Scientific Section

Papers Presented at the Sixty-First Annual Convention

INDIVIDUAL VARIATION IN BELLADONNA PLANTS AS A BASIS FOR IMPROVEMENT BY SELECTION.

A. F. SIEVERS, CHEMICAL BIOLOGIST, OFFICE OF DRUG PLANT INVESTIGATIONS, BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE.

The improvement of medicinal plants through the methods and principles of plant-breeding is beginning to receive considerable attention. The quality of many of our important drug plants as found on the market is far from satisfactory and the inability to secure drugs of sufficient strength has in some cases necessitated the lowering of the Pharmacopœial standard. Such a procedure was followed at the eighth decennial revision of the Pharmacopæia with regard to belladonna and stramonium. The supply of wild belladonna is rapidly becoming exhausted and the demands of the drug trade will soon have to be supplied from the cultivated plant. It is of great advantage, therefore, at this time to make concerted efforts toward securing a type of belladonna plant which, when extensive cultivation of the plant is finally resorted to, will produce a product which is of satisfactory and consistent medicinal strength.

With this object in view, the Office of Drug Plant Investigations of the U. S. Department of Agriculture has had in progress for several years a thorough investigation of the belladonna plant under cultivation with special reference to the variation of the alkaloidal content of the plants with a view toward obtaining data so that through the methods of plant-breeding or selection it might be possible to establish a desirable type of plant. This work is now being carried through the third season, and, while only a beginning has been made, the results are sufficiently illuminating to be worthy of presentation.

The investigation has been conducted mostly at the Arlington Experimental Farm at Arlington, Va. Some additional work has been done at the stations in Wisconsin and Maryland, but the largest number of plants have been under observation at Arlington. After some preliminary work, the investigation was finally started with fifty-nine individual plants in the spring of 1911. The plants were then in their second year. They were carefully watched as to growth and general development, and at five different stages samples of leaves were gathered from each plant. The leaves were taken from all parts of the plant, so as to insure a representative sample. After carefully drying the leaves in a well-ventilated room they were placed in light cloth bags until assayed.

The question of assaying required some special attention in order to devise a method suitable for assaying such small samples as those to be dealt with in this

work. Some samples weighed as little as four grams, dry weight, and since all assays were made in duplicate it was necessary to have a method applicable to two grams of material. After considerable experimentation, the Pharmacopœial method in a modified form was adopted. The principal modification of the method consisted in the use of much greater quantities of menstruum for the extraction of the alkaloids, experience having shown that the quantity recommended in the Pharmacopœia is not sufficient.

The stages of growth at which the leaves were gathered were as follows: The first, during the early part of May before the flowers appeared; the second, during the last of May when the plants were in full bloom; the third, about the middle of June when the berries were developing and in various stages of maturity; the fourth, early in September when the berries were mostly ripe; and the fifth, about the middle of October when the plants had grown considerable new leaves.

In a paper of this kind it is, of course, impossible to give all the analytical data of so many individual plants and only a summary will be given. The maximum, minimum, and average percentages of alkaloids in the leaves of these individual plants at each picking in 1911 were as follows:

Picking.	Percent of Alkaloids in Leaves.		
	Maximum.	Minimum.	Average.
	%	%	%
First	.852	.303	.472
Second	.879	.267	.528
Third	.925	.277	.517
Fourth	.908	.311	.633
Fifth	.733	.200	.519

In the following season, 1912, these same plants were again under observation and the leaves were picked at corresponding stages of growth. The maximum, minimum, and average percentages of alkaloids in these plants at the various stages were as follows:

Picking.	Percent of Alkaloids in Leaves.		
	Maximum.	Minimum.	Average.
	%	%	%
First	.869	.404	.601
Second	.747	.292	.503
Third	.882	.328	.553
Fourth	.806	.359	.568
Fifth	.678	.296	.447

It will be noticed that the range of variation in the individual plants is very great. It is this fact that seems to hold forth great promise of the successful application of plant-breeding and selection methods and it was for the purpose of definitely establishing the existence of such great variations that this investigation was started.

Another fact of vital importance has been established by the two years' work. It has been found that, in a general way, the plants which are very rich or very poor in alkaloids one year will display the same characteristic the following year. Furthermore, these same characteristics are evident at each picking throughout the season. Out of the fifty-nine plants examined, forty were found to retain their characteristics as regards alkaloidal content through both seasons. A few especially characteristic individuals may be given here in detail. Thus No. 3 assayed .384 percent, .375 percent, .277 percent, .549 percent, and .451 percent alkaloids at the different stages in 1911, and .393 percent, .448 percent, and .448 percent in 1912. Again, No. 39 assayed .303 percent, .262 percent, .327 percent, .614 percent, and .451 percent, in 1911, and .404 percent, .365 percent, .525 percent, and .600 percent in 1912. On the other hand, plant No. 7w assayed .558 percent, .831 percent, .832 percent, .727 percent, and .575 percent in 1911, and .782 percent, .666 percent, .646 percent, and .694 percent in 1912. Again, No. 6w assayed .596 percent, .879 percent, .925 percent, 711 percent, and .722 percent in 1911, and .847 percent, .882 percent, .804 percent, and .558 percent in 1912. These four plants illustrate what has been said in regard to the consistency of the individual type from season to season. Plants No. 3 and 39 are manifestly of poor medicinal quality, as compared with the average, while plant No. 6w and plant No. 7w are of exceptional quality. It is plants like the last two named which are being used as a basis for the propagation of a desirable type of belladonna. After the selection of a desirable parent plant, new generations are secured from these through seed or cuttings. The flower of the belladonna plant is usually cross-pollinated, so the question of close pollination must be considered. Thus far, only plants from cross-pollinated seeds have been tested. These plants made their first season's growth last year. Two pickings of leaves were made. These plants were not tested individually, but the leaf samples were taken collectively from all the plants secured from the same parent. It is interesting to note that the plants from the seed of No. 7w and No. 6w showed a high alkaloidal content. Thus plants from No. 7w averaged for the two pickings .804 percent, and those from No. 6w averaged 1.043 percent. It will be seen then that the first generation plants from the two richest parent plants selected showed conspicuous richness in their first season, even though the seeds from which they grew had probably been formed from cross-pollination. These plants, as well as many others, are being carefully observed and tested individually this year, 1913, and it is hoped that a few of very great medicinal strength will be obtained. At the same time, other first generation plants which were grown from inbred seeds from the same parent plants as those above are being grown this year and their leaves will be tested as soon as they have attained a suitable growth. The matter of propagation from cuttings is also being taken up in detail this year.

The plant breeder, in striving to evolve a valuable type of economically important plant, must reconcile two factors. The plant must yield a valuable product and at the same time it must yield enough of this product to make its production a commercial possibility. The problem with belladonna does not differ in this respect. If we are to depend on cultivation for its supply, then such cultivation must be made fairly profitable. The problem, therefore, be-

comes more difficult in that the desirable plant is not one which shows great medicinal qualities alone, but one which at the same time produces an abundance of leaves and roots. In the several years during which this work has been progressing, nothing has been found to indicate that any relationship exists between the physical appearance of the plant and its alkaloidal content. While no such general relationship may exist, there is always the possibility, however, of some of those unusually rich plants possessing at the same time the desired physical excellence which would make them the most valuable type from both the medicinal and the agricultural standpoint.

Much has been said and written concerning the production of alkaloids in belladonna. The influence of various soil constituents, excessive rainfall and drought, sunlight and shade have all been the subject of repeated investigations and the conclusions reached do not lack in variation. Of what value are all such investigations when the range of variation in individual plants is frequently greater than the difference in alkaloidal content attributed to different fertilizers or different climatic conditions? Until a type of plant is found which is at least fairly constant in the quality of alkaloids it produces, vital conclusions cannot be drawn from experiments such as those mentioned. The factor of individual variation must first be eliminated before the influence of environment can be definitely determined.

THE PREPARATION OF PURE SUCROSE AND DEXTROSE CARAMELS.

GEORGE D. BEAL AND HARPER F. ZOLLER, URBANA, ILL.

The attention of the authors was first directed to the preparation of caramels by the failure of the qualitative tests for caramel in a number of liquids to which it had been added as a coloring agent. In order to study these reactions we set out to prepare pure caramel and observe its behavior. A review of the literature on the subject interested us in the composition of caramels, and it is on this account that we have studied carefully the preparation of the caramels in the purest condition.

The recent communications of Beringer and others have induced us to report our findings to this section in the hope that they may be of some assistance. In nearly all papers caramel has been considered as a mixture of the products of the action of heat on sugars. Prof. Beringer has recently given us a method for the preparation of a purified caramel based on the use of alcohol and sodium carbonate, the latter to dissolve any water insoluble products. In our method we have avoided the formation of this insoluble product by a shorter time of heating, rendering unnecessary the use of the alkali.

Caramel may be defined as an intermediate product in the decomposition of a carbohydrate by heat, and its composition varies according to the source. This will be taken up in a later paper, now in preparation. In addition to the caramel a large amount of vapor is formed, consisting in part of water, formalde-