The remainder of sample B-1128, dried at 80° C. for five hours, which originally tested 0.0065, was divided into two portions, B-1131 and B-1132, and stored in a manner similar to that described above.

EXPERIMENT IV.

Digitalis Gloxiniaeflora, Single Plant.

No.	Manner of Storage.	Original Assay.	Assay 12 Months Later.	Moisture Content.
B-1131	Open Bottle	0.0065	0.0055	6.04
B-1132	Sealed Bottle	0.0065	0.0060	2.83

A third sample was secured by collecting leaves from several different plants of Digitalis gloxiniæflora. These leaves were dried at 30° to 55° C. for twenty hours; reduced to a No. 60 powder and gave a value of 0.006. Two lots were stored as in the preceding experiments.

EXPERIMENT V.

Digitalis Gloxiniaeflora, Several Plants.

No.	Manner of Storage.	Original Assay.	Assay 12 Months Later.	Moisture Content.
B-1134	Open Bottle	0.006	0.006	5.92
B-1133	Sealed Bottle	0.006	0.006	4.76

From these experiments, it is safe to conclude that in one year no deterioration occurred in our samples of leaf, irrespective of the manner of storage, even when the moisture content was as great as 6.04 percent.

In some instances, a higher value is secured on the second assay than was obtained on the fresh leaf. This is to be explained by the limits of experimental error inherent in the assay method, as in all except one case, this difference does not amount to 10 percent.

DIGITALIS LEAF.

1. Focke: Arch. d. Pharm., 1903, Vol. 241, p. 128.
2. Hale: Bull. No. 74, Hyg. Lab., U. S. P. H. and M. H. S.
3. Hart: Pharm. Jour., Vol. 26, p. 440.
4. Hatcher and Eggleston: Druggists' Circular, Vol. LVII, 1913, No. 6, p. 325.
5. Pratt: Bos. Med. and Surg. Jour., Vol. 163, p. 279, 1910.

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INFLUENCE OF SOIL COMPOSITION ON MEDICINAL PLANTS.

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Observations upon the influence of soil composition on medicinal plants have been made by several investigators. These observations have indicated that certain variations may occur in the percentage of active principles when the growing plants are subject to different soil conditions. Agriculturists have long recognized the influence of soil composition upon crop production. Their methods of measuring this influence, however, have differed from those employed with medicinal plants. Results have usually been determined by amount of total crop produced and not by chemical and biological assay. Recently some work has been done upon the chemical determination of certain constituents such as oil,

sugar, and protein, as a means of following this influence. A combination of these methods would be essential to the best results with medicinal plants.

Hooper (1) demonstrated this fact by his work upon Jalap. He found that by using superphosphate at the rate of 1600 pounds per acre, he could bring about an increase in the dry weight of tubers produced as well as an increase in the percentage of resin. While the increase in resin by this treatment was only 24 percent the increase in dry weight was 86 percent. Other investigators have given more attention to percentage of active principles than to crop production.

Carr (2) contends that although the addition of manure to a soil already suited to the growth of belladonna is no disadvantage and may be slightly beneficial, yet where large quantities of nitrogenous fertilizers are employed somewhat lower percentages of alkaloid are observed. This condition he attributes to the larger growth which results from such manuring. The author does not summarize his results with farmyard manure, nitrate, calcium cyanamide, basic slag, superphosphate, and potash. He concludes, however, by saying that in whatever latitude belladonna is grown, it will doubtless be found that the composition of the soil, the use of fertilizers, and seasonal conditions make for small variations. During the four years that he continued his experiment the results were not taken uniformly according to the fertilizers used.

Ransom and Henderson (3) have studied the effect of kainit, superphosphate, sodium nitrate, potato mixture, basic slag, and combinations of these upon yield of green weight and percentage of total alkaloids in belladonna. In their conclusions they state that although they have not obtained sufficient evidence that the percentage of alkaloid in the dried leaf is materially altered by artificial manures, it would appear that in several cases the yield of green plant per acre has been largely increased. They further state that it would also appear that in the case of belladonna it is useless to hope that the drug shall possess anything approaching uniformity in medicinal potency, even when carefully collected and dried. It would seem that we are entirely too young in this work to make predictions of what the future may or may not disclose. The possibilities of obtaining uniformity in cultivated belladonna have hardly been touched upon and will not be known until the numerous problems of propagation and breeding have been thoroughly investigated. No efforts worthy of mention have as yet been made to produce a strain or variety of belladonna which will exhibit a uniform yield of alkaloids.

Chevalier (4) records data indicating an increase of nearly 60 percent of total alkaloids in belladonna when treated with nitrogenous fertilizer in combination with manure. Acid phosphate and potash produced no increase.

Tschirch (5) makes the broad, general statement that an increase in the amount of valuable constituents has been affected in the case of almost all cultivated plants.

To gain more information upon the influence of soil composition upon medicinal plants, experiments have been performed, using digitalis, stramonium, and belladonna. These experiments have been performed both in the open field and in the greenhouse with various commercial fertilizers and soils of different mechanical mixture. Precautions have been taken to secure uniform conditions in selecting locations of plots and in the collection and curing of samples.

Digitalis. The plants for this experiment were grown from seed obtained in Oregon from wild plants. They were germinated December 8th, 1911, in the greenhouse, transplanted to flats and retained in the greenhouse until March 19th, 1912, when they were transferred to cold frames of double glass construction. Strong, hardy plants were thus obtained, eight hundred and seventy-five of which were used in the experiment. These were transplanted to the open field May 10th, 1912. Clean cultivation was practised throughout the summer. The plot was located in an apple orchard between two rows of trees upon a soil which had been cropped to potatoes for several consecutive years without fertilization. The plot was divided into six equal parts and the fertilizers applied at the rate of six hundred pounds per acre. The nitrate of soda was divided into three equal portions and applied at intervals of two weeks. The fertilizers were not applied until the plants were well established. They were then distributed by hand as uniformly as possible about the plants of each plot and immediately worked into the soil by cultivation. Mixed samples were collected for assay in early September, 1912, and cured at 100° centigrade. The following table gives the fertilizers used and the comparative results as obtained by the one hour frog heart method for assaying digitalis leaf.

Plot No.	Sample No.	Fertilizer Used.	Assay.
1	B-1232	Nitrate of Soda	0.0065
2	B-1233	Sulphate of Potash	0.0062
3	B-1234	Normal Fertilizer	0.0060
4	B-1235	Acid Phosphate	0.0062
5	B-1236	Nitrate of soda, Sulphate of potash (equal parts)	0.0060
6	B-1237	Control	0.0062

Little or no effect can be attributed to the use of these commercial fertilizers upon digitalis. This is evident from the fact that the difference in the comparative value of the samples from the different plots as indicated by the above figures is less than the possible experimental error.

Stramonium. The effects of a normal fertilizer upon the percentage of total alkaloids in stramonium when applied at the rate of 600 pounds per acre have been previously noted (6). The average results obtained are repeated below.

DATURA STRAMONIUM.

No Fertilizer.	Fertilizer.	No Fertilizer.	Fertilizer.
0.50%	0.61%	0.60%	0.64%

DATURA TATULA.

No Fertilizer.	Fertilizer.	No Fertilizer.	Fertilizer.
0.49	0.54	0.62	0.68

Belladonna. It is not only desirable to know the effects of commercial fertilizers upon medicinal plants but also the effects of various types and mixtures of soils. Only in this manner can the growth requirements of these plants be determined and their cultivation intelligently recommended. An open field experiment was performed upon first year belladonna plants using sodium nitrate, potassium sulphate, acid phosphate, and a normal fertilizer. The plants were started in the greenhouse March 8, 1912, from seed taken from a commercial shipment of crude belladonna leaves, assaying 0.62 percent total alkaloids. All

fertilizers were applied July 15, when the plants had reached a height of from twelve to twenty inches. They were applied by hand as uniformly as possible and immediately worked into the soil by cultivation. The total amount of sodium nitrate used was divided into three equal portions and applied at intervals of two weeks. All the fertilizers used were applied at the rate of six hundred pounds per acre. No commercial fertilizer other than stable manure had been used upon these plots for a period of at least twelve years. The samples for assay were collected September 19, 1912, from representative plants of each plot. The following results were obtained:

FIRST EXPERIMENT.

Plot No.	Sample No.	Fertilizer Used.	Assay.	
			Leaves.	Root.
1	B-1258	Sodium Nitrate	0.699%	0.510%
2	B-1259	Potassium Sulphate	0.858%	0.504%
3	B-1261	Acid Phosphate	0.911%	0.380%
4	B-1260	Normal Fertilizer	0.835%	0.575%
5	B-1262	Control	0.688%	0.420%

The results of the first experiment indicate that marked changes may be produced in the percentage of alkaloids through the application of certain commercial fertilizers. However, sufficient data have not been obtained to justify any statement concerning the regularity of these changes. It is not known whether the changes indicated are due to the direct or indirect action of the fertilizers and what their influence would be when applied to different types of soils, under varied climatic and meteorological conditions. The extent of the error as introduced by individual plant variation in an experiment of this kind is also not known but must be considerable. Ten individual plants from this plot showed a variation of 50 percent. True (7) in examining individual plants has found this variation to be as much as 70 percent.

Disregarding this possible source of error, the increase in percentage of total alkaloids in the leaf as indicated by these trials is 20 percent for potassium sulphate, 24 percent for acid phosphate, and 18 percent for the normal fertilizer, the sodium nitrate causing no change. The increase in the root is 18 percent for sodium nitrate, 17 percent for potassium sulphate, 27 percent for the normal and a decrease of 9 percent for acid phosphate.

To further test the effect of sodium nitrate and acid phosphate upon percentage of alkaloids another experiment has been performed upon second-year plants. It was hoped in this experiment to eliminate the factor of individual plant variation in percentage of alkaloids. To accomplish this, fifteen uniform plants were selected and marked with conspicuous tags. These were divided into three groups of five plants each, leaving considerable space between each of the three groups. All weeds were removed from around these plants and the soil thoroughly cultivated and pulverized. Before applying any fertilizer, samples were collected from the three groups of plants. In collecting these samples care was taken to obtain an equal number of leaves from each plant of a given group, and that these leaves should be of uniform size. This same proceedure was followed in collecting the samples after the fertilizers had been applied. In this manner and by the utilization of the same plants throughout the

experiment it is believed that the individual characteristics of the plants have been obviated and that the results should be more accurate than where no attention is given to the proportion of samples from different plants or to the utilization of identical plants throughout the experiment. After the original samples were collected plots number 1 and 2 were given a liberal application of sodium nitrate and acid phosphate, respectively. Plot number 3 received no fertilizer. After an interval of two weeks plots 1 and 2 were given a second application. At the end of another two weeks period samples were collected for assay in the manner designated. At this time the fifteen plants were removed from the soil and the principle roots from all plants of each group were taken for assay. At the beginning of the experiment a root sample was taken from three plants which were growing near the control group. Rain occurred after both applications of fertilizers thus insuring immediate availability.

SECOND EXPERIMENT.

Samples collected before applying fertilizers.

Plot No.	Sample No.	Fertilizer Used.	Assay Leaf.	Assay Root.
1.....	B-1624	Potassium Nitrate.....	0.558%	
2.....	B-1625	Acid Phosphate.....	0.569%	
3.....	B-1626	Control	0.598%	
	B-1627	Control, 3 plants.....		0.385%

Samples collected after applying fertilizer.

Plot No.	Sample No.	Fertilizer Used.	Assay Leaf.	Percentage Increase in Leaf.	Assay Root.
1.....	B-1712	Potassium Nitrate.....	0.724%	23%	0.345%
2.....	B-1713	Acid Phosphate.....	0.742%	23%	0.345%
3.....	B-1714	Control	0.724%	18%	0.322%

The results indicate a considerable increase in the percentage of alkaloids in the leaf samples from all plots. The percentage increase in the fertilized plots is but little greater than that in the control. Practically no change is indicated in the alkaloidal content of the root samples.

The third experiment upon belladonna was carried on in the greenhouse with soils of widely different types. The plants used in the experiment were obtained by vegetative propagation from individuals of known alkaloidal yield. These individual plants were field grown and were selected at random. Cuttings were made from them and placed in a propagating bed filled with sand. As soon as strong roots were developed the plants were potted in two-inch pots, using a loam soil. They were later transferred to four-inch pots. December 16, 1912, they were removed from these and after all soil was carefully washed from the roots they were placed in six-inch pots which had previously been filled with the different types of soils. In addition to the potted plants, ten were grown on a greenhouse bench, filled with rich fibrous loam.

All the plants made very poor growth during the winter, due to the low temperature of the greenhouse which was essential to the success of propagating work, being done at that time. The experiment was brought to a close September 7, 1913, and on account of their small size it was necessary to use the entire plants for assaying. The proportions of root to stem and leaves were about the same in all cases. On account of the presence of roots in the assay samples the

results would be expected to run lower than those of the original plants. The soils used in the experiment were as follows:

No. 1. From field in which original plants were grown.
No. 2. Equal parts clay and No. 1.
No. 3. Equal parts clay and sand.
No. 4. Equal parts clay and leaf mould.
No. 5. Leaf mould.

The clay was obtained from an excavation eight feet below the top soil. Sand was used in number 3 to improve the physical condition of the clay. The leaf mould consisted of well decayed vegetable substance obtained from a woodlot of mixed growth.

The following table gives the number of the original plants from which the vegetative cuttings were made, soil and sample numbers, percentage of total alkaloids as determined upon leaf samples from the original field grown plants. All numbers of the same denomination in the first column refer to the same original plants from which the cuttings were made. A complete series from each of these original plants was started in the different soils but many of them were lost before the completion of the experiment.

The results indicate a considerable decrease in percentage of alkaloids in all plants examined, regardless of soil treatment. Even when grown upon the same soil as the original plant the decrease has been as great as from 20 percent in

THIRD EXPERIMENT.

Number.	Soil Number.	Sample Number.	Assay.	Assay, Original Plant.
10	1	B-1664	0.351%	0.820%
10	2	B-1665	0.373%	0.820%
10	4	B-1666	0.364%	0.820%
13	3	B-1667	0.351%	0.870%
13	4	B-1668	0.382%	0.870%
13	5	B-1669	0.323%	0.870%
13	Bench	B-1670	0.498%	0.870%
13	Bench	B-1671	0.396%	0.870%
13	Bench	B-1672	0.477%	0.870%
14	1	B-1673	0.441%	0.664%
14	2	B-1674	0.365%	0.664%
14	Bench	B-1676	0.383%	0.664%
15	1	B-1677	0.283%	0.600%
15	Bench	B-1678	0.471%	0.600%
15	Bench	B-1679	0.501%	0.600%
15	Bench	B-1680	0.442%	0.600%
16	1	B-1681	0.412%	0.516%
16	Bench	B-1682	0.398%	0.516%
16	Bench	B-1683	0.436%	0.516%
17	1	B-1684	0.454%	0.616%
17	2	B-1685	0.404%	0.616%
17	3	B-1686	0.370%	0.616%
17	4	B-1687	0.412%	0.616%
17	Bench	B-1688	0.373%	0.616%

number 16, to 57 percent in number 10. Where two or more plants from the same individual parent were grown in the uniform soil on the greenhouse bench a variation is noted of from 9 percent in number 16, which is only slightly greater than the experimental error, to 20 percent in number 13. On the other hand, such widely different soils as number 1 and 4 produced a difference in alkaloidal

yield of only 4 percent in plants from number 10, and 9 percent in those from number 17, and 8 percent on soils 3 and 5 in plants from number 13. After allowing 5 percent for experimental error there is little difference in favor of soil influence in the foregoing instances. The results as a whole indicate that further work is necessary on the influence of soil composition upon medicinal plants, before any generalizations can be made. The second experiment indicates that seasonal variations in alkaloidal percentage may have to be investigated more thoroughly. In this instance the percentage of alkaloids in the control plants increased nearly as much as in the fertilized plants. Also the influence of the two fertilizers, though apparently slight, seem to be identical with reference to the percentage of alkaloids. It is believed that there have been considerable sources of error in most of the work upon soils and fertilizers. It is desirable in this respect to first locate these sources of error and then attempt to eliminate them before proceeding further upon the problems of soil composition.

LITERATURE CITED.

(1) Hopper: Pharm. Jour., July 11, 1896.
(2) Carr: Proc. 8th International Congress of Applied Chemistry, 1912, 17, 7.
(3) Ransom and Henderson: Proc. 8th International Congress of Applied Chemistry, 1912, 17, 63.
(4) Chevalier: Comptes Rendus, 1910, 150, 344.
(5) Tschirch: Pharm. Jour., 1909, 83, 420.
(6) Miller and Meader: The Assay of Individual Plants of Datura Stramonium, Datura Tatula, and Other Species and Varieties, presented at the semi-annual meeting of the American Chemical Society, March, 1913.
(7) True: Oil, Paint and Drug Reporter, 1911, 80, 29.

BOTANICAL DEPARTMENT, ELI LILLY & Co., Indianapolis, August 16, 1913.

FURTHER STUDY OF THE ALKALOID GELSEMININE.

L. E. SAYRE.

In previous papers read before this Section by the writer on the alkaloids of Gelsemium, it will be seen that there has been a progressive study of the uncrystallizable alkaloid, named by Thompson, Gelseminine.

The attempt has been to bring this alkaloid into such a state of purity that it will be available for making an ultimate chemical analysis.

The present year's work has been that resulting from an investigation of the alkaloids from 50 pounds of the crude drug. From this 50 pounds, there was obtained in an unpurified condition 21.5 gms. of Gelsemic acid, 75.7 gms. of crude Gelsemine and 36.4 gms. of crude Gelseminine, making in all 112.1 gms. of crude alkaloids and 21.5 gms. of crude Gelsemic Acid.

The effort in the present year's investigation was to purify the gelseminine for physiological testing and for the comparison with the alkaloid gelsemine. It should be stated in passing that hitherto we have found it very difficult to rid gelseminine absolutely from the contaminating impurity, gelsemine. We have found the separation of the two alkaloids to be made more complete by treating the crude gelseminine hydrochlorid first with alcohol which separates out most of the gelsemine hydrochlorid. The alcoholic extractive resulting from the evaporation of the alcoholic filtrate is now re-dissolved in acetone and a further separation of gelsemine hydrochlorid is possible.

By treating the extract resulting from the evaporation of the acetone solution of gelseminine hydrochlorid with water and precipitating the solution