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SOME EFFECTS OF SELECTION ON THE PRODUCTION OF ALKALOIDS IN BELLADONNA.

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INTRODUCTION.

The improvement of many of our important agricultural crops has been brought about by means of plant selection and breeding, and similar methods are being extended to various other fields of plant production in the hope of achieving similar results.

With a few exceptions, the growing of medicinal plants is still in its early stages, and it is a much-debated question whether these plants when cultivated lose any of their therapeutic properties; but with the constantly diminishing supply of many of our important native drug plants cultivation becomes more and more imperative. Also, the quality of some of the drugs on the market has deteriorated to such an extent that improvement is much to be desired, and it is hoped that this may be accomplished through the methods employed by the plant breeder.

Atropa belladonna is an important mydriatic drug, the supply of which has been of such inferior quality in recent years that the Office of Drug-Plant and Poisonous-Plant Investigations has been conducting experiments for some time with special reference to increasing the alkaloidal content of the plant. The first step was to make a general study of the plant with regard to individual variation

of the alkaloidal content; then to study the relation of physical and chemical characteristics—the influence of environmental factors, such as climate and soil—and the fluctuations in the alkaloidal content during the growing season. The results of these investigations have already been reported.¹

SELECTION OF TYPICAL PLANTS.

A number of plants were selected which from the data obtained in the preliminary investigation showed typical alkaloid-producing tendencies. Although only those plants which produce a high percentage of alkaloids are of commercial value, plants of the opposite type, namely, those which produce only small quantities, were also selected, in order to furnish more comprehensive data on the transmissibility of alkaloid-producing characteristics. An enumeration of the individual plants which were selected from the original lot, showing the alkaloidal content of their leaves at the various stages of growth during two seasons, is given in Table I.

TABLE I.—*Alkaloids in the leaves of selected belladonna plants during two seasons, at Arlington, Va.*

Plant No.	Yield of alkaloids on which selection was based.	Alkaloidal content (per cent).											
		Stage of growth, 1911.						Stage of growth, 1912.					
		First.	Second.	Thrd.	Fourth.	Fifth.	Average.	First.	Second.	Thrd.	Fourth.	Fifth.	Average.
34.	Low	0.335		0.526	0.532	0.200	0.414		0.292	0.320			0.406
3.	do	.384	0.375	.277	.549	.451	.407		.393	.448	0.448		.426
23.	do		.348	.354	.487	.425	.403	0.496	.366	.341			.401
46.	do	.337	.285	.308	.588	.431	.390	.418	.334	.480	.483	0.314	.406
13.	Medium	.614	.627	.626	.763	.563	.639	.495	.479	.655	.472	.488	.518
12.	High	.649	.782	.693	.497	.584	.641	.767	.631	.685	.715	.394	.638
7w	do	.558	.831	.832	.727	.571	.704	.782	.666	.646	.694	.573	.672
6w	do	.596	.879	.925	.711	.722	.766	.847	.747	.882	.804	.558	.768

METHOD OF CONTROLLING POLLINATION.

The flowers of *Atropa belladonna* are normally cross-pollinated and depend principally on insects, such as moths, beetles, and bumblebees, for the transportation of pollen. When insects are excluded the flowers fail to develop and soon drop off, which indicates that natural self-pollination occurs rarely, if at all.

In 1911 a large number of flower buds were inclosed in manila bags in the hope that close pollination would take place, but none of the flowers set seed. However, seeds resulting from the cross-pollination of the individual plants enumerated in Table I were collected and planted in flats in the greenhouse at Arlington, Va., in the following

¹ Sievers, A. F. Individual variation in the alkaloidal content of belladonna plants. Journal of Agricultural Research, v. 1, no. 2, p. 129-146, 1913.

January. Late in April, when the plants from these seeds were about 3 or 4 inches high, they were transplanted to the field.

In 1912 a second attempt was made to secure seeds from close-pollinated flowers, and in order to insure fertilization the pollen was transferred to the stigmas by hand before the flowers were inclosed. The method of bagging the flower buds used in the previous year again proved ineffectual, since a species of aphid bred in the bags in great numbers and destroyed the young plant tissues. Seeds were obtained from a number of selected plants which had been inclosed in cheesecloth cages, but even within those cages the aphides often multiplied to such an extent as to injure the plant. The seeds from these plants were sown in flats in the greenhouse and the seedlings therefrom transplanted to the field, the same as in 1911.

FIRST-GENERATION PLANTS FROM CROSS-POLLINATED PARENTS.

The plants from the seed of the cross-pollinated selected individuals in 1911 were transplanted to the field late in April. There were from 6 to 15 individual plants from each parent. These plants made a fairly rapid growth and by midsummer were in full bloom, but the growth they had made was not sufficient to make possible a picking from each individual plant. A collective sample was therefore secured from each set of individuals derived from the same parent. On August 30, when the berries were partially ripe, the second picking was made. In picking these samples care was taken to get leaves from all the individuals, so that the results of the assay would indicate as nearly as possible the average percentage of alkaloids in the leaves of the individuals. Table II shows the results of the assays.

TABLE II.—*Alkaloids in the leaves of first-generation belladonna plants grown from cross-pollinated selected parents at Arlington, Va.*

Parent plant.		Alkaloidal content (per cent.).			Parent plant.		Alkaloidal content (per cent.).		
No.	Yield of alkaloids.	First picking.	Second picking.	Average.	No.	Yield of alkaloids.	First picking.	Second picking.	Average.
34.....	Low....	0.524	0.693	0.609	12.....	High....	0.650	0.882	0.766
3.....	do.....	.479	.518	.498	7w.....	do.....	.617	1.063	.840
13.....	Medium	.640	.859	.750	6w.....	do.....	.805	1.282	1.043

From Table II it is evident that these first-generation plants displayed largely the characteristic of the parent plants as regards alkaloid production. Plants 7w and 6w merit special attention, their leaves containing more than 1 per cent of alkaloids.

The following season, 1913, these plants were large enough to furnish samples from each individual, and pickings were made at four stages of growth during the season. The first picking was on May 6, before flowering; the second on May 23, when the plants were in full

bloom; the third on June 21, when the first berries were developing; and the fourth on September 8, when most of the berries were ripe and much new growth had appeared in the form of shoots and suckers. The season was a very unfavorable one, and the plants made a rather small growth, so that during July and August the leaves were too small and sparse to secure samples. For the purpose of comparison, Table III gives the alkaloidal content of the various samples and the average for the season, the average alkaloidal content of each picking being summarized at the close.

TABLE III.—*Alkaloids in the leaves of individual first-generation belladonna plants at various stages of growth at Arlington, Va., during the second season, 1913.*

Plant No.	Alkaloidal content (per cent).					Plant No.	Alkaloidal content (per cent).				
	First stage, May 5.	Second stage, May 23.	Third stage, June 21.	Fourth stage, Sept. 8.	Average for season.		First stage, May 5.	Second stage, May 23.	Third stage, June 21.	Fourth stage, Sept. 8.	Average for season.
34 ₁		0.316	0.324		0.320	12 ₂	0.809	0.601	0.625	0.716	0.688
34 ₂	0.516	.473	.312	0.856	.539	12 ₇	.750	.608	.582	.574	.529
34 ₃	.608		.352	.863	.604	12 ₈	.587	.434	.569	.653	.561
34 ₄	.754	.434	.288		.492	12 ₉			.389	.549	.469
34 ₅	.622	.542	.356	.683	.551	7w ₁			.626	.737	.681
3 ₁	.538	.366	.430	.523	.466	7w ₂	.835	.550	.507	.759	.663
3 ₂	.565	.565	.630		.587	7w ₃	.620	.563	.431	.782	.599
3 ₃	.511	.552	.364	.790	.554	7w ₄	.792	.777	.588	.804	.740
3 ₄	.693	.607	.350	.833	.621	7w ₅		.521	.699	.998	.739
3 ₅	.513	.477	.533	.767	.573	7w ₆	.674	.721	.567	.663	.656
3 ₆	.438		.392		.415	7w ₇	.369	.765	.760		.631
3 ₇	.412	.415	.504		.444	7w ₈		.291		.645	.468
3 ₈		.300			.300	7w ₉	.714	.389	.544		.412
3 ₉		.479	.702	.782	.654	7w ₁₀	.634	.496	.489	.764	.596
13 ₁		.255	.459	.934	.549	7w ₁₁		.404	.626	.773	.601
13 ₂		.365	.596		.481	6w ₁	.541	.543	.501	.666	.561
13 ₃			.453		.453	6w ₂		.693	.881		.787
13 ₄	.492	.359	.318	.649	.455	6w ₃	.850	.641	.787	.885	.791
13 ₅		.466	.559	.563	.529	6w ₄		.866	.516	.529	.856
13 ₆			.470	.733	.601	6w ₅	.806	.640	.768	.829	.761
13 ₇		.341	.552	.766	.553	6w ₆	.780	.735	.663	.733	.728
13 ₈		.349	.664	.792	.601	6w ₇	.739	.917	1.313	.956	.981
13 ₉	.566	.452	.385	.815	.555	6w ₈	.780	.921	.937	1.020	.915
12 ₁	.854	.653	.737	.494	.688	6w ₉	.833	.921	.901	.919	.894
12 ₂	.480	.516	.661	.717	.594	6w ₁₀	.908	.925	.927	.876	.909
12 ₃	.760	.672	.675	.638	.686	6w ₁₁	.956	.973	.993	.924	.962
12 ₄	.605	.572	.626		.619	6w ₁₂	.681	.574	.670	.922	.712
12 ₅	.588		.675	.908	.543	6w ₁₃	1.143	.639	.852	.754	.847

SUMMARY OF ALL INDIVIDUAL PLANTS FROM EACH PARENT.

Parent plant.		Average alkaloidal content of leaves (per cent).				
No.	Yield of alkaloids.	Stage of growth.				Average for season.
		First stage.	Second stage.	Third stage.	Fourth stage.	
34.....	Low.....	0.625	0.441	0.326	0.800	0.501
3.....	do.....	.524	.470	.488	.739	.512
13.....	Medium.....	.529	.369	.495	.765	.531
12.....	High.....	.679	.573	.617	.662	.597
7w.....	do.....	.662	.547	.583	.769	.617
6w.....	do.....	.824	.740	.824	.862	.811
Average.....		.640	.523	.555	.766	

A careful study of Table III reveals the fact that the individual first-generation plants possess in large measure the same alkaloid-

FIG. 1.—Diagram showing the alkaloidal content of the leaves of first-generation belladonna plants from cross-pollinated parents at two stages of growth during the first season, 1912. The percentages indicated represent the average of all the individuals from each parent plant.

producing characteristic as the parent plant. Plant 6w, the richest of the original 59 individual plants under observation for two years, yielded from cross-pollination first-generation plants which were distinctly rich in the first season and again in the second season. Furthermore, the plants of the first generation from the cross-pollinated individuals selected from the original list for low alkaloidal content have shown a decidedly low percentage of alkaloids during each of the two seasons of growth. Figures 1 to 4 show graphically the average percentage of alkaloids in the leaves of all the first-generation plants from each parent at each of the pickings in 1912 and 1913. Besides showing at a glance what has already been said regarding the plants which are rich or poor in alkaloids, figure 1 also shows an interesting condition with regard to the relative richness of the plants at the

FIG. 2.—Diagram showing the average alkaloidal content of all the individual belladonna plants from each parent for the season, first year, 1912.

FIG. 3.—Diagram showing the alkaloidal content of the leaves of first-generation belladonna plants from cross-pollinated selected parents at four stages of growth during the first season. The percentages indicated represent the average of all the individuals from each parent plant.

various stages of growth. In the previous investigation¹ the plants were richest in alkaloids at the first and fourth stages; that is,

¹ Sievers, A. F. Op. cit.

the stage before flowering and the stages when the berries are ripe. In another season, however, the same plants were found to be richer in alkaloids at the second and fourth stages. In the present investigation we find the greatest percentage of alkaloids at a stage of growth which corresponds to the fourth stage in the

FIG. 4.—Diagram showing the average alkaloidal content of all the individual belladonna plants from each parent for the season, second year, 1913.

previous investigation. It seems, then, further established that the greatest concentration of alkaloids is at the period when the berries are mostly ripe and the leaves small and sparse.

COMPARISON OF FIRST-GENERATION PLANTS FROM CROSS-POLLINATED AND CLOSE-POLLINATED PARENTS.

From the parent plants, 6w and 7w, which yielded the progeny described in the foregoing pages, seeds were obtained in 1912 through both cross and close pollination and planted for purposes of comparison. Plants 12, 13, 3, and 34 were dead; therefore no seeds could be secured from them. In their stead, seeds were taken from plants 46 and 23, as these were examples of low alkaloid-yielding plants. The seeds were sown in the greenhouse and the seedlings transplanted to the field early in May, 1913. Owing to the hot weather and severe drought the plants made only a fair growth, and only two pickings were secured, the first on August 19, when the plants were in the flowering stage, and the second on September 23, when the berries were in various stages of ripeness. Table IV shows the percentage of alkaloids in the leaves of the individuals from each selected parent.

TABLE IV.—*Alkaloids in the leaves of first-generation belladonna plants from close pollination and cross-pollination of selected parents at two stages of growth at Arlington, Va., during the first season, 1913.*

Lot and number.	Alkaloidal content (per cent).			Lot and number.	Alkaloidal content (per cent).		
	First stage, Aug. 19.	Second stage, Sept. 23.	Average.		First stage, Aug. 19.	Second stage, Sept. 23.	Average.
Lot 6w, close pollinated:				Lot 6w, close pollinated—Contd.			
1.....	0.514	0.430	0.472	10.....	0.745	0.658	0.702
2.....	.687	.594	.640	11.....			
3.....	.421	.504	.463	12.....	.741	.705	.723
4.....	.834	.886	.860	13.....	.687	.613	.650
5.....	.773	.736	.755	14.....	.767	.750	.759
6.....	.731	.724	.728	15.....	.658	.760	.719
7.....	.689	.745	.717	16.....	.703	.722	.713
8.....	.735	.633	.684	17.....	.786	.741	.764
9.....	.696	.669	.683	18.....	.865	.515	.690

TABLE IV.—*Alkaloids in the leaves of first-generation belladonna plants from close pollination and cross-pollination of selected parents at two stages of growth at Arlington, Va., during the first season, 1913—Continued.*

Lot and number.	Alkaloidal content (per cent).			Lot and number.	Alkaloidal content (per cent).		
	First stage, Aug. 19.	Second stage, Sept. 23.	Average.		First stage, Aug. 19.	Second stage, Sept. 23.	Average.
Lot 6w, close pollinated—Contd.				Lot 7w, close pollinated—Contd.			
19.....	0.557	0.715	0.636	18.....	0.696	0.715	0.706
20.....		.503		19.....	.478	.669	.574
21.....	.753	.904	.829	20.....	.521		
22.....	.831	.841	.836	21.....			
23.....	.433	.562	.498	22.....	.829	.894	.862
24.....	.771	.693	.732	23.....	.574	.515	.545
25.....	.826	.789	.808	24.....	.717	.940	.829
26.....	.843	.799	.821	25.....	.813	.856	.835
27.....	.807	.657	.732	26.....	.730	.641	.686
28.....	.742	.848	.795	Average.....	.595	.632	
29.....		.565		Lot 7w, cross-pollinated:			
30.....	.794			1.....	.526	.771	.649
31.....		.662		2.....	.448	.468	.458
32.....	.690	.974	.832	3.....	.681	.586	.634
33.....	.679			4.....		.369	
34.....	.640	.748	.694	5.....	.512	.678	.595
35.....	.755	.848	.802	6.....	.813	.885	.849
36.....		.836		7.....	.430	.621	.526
37.....	.674	.526	.600	8.....	.658	.669	.664
Average.....	.672	.701		9.....	.676	.468	.572
Lot 6w, cross-pollinated:				10.....	.646	.646	.646
1.....		.724		11.....	.741	.631	.686
2.....	.609	.555	.582	12.....		.736	
3.....		.663		13.....	.598	.635	.617
4.....		.368		14.....		.621	
5.....	.705	.683	.694	15.....	.646	.799	.723
6.....	.665	.729	.697	16.....	.675	.526	.601
7.....	.557	.729	.643	17.....	.729	.633	.681
8.....	.598	.447	.523	18.....	.669	.609	.639
9.....	.504	.565	.535	19.....	.681	.511	.596
10.....	.688	.729	.709	20.....		.369	
11.....	.759	.543	.651	21.....	.504		
12.....	.574	.574	.574	22.....			
13.....	.594			23.....	.609		
14.....	.693	.550	.622	24.....	.500		
15.....	.391			Average.....	.618	.611	
16.....	.646			Lot 23, cross-pollinated:			
17.....	.478			1.....	.199		
18.....	.486	.505	.496	2.....	.369		
19.....	.450			3.....			
20.....	.621	.526	.574	4.....	.426		
21.....	.535	.726	.681	5.....	.305		
23.....		.613		6.....	.332		
Average.....	.586	.602		7.....	.494		
Lot 7w, close pollinated:				8.....	.538		
1.....	.717	.591	.654	9.....	.455		
2.....	.645	.545	.595	10.....	.393		
3.....	.632	.515	.574	Average.....	.408		
4.....		.394		Lot 46, cross-pollinated:			
5.....	.526	.468	.497	1.....	.383	.406	.395
6.....	.654	.658	.656	2.....	.369	.382	.376
7.....	.442	.492	.467	3.....	.451	.480	.466
8.....	.591	.646	.619	4.....	.314		
9.....		.602		5.....	.528		
10.....	.409	.550	.480	6.....	.480	.741	.611
11.....	.427	.470	.449	7.....	.400	.366	.383
12.....	.510	.586	.548	8.....	.332	.316	.324
13.....	.685	.753	.719	Average.....	.407	.448	
14.....	.650	.712	.681				
15.....	.340	.626	.483				
16.....	.626	.705	.666				
17.....	.492	.633	.563				

TABLE IV.—*Alkaloids in the leaves of first-generation belladonna plants from close pollination and cross-pollination of selected parents at two stages of growth at Arlington, Va., during the first season, 1913—Continued.*

SUMMARY OF AVERAGES FOR THE VARIOUS LOTS.

Lot.	Alkaloidal yield of parent.	Average alkaloidal content (per cent).		
		First stage.	Second stage.	Season.
Lot 6w, close pollinated.....	High.....	0.672	0.701	0.686
Lot 6w, cross-pollinated.....	do.....	.586	.602	.594
Lot 7w, close pollinated.....	do.....	.595	.632	.614
Lot 7w, cross-pollinated.....	do.....	.618	.611	.615
Lot 23, cross-pollinated.....	Low.....	.408		.408
Lot 46, cross-pollinated.....	do.....	.407	.448	.428

The following year, 1914, these plants were again analyzed. The first picking was made on August 1, when the berries were beginning to ripen. This is the stage at which, as shown by other experiments, the greatest percentage of alkaloids is present. The second picking was made on September 5, when the berries had all ripened. Table V shows the percentage of alkaloids in each case, the averages being summarized at the close.

TABLE V.—*Alkaloids in the leaves of first-generation belladonna plants from close pollination and cross-pollination of selected parents at two stages of growth at Arlington, Va., during the second season, 1914.*

Lot and number.	Alkaloidal content (per cent.).			Lot and number.	Alkaloidal content (per cent.).		
	First stage, Aug. 1.	Second stage, Sept. 5.	Average.		First stage, Aug. 1.	Second stage, Sept. 5.	Average.
Lot 6w, close pollinated:				Lot 6w, close pollinated—Contd.			
1.....	0.864	0.882	0.873	31.....	1.030	0.783	0.906
2.....	.770	.611	.690	32.....	.902	.792	.847
3.....	.746	.581	.663	33.....	1.031		
4.....	.858	.858	.858	34.....	1.096	.908	1.002
5.....	.771	.829	.800	35.....	1.043	.765	.904
6.....	.761	.956	.858	36.....	.860	.657	.908
7.....		.924		37.....	.744	.454	.549
8.....	.924	.854	.889	Average.....	.906	.765	
9.....	.916	.864	.890				
10.....	.859	.618	.738	Lot 6w, cross-pollinated:			
11.....				1.....		.753	
12.....	1.045	.953	.999	2.....	1.169	.687	.923
13.....		.831		3.....	.969	.717	.843
14.....				4.....			
15.....	.646	.693	.669	5.....	1.074	.756	.915
16.....				6.....	.990	.575	.782
17.....	.739	.795	.767	7.....	.987	.530	.758
18.....	.943	.612	.627	8.....			
19.....	.930	.708	.819	9.....	.790	.565	.677
20.....	.681	.882	.781	10.....	.804	.663	.733
21.....	.935	.813	.874	11.....	1.062	.647	.854
22.....	1.016	.747	.881	12.....	.771	.712	.741
23.....		.777		13.....	.943	.626	.784
24.....	1.193	.753	.973	14.....	.903	.572	.737
25.....	.969	.837	.905	15.....	.810	.657	.733
26.....	.990	.833	.911	16.....			
27.....	1.103	.699	.856	17.....	.929		
28.....	.976	.691	.833	18.....	.728	.621	.674
29.....	.797	.599	.698				
30.....	.941	.742	.841				

TABLE V.—*Alkaloids in the leaves of first-generation belladonna plants from close pollination and cross-pollination of selected parents at two stages of growth at Arlington, Va., during the second season, 1914—Continued.*

Lot and number.	Alkaloidal content (per cent).			Lot and number.	Alkaloidal content (per cent).		
	First stage, Aug. 1.	Second stage, Sept. 5.	Average.		First stage, Aug. 1.	Second stage, Sept. 5.	Average.
Lot 6w, cross-pollinated—Contd.				Lot 7w, cross-pollinated—Contd.			
19.....	0.997	0.520	0.758	8.....	0.816		
20.....		.559		9.....	1.062	0.596	0.579
21.....	1.124	.644	.884	10.....	.690		
22.....	1.138	.608	.873	11.....	.868		
Average.....	.952	.634		12.....	.838		
Lot 7w, close pollinated:				13.....	1.062	.574	.818
1.....	.948	.771	.859	14.....	.990	.672	.831
2.....	.626	.672	.649	15.....	.758	.425	.591
3.....	.898	.693	.795	16.....	.885	.641	.763
4.....		.777		17.....	.704	.535	.619
5.....	.704	1.098	.906	18.....	.983		.983
6.....	.953	.777	.865	19.....	.739	.394	.566
7.....	.624	.594	.609	20.....	.758	.897	.827
8.....	.851	.834	.842	21.....	1.015	.732	.873
9.....	.756	.926	.841	22.....	.784	.813	.798
10.....	.927	.822	.874	23.....	.872	.620	.746
11.....		.873		24.....	.877	.798	.838
12.....	.950	.657	.803	25.....	1.036	.823	.929
13.....	.744	.762	.753	26.....	1.036	.823	.929
14.....	1.193	.837	1.015	27.....	.631	.345	.488
15.....	.997	.599	.798	Average.....	.865	.619	
16.....	.882	.809	.845	Lot 3:			
17.....	.997	.632	.814	1.....	.452	.319	
18.....	.694	.707	.650	2.....	.452	.528	
19.....	.964			3.....	.306	.681	
20.....	.709			4.....	.425	.365	
21.....	1.010			Average.....	.409	.473	
22.....	.797			Lot 23:			
23.....	.810	.687	.748	1.....		.344	
24.....	.790	.651	.665	2.....		.472	
Average.....	.855	.753		3.....		.517	
Lot 7w, cross-pollinated:				4.....		.532	
1.....	.992	.672	.832	5.....		.448	
2.....	1.062			6.....		.581	
3.....	.864	.596	.730	7.....		.505	
4.....	.810	.523	.666	8.....		.588	
5.....	.911	.553	.732	9.....		.502	
6.....	1.036	.696	.866	10.....		.487	
7.....	.568	.463	.515	Average.....		.497	

SUMMARY OF AVERAGES FOR THE VARIOUS LOTS.

Lot.	Alkaloidal yield of parent.	Average alkaloidal content (per cent).		
		First stage.	Second stage.	Season.
Lot 6w, close pollinated.....	High.....	0.906	0.765	0.835
Lot 6w, cross-pollinated.....	do.....	.952	.634	.793
Lot 7w, close pollinated.....	do.....	.855	.753	.804
Lot 7w, cross-pollinated.....	do.....	.865	.619	.742
Lot 3, cross-pollinated.....	Low.....	.409	.473	.441
Lot 23, cross-pollinated.....	do.....		.497	.497

From Tables IV and V it becomes apparent that during both the first and second seasons these first-generation plants display very markedly the characteristic of the parents as regards alkaloid production. Thus, in 1913 the average percentage of alkaloids found in the plants from the original parent 6w and the parent 7w, which were selected for high alkaloid-producing tendencies, was 0.640 and

FIG. 5.—Diagram showing the alkaloidal content of the leaves of first-generation belladonna plants from close-pollinated and cross-pollinated selected parents at two stages of growth during the first season, 1913. The percentages indicated represent the average of all the individuals from each parent plant: a, Plants from close-pollinated parents; b, plants from cross-pollinated parents.

0.615 per cent, respectively. On the other hand, the averages from the plants 23 and 46, which were produced from parents of the opposite tendencies, are 0.408 and 0.428 per cent, respectively. Again the following year we find the average of plants 6w and 7w to be 0.814 and 0.773 per cent, respectively. Lot 46 was not picked that year, but lot 23 averaged 0.497 per cent.

It will be seen from the tables that the effect of close pollination as compared with cross-pollination is not as great as would be expected.

FIG. 6.—Diagram showing the average alkaloidal content of all the individual belladonna plants from each parent for the first season, 1913: a, Plants from close-pollinated parents; b, plants from cross-pollinated parents.

In the case of 6w the plants from close pollination are on the average 0.091 per cent richer than those from cross-pollination in 1913, and 0.042 per cent richer in 1914. The 7w plants show a difference of 0.001 per cent in favor of cross-pollination in 1913 and a difference of 0.062 per cent in 1914. Figures 5 to 8 graphically illustrate these conditions. Sufficient information has not been obtained to indicate with any degree of certainty the influence of cross-pollination and close pollination on the transmissibility of the alkaloid-producing characteristic. As a possible explanation of the large percentage of plants with high alkaloidal

content among the first-generation plants secured from cross-pollinated selected parents, Dr. W. Van Fleet, of the Office of Drug-Plant and Poisonous-Plant Investigations, has prepared the following statement:

The arrangement of anthers and stigma in the belladonna bloom and the respective periods of their maturity and receptivity are such as to fit the plant in a high degree

for cross-pollination and to almost exclude the probability of close fertilization in individual flowers. However, the feeding habits of the principal pollinating insects (mostly night and day flying lepidoptera), which visit in turn many open blooms on the same plant, may tend after all to promote self or close pollination on a considerable proportion of blooms on any given plant. If all the seeds in a single fruit should mature and produce plants with a high alkaloidal content or any other transmissible

FIG. 7.—Diagram showing the alkaloidal content of the leaves of first-generation belladonna plants from close-pollinated and cross-pollinated selected parents at two stages of growth during the second season, 1914. The percentages indicated represent the average of all the individuals from each parent plant: a, Plants from close-pollinated parents; b, plants from cross-pollinated parents.

feature of the seed parent, it does not necessarily follow that the pollen of another individual possessing this feature in a lesser degree might not have a reducing effect on the desired characteristic, as the seeds planted may easily be the result of actual close pollination between different blooms of the maternal plant or even of self-fertilization of an individual bloom. It is but fair to suppose that a majority of the seeds of a given plant may thus be self-pollinated, notwithstanding nature's adaptation of the blooms for crossing, unless it has been made apparent that belladonna blooms are not receptive to their own pollen or to that of flowers on the same plant, but must be fertilized by pollen produced by another individual. This conclusion has not been demonstrated by any controlled experiments that have come to my knowledge.

SECOND-GENERATION PLANTS FROM CROSS-POLLINATION.

Up to this point the investigation has dealt entirely with first-generation plants. During the season of 1914 analyses were made of plants which represented the second generation of the original selected individuals. The seed which furnished these plants was secured from the following first-generation plants in the fall of 1913: 6W₇, 6W₁₀, 6W₁₁, 7W₅, 2₂, 2₃, 3₁, 3₆, 3₇, 3₄, 3₄, 3₄, 3₅. The plants from 6w and 7w were selected as types of high alkaloid-yielding plants, those from 2 as medium, and those from 3 and 34 as low alkaloid-yielding types. The seed was planted in the greenhouse at Arlington, Va., in January, 1914, and the plants were transferred to small pots when of suitable size.

FIG. 8.—Diagram showing the average alkaloidal content of all the individual belladonna plants from each parent for the second season, 1914: a, Plants from close-pollinated parents; b, plants from cross-pollinated parents.

When the plants were about 3 inches high they were divided into three lots. One lot was transplanted to the gardens at Arlington, Va., another lot was shipped to Timmons ville, S. C., and a third lot to Madison, Wis. The object was to grow at these widely separated locations under different soil and climatic conditions plants which were obtained from a definite source. Data would thus be secured which would serve to indicate whether the transmissibility of the alkaloid-producing quality would be affected by external influences. Furthermore, it would throw some light on the question of alkaloid production as dependent on sunlight, rainfall, and soil conditions, which is a question still unsettled. At Arlington and at Madison two pickings were made of all the individuals, while at Timmons ville only one collective sample was secured from the plants from each individual parent. In Table VI the percentage of alkaloids in the individual plants at Arlington and Madison is presented, the summary of averages and the results from Timmons ville being added at the close.

TABLE VI.—*Alkaloids in the leaves of second-generation belladonna plants from cross-pollinated parents at two stages of growth at Arlington, Va., and Madison, Wis., in 1914.*

Lot and number.	Arlington, Va.			Madison, Wis.		
	First stage, Aug. 18.	Second stage, Sept. 12.	Average.	First stage, Aug. 4.	Second stage, Sept. 5.	Average.
Lot 6w ₇ :						
1.....	0.783	0.461	0.622	0.962	0.956	0.959
2.....	.542	.570	.556	.707	.813	.760
3.....		.488	.488		.781	.781
4.....	.791	.666	.728	.653	.653	.653
5.....	.414	.558	.486	.791	.530	.710
6.....		.851	.851	.765	.707	.736
7.....	.574	.620	.597	.580	.791	.685
8.....	.716	.646	.681	.599	.861	.730
9.....	.837	.835	.836	.794	.583	.688
10.....	.554	.653	.603	.478	.461	.470
11.....	.708	.522	.615	1.020	.727	.873
12.....	.674	.340	.557	1.322	1.027	1.174
13.....	.695	.605	.650	.867	.813	.840
14.....	.588	.669	.628	.956	.908	.932
15.....		.525	.525	.538	.949	.793
Lot 6w ₁₀ :						
1.....	.669	.541	.605	.736	.557	.646
2.....	.755	.691	.723	.653		.653
3.....	.639	.516	.577	.685	.829	.757
4.....				.806	.759	.783
5.....	.641	.573	.607	.822	1.068	.945
6.....	.550	.541	.546	.762	.876	.819
7.....	.748		.748	.740	.813	.776
8.....	.557		.557	.803	.733	.768
9.....				1.062	.908	.985
10.....	.327	.398	.362	.695	.838	.766
11.....				.905	1.115	1.010
12.....				.752	.813	.783
13.....				.775	.943	.859
14.....				.554	.605	.579
15.....				.806	.752	.779
Lot 6w ₁₁ :						
1.....				.554	.488	.521
2.....	.557		.557	.573		.573
3.....	.630	.636	.633	.580	.759	.669
4.....	.597	.504	.547	.781	.749	.765
5.....				.630		.630

TABLE VI.—*Alkaloids in the leaves of second-generation belladonna plants from cross-pollinated parents at two stages of growth at Arlington, Va., and Madison, Wis., in 1914—Continued.*

Lot and number.	Arlington, Va.			Madison, Wis.		
	First stage, Aug. 18.	Second stage, Sept. 12.	Average.	First stage, Aug. 4.	Second stage, Sept. 5.	Average.
Lot 6W ₁₁ —Continued.						
6.....	0.800	0.583	0.692	0.959	0.988	0.973
7.....	.840	.513	.676	.914	.908	.911
8.....		.701	.701			
9.....						
10.....		.408	.408			
11.....	.359	.541	.450			
12.....						
13.....		.392	.392			
14.....	.574	.775	.679			
15.....						
16.....		.488	.488			
17.....	.587	.433	.510			
18.....	.526	.318	.422			
Lot 7W ₃ :						
1.....	.653	.653	.653	.521	.560	.551
2.....	.659	.557	.608	.513	.484	.498
3.....	.543		.543			
4.....	.554	.541	.548			
Lot 2 ₂ :						
1.....	.545		.545	.580	.775	.677
2.....	.365	.408	.386	1.170	1.221	1.196
3.....	.369	.494	.431	.864	.723	.794
4.....	.310	.478	.394	.765	.617	.691
5.....		.295	.295		.605	.605
6.....	.557	.513	.535	.642	1.099	.870
7.....		.324	.324			
8.....	.426	.337	.382			
Lot 2 ₃ :						
1.....				.461	.669	.568
2.....				.478	1.086	.782
3.....	.573	.547	.560	.573	1.250	.911
4.....				.620	.535	.577
5.....	.423	.372	.397	.870	1.309	.589
6.....		.382	.382	.625	.972	.799
7.....	.489	.646	.567	.478	.924	.701
8.....				.532	.679	.605
9.....	.536	.532	.534	.478	.781	.629
10.....	.462	.455	.459	.491	.636	.564
11.....	.418	.538	.478	.455	.691	.573
12.....		.382	.382	.510	.924	.717
13.....	.511	.420	.465	.494	.599	.547
14.....	.519	.442	.482		.892	.892
15.....	.420	.567	.494	.630	.723	.676
16.....	.456	.525	.492	.324	.366	.345
17.....	.510	.318	.414			
Lot 3 ₁ :						
1.....				.474	.617	.546
2.....	.532		.532	.318	.669	.493
3.....				.312	.423	.367
4.....	.522	.510	.516	.350	.510	.430
5.....				.504	.803	.654
6.....	.197		.197	.541	.852	.697
7.....	.433	.263	.348	.445	.510	.417
8.....						
Lot 3 ₂ :						
1.....		.292	.292	.350	.424	.387
2.....				.484	.502	.493
3.....					.682	.682
Lot 3 ₃ :						
1.....	.457	.350	.403	.488		.488
2.....	.502	.324	.413	.717	.978	.847
3.....	.412	.334	.373	.494	.539	.516
4.....	.420	.414	.417	.573	.630	.601
5.....				.653		.653
6.....	.444	.408	.426	.717	.813	.765
7.....	.448	.636	.542	.488	.672	.580
8.....	.463	.455	.459		.701	.701
9.....	.452	.550	.501			
10.....	.421	.318	.369			

TABLE VI.—*Alkaloids in the leaves of second-generation belladonna plants from cross-pollinated parents at two stages of growth at Arlington, Va., and Madison, Wis., in 1914—Continued.*

Lot and number.	Arlington, Va.			Madison, Wis.		
	First stage, Aug. 18.	Second stage, Sept. 12.	Average.	First stage, Aug. 4.	Second stage, Sept. 5.	Average.
Lot 34 ₂ :						
1.....	0.621		0.621	0.940	0.707	0.823
2.....				.729	.646	.687
3.....	.630		.630	.822		.822
4.....	.646		.646	.478	.439	.458
5.....				.797	.605	.701
6.....				.663	.445	.554
7.....	.440	0.350	.395	.605	.510	.558
8.....	.562	.376	.479	.756	.845	.800
9.....	.369	.366	.368	.636	.669	.652
10.....	.430	.254	.342	.717	.749	.733
11.....	.459	.382	.421	.710	.886	.798
12.....	.507	.343	.425	.797	.541	.669
13.....	.483	.461	.472	.714	.663	.689
14.....	.521		.521	.599	.797	.698
15.....	.720	.626	.673	.685	.876	.782
16.....	.550	.461	.505	.302	.701	.501
17.....	.618	.324	.471		.491	.491
18.....	.511	.382	.447			
Lot 34 ₃ :						
1.....		.546	.546	.596	.832	.714
2.....	.659	.589	.624	.532	.765	.648
3.....	.650	.501	.576	.573	.791	.682
4.....	.626	.580	.603		.876	.876
5.....				.559	.803	.681
6.....				.593	.861	.727
Lot 34 ₄ :						
1.....	.482	.356	.419	.414	.681	.547
2.....	.641		.641	.350	.752	.556
3.....	.503	.504	.504	.350	.532	.441
4.....	.450	.142	.296	.398	.508	.453
5.....	.489	.458	.474	.461		.461
6.....	.515	.593	.554	.471	.478	.475
7.....					.699	.699
8.....	.517	.330	.424	.346	.792	.569
9.....				.359	.732	.596
10.....	.512	.494	.503	.455	.756	.605
11.....	.458	.438	.458			
12.....						
13.....	.398		.398			
Lot 34 ₅ :						
1.....		.461	.461	.398	.414	.406
2.....				.445	.541	.493
3.....	.558	.445	.501	.484	.605	.545
4.....		.458	.458	.414	.541	.478
5.....		.343	.343		.478	.478
6.....		.478	.478	.541	.911	.726
7.....	.421	.445	.433	.392	.956	.674
8.....		.318	.318	.583	.573	.578
9.....					.350	.350
10.....				.414	.468	.441
11.....					.318	.318
12.....						
13.....						
14.....	.674	.532	.603			
15.....	.263	.308	.286			

TABLE VI.—*Alkaloids in the leaves of second-generation belladonna plants from cross-pollinated parents at two stages of growth at Arlington, Va., and Madison, Wis., in 1914—Continued.*

SUMMARY OF AVERAGES FOR EACH LOT, INCLUDING RESULTS AT TIMMONSVILLE, S. C.

Lot.	Alkaloidal content (per cent).							
	Arlington, Va.			Madison, Wis.			Timmons ville, S. C.	
	First stage, Aug. 18.	Second stage, Sept. 12.	Average.	First stage, Aug. 4.	Second stage, Sept. 5.	Average.	First stage.	Average.
Lot 6w ₇	0.656	0.600		0.783	0.777		0.992	
Lot 6w ₁₀	.611	.543		.800	.829		.906	
Lot 6w ₁₁	.608	.524		.729	.745		.849	
Average.....	.625	.556	0.592	.771	.784	0.778	.916	0.916
Lot 7w ₃	.602	.584	.593	.524	.532	.528	.950	.950
Lot 2 ₂	.429	.407		.822	.840			
Lot 2 ₃	.483	.471		.674	.814		.771	
Average.....	.456	.436	.446	.748	.827	.788	.771	.771
Lot 34 ₂	.538	.384		.673	.661		.801	
Lot 34 ₃	.645	.554		.696	.821		.852	
Lot 34 ₄	.496	.414		.530	.660		.663	
Lot 34 ₅	.479	.421		.514	.569		.705	
Average.....	.539	.443	.491	.603	.678	.642	.755	.755
Lot 3 ₁	.496	.386		.524	.627		.702	
Lot 3 ₂				.476	.536		.532	
Lot 3 ₇	.446	.421		.602	.722		.669	
Average.....	.471	.402	.437	.534	.628	.581	.634	.634

A study of Table VI will reveal the fact that the plants showed marked differences in the percentage of alkaloids produced at the different stations. Thus, the general season average of all the plants at Arlington was 0.512 per cent, at Madison 0.663 per cent, and at Timmons ville 0.804 per cent. At the last-mentioned station, however, only one picking was made, which may account in some measure for the unusually high percentage of alkaloids found. At Arlington the first picking assayed considerably higher than the second, while at Madison the second picking was richer. It should be remembered, however, in this connection, that the season at Madison is somewhat later than at Arlington. Previous experiments have shown that the plants are richest in alkaloids when the flowering period is over and the berries are beginning to ripen. At this stage the leaves are small but very rich in alkaloids. At Arlington the first picking was made August 18, when the plants were in the stage just described, and the second picking was made later in the season, when the new growth had reduced the percentage of alkaloids considerably. At Madison, on the other hand, the first picking was made August 4, before the

rich stage was reached, and consequently the second picking, September 5, corresponds very closely to the first picking at Arlington

FIG. 9.—Diagram showing the alkaloidal content of the leaves of second-generation belladonna plants from cross-pollinated parents at two stages of growth at Arlington, Va., and Madison, Wis., and at one stage of growth at Timmons ville, S. C., during the first season, 1914.

in so far as the stage of growth is concerned. It is evident from the results obtained that at all three stations these second-generation

FIG. 10.—Diagram showing the average alkaloidal content of all the individual second-generation belladonna plants from each parent at Arlington, Va., Madison, Wis., and Timmons ville, S. C., during the first season, 1914.

plants show the characteristics of the parents as regards their alkaloidal content. Naturally, there are individual variations, and the plants at one station may be uniformly lower in alkaloidal content than at another station, but the point to be observed here is that at all the stations it is true in a general way that the plants selected originally from parents of high alkaloid-yielding tendencies show generally a greater percentage of alkaloids than those from parents of the opposite tendency. Table VI and figures 9 and 10 show this very plainly. It must be pointed out, however, that only a few plants were included under lot 7W₅, and this must be borne in mind when studying the averages.

In order to gather some information regarding the influence of climatic conditions upon alkaloid production, a tabulation was made of the principal weather characteristics at or near the three stations for the four months, May to August, inclusive, of the year 1914. This compilation is shown in Table VII.

TABLE VII.—*Temperature, precipitation, and days of sunshine at Arlington, Va., Madison, Wis., and Timmons ville, S. C., during May, June, July, and August, 1914.*

Station and month.	Temperature (° F.).			Precipitation.			Aspect of sky (days).		
	Mean maximum.	Mean minimum.	Mean.	Total.	Departure from normal.	Days with 0.01 or more.	Clear.	Partly cloudy.	Cloudy.
Washington, D. C.: ¹				<i>Inches.</i>	<i>Inches.</i>				
May.....	78	55	67	1.72	-2.1	7	14	11	6
June.....	84	64	73.8	6.20	2	11	12	12	6
July.....	85	67	75.9	2.32	-2.3	12	7	12	12
August.....	86	66	76.4	6.00	1.6	10	10	13	8
Total.....				16.24			43	48	32
Florence, S. C.: ²									
May.....	87.2	57.4	72.3	1.70		2	14	13	4
June.....	92.1	68.9	80.5	3.88		7	12	12	6
July.....	92.8	68.5	80.6	3.31		6	12	16	3
August.....	89.9	69.3	79.6	4.19		10	8	15	8
Total.....				13.08			46	56	21
Madison, Wis.:									
May.....	69.7	50.9	60.3	5.97	2.35	13	9	14	8
June.....	75.7	57.6	66.6	3.46	-0.64	14	8	12	10
July.....	83	64.6	73.8	1.49	-2.50	8	13	13	5
August.....	79.6	61	62.5	3.60	.39	7	11	12	8
Total.....				14.52			41	51	31

¹ The conditions at Washington should approximate very closely those at Arlington, Va.² About 7 miles from Timmons ville, S. C.; volunteer observer.

It has been claimed by some investigators that dry and sunny seasons will result in greater alkaloid production than wet and cloudy seasons. The data in Table VII will serve to throw some light on this point. The plants from the three localities specified were all grown from the same seed, and it would appear that if the contention regarding climate were correct there should be some correlation between the quantity of alkaloids found and the seasonal conditions. However, in the four months there is a difference of only about 3 inches in the rainfall and a difference of only five in the number of sunny days. It is true that at Timmons ville the precipitation was the least and the number of sunny days the greatest, and the percentage of alkaloids was also the greatest. It has already been pointed out, however, that only one picking of leaves was secured at this place, which may account for the high average percentage of alkaloids found; and, furthermore, the difference in climatic conditions is so small that little significance can be attached to it. It is possible, of course, that differences in soil conditions at the three stations may be sufficiently great to influence any relationship due to climatic conditions which might otherwise be apparent.

REPRODUCTION OF SELECTED PLANTS FROM CUTTINGS.

In the fall of 1913 cuttings were secured from the first-generation plants and rooted in the greenhouse. Although the season had been bad and the condition of the cuttings was rather poor, a good percentage of them rooted and by spring furnished plants 3 to 4 inches high. These were transplanted to the field early in May. The severe drought during that month caused a considerable number of the plants to die, although they had developed a good root system. On August 18, when the flowering was mostly over, the first picking was made. On October 12, after most of the berries were ripe and some new leaves had developed, a second picking was secured. In Table VIII are given the percentages of alkaloids found in each individual plant, the summary of seasonal averages being added at the close.

TABLE VIII.—*Alkaloids, at two different stages, in the leaves of belladonna plants grown from cuttings from selected parents at Arlington, Va.*

Lot and number.	Alkaloidal content (per cent).			Lot and number.	Alkaloidal content (per cent).		
	First stage, Aug. 18.	Second stage, Oct. 12.	Average for season.		First stage, Aug. 18.	Second stage, Oct. 12.	Average for season.
Lot 6w ₁ :				Lot 6w ₁₁ —Contd.			
5.....	0.573	0.491	0.532	9.....	0.602	0.613	0.608
Lot 6w ₂ :				11.....	.871	.669	.770
2.....	.512	.525	.517	12.....	.801	.829	.815
Lot 6w ₃ :				13.....	.680	.420	.550
1.....		.525	.525	15.....		.630	.630
2.....	.725	.593	.659	16.....	.510	.238	.374
3.....		.496	.496	17.....		.537	.537
4.....	.606	.620	.613	19.....	.665		
5.....	.604	.488	.546	Average.....	.687	.611	.649
6.....	.837	.749	.493				
Average.....	.693	.578	.634	Lot 6w ₁₂ :			
Lot 6w ₄ :				2.....	.624	.610	.617
1.....	.573	.522	.548	3.....	.731	.398	.565
2.....	.615	.580	.597	4.....		.445	.445
3.....	.585	.343	.464	Average.....	.677	.484	.581
4.....	.607	.478	.543				
5.....		.589	.589	Lot 7w ₃ :			
5.....		.315	.315	2.....	.477	.429	.453
7.....	.573	.442	.508	5.....		.794	.794
8.....	.516	.372	.444	Average.....	.477	.611	.544
Average.....	.575	.455	.565				
Lot 6w ₅ :				Lot 7w ₄ :			
1.....	.795	.797	.796	1.....	.587	.567	.577
2.....	.745	.749	.747	2.....		.557	.557
Average.....	.770	.773	.772	3.....	.562	.525	.544
Lot 6w ₉ :				5.....	.631	.666	.648
6.....	.770	.516	.643	6.....			
Lot 6w ₁₀ :				7.....	.436	.669	.553
1.....	.623	.618	.621	Average.....	.553	.597	.575
3.....	.660	.691	.676				
Average.....	.641	.654	.648	Lot 7w ₅ :			
Lot 6w ₁₁ :				1.....		.569	.569
1.....	.774	.653	.714	2.....		.636	.636
2.....	.602		.602	4.....	.584	.784	.684
5.....	.598		.598	5.....	.837	.759	.798
6.....	.682	.752	.717	6.....	.734	.620	.677
8.....	.661	.775	.718	7.....	.639	.700	.669
				8.....	.657	.578	.668
				Average.....	.690	.664	.677

TABLE VIII.—*Alkaloids, at two different stages, in the leaves of belladonna plants grown from cuttings from selected parents at Arlington, Va.—Continued.*

Lot and number.	Alkaloidal content (per cent).			Lot and number.	Alkaloidal content (per cent).		
	First stage, Aug. 18.	Second stage, Oct. 12.	Average for season.		First stage, Aug. 18.	Second stage, Oct. 12.	Average for season.
Lot 7w ₁₀ :				Lot 34 ₃ :			
3.....	.516	.688	.602	1.....	0.623		0.623
6.....		.864	.864	2.....	.539	0.596	.567
Average.....	.516	.776	.646	Average.....	.581	.596	.588
Lot 34 ₂ :				Lot 34 ₃ :			
1.....		.636	.636	1.....	.514	.439	.476
3.....	.499	.391	.445	2.....	.458	.440	.449
4.....	.598	.525	.562	Average.....	.486	.440	.463
5.....	.558	.408	.483				
6.....	.601	.590	.596				
Average.....	.564	.510	.537				

SUMMARY OF SEASONAL AND GROUP AVERAGES.

Lot.	Alkaloid yield of parent.	Average alkaloidal content (per cent).	
		For season.	For all plants from same parent.
Lot 6w ₁	High.....	0.532	0.616
Lot 6w ₂	do.....	.517	
Lot 6w ₃	do.....	.634	
Lot 6w ₄	do.....	.565	
Lot 6w ₈	do.....	.772	
Lot 6w ₉	do.....	.643	
Lot 6w ₁₀	do.....	.648	
Lot 6w ₁₁	do.....	.649	
Lot 6w ₁₂	do.....	.581	
Lot 7w ₃	do.....	.544	0.610
Lot 7w ₄	do.....	.575	
Lot 7w ₅	do.....	.677	
Lot 7w ₁₀	do.....	.646	
Lot 34 ₂	Low.....	.537	0.529
Lot 34 ₃	do.....	.588	
Lot 34 ₃	do.....	.463	

Here, again, the plants from lots 6w and 7w show marked superiority over those of lot 34, the parent plant of which was poor in alkaloids. These plants will be observed and studied carefully through another season, to determine whether the same relationship holds true, after which desired individuals will be selected and further propagated through cuttings.

CONCLUSIONS.

It having been established in the previous investigation that a wide range of variation exists in the alkaloidal content of belladonna plants, the present investigation was undertaken to determine whether the characteristic of alkaloid production is transmissible to

the progeny through seed and whether the character is changed by vegetative propagation. The results thus far show that the first-generation plants secured from seed of cross-pollinated selected individuals display the characteristic of the maternal parent with regard to alkaloid productivity. This condition is generally true at all stages of growth during a season and also for at least two successive seasons. Close pollination of the parent plant has shown only a moderate influence on the transmission of this characteristic.

Second-generation plants from cross-pollination have been grown at Arlington, Va., Madison, Wis., and Timmons ville, S. C., and at all three stations they have displayed the relative alkaloid-producing tendencies evident in the original parent plant and the generation preceding.

While the plants at the different localities showed a parallel relationship toward each other, there was considerable difference in the general quantity of alkaloids produced. Thus, in the case of Madison and Arlington, where two pickings were made at fairly corresponding stages of growth, it was found that the Madison plants yielded more alkaloids than those at Arlington. At Timmons ville the yield was still greater than at Madison, but here only one picking was made, and it is hardly possible to make a true comparison. Nothing definite developed to indicate that a relationship exists between the amount of precipitation and sunshine and the percentage of alkaloids produced.

Plants were grown from cuttings, and at two stages of their growth these plants showed a marked tendency to display the same characteristic regarding alkaloid production as the plants from which they were propagated and the original parents of those plants.

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