BREEDING LOW-NICOTINE TOBACCO[1]

By W. D. VALLEAU

Plant pathologist, Kentucky Agricultural Experiment Station

INTRODUCTION

In 1933 the Kentucky Agricultural Experiment Station received from J. B. Hutson, of the United States Department of Agriculture, then stationed in Germany, seven lots of Havana and Cuba cigar tobacco varieties low to very low in nicotine content. These seeds had been obtained from Dr. Erwin Baur, director of the Kaiser Wilhelm Institute. So far as the writer was aware, there were at that time no varieties of cigarette or pipe tobacco of low nicotine content. It was decided, therefore, to develop a variety or varieties of cigarette tobacco of low nicotine content to meet a possible demand for such a tobacco as a health measure. It was further decided that this variety should carry the recessive color factors of burley because burley is a light-colored tobacco when cured and light color is commonly associated in the mind of the public with mildness.

MATERIALS AND METHODS

Three strains of Cuba, designated 30/33, 31/33, and 32/33, each listed as very low in nicotine, were used as the source of the low-nicotine character. In 1934, analyses of these varieties showed that they contained 0.19, 0.05, and 0.11 percent nicotine, and in 1935, 0.18, 0.22, and 0.21 percent, respectively. These varieties were crossed with Ky 5, Ky 7, Ky 14, and Ky 16—all black-root-rot-resistant varieties of burley tobacco. In 1934 these varieties analyzed 2.18, 2.10, 1.37, and 2.18 percent nicotine, respectively.

All nicotine determinations were made on leaves from the middle of field-grown plants as these leaves are usually the highest in nicotine in tobacco harvested and cured like burley. During the past several years the tobacco has been grown on more fertile soil than in the early years, and the nicotine content of Ky 16, used as a control, has been higher. From 1934 to 1942 the nicotine determinations were made by members of the Department of Chemistry of the experiment station, who used the method of the Association of Official Agricultural Chemists (1, *p.* 64).[2] In 1943 and 1944 Markwood's (*10*) green disk method was used and determinations were made by E. M. Johnson. Since then the determinations have been made under the

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[2] Italic numbers in parentheses refer to Literature Cited, p. 180.

direction of R. B. Griffith who has used a rapid modification of the A. O. A. C. method (*6*). In 1947 determinations were made by the method of Bowen and Barthel (*3*).

EXPERIMENTAL RESULTS

CROSSES

The F_1 generation of the dark cigar tobacco by the light-colored burley was all dark, as would be expected. A single determination of nicotine in an F_1 30/33 × Ky 7 showed that it had 3.18 percent when Ky 16 had 1.73 percent and the low-nicotine parent had 0.22 percent. This suggests that the high-nicotine factor is completely dominant over low nicotine. The difference in nicotine content between the F_1 hybrid and Ky 16 is not significant.

The F_2 generation segregated with respect to plant color, producing approximately 15 dark plants to 1 light-colored plant. There was also segregation with respect to nicotine content, with a range from plants with no nicotine to 2.82 percent in a total of 345 burley-colored plants tested (table 1).

TABLE 1.—Nicotine content of burley-colored F_2 plants from low-nicotine cigar tobaccos crossed with burley, 1935

Nicotine (percent)	31/33×Ky 5	30/33×Ky 7	31/33×Ky 14	30/33×Ky 16	32/33×Ky 14	Total
0	2	3	4	2	14	25
.1	14	11	20	10	11	66
.5	9	20	23	26	21	99
1.0	22	17	22	18	12	91
1.5	11	15	4	6	3	39
2.0	10	8	5	2	0	25
Total plants	68	74	78	64	61	345

There appears to be no sharp segregation into low-nicotine and high-nicotine content plants as claimed by Hackbarth and Sengbusch (*9*), who concluded that the nicotine content of tobacco is dependent upon a single factor pair. However, these results do not disprove their contention, for with as variable a factor as nicotine, considerable variation might occur within any one class so that the three classes, homozygous low, heterozygous, and homozygous high, might run together. There are also the complicating factors of nicotine and nornicotine. It will be shown later that in some segregates much of the nicotine seems to change to nornicotine, some of which is determined as nicotine by the A. O. A. C. method.

In 1936 the F_3 generation of some of the very low nicotine plants from the previous year were grown. Of these a few were selected for plant type and nicotine determinations made. The results are given in table 2.

It is evident that burley-colored plants low in nicotine in the F_2 generation do not always produce plants of equally low nicotine content in the F_3 generation; but the F_3 plants from selected low-nicotine F_2 plants are sometimes all relatively low in nicotine and on an average are much lower than burley.

TABLE 2.—*Nicotine content of F_2 plants and of F_3 burleylike plants of low nicotine × high nicotine hybrids*

F_3 generation of cross—	Nicotine content of F_2 plants	F_3 plants	Range in nicotine content of F_3 plants	Average nicotine content of F_3 plants
	Percent	*Number*	*Percent*	*Percent*
30/33×Ky 7	0.07	4	0.24–.30	0.27
31/33×Ky 14	.01	1	.02	.02
Do	.06	5	1.00–1.56	1.23
Do	.07	5	.32–.98	.70
32/33×Ky 14	.03	5	.46–1.77	1.34
Do	.06	7	.07–1.56	.66
30/33×Ky 7	.01	7	.03–1.11	.60
Do	0	6	1.15–1.78	1.45
32/33×Ky 14	.02	7	.16–.98	.48
Do	.02	9	.52–3.60	1.78
Do	.02	14	.13–2.45	1.26
Do	.02	5	.28–.46	.38

In 1937 determinations of nicotine in seven F_4 plants of a strain from a 0.02-percent F_3 plant showed all to contain 0.05 percent or less; progeny of a 0.07-percent plant contained less than 0.3 percent; progeny of a 0.02-percent plant contained 0.45 percent or less; while in some groups of plants from very low nicotine parents the nicotine content ranged from 0.20 to 2.45 percent. The results seemed to show, however, that extremely low nicotine content strains of large-leafed tobacco having the color of burley could be isolated.

BACKCROSSES

In 1936 it became evident that it would not be possible to select desirable types of tobacco without further backcrossing. Therefore, several backcrosses were made in which Ky 16 was used as the pollen parent and progeny of several very low nicotine plants were used as the seed parents. There was no assurance, however, that the seed parents were all low in nicotine. The next year a good type plant of the F_2 backcross, later shown to contain 1.08 percent nicotine, was crossed with several F_3 32/33×Ky 14, the parent of which tested 0.02 percent nicotine, and with F_4 31/33×Ky 14, of which seven were tested for nicotine and the highest was found to contain 0.05 percent. Another F_2 segregate containing 3.25 percent nicotine was also used (table 3).

TABLE 3.—*Result of crossing F_2 segregates of low-nicotine burleylike plants × Ky 16, with low-nicotine plants*

Nicotine content of parents (percent)	Plants tested	Nicotine content	
		Range	Average
	Number	*Percent*	*Percent*
0.03×1.08	1	0.05	
0.01×1.08	19	.01–.60	0.27
0.01×1.08	19	.01–.58	.06
0.06×1.08	13	.02–1.04	.36
0.13×1.08	13	.01–1.21	.21
0.07×3.25	17	.05–2.09	1.49
Progeny of 1.08 percent plant used in above crosses	15	.29–.89	.49
0.45-percent —— selection of 1.08-percent plant	6	.71–1.05	.88
0.33-percent —— selection of 1.08-percent plant	3	.45–.94	.74

The results of these backcrosses indicate that a plant testing as high as 1.08 percent, when crossed with established low-nicotine strains, produces progeny low in nicotine; but where a high-nicotine plant possibly carrying a factor or factors for low nicotine was used as one parent the F_2 progeny ranged from 0.05 percent to 2.09 percent, with an average of 1.49 percent nicotine.

In 1939, F_3 progeny of plants crossed with the 1.08-percent nicotine plant (table 3) were grown from F_2 plants testing 0.06 percent nicotine or less. Of 52 F_3 plants tested, the nicotine content ranged from 0.01 percent to 2.41, with an average of 0.34 percent. Progenies of several plants were all below 0.1 percent, indicating that very low nicotine strains could be selected following backcrossing with a high-nicotine parent.

In 1940, eight F_4 progeny of a 0.02-percent F_3 plant averaged 0.025 percent nicotine. A selection with 0.9 percent nicotine from the 0.07- × 3.25-percent nicotine cross (table 3, line 6) gave progeny (seven tested) ranging from 1.06 to 1.59 percent nicotine, with an averge of 1.29 percent, a figure definitely below the nicotine content of burley tobacco grown under the same conditions.

In 1940, the F_2 generation of a second backcross with Ky 16 on a 0.02-percent plant was grown. Nicotine content ranged from 1.11–4.50 in 26 plants tested. While the majority were high, 4 contained less than 1.75 percent nicotine. Four F_3 plants of the plant testing 1.11 ranged from 0.71 to 1.20 percent nicotine.

In 1942, plants from 10 low-nicotine selections from the F_5 generation of crosses listed in table 3 and F_3 second backcrosses of low-nicotine plants of these with Ky 16 were grown. In all, 80 plants were tested for nicotine content. Nicotine ranged from 0.01 percent to 0.90 percent, when Ky 16 grown under the same conditions contained 3.8 percent. The average nicotine content of the 80 plants was 0.246 percent, showing that low-nicotine strains could be isolated after 2 or 3 crosses with burley.

NICOTINE CONTENT OF CROPS OF LOW-NICOTINE TOBACCO

Four small commercial crops of low-nicotine burleylike tobacco were studied for nicotine content in 1945. They were grown on fertile soil and all made rapid, vigorous growth. Nicotine determinations were made of individual seed plants and the nicotine content of a composite sample of a middle leaf from each of 25 topped and suckered plants was determined. A few upper leaves had been stripped from the seed plants, and the plants had been suckered. Samples were collected at the time the remainder of the crop was cut, taking 4 leaves from near the center of the plant from each seed plant and 1 similarly placed leaf from each of 25 topped and suckered plants. Nicotine determinations were made by the quick method of Griffith and Jeffrey (*6*) after the tobacco was air-cured. Distillations were made from MgO and from NaOH–NaCl.

Three of the strains appear to have been low-nicotine strains (table 4), while one (S 2) apparently contained an occasional plant of higher nicotine content that increased the average nicotine content of this strain above that of the others.

TABLE 4.—*Alkaloid determinations of 4 crops of low-nicotine tobacco, 1945*

Strain	Seed plants	Range in nicotine content determined with MgO	Average	Range in nicotine content determined with NaOH-NaCl	Average
	Number	*Percent*	*Percent*	*Percent*	*Percent*
S 1	25	0.002–0.435	0.109	0.002–0.862	0.231
S 2	24	.001–1.375	.498	.017–1.414	.670
S 3	25	.031– .352	.130	.055– .932	.402
S 4	24	.051– .334	.140	.059– .874	.394
S 1	Composite of 25 topped plants.	.035	----------	.276	----------
S 2		.675	----------	.875	----------
S 3		.173	----------	.489	----------
S 4		.125	----------	.427	----------

A half-acre crop of another strain was grown in 1946 for Dr. H. B. Haag, of the University of Virginia, for his pharmacological studies with low-nicotine tobacco. Dr. Haag reported that after redrying, a thieved sample from a hogshead gave, on analysis, 0.08 percent nicotine, 0.06 percent nornicotine, and 0.14 percent total alkaloids. A previous half-acre crop of low-nicotine tobacco raised for Dr. Haag for studies on the role of nicotine in the cigarette habit contained 0.23 percent nicotine (*4*).

There seems to be no doubt from these results that commercial crops of low-nicotine tobacco very low in nicotine content can be grown under ordinary field conditions.

NICOTINE AND NORNICOTINE CONTENT OF LOW-NICOTINE TOBACCO

In 1945 and 1946 a modification of the A. O. A. C. method was used for nicotine determinations with the quick distillation apparatus of Griffith and Jeffrey (*6*). Distillations were made with both MgO and NaOH-NaCl. There is a marked difference in alkaloid content as determined by these methods. So far as Griffith has been able to determine, MgO distillation gives a high value for nicotine since 35 to 40 percent of the nornicotine present in pure or mixed solutions is obtained in the distillate. The NaOH-NaCl concentrations used gave the total steam volatile alkaloids as nicotine. The difference between the MgO and the NaOH-NaCl results gives an approximation of alkaloids other than nicotine, usually assumed to be nornicotine The A. O. A. C. method as used in the early studies (*1, p. 64*), according to unpublished work of Griffith, measures nicotine and from 10 to 30 percent of nornicotine or other compounds and so is not accurate for low-nicotine varieties, some of which appear to contain considerable nornicotine. The present A. O. A. C. (*2, p. 74*) method measures nicotine plus nornicotine and so is not as satisfactory for low-nicotine varieties as the older method.

If the MgO values of all low-nicotine selections are arranged in order, from low to high, and divided by the respective NaOH-NaCl values to determine percentage of alkaloids that is largely nicotine, the figure rises from a very low one (less than 7 percent at the 0–0.01 level) to over 90 percent at the 3-percent level[3] (table 5). That is,

[3] This relationship was called to the writer's attention by Dr. R. N. Jeffrey.

when nicotine is very low the percentage of total alkaloids that is nicotine is correspondingly low, while as the MgO value rises it becomes a greater and greater proportion of the total alkaloids until in ordinary tobacco 85 to 95 percent of the total alkaloid is nicotine or at least is distilled over from MgO.

TABLE 5.—$\frac{MgO}{NaOH-NaCl}$ *values (approximate percent of total alkaloids that is nicotine) in low- and high-nicotine content tobaccos in 1945 and 1946*

MgO value	1945		1946	
	Number of plants	MgO / NaOH−NaCl	Number of plants	MgO / NaOH−NaCl
0. − .01	13	0.0660	9	0.0085
.01− .05	14	.2189	23	.0868
.05− .10	26	.3074	13	.1298
.1 − .5	103	.4641	39	.2530
.5 −1.0	44	.5903	14	.6328
1.0 −1.5	27	.8552	10	.8676
1.5 −2.0	27	.8995	2	.8759
2.0 −2.5	8	.8582	--------	----------
2.5 −3.0	2	.8665	--------	----------
3.0 −3.5	6	.9392	2	.8778
3.5 −4.0	1	.9411	--------	----------
4.0 −5.5	3	.9399	--------	----------

Table 5 also shows that the MgO/NaOH-NaCl value, or approximate percent of total alkaloids that is nicotine, in low-nicotine tobaccos was decidedly higher in 1945 than in 1946.

While in general the proportion of nicotine to total alkaloids in the tobaccos studied increases as the total alkaloid content increases (table 5), this does not mean that strains of tobacco cannot be selected that differ markedly in the relation of nicotine to total alkaloids at any given level of the latter. In the progeny of a cross of a 0.02-percent nicotine content burleylike plant × Turkish (Basma) backcrossed with a 0.02 percent burleylike plant, an F_2 of the backcross was selected that tested 0.43 percent nicotine when plants of Ky 16 at the same stage of growth tested 2 percent. The F_3 progeny of this plant ranged in MgO value from 0.082 to 1.007 (average of 24 plants, 0.412), and in NaOH-NaCl value from 0.193 to 1.131 (average of 24 plants, 0.612). The F_4 plants of selected F_3's showed distinct segregation as far as the MgO value was concerned, while the NaOH-NaCl value for the group was remarkably uniform, ranging from 0.38 to 2.23 percent, with 53 of the 75 plants tested lying between 0.85 and 1.8 percent. The range and average of the MgO value, the average of the NaOH-NaCl value, and the ratio between the two values are given in table 6.

In this table the MgO/NaOH-NaCl values have been arranged in descending order, indicating that strains have been isolated having an NaOH-NaCl or total alkaloid content of about 1.5 percent but an MgO value or approximate nicotine content ranging from 94 percent of the total alkaloid content to only 16.6 percent.

TABLE 6.—*Alkaloid content of strains of tobacco resulting from a cross of low-nicotine burleylike tobacco with Turkish tobacco, and Ky 16, as determined by distillation with MgO and NaOH-NaCl, 1946 crop*

Plants (number)	MgO value		NaOH-NaCl average	MgO/NaOH-NaCl
	Range	Average		
	Percent	*Percent*	*Percent*	*Percent*
7	0.58 -1.17	0.89	0.94	0.9408
7	.91 -1.85	1.44	1.59	.9034
3	.19 - .64	.47	.58	.8057
8	.401-1.58	1.06	1.33	.8014
7	.50 -1.10	.77	.98	.7779
4	.18 - .93	.66	.87	.7557
6	.37 -1.50	.94	1.64	.5705
7	.32 - .67	.52	1.21	.4329
9	.11 - .90	.51	1.29	.3945
7	.29 - .71	.49	1.29	.3837
6	.13 - .65	.31	.81	.3830
3	.14 - .27	.22	1.32	.1661
Ky 16	3.08 -3.74	3.30	3.68	.8951

While these results are based on small numbers, they give proof that strains of tobacco closely similar to burley in general characteristics can be isolated that have a total alkaloid content of approximately 1 to 1.5 percent when ordinary burley has about 3.5 percent. The results suggest further that in this range strains can be isolated with a large proportion of the alkaloid in the form of nicotine, while other strains appear to have a large proportion of the alkaloid as nornicotine. Strains of cigarette tobacco that are not likely to produce over 1.5 to 2 percent nicotine under conditions that result in 3.5 percent or more nicotine in ordinary burley may prove of great value to the trade.

In 1947 strains derived either directly from the low nicotine × burley backcrosses or from crosses of low nicotine burleylike plants with Turkish tobacco were grown for nicotine determination. The plants from which samples were to be collected were bagged for seed but were kept suckered. Leaf samples were collected from the middle of the plants, at normal cutting time, and air-cured. Nicotine and nornicotine determinations were made by the Bowen-Barthel method (*3*). Ten plants of Ky 16 and 15 plants each of 19 low nicotine strains were saved for analysis.

The majority of the strains appeared to be fairly homozygous with respect to nicotine and nornicotine. In table 7 the strains are arranged in order of increasing nicotine content. Ten strains proved to be very low in nicotine, the highest averaging 0.05 percent. The other 9 strains ranged from a low average of 0.319 percent nicotine to 0.813. One of the low nicotine strains (range 0 to 0.042) had a range of nornicotine from 0.100 to 1.61 with an average of 0.670. This furnishes the best evidence so far obtained in these studies that strains might be isolated with a relatively high total alkaloid content (about 1 percent) nearly all of which is nornicotine. There is abundant evidence that strains can be isolated with very low nicotine as well as low total alkaloid content.

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TABLE 7.—*Nicotine and nornicotine content of 19 strains of low-nicotine tobacco and Ky 16, grown in 1947, as determined by the Bowen-Barthel method*

Nornicotine		Nicotine		Total Alkaloids		Nicotine Divided by
Range	Average	Range	Average	Range	Average	Total alkaloids
Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
0.005–.058	0.028	0 –.006	0.0004	0.005–.064	0.028	.01
.242–.648	.384	0 –.015	.003	.242–.648	.388	.008
.019–.257	.138	0 –.069	.006	.083–.257	.144	.04
.026–.268	.164	0 –.018	.007	.034–.268	.162	.04
.001–.062	.031	0 –.027	.010	.001–.077	.041	.24
.211–.655	.412	0 –.039	.010	.230–.681	.422	.02
.100–1.61	.670	0 –.042	.013	.116–.64	.683	.02
0 –.299	.049	0 –.065	.014	0 –.305	.063	.22
.195–.582	.379	0 –.349	.044	.207–.544	.422	.10
.046–.201	.128	0 –.254	.050	.098–.300	.179	.28
.008–.098	.052	.256–.447	.319	.298–.506	.371	.86
.042–.373	.144	.165–.615	.391	.337–.832	.535	.73
.035–.373	.154	.137–.880	.527	.280–1.075	.654	.81
.025–.133	.074	.447–.705	.589	.525–.830	.656	.89
.070–.253	.126	.111–.855	.617	.364–1.005	.810	.76
.006–.339	.139	.118–.905	.642	.181–1.174	.777	.83
.043–.248	.106	.381–.946	.663	.556–.995	.770	.86
.062–.159	.122	.475–.883	.690	.690–1.022	.811	.85
.040–.317	.135	.457–1.690	.813	.581–1.852	.944	.86
[1].106–.472	.290	1.08 –1.75	1.457	1.38–1.96	1.760	.83

[1] Ky.16

When the average nicotine content of each strain was divided by the average total alkaloid content the 19 strains were again divided sharply into 2 groups, one with 28 percent or less of the total alkaloids in the form of nicotine and the other with 73 percent or more of the total alkaloids as nicotine. All of the latter group contained over 0.3 percent average nicotine while all of the former group contained an average of 0.05 percent or less. These low-nicotine strains could all be considered primarily nornicotine strains as most of the alkaloid appears to be nornicotine. Four of the strains in the low-nicotine group contained a higher average total alkaloid content than the lowest of the higher nicotine group. These results suggest that in breeding for low-nicotine varieties of tobacco, 2 sets of factors are concerned: (1) those that control total alkaloid production and (2) those that control the change of nicotine in the leaf to nornicotine or other products of nicotine. It is probably desirable to have varieties in which both sets of factors are operating, if the desired product is to be uniformly low in nicotine year after year. If complete dependence were placed on low total alkaloids these would undoubtedly vary from year to year and the nicotine content might be higher than desirable following, perhaps, a dry year. Moreover, if a factor is operating to change nicotine to some other product, the nicotine content of the tobacco will have much more opportunity to remain constant from year to year.

DISCUSSION

The object in developing a low-nicotine cigarette tobacco was to meet a possible demand for tobacco so low in nicotine that it could be smoked, without harmful effects from nicotine, by people who are sensitive to this alkaloid.

As to the immediate effects of nicotine on the smoker, there are at least two: an increase in blood pressure resulting from absorption of nicotine into the blood stream (*7*) and irritation of the mucous membranes with which the smoke comes in contact (*5*). Haag and Larson (*8*) in preliminary tests showed that—

> smoke from the low nicotine cigarettes on an average produced effects comparable to those observed after the smoking of nicotine-free cigarettes, both types of cigarettes evoking circulatory responses, markedly less than those effected by ordinary cigarettes.

Smoke from cigarettes made from low-nicotine tobacco was inhaled by persons with very sensitive throats with only the slightest feeling of irritation, smoke from a low-nicotine cigar with wrapper and binder of the usual type caused distinct but mild irritation, while smoke from an ordinary cigarette caused immediate coughing and left the throat very uncomfortable for several minutes. Cigarettes prepared from low-nicotine tobacco to which about 2 percent nicotine malate had been added (*5*) affected sensitive throats in exactly the same way as an ordinary cigarette. There seems to be little doubt that the immediate irritation of the throat by cigarette smoke is almost entirely caused by its nicotine content.

While the principal object of the present study was to produce a burley-colored cigarette tobacco of low nicotine content, yet the results have contributed something to the knowledge of the inheritance of nicotine-controlling factors. It seems certain, as has been pointed out by others, that high nicotine is dominant over low nicotine in F_1 crosses. In F_2 crosses the problem of classifying the individual plants is complicated by methods of nicotine determination. The A.O.A.C. (*1*, *2*) method is adapted to high-nicotine varieties in which most of the alkaloid is nicotine so that in the early studies not only nicotine but but nornicotine and perhaps other substances were reported as nicotine. While this did not delay work on selection for low nicotine, it undoubtedly contributed to confusion in understanding results from the genetic standpoint. It is obvious that in the low-nicotine tobaccos the inheritance of at least two sets of factors are involved, those concerned with the rate of nicotine production and those that control conversion of nicotine to nornicotine or other products. If the original alkaloid produced is nicotine, then there must be control mechanisms not only over the rate of nicotine production but also over its conversion to nornicotine, or other products.

The present study has demonstrated that the ability to produce only a small amount of nicotine and a low total alkaloid content may be transferred from low-nicotine cigar tobacco to a burleylike variety of tobacco. Assuming that nicotine is the alkaloid produced by the roots of these strains, then in some strains of low-nicotine tobacco there must be a mechanism for changing nicotine to some other product, presumably nornicotine, while, in other strains this ability occurs only to a very slight extent. If the ability to convert a considerable porportion of nicotine to some other product can be transferred to ordinary varieties of high-nicotine content tobacco without disturbing the variety too much otherwise, it is possible that the nicotine content of ordinary tobaccos might be kept at a more desirable level year after year.

The low-nicotine burleylike varieties of tobacco described herein have been recognized by the United States Department of Agriculture (*11*) as a subtype of burley and have been designated Type 31-V.

SUMMARY

Varieties of burleylike tobacco of very low nicotine content have been developed by crossing and backcrossing low-nicotine cigar tobacco with burley. The low-nicotine burleylike tobacco has been recognized by the United States Department of Agriculture as a subtype of burley and has been designated Type 31–V.

First generation crosses between burley and low-nicotine cigar tobacco were high in nicotine. In the F_2 generation there was segregation with respect to nicotine content. The percentage of nicotine ranged from 0 to 2.82, with no sharp line between low- and high-nicotine content. This may have been because both nicotine and nornicotine were reported as nicotine. Progeny of some low-nicotine F_2 plants produced only low-nicotine plants, but this was not always the case.

Commercial crops have been grown with an average nicotine content below 0.10.

In general, when the nicotine content of segregates is very low the percentage of total alkaloids that is nicotine is correspondingly low, while as the nicotine content rises the percentage of total alkaloids that is nicotine also rises until, in ordinary burley tobacco, 85 to 95 percent of the total alkaloid is nicotine. However, evidence was obtained that strains could be isolated with about 1 percent total alkaloid nearly all of which was nornicotine.

When the average nicotine content of low-nicotine strains was divided by the average total alkaloid content 19 strains were divided sharply into 2 groups; one with 28 percent or less of the total alkaloid in the form of nicotine, and the other with 73 percent or more of the total alkaloid as nicotine.

There appear to be two sets of factors controlling the inheritance of nicotine and nornicotine: those that control total alkaloid production, and those that control the change of nicotine in the leaf to nornicotine.

The immediate irritation of the throat by cigarette smoke appears to be caused almost entirely by its nicotine content.

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