11. A. Störck, "Essay on the Medicinal use of Hemlock," 1761. Original not seen; see Harley (5), p. 711.

12. Tanret. A communication made to the *Societe de Pharmacie* of Paris, reported by G. Planchon and E. Collin. *Les Drogues Simples d'Origine Vegetale*, Paris, 1896, Tome II, p. 221, date and title not given.

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ATROPA BELLADONNA.*

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INTRODUCTION.†

In the last three years the cultivation of belladonna in the United States has been greatly stimulated by the high prices paid for the crude drug. The advance in price was due to the lack of shipping, hampering importations, and to the fact that foreign countries which previously supplied our markets were at war, and were utilizing their land and time in the production of human food.

Many plants are utilized for medicinal purposes, but the relative amounts are small when compared with staple crops, such as corn, wheat and potatoes. Stockberger[14] states that in 1917 approximately 100 acres of belladonna were grown in the United States, and that the cultivation of more than 500 acres of this crop might cause over-production. Since the market price for belladonna is 500 to 600 percent higher now (September 1918) than it was before the war, we would infer that we had as yet not reached the point of over-production. Stockberger[13] also summarizes drug plant culture, which may well apply to belladonna—"the problems presented by the cultivation of drug plants are not less difficult than those encountered in the production of many other crops. Drug plants are subject to the same diseases and risks as other crops, and are similarly affected by variations in soil and climatic conditions. They require a considerable outlay of labor, the same as other crops, and likewise require intelligent care and handling."

Scientific investigations dealing with the cultivation of belladonna, as with other medicinal plants, have been carried out principally by pharmaceutical houses, state experiment stations and universities, which maintain drug gardens. While published scientific investigations deal with all phases of belladonna culture, from a practical standpoint, much information is still necessary in order to be successful with this crop.

The author has studied various phases of belladonna culture with the thought of using the results in practice.

STUDY OF THE GERMINATION OF BELLADONNA SEEDS.

Belladonna seeds germinate very slowly and irregularly. Haynes and Newcomb[6] state that a small part of the seed germinated in two or three weeks, while the remainder germinated in four to five weeks. Sievers[11] concluded that late fall sown seeds germinate much sooner in the spring than seeds sown in the spring.

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He also states the first seeds germinate in about three weeks while the bulk begin to germinate between the fourth and fifth week. Sievers[11] found that treating belladonna seeds with sulphuric acid was of no great value in hastening the germination. Treatment with hydrogen peroxide was found to be very beneficial. Moistening the seeds and then subjecting them to a temperature of —12° C. for five hours materially hastened germination.

To determine the length of time required for belladonna seeds to germinate, the following experiment was made: In each of two flats (12 × 20″) of soil, one thousand viable belladonna seeds were planted at a depth of one-fourth inch. The number of seeds that germinated was counted after two, three and five weeks. The results are shown below.

TABLE 1.—TABLE SHOWING THE PERCENTAGE OF GERMINATION OF BELLADONNA SEEDS.

	Percentage Germination.		
Flat No.	2 Weeks.	3 Weeks.	5 Weeks.
1	3.7	7.6	10.4
2	3.0	6.6	9.0

The results show that very few belladonna seeds germinate in the first three weeks and only ten percent after five weeks.

Since Sievers[11] found that freezing belladonna seeds for a short time was effective in hastening germination, an experiment including this factor was carried out. About 25 grammes of belladonna seeds were moistened and then placed in a large test tube. After subjecting these seeds to a temperature of —12° C. for six hours they were dried at room temperature. One thousand of these seeds were planted in each of two flats.

TABLE 2.—TABLE SHOWING THE EFFECT OF FREEZING BELLADONNA SEEDS UPON THE PERCENT OF GERMINATION.

		Percentage Germination.		
Flat No.	Treatment.	2 Weeks.	3 Weeks.	5 Weeks.
1	not frozen	3.7	7.6	10.4
2	not frozen	3.0	6.6	9.0
5	frozen	3.4	10.8	12.7
6	frozen	3.6	6.0	9.8

The above results demonstrate that freezing this particular sample of belladonna seeds did not appreciably benefit the extent of germination.

The effect of increasing the humidity and preventing excessive evaporation from the surface of the soil during the germination of belladonna seeds was determined.

The same methods as followed in the previous experiments were carried out, with the exception that two flats were covered tightly with glass plates, thus preventing excessive evaporation.

TABLE 3.—TABLE SHOWING THE EFFECT OF INCREASED HUMIDITY UPON THE GERMINATION OF BELLADONNA SEEDS.

		Percentage Germination.		
Flat No.	Treatment.	2 Weeks.	3 Weeks.	5 Weeks.
1	None	3.7	7.6	10.4
2	None	3.0	6.6	9.0
3	Increased Humidity	7.0	10.4	9.0
4	Increased Humidity	7.3	10.2	12.5

Increasing the humidity appreciably hastened the germination of belladonna seeds during the second and third weeks. At the five-week period, however, there was no appreciable increase. By increasing the humidity another factor presented itself, namely, that of "damping-off" fungi. This factor was responsible for the low results in flats Nos. 3 and 4 at five weeks.

All soils contain organisms and spores and cysts of many different kinds, some of which, when the conditions are favorable, become destructive to higher plants. An example of this kind was shown above in the case of the "damping off" fungi. By destroying these factors by sterilization a much more suitable medium is obtained.

To determine the effect of sterilization of a soil upon the extent of germination, a series of four flats of soil were prepared. Two of these flats of moistened soil were carefully wrapped with heavy Manila paper and sterilized in the autoclave at 15 lbs. pressure for $2^1/_2$ hours on two successive days. The four flats (two sterilized and two unsterilized) were planted with one thousand belladonna seeds. They were all covered with glass plates to prevent excessive evaporation. After three, four and five weeks, the number of seeds that had germinated was recorded.

TABLE 4.—TABLE SHOWING THE EFFECT OF STERILIZATION OF SOIL UPON THE EXTENT OF GERMINATION OF BELLADONNA SEEDS.

		Percentage Germination.		
Flat No.	Treatment.	3 Weeks.	4 Weeks.	5 Weeks.
1.....	No treatment	6.5	9.9	10.5
2.....	No treatment	2.5	3.5	3.5
3.....	Sterilized	15.2	16.7	17.3
4.....	Sterilized	22.0	37.6	41.6

The above data shows conclusively that sterilizing the soil was effective in producing a larger germination of belladonna seeds.

STUDY OF THE PLANTING OF BELLADONNA.

Practically all authorities on belladonna culture conclude that, to be successful with this crop, the seed should be planted in the hot house or cold frame, and when the plants have attained a sufficient growth they should be potted into small pots and grown under these conditions until they are transplanted into the field. It is essential to know just how long a period elapses from the time when the seed is planted until the plants are large enough to be transplanted into the field, as under practical conditions the seeds must be sown long enough in advance so that the plants will be sufficiently large when the field planting season begins. To determine the length of time from the period when belladonna seeds are planted until the plants are ready for potting each of eight flats of soil was planted with one thousand belladonna seeds. The development of the small plants was carefully noted and the results recorded. The germination, as before noted, was very irregular, many not having germinated eight weeks after planting. In this experiment almost all of the plants were large enough to pot five weeks after the seeds were planted. Fifty percent had the fifth leaf formed. Many plants were large enough to transplant (having four leaves formed) four weeks after the seeds were planted.

To determine the approximate length of time after the plants were potted until they were large enough for planting in the field, forty-five $1^1/_2$-inch pots were

filled with a light compost soil. Each pot was planted with a small belladonna plant, being careful that plants of about the same size were selected. Three months after potting, fifteen average sized plants were measured. The results were $4^3/_4$, 4, 4, 4, 5, 5, 5, 5, $3^1/_2$, $4^1/_2$, 5, 5, 5, 4 and $3^1/_2$ inches. The average height of these fifteen plants was $4^1/_2$ inches. It is apparent that three months after the small plants are potted they are large enough for transplanting into the field.

To ascertain to what extent added inorganic fertilizers hasten the growth of small belladonna plants when they are potted, at the same time that the above experiment was made, a series of pots having the same compost soil, with the addition of 3000 lbs. of sodium nitrate, 1200 lbs. of potassium sulphate and 2400 lbs. of calcium phosphate (mono-basic) per acre of 2,000,000 lbs., were planted. The measurements of fifteen average sized plants grown in the fertilized soil in three months were $7^1/_2$, 7, 7, 7, $6^3/_4$, $5^1/_2$, 6, 6, 8, $8^1/_4$, 8, 8, $7^1/_2$, $8^1/_2$, and $8^3/_4$ inches. The average height was $7^1/_4$ inches. Upon comparing the height of plants grown in fertilized and unfertilized soil ($7^1/_4$ vs. $4^1/_2$ inches), it is apparent that fertilization greatly stiumulated the growth.

EFFECT OF FERTILIZATION UPON THE GROWTH OF BELLADONNA PLANTS.

In the northern parts of the United States the growing season in the field is comparatively short, hence where it is possible to control them, the cultural conditions should be as near the optimum as possible in order to get the greatest yield. In the cultivation of belladonna to what extent is fertilization necessary? Schneider[10] states that the experiments with fertilizers were not successful, and that a fairly rich soil supplied with plenty of lime gave the best results. Carr[4] found that cultivated plants contained little more alkaloid than wild belladonna. He[2] states that fertilizers lower the alkaloid content, especially where nitrogenous manures are applied, this being due to the larger leaf growth. Carr[2] finds, further, that if the soil is not already rich, manure as well as complete fertilizers increase the yield considerably.

As it has been found that to secure a successful crop of belladonna, in almost all sections of the United States, it is essential that the seeds be germinated and the plants developed under glass, what fertilizers and in what amounts is it necessary to apply to secure the largest yields and best plants in the shortest time? For information on this point, the following experiment was made: About 350 lbs. of a light manure compost soil was divided into seven lots of 50 lbs. each. Fertilizer applications were made to the various lots as shown in the table below. Forty-five $1^3/_4$" pots were filled from each lot of soil. The pots were then planted with small belladonna plants. In order that the experimental error should be small, plants of as nearly the same size as possible were planted. After these had grown for three months, ten average sized plants of each lot were carefully taken from the pots. The adhering soil was washed from the roots and the moisture dried off by means of filter paper. The plants were weighed and the results are presented on following page.

Studying the results presented in Table 5, it is apparent that large applications of inorganic fertilizers greatly increased the size and weight of belladonna plants, since two and a half times as much growth was realized in series No. 5 as in Series 7. Smaller amounts of fertilizer, namely, one-third as much as

TABLE 5.—TABLE SHOWING THE EFFECT OF FERTILIZERS UPON SMALL BELLADONNA PLANTS GROWN IN POTS.

Series No.	Fertilizer. Lbs. per Acre of 2,000,000 Lbs.	Weight of Plants. Gms.		Average. Gms.
1	1000 $NaNO_3$	3.8	3.0	
	400 K_2SO_4	2.3	3.7	
	800 $Ca(H_2PO_4)_22H_2O$	2.6	2.8	
	1000 $CaCO_3$	2.2	3.2	
		2.1	2.4	2.81
2	1000 $NaNO_3$	3.9	3.4	
	400 K_2SO_4	2.2	2.6	
		2.7	3.3	
	800 $Ca(H_2PO_4)_22H_2O$	2.7	2.7	
		1.5	3.2	2.82
3	400 K_2SO_4	2.1	1.7	
		2.2	1.7	
	800 $Ca(H_2PO_4)_22H_2O$	1.8	2.2	
		1.9	2.3	
	1000 $CaCO_3$	2.0	2.1	2.00
4	3000 $NaNO_3$	4.4	4.6	
	1200 K_2SO_4	4.7	5.0	
	2400 $Ca(H_2PO_4)_22H_2O$	4.7	4.7	
	3000 $CaCO_3$	4.8	4.1	
		5.5	4.0	4.65
5	3000 $NaNO_3$	4.9	5.6	
	1200 K_2SO_4	4.0	6.6	
		6.5	5.2	
	2400 $Ca(H_2PO_4)_22H_2O$	6.0	4.2	
		7.7	4.7	5.54
6	1200 K_2SO_4	1.1	1.5	
	2400 $Ca(H_2PO_4)_22H_2O$	1.6	2.1	
		2.0	1.3	
	3000 $CaCO_3$	1.6	1.3	
		1.8	1.7	1.60
7	No Fertilizer	2.7	1.8	
		1.8	1.9	
		2.3	1.6	
		1.4	1.7	
		1.5	2.8	2.05

the largest, did not materially increase the yields. That sodium nitrate was very essential for the maximum development of small belladonna plants is seen on comparing the results of Series 4 with 6. About one-third as much growth was realized in the determinations when sodium nitrate was not added. Comparing the results of Series 4 and 5, we note that calcium carbonate was not an important factor for the largest yield of these small plants, as there was a slightly larger amount of growth in the series where calcium carbonate was not applied.

To more fully determine the absolute fertilizer requirements of belladonna plants, an experiment was made in which sand instead of soil was used. Nine series of pots, using different combinations and amounts of fertilizers, were made. As in the above experiment there were forty-five $1^3/_4$-inch pots in each series. A small belladonna plant was planted in each pot, and after two and a half months

ten average size plants were selected. As before, these were washed and the moisture removed with filter paper.

TABLE 6.—SHOWING THE EFFECT OF INORGANIC SALTS UPON THE GROWTH OF BELLADONNA PLANTS WHEN GROWN IN SAND.

		Development of small plants.			Development of plants to maturity.		
Series No.	Fertilizer applications per acre of 2,000,000 lbs. Lbs. per acre.	Wt. of plants.		Average wt. of plants. Gms.	Pots No.	Wt. of plants. Gms.	Average weight. Gms.
					801	3.1	
21.....	776 $(NH_4)_2SO_4$	1.65	1.1				
	400 K_2SO_4	1.3	1.2	1.24	802	2.9	2.8
	800 $Ca(H_2PO_4)_22H_2O$	1.3	1.1				
	1000 $CaCO_3$	1.25	1.1		803	2.5	
		1.3	1.4				
22.....	1000 $NaNO_3$	1.4	1.2		804	2.4	
	400 K_2SO_4	1.2	1.1				
	800 $Ca(H_2PO_4)_22H_2O$	1.5	1.3	1.23	805	2.0	2.7
	1000 $CaCO_3$	1.2	1.1				
		1.2	1.1		806	3.7	
23.....	400 K_2SO_4	0.35	0.30		807	1.2	
	800 $Ca(H_2PO_4)_22H_2O$	0.33	0.35				
	1000 $CaCO_3$	0.35	0.30	0.32	808	1.9	1.3
		0.31	0.30				
		0.32	0.32		809	0.9	
24.....	1000 $NaNO_3$	3.3	2.1		810	4.2	
	400 K_2SO_4	3.15	2.2				
	800 $Ca(H_2PO_4)_22H_2O$	2.40	2.15	2.36	811	2.3	2.9
		2.7	1.9				
		1.9	1.8		812	2.4	
25.....	2328 $(NH_4)_2SO_4$	4.4	2.0		821		
	1200 K_2SO_4	4.2	2.4			4.9	
	2400 $Ca(H_2PO_4)_22H_2O$	3.3	1.3	2.58	822	4.1	4.5
	3000 $CaCO_3$	2.6	2.0				
		1.8	1.8		823		
26.....	3000 $NaNO_3$	2.7	1.7		824		
	1200 K_2SO_4	2.0	1.8			4.4	
	2400 $Ca(H_2PO_4)_22H_2O$	2.0	1.8	1.95	825	5.1	5.3
	3000 $CaCO_3$	1.8	2.1		826	6.5	
		2.0	1.6				
27.....	1200 K_2SO_4	0.40	0.40		827	1.7	
	2400 $Ca(H_2PO_4)_22H_2O$	0.40	0.38				
	3000 $CaCO_3$	0.42	0.39	0.39	828	1.2	1.5
		0.35	0.36				
		0.43	0.35		829	1.5	
28.....	3000 $NaNO_3$	4.4	1.9		830	3.4	
	1200 K_2SO_4	3.7	2.0				
	2400 $Ca(H_2PO_4)_22H_2O$	3.2	3.1	2.73	831	5.4	4.5
		2.7	1.9				
		2.0	2.4		832	4.7	
29.....	No Fertilizer	0.32	0.25		833	0.9	
		0.35	0.31				
		0.30	0.34	0.27	834	1.0	1.0
		0.20	0.21				
		0.23	0.24		835	1.1	

The effect of inorganic salts upon the growth of small belladonna plants is clearly demonstrated in the results presented above. In the determinations where a complete fertilizer was applied, as in Series 21, 22, 25 and 26, eight times as much growth was appreciated as was in the check (Series 29) where no fertilizers were added. Where complete fertilizers were applied, those receiving the largest applications made the largest yields. On comparing the results of Series 23 with 21 and 22, and 27 with 25 and 26, the importance of the nitrogen is appreciated, as only from one-fourth to one-sixth the growth was realized where no nitrogen was supplied. As in the previous experiments with soil, larger growths were realized where no calcium carbonate was applied than where it had been furnished.

To determine the effect of inorganic salts upon belladonna plants from the small plant stage to maturity, twenty-seven 6″ pots were filled with sand and to each series of three pots inorganic salts were added in amounts (per acre), and in the same proportion, as in the nine series of small pots above described. For instance, as seen in Table 6, pots Nos. 801, 802 and 803 received the same amount of fertilizer in proportion as the forty-five small pots of Series 21. Nos. 804, 805 and 806 received the same as No. 22, etc. Each of the pots Nos. 801, 802 and 803 were planted with one representative plant of Series 21. Likewise Nos. 804, 805 and 806 were planted with plants from Series 22, and so on throughout the series. The moisture condition of each of these pots was carefully maintained at the physical optimum of the sand. When the plants had reached the blossoming stage they were harvested and dried for 48 hours at 90° C. The weight of each individual plant is shown in Table 6, Part 2. In the last column of figures is the average weight of the three plants. Upon studying the results in Table 6, it will be seen that in practically all cases the fertilizers produced increases in yield with mature plants corresponding to those produced with small plants in the first stage of growth. Again in all cases where the largest applications of fertilizer were made the yields were largest. As before, one-fourth to one-half the yield was realized in the determinations receiving no nitrogen.

Since we had found the effect of inorganic salts upon the growth of belladonna plants in a light compost and also in sand, it was desired to find how much inorganic fertilizers influenced belladonna when applied to field soils. A large sample of clay loam soil was taken from a cultivated field on the premises of the Mulford Biological laboratories. Into each of twenty-one 6″ pots, 1,750 grammes of this soil was weighed. Fertilizer applications as shown in Table 7 were carefully made to each pot. The moisture conditions were made up to the physical optimum of the soil and maintained at this throughout the experiment by weighing the pots every day and restoring the loss due to evaporation and transpiration. Three small belladonna plants of the same size were planted in each pot. After they had grown for five weeks, two of the plants were pulled out. The remaining plants were allowed to grow until they had all reached the blossoming stage, when they were harvested and dried. The results are recorded on next page.

Inorganic fertilizers applied to this soil were effective in increasing the growth of belladonna. In every case where fertilizers were applied the yield was $2^1/_2$ to 3 times the growth of that in the control pots, where no fertilizers were applied. Comparing the results of pots Nos. 6, 7 and 8, with Nos. 31, 32 and 33, we found that where ammonium sulphate was the source of nitrogen, a slightly higher yield

TABLE 7.—TABLE SHOWING THE EFFECT OF INORGANIC FERTILIZER APPLIED TO A CLAY LOAM SOIL UPON THE GROWTH AND DEVELOPMENT OF BELLADONNA PLANTS.

Pot No.	Fertilizer Treatment. Lbs. per acre of 2,000,000 lbs.	Weight of stems and leaves. Gms.	Average. Gms.
1.....	No fertilizer	1.2	
2.....	No fertilizer	2.1	
3.....	No fertilizer	1.1	1.46
6.....	466 $(NH_4)_2SO_4$	3.8	
7.....	400 K_2SO_4	5.0	
8.....	800 $Ca(H_2PO_4)_22H_2O$		5.00
	1000 $CaCO_3$	6.3	
	100 $MgSO_4$		
	400 K_2SO_4	4.3	
11.....	800 $Ca(H_2PO_4)_22H_2O$	3.4	
12.....	1000 $CaCO_3$		3.80
13.....	100 $MgSO_4$	3.7	
	466 $(NH_4)_2SO_4$	3.8	
16.....	400 K_2SO_4	4.2	3.80
17.....	1000 $CaCO_3$	3.5	
18.....	100 $MgSO_4$		
	466 $(NH_4)_2SO_4$		
21.....	400 K_2SO_4	3.1	
22.....	800 $Ca(H_2PO_4)_22H_2O$	3.6	3.60
23.....	100 $MgSO_4$	4.3	
	466 $(NH_4)_2SO_4$	5.6	
26.....	800 $Ca(H_2PO_4)_22H_2O$	3.0	4.60
27.....	1000 $CaCO_3$	5.2	
28.....	100 $MgSO_4$		
	600 $NaNO_3$		
31.....	400 K_2SO_4	3.6	
32.....	800 $Ca(H_2PO_4)_22H_2O$	5.6	4.10
33.....	1000 $CaCO_3$	3.1	
	100 $MgSO_4$		

was realized than where sodium nitrate was employed. The results of pots Nos. 11, 12 and 13, 16, 17 and 18, and 21, 22 and 23, the series where nitrogen, phosphorus and lime were not supplied, respectively, were all about the same, namely, $2^1/_2$ times the results of the controls. These results show that there was a certain amount of available nitrogen, phosphorous and lime in this particular soil, and that while the plants did not make their maximum growth, they could utilize the other salts that were supplied.

EFFECT OF MOISTURE UPON THE GROWTH OF BELLADONNA PLANTS.

There is a very great difference in the amount of rainfall in various sections of the country and at different seasons. Likewise, in certain sections of the country irrigation is practiced, thus the moisture conditions can be controlled. Under controlled conditions, how much moisture do belladonna plants require? To gain some information upon the effect of conditions of moisture upon the growth and development of belladonna, the following experiment was made. Each of nine 6″ pots was filled with 1750 grammes of the same clay loam soil as was used in the above experiment. In order that the lack of fertilizers would not be a factor, a complete fertilizer, equivalent to 1000 lbs. of $CaCO_3$, 800 lbs. of $Ca(H_2PO_4)_2$-

$2H_2O$, 400 lbs. of K_2SO_4, 600 lbs. of $NaNO_3$ and 100 lbs. of $MgSO_4$, per acre of 2,000,000 lbs., was added to each pot. To one series of three pots distilled water was added so that the conditions of moisture were made up to the physical optimum of the soil. The moisture in the soil of the second series of three pots was made up to one-half the physical optimum and that of the third series was made to one and a half of the optimum. One small belladonna plant was planted in each of the nine pots. Plants of nearly the same size were planted. In all cases the moisture was maintained in the above-mentioned conditions by carefully weighing the pots every day and restoring the loss due to evaporation and transpiration. After the plants had grown for three and a half months they were harvested. The plant of each pot was cut off even with the surface of the soil, placed in a Manila bag and dried at 60° C. for four days. The roots were also carefully harvested, washed and dried.

TABLE 8.—TABLE SHOWING THE INFLUENCE OF MOISTURE UPON THE GROWTH OF BELLADONNA PLANTS GROWN IN A CLAY LOAM SOIL.

Pot No.	Moisture conditions.	Weight of leaves and stems. Gms.	Average. Gms.	Wt. of roots. Gms.	Average. Gms.
101	optimum	6.0		1.2	
102	optimum	6.5	6.5	1.4	1.6
103	optimum	6.9		2.2	
104	1/2 optimum	4.3		1.6	
105	1/2 optimum	4.0	4.1	1.0	1.4
106	1/2 optimum	4.0		1.6	
107	1 1/2 optimum	2.6		0.5	
108	1 1/2 optimum	1.1	1.9	0.2	0.33
109	1 1/2 optimum	2.2		0.3	

The results presented in Table 8 demonstrate conclusively that the moisture conditions of the soil in which belladonna grows is a very important factor in influencing the yield of belladonna leaves, stems and roots. In the pots where moisture to the extent of one-half the physical optimum of the soil was applied, two-thirds the weight of leaves and stems was realized compared with the pots where moisture was at the physical optimum, while, where one and a half times the optimum was applied, the growth was less than one-third of that where the optimum moisture was maintained. The root growth of the optimum and one-half optimum series was practically the same, while that of the one and a half optimum series was only one-fifth as large.

EFFECT OF SHADE UPON THE GROWTH AND DEVELOPMENT OF BELLADONNA PLANTS.

In the case of certain medicinal plants, shade is essential for growing a successful crop. Some investigators are of the opinion that belladonna plants require a certain amount of shade for maximum growths and yields. Gordon[5] states that belladonna plants did best when they were shaded part of the day. Carr,[3] testing the effect of various colors upon the yield and alkaloid content of belladonna, found that a green shade reduced the amount of inflorescence and at the same time increased the alkaloid content.

The effect of shade upon the growth and development of belladonna plants was determined. At the time the above experiment, testing the effect of mois-

ture on the growth of belladonna, was made, three additional 6″ pots were filled with soil. Fertilizers were added in the same amount as in the nine pots. The moisture was made up to the physical optimum, hence the conditions were identical with the optimal series of the moisture experiment, and that series served as a check for this shade experiment. After the pots were planted with the small plants, each of the three shade pots was covered with a frame 12 × 12 × 18 inches, which was covered with one thickness of the ordinary gauze cloth. These three pots received the same treatment as the optimal series of the moisture experiment, with the exception that they were shaded.

TABLE 9.—TABLE SHOWING THE EFFECTS OF SHADE UPON THE GROWTH AND DEVELOPMENT OF BELLADONNA PLANTS.

Pot No.	Treatment.	Weight of leaves and stems. Gms.	Average. Gms.	Wt. of roots. Gms.	Average. Gms.
101	Not shaded	6.0		1.2	
102	Not shaded	6.5	6.5	1.4	1.6
103	Not shaded	6.9		2.2	
201	Shaded	3.5		0.7	
202	Shaded	4.9	3.8	0.9	0.73
203	Shaded	3.0		0.6	

The effect of shade produced by the ordinary gauze cloth is readily seen by noting the results of Table 9. With all the conditions as to fertilizers, moisture, temperature and plants identical, shading the plants resulted in the production of about one-half of the yield of the unshaded series. From the practical standpoint, this is a very important factor. It is true, the alkaloid content of the shaded plants might have been higher than that in the unshaded series, as was found by Carr,[3] but in this case the alkaloid content of the shaded plant would have to be about twice that of the unshaded, to yield a like amount of alkaloid. Alkaloid determinations were not made on account of the small amounts of leaves and stems obtained.

STUDY OF THE MEANS OF COMBATING INSECTS ON BELLADONNA PLANTS.

Probably the greatest difficulty in the cultivation of belladonna is the control of this plant against the attacks of insects. Practically all of the chewing and sucking insects that are injurious to the various truck crops are destructive to belladonna. The most destructive insects, however, are the Colorado potato beetle and the green and pink aphids. To keep young plants free from insects, Borneman[1] recommends lemon oil and whale oil soap, while, as the plant becomes stronger, a weak solution of lead arsenate is recommended. To control the chewing insects, both Paris green and lead arsenate are recommended. As there was very little data available which showed carefully outlined experiments on the control of insects, several experiments were made. Twenty-four belladonna plants that were grown in the field, and that were badly infested with Colorado potato beetle, were selected for experimental purposes. Six plants were sprayed with Paris green 1 : 100, six with Paris green 1 : 50, a like number with lead arsenate 5 lbs. to 100 gallons of water, and the remainder left as controls. A week later they were again sprayed, and two weeks later a third time.

TABLE 10.—SHOWING THE CONTROL OF THE DESTRUCTIVE EFFECTS OF THE COLORADO POTATO BEETLE ON BELLADONNA PLANTS BY MEANS OF VARIOUS SPRAYS.

	Treatment.	Effect of spray on insects.	Effect of spray on plant.	Effect of Insect on plants.
1.	No treatment	..	..	plant destroyed
2.	Paris green 1 : 100	destroyed	no injury	no injury
3.	Paris green 1 : 50	destroyed	badly injured	no injury
4.	Lead arsenate	destroyed	no injury	no injury

The results of the experiment given above demonstrate that belladonna plants cannot withstand the attack of potato beetles if they are not suitably protected by means of some poison. Both Paris green, of a concentration of 1 pound to 100 gallons of water, and lead arsenate 5 pounds to 100 gallons, proved effective in controlling the attacks of this insect. No spray injury was perceptible on the plants where these sprays were applied. Paris green, 1 pound to 50 gallons of water, proved too concentrated for belladonna plants, as all the plants which were sprayed with this solution were destroyed by the effects of the spray.

For controlling various aphids on the belladonna plants, several combinations of soap and kerosene and soap and tobacco extract were tried. The results with the first mixture (soap and kerosene) were unsatisfactory. Soap and tobacco combinations were only partially successful. The principal difficulty experienced in the latter case was controlling the concentration of the extract of tobacco.

Experiments using "nicotine sulphate" (Black Leaf 40) with soap proved very satisfactory in controlling the various forms of aphids. A field experiment was performed, in which case a series of 12 plants were sprayed with a nicotine soap solution made in the proportion of 4 pounds of soap, 1/2 pint "nicotine sulphate" (Black Leaf 40) and 100 gallons of water; a second series of 12 plants with 4 pounds soap and 3/4 pint of "nicotine sulphate;" a third series with 4 pounds of soap and 1 pint of "nicotine sulphate" to 100 gallons of water. A series of 12 plants were reserved as checks, receiving no spray treatment.

TABLE 11.—TABLE SHOWING THE CONTROL OF APHIDS ON BELLADONNA PLANTS.

Series No.	Treatment.	Effect on Insects.	Effect on plants.
1.	Soap + 1/2 pt. "nicotine sulphate"	aphids 75% destroyed	no effect
2.	Soap + 3/4 pt. "nicotine sulphate"	aphids 100% destroyed	no effect
3.	Soap + 1 pt. "nicotine sulphate"	aphids 100% destroyed	very slight injury
4.	No treatment		All plants badly damaged by aphids

The results of this experiment show conclusively that "nicotine sulphate" (Black Leaf 40) in combination with soap is very effective in destroying aphids on belladonna. "Nicotine sulphate" 1/2 pint in combination with the soap was not as effective in controlling the aphids as the solution made with either the 3/4 pt. or the 1 pint per 100 gallon. The latter solution, while very effective in destroying the aphids, produced slight spray injury.

STUDY OF THE EFFECT OF DRYING BELLADONNA LEAVES AT DIFFERENT TEMPERATURES UPON THE ALKALOID CONTENT.

Drying medicinal plants is a step in the practical phase of drug culture that requires a certain amount of consideration. No definite temperature at which this plant should be dried has been adopted. Sievers[12] after air drying his samples,

dried them to constant weight in a hot air oven at a maximum temperature of 60° C. Roberts[9] reports results of experiments on drying fresh belladonna leaves, which show loss of 60% on drying in vacuum at 45° C.

To determine to what extent drying at various temperatures, and heating first and then drying, influences the alkaloid content of belladonna leaves and the color of the final product, an experiment covering this phase of the work was made.

A large sample of about 2000 Gm. of belladonna leaves was collected. In order to reduce the possibilities of errors incurred in sampling, these leaves were cut into small pieces. After this mass of small particles of leaves was very carefully mixed it was divided into five samples of about 400 Gm. each. One sample was dried under natural conditions in the greenhouse, at a temperature of 30° C.; one sample was dried in a large incubator where the temperature was maintained at 55–60° C. Another was dried at 100°C., a fourth sample was heated to 100°C., and then the drying completed at 55 to 60°C., and the other was placed in an autoclave, which was maintained at 15 lbs. pressure for 1½ hours. It was then dried at 55–60° C. The samples, when thoroughly dry, were submitted for analysis. The results were as follows:

TABLE 12.—TABLE SHOWING THE EFFECT OF DRYING AT VARIOUS TEMPERATURES UPON THE ALKALOID CONTENT AND CHARACTER OF BELLADONNA LEAVES.

Sample No.	Treatment.	Mydriatic Alkaloid.	Color of leaves.
401.....	Dried in greenhouse at 30° C.	0.327	fine green
402.....	Dried at 55–60° C.	0.345	green
403.....	Dried at 100° C.	0.369	brown
404.....	Heated to 100° C. then dried at 55–60° C.	0.366	brown
405.....	Autoclaved, then dried at 55–60° C.	0.264	brown

The results presented above show that the temperature at which belladonna leaves are dried is a very important factor. The samples dried at 55–60° C., 100° C. and heated to 100° C., then dried at 55–60° C., showed a slightly higher percent of mydriatic alkaloid than the sample dried at 30° C. When a higher temperature than 30° C. was employed, the fine green color of the leaves was somewhat lost. The sample dried at 55–60° C. was still green, but those dried at a temperature above this or heated at 100° C., or in the autoclave and then dried at 55–60° C., were of an undesirable brown color. By heating in the autoclave and then drying at 55–60° C., 20 percent of the total mydriatic alkaloid content was lost. From these results we would conclude that when artificial heat is employed in drying leaves of belladonna, it is not advisable to employ a temperature higher than 55–60° C.

STUDY OF THE PRODUCTION OF SEEDS BY BELLADONNA PLANTS.

One of the principal reasons for the lack of advance in cultivation of belladonna in the United States is because of the difficulty in securing good viable seed. Up to 1914 most of the belladonna seeds were obtained from abroad. Since that time individuals interested and engaged in the cultivation of belladonna have learned how to harvest their own seed.

Belladonna plants are very prolific seed producers, each plant yielding a very large number of seeds. As it was desirable to know how much seed belladonna plants produced, the seed from each of sixteen plants was collected at the time when

most of the seed-pods were black. The following are the weights in grammes of seeds harvested from the sixteen plants: 65.0, 32.0, 21.0, 20.0, 27.0, 18.0, 63.5, 28.5, 18.5, 9.0, 9.5, 29.7, 27.2, 26.2, 39.0, and 15.5. The average of these sixteen weights was 28.1 grammes. Under field conditions twenty-two pounds of good viable seeds of belladonna were harvested from one-tenth of an acre. Formerly when seeds were collected from belladonna plants, it was the custom not to molest the plant and utilize no portion of it other than the seeds. It has been found here, with belladonna plants, that if they are planted sufficiently early in the season, and the first growth of leaves harvested, a larger number of leaves and seed-pods will form than if the first crop of leaves is not picked. The above reported figures represent seed taken from plants, the first crop of leaves of which had been harvested.

INFLUENCE OF THE PRESENCE OF STEMS UPON THE ALKALOID CONTENT OF BELLADONNA LEAVES.

It is usually the custom to utilize only the leaves of the belladonna plants, as it is generally considered that the alkaloid content of the stems would be far below the U. S. Pharmacopoeia requirement. It has been previously shown by the writer[7] in the case of stramonium that the stems can be used in conjunction with the leaves in the proportion in which they exist at harvesting time and the U. S. P. requirements still met. Similarly the writer[8] has demonstrated the fact that stems and roots of hyoscyamus collected after harvesting the seed contain a relatively high percentage of alkaloid. As was stated before,[7] if we can utilize the stems it will greatly reduce the cost of harvesting.

To secure information on the extent to which the presence of stems of belladonna influences the total alkaloid content of leaves, six representative belladonna plants were harvested. The leaves, stems and roots of each plant were separated, placed in separate Manila paper bags, and dried in the oven for four days at 55 to 60° C.

TABLE 14.—TABLE SHOWING THE PROPORTION OF STEMS TO LEAVES IN BELLADONNA PLANTS.

Plant No.	Part of plant.	Wt. of Material. Gms.	Proportion of leaves to stems. Percent.
1	Leaves	88.8	68.3
1	Stems	41.1	31.7
2	Leaves	47.8	65.8
2	Stems	24.8	34.2
3	Leaves	71.8	66.7
3	Stems	35.8	33.3
4	Leaves	49.8	65.0
4	Stems	26.8	35.0
5	Leaves	50.4	66.6
5	Stems	25.3	33.4
6	Leaves	63.8	63.6
6	Stems	36.5	36.4

The results with six plants, as presented in Table 14, show that under the ordinary field conditions the proportion of leaves to stems of belladonna is as 2 to 1.

To determine the effect of the presence of stems upon the total alkaloid content of belladonna, samples of the unground parts of the plants were made up

as shown in the following table. After each sample was ground and carefully mixed, mydriatic alkaloid determinations were made.

TABLE 15.—TABLE SHOWING THE EFFECT OF THE PRESENCE OF STEMS UPON THE TOTAL ALKALOID CONTENT OF BELLADONNA.

Sample No.	Kind of material.	Mydriatic alkaloids.
1	Leaves of plants Nos. 1 and 2	0.466
2	Leaves of plants Nos. 3 and 4	0.488
3	Leaves of plants Nos. 5 and 6	0.434
4	Leaves of plants Nos. 1 and 2 + 10% Stems of plants Nos. 1 and 2	0.508
5	Leaves of plants Nos. 3 and 4 + 10% Stems of plants Nos. 3 and 4	0.513
6	Leaves of plants Nos. 5 and 6 + 10% Stems of plants Nos. 5 and 6	0.459
7	Leaves of plants Nos. 1 and 2 + Stems of plants Nos. 1 and 2 in prop. 67.4 : 32.4	0.600
8	Leaves of plants Nos. 3 and 4 + Stems of plants Nos. 3 and 4 in prop. 66.0 : 34.0	0.508
9	Leaves of plants Nos. 5 and 6 + Stems of plants Nos. 5 and 6 in prop. 64.9 : 35.1	0.479
10	Stems of plants Nos. 1 and 2	0.334
11	Stems of plants Nos. 3 and 4	0.291
12	Stems of plants Nos. 5 and 6	0.216

The results of the above experiment demonstrate the fact that all parts of these belladonna plants showed a high content of mydriatic alkaloid. The alkaloid determination of the stems in but one case showed a content of an appreciable amount below the U. S. P. requirement of 0.30 percent. It is seen that the results of samples Nos. 4, 5 and 6, which were made allowing the presence of the maximum amounts of stems which the U. S. P. requirement ordinarily permits (that is, if stems are considered as foreign matter), and Nos. 7, 8 and 9, in which cases there was 33% stems used, run as high or higher in mydriatic alkaloid than did the samples of leaves Nos. 1, 2 and 3. Theoretically it would seem that Samples 7, 8 and 9 should be considerably lower in alkaloid content than Nos. 1, 2 and 3, due to the presence of the stems, but practically, on taking samples for analysis, deviations of this kind would enter into such determinations, because of taking unground parts of the plants, conforming as nearly as possible to the practical condition. The results with these plants show conclusively that the stems can be harvested and used in conjunction directly with the leaves as they exist in the field without danger of the final product falling below the U. S. P. requirement.

SUMMARY.

The following is a brief summary of the results of experiments carried out in this work.

1. Under ordinary conditions, using good viable belladonna seeds in soil, about 7 percent will germinate in three weeks and 10 percent in five weeks.

2. Freezing belladonna seed at —12° C. for six hours does not appreciably hasten or increase the germination in soil.

3. Increasing the humidity of soil increased the germination of belladonna seed about 4 percent in 2 and 3 weeks, but it encouraged the development of "damping off" fungi in unsterilized soil.

4. Sterilizing the soil in the autoclave encouraged the germination of about twice the ordinary number of belladonna seeds and prevented the destructive effects of the "damping off" fungi.

5. Most of the plants resulting from the seeds which germinated in the first three weeks after the seed is planted will have reached a large enough height to transplant into small pots in five weeks.

6. When using ordinary compost soil, to which no inorganic salts are added, three months after potting the plants will be large enough to transplant into the field. When inorganic salts, such as $NaNO_3$, K_2SO_4 and $Ca(H_2PO_4)_22H_2O$ are added to the same compost soils, it will not be necessary to grow them in the pots for as long a period as three months before planting in the field.

7. In forcing small belladonna plants, when growing them in the small pots, to secure suitable plants for the field, applying a combination of 3000 pounds $NaNO_3$, 1200 pounds K_2SO_4 and 2400 pounds $Ca(H_2PO_4)_22H_2O$ on an acre basis of 2,000,000 pounds, gave the best results in cases where a clay-loam compost was used and also where sand was employed.

8. Growing belladonna in sand to which no nitrogen fertilizers were supplied resulted in one-half to one-third the yield which was obtained when nitrogen was supplied. There was little difference between the growth of belladonna when nitrogen was applied in the form of $NaNO_3$ or $(NH_4)_2SO_4$.

9. The application of $CaCO_3$ did not seem to be an effective factor in increasing the growth of the belladonna plant in sand.

10. Applying inorganic fertilizers to a clay loam soil was very effective in producing increased yield. Where the complete fertilizer was added there was $3^1/_2$ times as much growth as where no fertilizers were applied.

11. Two-thirds as much weight, of leaves and stems, was harvested where moisture to the extent of one-half the physical optimum of the soil was applied as was produced where the conditions of moisture were at the optimum. Applying moisture of $1^1/_2$ times the optimum produced less than one-third of the growth where the optimum was employed.

12. Shading belladonna plants with gauze resulted in the growth of about one-half as much weight of leaves, stems and roots as was obtained in unshaded plants.

13. Colorado potato beetles were controlled on belladonna plants by applying Paris green 1 : 100, or lead arsenate 5 pounds to 100 gallons and no spray injury was realized.

14. "Nicotine sulphate" (Black Leaf 40), $^3/_4$ pint in addition to 4 pounds of soap per 100 gallons of water was effective in destroying and controlling aphids on belladonna.

15. To secure the most desirable product, leaves of belladonna should not be dried at a temperature higher than 55–60° C.

16. An ordinary belladonna plant will easily yield 28.0 Gm. (approximately 1 ounce) of viable seed, if collected at the proper time.

17. Under field conditions the proportion of leaves to stems of belladonna is about 2 : 1.

18. Alkaloid determinations of samples of belladonna made with 90-percent leaves and 10 percent stems, which is the largest amount of stems that the U. S. P. permits, were not any lower in mydriatic alkaloid than samples of leaves. Likewise, samples containing $33^1/_3$ percent stems and $66^2/_3$ percent leaves were no lower in alkaloid than the samples made of leaves.

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SUBJECTS.

Study of the Germination of Belladonna Seeds.
Study of the Planting of Belladonna.
Effect of Fertilization upon the Growth of Belladonna Plants.
Effect of Moisture upon the Growth of Belladonna Plants.
Effect of Shade upon the Growth and Development of Belladonna Plants.
Study of the Means of Combating Insects on Belladonna Plants.
Study of the Effect of Drying Belladonna Leaves at Different Temperatures upon the Alkaloid Content.
Study of the Production of Seeds by Belladonna Plants.
Influence of the Presence of Stems upon the Alkaloid Content of Belladonna Leaves.
Summary.
Bibliography.

A STUDY OF VARIOUS PNEUMOCOCCIDAL SOLUTIONS FOR MOUTH WASHES.*

BY S. SOLIS COHEN, M.D., AND EDWARD STEINFIELD, M.D.

Dochez and Avery,[1] Stillman[2] and Sydenstricker and Sutton[3] have demonstrated that infection in pneumonia occurs by way of the upper respiratory tract, through contact with pneumonia patients, convalescents or carriers, harboring virulent fixed strains of pneumococci in the mouth.

At the request of one of us (S. S. C.) Kolmer and Steinfield[4] investigated the use of specific pneumococcidal substances such as ethylhydrocuprein hydrochloride, quinine and urea hydrochloride, quinine bisulphate and other cinchonics incorporated in a mouth wash. They found that ethylhydrocuprein was effective as a pneumococcicide in solution so dilute as 1 : 160,000, and the quinine salts in solutions of about 1 : 20,000. The strongest solution they could induce patients to use was 1 : 10,000. They recommend, accordingly, the use of quinine hydrochloride 1 : 10,000 in the "Liquor Thymolis" of the House Pharmacopoeia of the Philadelphia Polyclinic, as a means of diminishing the chances of infection in nurses and physicians and minimizing the dangers of spread by carriers. In their experiments, however, this solution could not keep the mouth and throat secretions continuously sterile.

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* From the Clinical Laboratories of the Jewish Hospital, Philadelphia.