BREEDING TOBACCO FOR HIGH AND LOW NICOTINE CONTENT.

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THE RELATION OF NICOTINE TO THE QUALITY OF TOBACCO.

Nicotine has long been regarded as the characteristic alkaloid of tobacco, but what part, if any, it plays in the nutrition of the plant is not known. The physiological effects on the human system resulting from the use of tobacco are undoubtedly due to the nicotine, but experiments have repeatedly shown that the burn, flavor, aroma and other important qualities are in no sense proportional to the amount of nicotine present. Nicotine thus appears to bear essentially the same relation to tobacco that alcohol bears to alcoholic beverages, for while the physiological action of the latter is due to the alcohol, this does not affect their flavor.

Some individuals, however, are much more sensitive to the effects of nicotine than are others and for this reason numerous attempts have been made to devise a process for the partial removal of the nicotine from tobacco by artificial means. A very large number of patents have been issued, especially in Germany, for processes supposed to accomplish this result, but thus far none of these have met with any considerable success commercially. The flavor and aroma of tobacco are comparable in delicacy to those of tea and coffee, and even the mildest treatment for the removal of the nicotine is almost certain to injure these qualities.

There can be no doubt that there is a genuine demand for tobacco containing only a very small quantity of nicotine, provided the other attributes of the best grades of the crop can be retained. The most likely means of attaining this object would seem to lie in the systematic breeding of types characterized by their low nicotine content, together with the proper control of environmental influences. With this object in view, experiments have been carried out during the past two years in connection with the tobacco breeding investigations of the Bureau of Plant Industry.

VARIATION IN THE NICOTINE CONTENT OF TOBACCO.

Nicotine is present in the plant from its earliest stages of growth, and is found in the roots, stalks, stems, and leaves. As the development of the plant proceeds the percentage of nicotine in the leaf increases,

reaching its maximum at maturity. The amount of the alkaloid contained in the leaves of tobacco from different sources varies very widely, amounting to more than 5 per cent in some cases, while in others it falls below 1 per cent. Our breeding experiments have served to demonstrate that the question of the relative nicotine content is a complex one, and the amount of alkaloid produced and the amount contained in a given plant is greatly influenced by a variety of circumstances. The more important factors, other than heredity, concerned in this question will be mentioned in a succeeding paragraph.

When it is desired to compare the relative nicotine content of different types or of selections within the type, the external influences of soil, climate, fertilizers, etc., must be rigidly controlled. Our experiments tend to show that apart from the effects produced by these external conditions there is a distinct influence of heredity in operation and that by properly controlling extraneous factors, it is possible to secure strains showing decided uniformity and characterized by a high or low nicotine content, as the case may be. In the case of thoroughly acclimated types showing uniformity in other characters, the variation in nicotine content from the mean value among individuals rarely exceeds ten per cent of the total.

We have worked with a number of hybrids and have obtained variations in the first generation usually amounting to from 40 to 60 per cent. In the second generation, these variations seem to increase, often amounting to 100 per cent, and in the third and even the fourth generations the variation continues. For our purposes it has been necessary to consider a number of different types and hybrids so that it has not been possible in any one case to study a sufficiently large number of individuals to determine whether such laws as Mendel's can be applied.

INFLUENCE OF ENVIRONMENT ON THE PRODUCTION OF NICOTINE.

Early in our studies on nicotine content, it became apparent that a number of factors come into play which must be taken into account in any systematic breeding with reference to this property. First of all must be considered the distribution of the alkaloid in the parts of the plant so that samples used for analysis may be truly representative. Of the parts of the plant the leaves contain most nicotine, while the upper leaves contain more than the lower ones and sucker leaves contain least of all. The question of fertilizers needs to be considered, especially those containing nitrogen, but this phase of the problem has not been investigated.

The effect of changing the latitude on the nicotine production has received attention, including the interesting question of whether change from northern to southern latitudes or the reverse exerts the greater influence on the production of nicotine. A great deal of material has been collected for studying this problem, but has not been examined as yet, so that no statement can be made on this point. We have available considerable data bearing on seasonal influences, more particularly the amount of moisture available to the plant during the growing season, and these data show that these influences are surprisingly large. In the case of some strains of high nicotine content, which normally showed marked uniformity in characters, having been grown in the same locality for many years, the average nicotine content of the plants grown in the season of 1907 was about one-half of that contained in those grown in 1906.

Another consideration of importance bearing on nicotine production has to do with the cultural methods used in the production of the tobacco crop. The practices of removing the lateral shoots from the plant as they appear, known as "suckering" and of breaking out the bud just before the plant blooms, known as "topping," both influence the amount of nicotine contained in the leaf. No definite statement of the extent of these influences can be made at the present time.

SOURCES OF LOSS OF NICOTINE AFTER HARVESTING TOBACCO.

As has already been stated, the maximum content of nicotine in the leaf is attained at maturity and as soon as the curing begins, a loss of nicotine sets in which continues indefinitely. There are two causes for this progressive loss of nicotine, one of a purely physical nature, while the other is a chemical one. In the course of our investigations on this problem, it has been found that a portion of the nicotine is in combination with malic and citric acids, forming comparatively stable salts which do not readily volatilize. A second portion of the nicotine, however, occurs in the form of very weak salts, which volatilize very readily. This second portion begins to escape in the early stages of the curing process and is especially marked during the subsequent fermentation due to the rise in temperature. Loss from this source continues throughout the aging period, but at a decreased rate.

Aside from the loss of nicotine by simple volatilization, a portion of the base is slowly oxidized forming dark colored tarry products. This chemical change is a slow but continuous one and is of special importance

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in the case of old tobaccos. Exposure to moisture and light seems to hasten this change. It is evident that these facts must be taken into account in studying the relative nicotine content. Samples of leaf which have been cured by the use of artificial heat should not be compared with those cured without heat nor should those which have been fermented be compared with those unfermented. All samples should be examined as soon after curing as possible. The amount of nicotine lost during the curing process has never been determined but it must be considerable, even when no heat is used. The loss during the fermentation process has been found to amount to as much as a third of the total.

SOME RESULTS OBTAINED IN THE BREEDING EXPERIMENTS.

It is hardly necessary to state that results thus far obtained are principally of a preliminary nature, since results of final character can at best be obtained only from experiments continued for a number of years. At the present time there are not available sufficient data for establishing any definite correlation between visible field characters and the nicotine content of the plant, so that it has been necessary to resort to chemical analysis to obtain the necessary information. The first problem, therefore, was to devise a laboratory method of analysis which should be both rapid and accurate and this has been accomplished.*

The occurrence and distribution of nicotine in the different parts of the plant and especially the relative amounts contained in the leaves on different parts of the stalk has been investigated. This information is necessary for guidance in drawing representative samples for analysis. The effects of topping and suckering are now being studied. A matter of prime importance in breeding tobacco is due consideration of the influences of various factors included under the term environment on the character of the product. With reference to the content of nicotine, our experiments on this problem are not complete, but results already obtained emphasize the necessity of eliminating or controlling these extraneous factors in carrying on the breeding intelligently.

In addition to the factors influencing the production of nicotine in the plant, consideration must also be given to the sources of loss of this constituent during the curing and subsequent handling of the leaf to avoid erroneous conclusions.

*U. S. Department of Agriculture, Bureau of Plant Industry. Bulletin 102, Part vii.

Much data has been collected which goes to show that, apart from the various influences mentioned above, heredity plays a fundamental part in the production of nicotine in the individual. Strains have been obtained which are characterized by high and low nicotine content, respectively, and it now remains to determine whether these characteristics can be fixed under practical conditions and yield high grade products.

TOBACCO SEED SEPARATION, SELECTION AND PROPAGATION.

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The tobacco industry of Connecticut is one of the most profitable as well as the most unprofitable industries of the state. In 1896 we commenced selecting seed plants to produce a better shaped leaf as well as more leaves to the plant, with varied success. One year we would think we had made great progress while the next year would meet with complete failure. In 1903 we commenced saving seed under a paper bag. The next season we could see some gain, but there were still a good many small and undesirable plants which materially reduced the weight of the crop as well as showed up badly at the time of sale. On examining the seed closely a great many small seeds which were light in weight and low in vitality and very slow in germinating were found. Some seed would not germinate until the following spring, so it was possible to set plants from seed sown the year previous.

In the winter of 1904 I made a machine for separating the seed. This apparatus takes out all the light and undesirable seed and retains the heavy ones. This separation is done by placing the seed in a funnel shaped hopper at the top of the machine where they are fed into a tube which takes them down into a glass tube where they pass on to a spreader and into an air blast. There the light seed is blown out over the top of the tube, while the heavy seed is caught in a receiving funnel below and pass out of the machine through a tube at the bottom. In the spring of 1904 in sprouting my seed for sowing (which had been separated) I was greatly surprised to find it all sprouting at the same time, whereas I had been accustomed to having it sprout a few seeds at a time extending over several days, while with separated seed it all sprouted within twenty-four hours of the time the first seed sprouted and in from two or three days the bed was all green with plants. From 1904 up to the present time I have had no trouble with small undesirable plants and but little difficulty with what are called calico plants. The weight