Stock = 2n plants

ALKALOIDAL CONTENT

(Per Cent. Dry Weight)

Plant sample	Aerial parts	Roots
1951.		
DSA Control 2n plants ..	0·10–0·17–0·25	0·06–0·11–0·17
Grafts 1–8	0·14–0·17–0·20	0·06–0·09–0·13
DSC Control 2n plants ..	0·19–0·23–0·31	0·07–0·10–0·13
Graft 1	0·33*	0·37*
" 2–8	0·17–0·21–0·25	0·08–0·12–0·16
DSE Control 2n plants ..	0·27–0·29–0·33	0·09–0·12–0·15
Graft 1	0·34*	0·15
" 2	0·38*	0·21*
" 3	0·22	0·10
" 4	0·21	0·11
1952.		
DSA Control 2n plants ..	0·30–0·48–0·59	0·05–0·12–0·16
Graft 1	0·67*	0·16
" 2–6	0·35–0·46–0·50	0·12–0·14–0·17
DSBI Control 2n plants ..	0·12–0·15–0·20	0·04–0·06–0·08
(F_1) Graft 1	0·28*	0·04
" 2	0·18	0·06
DTS Control 2n plants ..	0·16–0·23–0·32	0·08–0·11–0·14
(F_1) Graft 1	0·14	0·16*
1952–53.		
DSB Control 2n plants ..	0·14–0·15–0·16	0·08–0·10–0·12
Graft 1	0·16	0·11
" 2	0·26*	0·14*
" 3	0·33*	0·08
" 4	0·32*	0·13*
DTR Control 2n plants ..	0·19–0·22–0·24	0·09–0·10–0·11
(F_1) Graft 1	0·23	0·09

* Indicates significant increase compared with 2n control.

D. tatula when compared with diploid controls; this increase was found to occur both in tetraploid plants produced by colchicine treatment of the seed, and in the tetraploid plants of the first filial generation (F_1). Occasional abnormal behaviour was observed; for example, very little increase in alkaloid content was found in the aerial parts of any of the induced tetraploid plants of the DSB series grown in 1951, although significant increases occurred in 1952 and 1952–53, and the F_1 generations from two tetraploid plants (not analysed in 1951) when grown in 1952 showed considerable increases (Tables I and II). The DSE series in 1951 showed

no alkaloid increase in tetraploid plants grown on the plot, but a single plant grown in the greenhouse showed considerable increase; no increases occurred in induced tetraploid plants of the same series in 1952 (Table II). Similarly, induced tetraploid plants of the DTQ series grown in 1952 showed no alkaloid increase in the aerial parts. Differences between the behaviour of plants grown on the plot and those grown in the greenhouse have already been indicated for the DSE series, and Table II gives comparable results for the DSA and DSC series also. Thus while the DSA

TABLE VI

TOMATO: STRAMONIUM GRAFTS I

Control Grafts:—Scion = 2n tomato, Stock = 2n stramonium
Test Grafts Scion = 4n tomato, Stock = 2n stramonium

ALKALOIDAL CONTENT
(Per Cent. Dry Weight)

Plant sample	Aerial parts	Roots
1951.		
DSA Control 2n stramonium plants	0·10–**0·17**–0·25	0·06–**0·11**–0·17
Control grafts 1–5	0·17–0·22–0·28	0·05–**0·07**–0·08
Test graft 1	0·36*	0·12*
" " 2	0·17	0·11*
" " 3	0·17	0·08
DSC Control 2n stramonium plants	0·19–**0·23**–0·31	0·07–**0·10**–0·13
Control grafts 1–6	0·21–0·27–0·35	0·08–**0·10**–0·15
Test graft 1	0·44*	0·20*
DSE Control 2n stramonium plants	0·27–**0·29**–0·33	0·09–**0·12**–0·15
Control grafts 1–3	0·18–**0·22**–0·24	0·15–**0·17**–0·18
1952.		
DSA Control 2n stramonium plants	0·30–**0·48**–0·59	0·05–**0·12**–0·16
Control grafts 1–4	0·20–**0·21**–0·23	0·09–**0·12**–0·14
Test graft 1–2	0·14–**0·16**–0·18	0·12
DSC Control 2n stramonium plants	0·23–**0·32**–0·39	0·09–**0·11**–0·15
Control grafts 1–4	0·20–**0·23**–0·25	0·06–**0·09**–0·12
Test graft 1–2	0·10–**0·12**–0·14	0·06–**0·07**–0·08
1952–53.		
DSB Control 2n stramonium plants	0·14–**0·15**–0·16	0·08–**0·10**–0·11
Control grafts 1–2	**0·10**	**0·08**
Test graft 1	0·16*	0·12*
" " 2	0·21*	0·09

* Indicates significant increase compared with 2n control.

plants showed normal increases in alkaloid content in the aerial parts of tetraploid plants grown both on the plot and in the greenhouse when compared with diploid controls, tetraploid plants of the DSC series showed no increases when grown in the greenhouse, but significant increases when grown on the plot.

The results obtained by analyses of the scions and stocks of the reciprocal grafts between diploid stramonium and diploid tomato plants support those already reported by other workers[5–9]. Alkaloids were present in all the tomato scions and in the majority of them the amount was approximately equal to that found in normal diploid *Datura* plants (Tables VI and VII). This, and the absence of alkaloids in any of the *Datura* scions

grown on tomato stocks, further support the hypothesis that the alkaloids are synthesised in the root in these species.

Comparison of the total alkaloid content of the roots of tetraploid and diploid plants shows that in general the roots of tetraploid plants were considerably richer in alkaloids than the corresponding diploid controls (Tables I and II). Among the tetraploid plants which showed no increase in alkaloid content in the aerial parts some likewise showed no increase

TABLE VII

TOMATO : STRAMONIUM GRAFTS II

Control Grafts :—Scion = 2n tomato, stock = 2n stramonium
2:4 Test Grafts :—Scion = 2n tomato, stock = 4n stramonium
4:4 Test Grafts :—Scion = 4n tomato, stock = 4n stramonium

ALKALOIDAL CONTENT
(Per Cent. Dry Weight)

Plant sample	Aerial parts	Roots
1951.		
DSA Control 2n stramonium plants	0·10–0·17–0·25	0·06–0·11–0·17
Control grafts 1–5	0·17–0·22–0·29	0·05–0·07–0·08
2:4 Test graft 1	0·22	0·10*
4:4 Test graft 1	0·16	0·18*
DSC Control 2n stramonium plants	0·19–0·23–0·31	0·07–0·10–0·13
Control grafts 1–6	0·21–0·27–0·35	0·08–0·10–0·15
2:4 Test grafts 1–2	0·21	0·07
1952.		
DSA Control 2n stramonium plants	0·30–0·48–0·59	0·05–0·12–0·16
Control grafts 1–4	0·20–0·21–0·23	0·09–0·12–0·14
2:4 Test grafts 1–2	0·12–0·17–0·21	0·11–0·13–0·14
DTS Control 2n stramonium plants	0·16–0·23–0·32	0·08–0·11–0·14
(F_1) Control grafts 1–5	0·09–0·15–0·32	0·11–0·14–0·17
2:4 Test grafts 1–2	0·11	0·09
4:4 Test graft 1	0·29*	0·16*
1952–53.		
DSB Control 2n stramonium plants	0·14–0·15–0·16	0·08–0·10–0·12
Control grafts 1–2 ..	0·10	0·08
2:4 Test graft 1	0·19*	0·15*
DTR Control 2n stramonium plants	0·19–0·22–0·24	0·09–0·10–0·11
(F_1) Control grafts 1–2	0·05	0·07–0·08–0·09
2:4 Test grafts 1	0·05	0·07
2	0·11*	0·05*
4:4 Test graft 1	0·13*	0·12*

* Indicates significant increase compared with 2n control.

in the roots (DSB 1951, DSC greenhouse, Table II); others showed considerable increases (DSE plot, 1951, DTQ 1952, Table II). Conversely some of the tetraploid plants which were richer in alkaloid in the aerial parts did not show a corresponding increase in the roots (DSA plot, 1951, DSB 1952, Table II).

On the hypothesis that the root is the site of alkaloid synthesis it would appear, therefore, that in tetraploid plants there is increased alkaloid production by the tetraploid roots; this is accompanied by an increase in the alkaloid content of the aerial parts. Examination of the graft experiments in which tetraploid stramonium plants were used as stocks shows that in the 2ns/4ns series, 11 out of the 16 diploid scions had increases in

alkaloid content when compared with control plants grown on diploid roots (Table IV); these increases would appear to be due to the influence of the tetraploid stocks. A smaller number of the stocks showed definite increases in alkaloid content when compared with diploid controls, but the amounts present are comparable in all cases with those found in normal tetraploid plants (cf. Tables I and II). In the tomato on stramonium grafts, 2 tomato scions of the 2nt/4ns series were somewhat richer in alkaloid content than those of the control grafts 2nt/2ns (Table VII); one of these showed an increase also in the alkaloid content of the tetraploid stock. The remaining 7 grafts showed no increase in either the scions or stocks. These results suggest, therefore, that a diploid tomato scion when grown on a tetraploid stramonium stock has an alkaloid content equivalent to that found when grown on a diploid stramonium stock and that in addition the diploid tomato scion has a depressant effect on the alkaloid content of the tetraploid stramonium stock. This contrasts markedly with the results obtained in the 2ns/4ns series. Analyses of the 4nt/4ns grafts (Table VII) showed some increase in alkaloid content in 2 of the scions and in all the stocks; hence it appears that a tetraploid tomato scion does not depress the alkaloid content of a tetraploid stramonium stock to the same extent as does a diploid tomato scion.

The results of the graft experiments in which diploid stramonium plants were used as stocks are given in Tables V and VI. In the 4ns/2ns series (Table V) 25 grafts showed alkaloid contents in both scions and stocks equal to those found in normal diploid plants; one graft (DSC 1) showed remarkable increase in alkaloid content in the stock and some increase in the scion; 7 other grafts showed increases in the scions, and 4 of these had corresponding increases in the stocks; one other had alkaloid enrichment in the stock but not in the scion. Among the 4nt/2ns grafts (Table VI), 5 had alkaloid contents equivalent to diploid controls in both scions and stocks; 4 showed alkaloid enrichment in the scions and 3 of these also showed increases in the stocks; one other had increased alkaloid content in the stock but not in the scion. The general inference from these results, therefore is that when diploid stramonium plants are used as stocks the alkaloid content in both scions and stocks is equivalent to that of normal diploid plants although there is some indication of an influence by tetraploid scions, resulting in increased alkaloid content in both scions and stocks in some of the grafts.

The results of the analyses of the branch chimeras, given in Table III, further suggest that the tetraploid or diploid nature of the aerial parts influences the alkaloid content; in all 3 plants in which tetraploid and diploid branches were growing on the same root system the tetraploid branch was found to have a higher alkaloid content than the diploid branch. This is possibly due to an increased storage capacity by the tetraploid branch, although it does not preclude the possibility of differential rates of synthesis occurring in the branches.

Recent work by Evans and Partridge[19] using reciprocal grafts between *Datura tatula* and *D. ferox* has indicated that the aerial parts of *D. tatula* exhibit some faculty for the synthesis of hyoscine and hyoscyamine. While

the results of our graft experiments strongly suggest that the main alkaloid synthesis does take place in the roots of *D. stramonium* and *D. tatula*, the capacity of the aerial parts to synthesise may be an explanation of the scion influence if it is assumed that this capacity is increased by tetraploidy. It was shown by Warren-Wilson[9] that downward translocation of alkaloids does occur in *Atropa belladonna*; the presence of alkaloids in the tomato roots growing from the graft union on the 2nt/2ns grafts shows that downwards translocation also occurs in the *Datura* species. Thus the alkaloid content of the root system must be regarded as a balance between rate of synthesis therein, removal upwards in the transpiration stream, and downward translocation with other elaborated storage products. Similarly the leaf content of alkaloid is a balance between alkaloid received in the transpiration stream and that removed by downward translocation, with the possibility, not yet proved, of some alkaloid synthesis in the leaf.

Summary

1. Previous work showing a considerable increase in alkaloid content in the aerial parts of tetraploid plants of *Datura stramonium* and *D. tatula* compared with diploid controls has been confirmed, a maximum increase of 286 per cent. above 2n mean being recorded in an F_1 plant.

2. When diploid *D. stramonium* scions were side-grafted on diploid tomato stocks no trace of solanaceous alkaloid was found in either the scions or stocks. In the reciprocal grafts alkaloids were present in both stocks and scions in amounts equivalent to those found in normal diploid *Datura* plants.

3. Comparison of the total alkaloid content of the roots of tetraploid and diploid plants of *D. stramonium* and *D. tatula* showed that the roots of tetraploid plants were considerably richer in alkaloids than the corresponding diploid controls, the increase being up to threefold.

4. Reciprocal grafts between tetraploid and diploid *D. stramonium* suggest that the 4n or 2n nature of the stock determines the alkaloid content of the scion and stock. Thus in 2ns/4ns grafts the alkaloid content of scion and stock was equivalent to that found in normal 4n plants; in 4ns/2ns the alkaloid content was equivalent to that found in normal 2n plants. Some divergent results were obtained indicating possible influence by the scion on alkaloid content of both stock and scion.

5. In grafts 2nt/4ns the tomato scion appears to have a depressant effect on the alkaloid content of the 4n stramonium stock; this effect is less marked when 4n tomato is used as the scion.

6. In branch chimeras the 4n branch is richer in alkaloid than the 2n branch growing on the same root.

7. The possible capacity of the aerial parts to synthesise alkaloids is discussed.

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This communication forms part of the subject-matter of a thesis to be presented by one of us (B. P. J.) for the degree of Doctor of Philosophy in the Univeristy of London.

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DISCUSSION

The two papers on Alkaloid Biogenesis were taken together, the first being presented by DR. W. C. EVANS and the second by MISS B. P. JACKSON.

DR. W. MITCHELL (London) pointed out that in their concluding sentence the authors of the first paper suggested that alkaloid formation was probably not related simply to the metabolic processes of the plant. Was it, then, their opinion that alkaloids were waste products, as had so long been the general view? Presumably if the relation were not direct they were not waste products but more probably were utilised in some way in the metabolic cycle. The fact that the alkaloid content often reached a maximum at flowering suggested that they played an active part, especially since the content thereafter usually diminished. Again, why should solanaceous plants pack their seeds with alkaloids if they were waste products? He asked the age of the belladonna seedlings when it was found that 89 per cent. of the total alkaloids was hyoscine, and at what stage the drop in alkaloidal content took place. The quantities of hyoscine in *Atropa belladonna* at all stages were very small and they appeared to have been determined by titrimetric methods. Could the authors be certain that that material really was hyoscine? In particular, had they been able to collect enough to prepare a crystalline derivative such as the aurichloride? Only thus could one be really satisfied as to the identity of the alleged hyoscine. In the last sentences of the second paper, reference was made to alkaloids being translocated to the root amongst other "storage products." Could it be inferred

that these authors also did not class vegetable alkaloids as waste products in all cases?

DR. K. BULLOCK (Manchester) asked whether the authors of the second of the papers had yet reached the stage of being able to compare the ordinary and tetraploid plants in terms of yield of alkaloid per acre. Secondly, had they ever tried expressing their results in terms of alkaloidal nitrogen as a percentage of total nitrogen, and, if so, had that yielded any interesting results?

DR. T. E. WALLIS (London) said that the authors confirmed the customary method of collecting belladonna when flowering commenced. Possibly the plant made some use of the alkaloids in its metabolism, and in that connection he noticed how differently *D. tatula* and *D. erox* behaved when they were germinating. *D. tatula* seemed to accumulate hyoscine during germination, whereas in *D. ferox* the hyoscine decreased. That looked as though one of the plants was using hyoscyamine to do something in its metabolism and the other was using hyoscine for a similar purpose. Apparently the authors of the first paper found that *Atropa belladonna* on the whole produced its alkaloid in the roots, whereas the *D. tatula* plants produced their alkaloid both in the stems and the root. He wondered whether the authors had considered the fact that belladonna was a perennial plant and *D. tatula* and *D. ferox* were annual plants.

DR. R. RUYSSEN (Belgium) said that one of his workers had discovered that the rate of biosynthesis of the alkaloids of cinchona in leaves of young plants was almost parallel to that of carbohydrates, which were studied using ^{14}C, under the same conditions of light, temperature and season.

DR. W. C. EVANS, commenting on the other paper, said that the problem of the site of the biogenesis of alkaloids was associated with difficulties in experimentation as well as in interpreting the results. In a previous publication they (Evans and Partridge) had shown that *D. ferox* scions grafted on to *D. tatula* roots accumulated hyoscyamine in the leaves, and that had been taken as direct evidence for the synthesis of hyoscyamine in *D. tatula* leaves. He considered that the paper under discussion afforded considerable evidence of the formation of alkaloids in the leaves of *D. tatula* and *D. stramonium*. In that respect the branch chimeras were very interesting plants. Here there were plants which had both $4n$ and $2n$ branches on $4n$ stocks. From the analysis of those plants the authors suggested that increased storage capacity was possibly the reason for the increase in alkaloid content, but he did not agree because solanaceous leaves were capable of storing large quantities of injected hyoscine or hyoscyamine without any ill effects on the plant itself. Tables VI and VII served to emphasise the necessity for extreme caution in interpreting results based on grafts between plants of different genera and plants which normally produced alkaloids and those which did not. In fact, all the results in the two tables could be used as evidence that it was the tomato leaves which synthesised the alkaloids as a result of precursors or stimulation from the stramonium stock, and that would

be particularly well borne out by the fact that 4*n* tomato scions when grafted on to 4*n* stocks produced more alkaloid than when the 2*n* scions were grafted on to the same stocks. This hypothesis was not very probable, but it showed that reliable information on the site of synthesis could not be obtained from somewhat unnatural material. Turning to the experimental procedure, he considered it a great pity that the authors did not determine both the hyoscine and the hyoscyamine present in the plants rather than just total alkaloid, because the crucial point was that it had not yet been proved that both hyoscine and hyoscyamine were in fact synthesised in the same organ in the stramonium plants. Their previous work had shown that in *D. tatula* different alkaloids were synthesised in different organs, and therefore estimations of total alkaloids were liable to give confusing results. That could be one reason for the rather numerous inexplicable results which the authors had reported in their paper.

MR. H. B. WOODHEAD (Manchester) said that it would be interesting to investigate the transpiration streams to find out whether the alkaloids or their precursors were transported in the plant.

DR. W. C. EVANS in reply said that so far as solanaceous alkaloids were concerned it was not known whether they played any part in the general metabolism or were merely waste products. They might exist in equilibrium with other substances, and as soon as they were formed perhaps they might be changed into something else, in which case, of course, they were important so far as the metabolism of the plant was concerned. The age of the belladonna seedlings when the alkaloidal content consisted of 89 per cent. hyoscine was about 6 or 8 weeks. They had no doubt that the alkaloid expressed as hyoscine was, in fact, hyoscine. In a fair number of cases this had been proved by the preparation of the aurichloride or picrate. Dr. Wallis's point was very interesting and had engaged the attention of the authors. They were aware of the fact that *D. tatula* seemed to build up hyoscine whereas in *D. ferox* the amount of alkaloid tended to decrease. It had to be borne in mind in recording such results that the percentage of alkaloids expressed as dry weight might give misleading results from the point of view of the amount of alkaloid which was actually in the plant. He doubted whether reliable conclusions could be drawn from analyses of the alkaloids found in the transpiration stream.

MISS B. P. JACKSON, in reply, said they had not themselves determined the yield per acre of the diploid and tetraploid plants, but this was being investigated by other workers and there was little doubt that the yield of alkaloid per acre was considerably higher in tetraploid plants than in diploid plants. The alkaloidal nitrogen had not been determined as a percentage of total nitrogen. Evans and Partridge in an earlier paper stated that hyoscyamine was synthesised in the leaves of *D. tatula*, but examination of their results showed that the quantity of hyoscyamine found in the grafts of *D. tatula* grown on *D. ferox* stocks was very small in two cases and moderate in the third. It seemed possible that the hyoscyamine had been carried over during the process of grafting, as

she had noted that in the method used the scions were allowed to remain on their own roots for a period of 5 days before being severed. She agreed that the results with the branch chimeras were indicative of there being a differential rate of synthesis of the alkaloids in the aerial parts. However, the results of the grafts using tomato seemed to provide overwhelming evidence of synthesis in the roots, and the complete absence of alkaloid in *D. stramonium* when grown on tomato root did, in her view, suggest that a *D. stramonium* top was incapable by itself of producing alkaloid.

DR. J. M. ROWSON pointed out that he and Miss Jackson had made use of interspecific and intergeneric grafts, whereas Dr. Evans had omitted in his earlier paper to include any intergeneric grafts. It would be interesting to investigate the effect of grafting *D. ferox* on a non-alkaloid producing plant.