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ALKALOID COMPOSITION OF NICOTIANA RUSTICA STRAINS

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Contents

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ALKALOID COMPOSITION OF *NICOTIANA RUSTICA* STRAINS

R. N. Jeffrey 1/

Nicotine is generally considered to be the typical alkaloid of the genus Nicotiana, though it is well known (5)2/ that different species contain different alkaloids. Studies conducted in this (6) and other (2, 7, 8) laboratories since more sensitive means of differentiation of alkaloids have become available, have shown that in *N. tabacum* strains exist which differ in the proportion of nornicotine to nicotine in the green or in the cured leaf. We are not aware of any published report of a similar situation in *N. rustica*, so when samples of a considerable number of strains of *N. rustica* became available in connection with other work of the Tobacco Section, they were analyzed, both in the green state and after curing, to determine whether strains which differed significantly in alkaloid composition either before or after curing were present in this collection.

Certain information concerning the strains grown is given in Table 1. This includes the source of the seed used and some information as to the previous history of the strain. In most instances additional information is available if needed by an investigator, but nearly all samples lead back to some point where the record is incomplete.

Methods

The seed was sown in the greenhouse about the middle of April 1956 in pots, and the seedlings were transplanted into 2-inch thumb pots when large enough. The contents of 12 pots of each strain were planted the end of May in a plot located in the Sunnyside Field on the East Farm at Plant Industry Station, Beltsville, Md. The plants of a given strain were all located in one row with

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2/ Numbers in parentheses refer to Literature Cited, at the end of this report.

no plot replication, but since the entire plot was only 72 x 35 feet, not much variability due to location is to be expected. The planting distance was 42 x 18 inches.

When each strain bloomed it was topped and kept suckered; it was harvested about 4 weeks after topping. The dates of topping and harvest of each strain are shown in Table 1. With a few exceptions, the sample for immediate analysis was taken on the same day that the plants were harvested for air curing. When a large number of strains were harvested the same day, part of the samples for immediate analysis were taken on the preceding or following day to decrease the time between sampling and preservation for analysis. At time of sampling, all 12 of the plants of each strain were examined and any obviously off-type plants were eliminated. A group of 3 or 4 plants covering the range of sizes present was selected for immediate analysis and another group of 7 or 8 plants averaging about the same size was selected for harvest, curing, and analysis after curing.

The plants of the first group were immediately taken to the laboratory, where the leaves were removed and weighed. Subsamples were taken in such a way as to include a proportional weight of leaves from all plants sampled and from all leaf heights; also a proportion of petiole was taken corresponding to its proportion by weight in the original leaf sample. One of these subsamples of each strain was macerated in a Waring Blendor[2] with 50% acetone as previously described (4), and another was used for moisture determination. A portion of the acetone solution was used for paper chromatographic estimation of the kinds and relative amounts of alkaloids present (4) and another portion was analyzed for total alkaloid by pipetting 10 ml. of it into a steam still (1), adding 1 ml. of 1 + 4 HCl, distilling until the acetone was removed, adding 4 ml. 30% NaOH and 1 gm. of NaCl and distilling into a receiver containing 5 ml. of HCl and determining the total

2/ Mention of a product in this paper does not imply recommendation or endorsement by the USDA over others not mentioned.

alkaloid content spectrophotometrically (9). The stalks were also weighed and subsampled so as to include proportional parts of each plant and of each height on the stalk, and similarly used for the determination of moisture, alkaloid quality, and total alkaloids.

The harvested plants were speared onto sticks and cured in a tobacco air-curing barn without heat. They were stripped, air dried by being exposed in a warm room in the winter, ground, and analyzed by the chromatographic method (4), and by direct steam distillation from the powder, using NaOH and NaCl (3), followed by spectrophotometric determination (9).

Results

The results obtained on the leaves are shown in Table 2 and on the stalks in Table 3. The primary purpose of this study was to determine whether any of the available strains of N. rustica possessed the factor for the conversion of nicotine to nornicotine. Whenever an N. tabacum strain or a Nicotiana species has been found to contain a large proportion of nornicotine, the largest proportion of nornicotine to other alkaloids has always been found in the leaves. When the completion of leaf analysis failed to disclose any such strains among those available, the analysis of the stalks was discontinued. Thus, Table 3 is incomplete on the composition of the cured stalks of certain strains.

No claims are made for a high degree of precision for the paper chromatographic methods, but it is evident that in all N. rustica strains included in this study the predominant alkaloid was nicotine, both at time of harvest and after curing. At harvest, when the two determinations were run on the same solution, the average nicotine content by chromatography was 98% of the total alkaloid value. The maximum proportion of nornicotine found in any sample was about 4% of the total alkaloid value. The results given as T (trace) on the freshly harvested samples represent about 0.1% nornicotine or of the order of 1-2% of the total alkaloid.

Table 1. Strains of N. rustica Tested and Certain of Their Growth Characteristics.

Number 56-B-	Source of Strains	Designation	Source of Seed Where Prod.	Source of Seed When Prod.	Date Topped 1956	Date Harvested 1956
101	HHS[1/]	4384	GH[2/]	1943	8/6	9/5
102	HHS	4385 L5-6	GH	1943	8/6	9/5
103	HHS	4386 L5-6	GH	1943	8/6	9/5
104	HHS	4390 L5-2-1	GH	1943	8/6	9/5
105	HHS	4398 L5-2-1	GH	1943	8/2	8/31
106	HHS	4399 L5-2-1	GH	1943	8/6	9/5
107	HHS	43054 L5-2-1	GH	1943	8/2	8/31
108	HHS	43101 L-2-1B x L-6-2-1	GH	1943	8/2	8/31
109	HHS	43102-1 L6-2-1 x L-5-2-1B	"	1943	8/2	8/31
110	HHS	43103-5 L-5-2-1 x L-6-2-1	"	1945	8/6	9/5
111	HHS	43104-1 L-5-2-1B x L-6-2-1	"	1945	8/6	9/5
112	HHS	4401 L-5-2-1B	"	1945	8/2	8/31
113	EGB[3/]	Brasilia #7	GH	1934	8/2	8/30
114	OO[4/]	Brasilia #23	GH	1934	8/2	8/30
115	SPI [5/]	34753 Brasilia	GH	1946	8/6	9/5
116	SPI	34752	GH	1934	8/2	8/31
117	SPI	34754	GH	1934	7/20	8/16
118	OO	68	GH	1952	8/2	8/30
119	HHS	Mammoth "non-flowering"	GH	1943	8/2	8/30
120	HHS	C39-193 Adv.gen. tabacum x rustica	"	1952	8/2	8/31
121	EGB	German #2	GH	1947	7/16	8/13
122	EGB	German #1	GH	1947	7/20	8/16
123	Sept. 1944 (From Russia)	Mahorha #1, Ac 18/7	GH	1946	7/3	7/30
124	Sept. 1944	" #2, Armenia	GH	1945	7/13	8/9
125	" "	" #3, Voronezhskaia	"	1945	7/3	7/30
126	" "	" #4, Tall green	"	1945	7/6	8/7
128	" "	" #6, Yellow 109	"	1945	7/6	8/7
129	" "	" #7, Pekhletz local	"	1945	6/27	7/25
130	" "	" #8, Prosechenskaia	"	1945	7/13	8/9
131	" "	" #9, Slepukhinskaia	"	1945	7/9	8/7
132	" "	" #10, Saratorskaia	"	1945	7/16	8/13
133	" "	" #11, Stalin-gradskaia	Original seed	1944	7/16	8/13
134	" "	" #12, Iurievskaia	"	1944	7/3	7/30
135	Kortoff-Russia	White seed #41	GH	1934	7/3	7/30
136	Russia	Bahhoun Sesnitza #46	GH	1934	7/20	8/16
138	"	Jainkaya Soldada #40	GH	1934	7/3	7/30
140	"	Drongi #41 Blue black	GH	1934	7/3	7/30
146	Harbin, Manchuria	#6	GH	1934	7/20	8/16

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2/ Greenhouse.
3/ E. G. Beinhart, formerly Eastern Regional Research Laboratory, ARS, USDA.
4/ Otto Olson, formerly Office of Tobacco Investigations, USDA.
5/ Seed and Plant Introduction number.

Table 2. Fresh Weight per Plant of Leaves of Various Strains of *N. rustica* and the Amounts of Alkaloids in the Leaves (expressed on a Dry Weight Basis) Before and After Curing.

Number	Leaf Wt. per Plant	At Harvest					After Curing				
		Total Alkaloid		Nic	Nor	Anab	T.A.	Nic	Nor	Anab	Unknown
56-B-	Gm.	%	Gm./Plant	%	%	%	%	%	%	%	%
101	682	4.67	6.07	4.0	T	T	4.27	4.0	.05	.05	T
102	597	5.30	6.39	4.7	.15	T	5.56	4.5	.05	.05	T
103	793	6.26	8.32	8.2	T	T	4.51	4.0	.08	.05	T
104	853	7.32	10.84	7.9	T	T	6.94	6.4	.05	.05	T
105	413	9.38	6.57	7.8	T	T	5.81	5.0	.03	.03	T
106	846	5.98	7.53	5.1	T	T	6.20	5.0	.03	.03	T
107	905	10.56	15.02	7.4	.19	T	5.62	4.3	.03	T	T
108	854	6.28	7.77	5.7	T	0	5.10	4.8	.03	.03	T
109	650	4.98	6.33	3.9	0	0	6.16	6.8	.03	.03	T
110	652	6.30	7.30	6.9	T	0	5.17	3.8	.05	T	T
111	571	6.74	6.80	7.4	.17	T	6.54	5.6	.05	.03	T
112	1116	6.04	10.72	5.5	.19	T	6.01	6.5	.03	.05	T
113	546	8.79	8.73	8.6	T	T	8.05	9.0	.03	.05	T
114	715	7.64	8.44	7.5	T	T	7.97	8.0	.05	.05	T
115	728	8.28	10.55	8.6	T	T	6.09	6.0	T	.03	T
116	483	10.14	7.09	7.7	.21	T	6.94	6.1	.05	.03	T
117	483	9.61	6.81	8.0	.21	T	7.19	6.0	.08	.05	T
118	782	6.82	10.01	7.9	T	T	7.90	6.9	.05	.03	T
119	732	7.17	8.34	7.5	T	T	6.40	5.5	.05	.03	T
120	385	5.77	3.35	6.0	T	T	4.56	4.5	T	T	T
121	176	11.12	2.83	11.9	.41	?	10.14	8.0	.10	.02	0
122	283	13.13	5.75	11.4	.58	T	9.44	7.5	.15	T	T
123	330	11.03	3.76	10.7	0	0	7.96	6.5	.05	.09	.05
124	341	7.44	2.90	7.5	T	0	6.21	4.1	.05	.05	.03
125	115	9.28	1.62	9.6	T	0	8.02	6.8	.07	.07	.05
126	453	7.60	4.03	8.6	0	0	5.93	4.0	.02	T	T
128	304	4.89	1.67	5.2	0	0	5.36	3.3	.03	.05	.03
129	142	9.90	1.74	9.1	.24	0	8.98	7.0	.10	.10	.02
130	456	7.46	3.87	8.2	.27	?	6.43	5.3	.10	.03	T
131	259	4.55	1.76	5.6	0	0	6.30	6.0	.10	.05	T
132	457	8.50	4.75	7.9	.25	0	7.38	4.5	.10	T	T
133	611	10.20	6.11	10.6	T	0	6.95	6.8	.08	.05	.03
134	128	8.96	1.45	8.9	0	0	6.06	5.5	.03	.03	.03
135	118	11.49	1.78	12.1	0	0	5.22	3.4	.05	.05	T
136	544	7.38	4.95	7.4	.24	0	5.99	4.0	.03	.03	T
138	106	12.16	1.61	12.1	0	0	8.44	8.0	.10	.05	T
140	81	8.22	.81	8.3	0	0	6.42	5.3	.03	.03	T
146	352	8.20	4.26	7.6	.26	T	6.46	3.9	.10	.05	T

Table 3. Fresh Weight per Plant of Stalks of Various Strains of N. rustica and the Amounts of Alkaloids in the Stalks (Expressed on a Dry Weight Basis) Before and After Curing.

Number 56-B-	Fresh Weight per Plant Gm.	At Harvest: Total Alkaloid %	Total Alkaloid Gm. in stalk per plant	Total Alkaloid Gm. in top per plant	Nic %	Nor %	Anab %	After Curing: T.A. %	Nic %	Nor %	Anab %	Unknown %
101	476	1.51	1.16	7.23	1.5	T	T					
102	482	1.37	1.08	7.47	1.4	T	0					
103	579	1.25	1.26	9.58	1.5	T	0					
104	305	2.48	1.30	12.14	2.4	T	0					
105	276	.96	.48	7.05	.8	T	T					
106	350	1.95	.96	8.49	2.4	T	0					
107	383	.82	.64	15.66	.7	T	0					
108	517	.98	.75	8.52	1.8	T	0					
109	295	1.23	.67	7.00	1.2	0	0					
110	359	1.14	.71	8.01	1.5	T	0					
111	333	1.36	.80	7.60	1.4	.06	T					
112	350	1.31	.70	11.42	1.9	0	0					
113	220	1.71	.74	9.47	2.4	.05	T					
114	269	1.63	.80	9.24	1.6	T	T					
115	303	2.24	1.10	11.65	2.7	T	T					
116	132	3.50	.68	7.77	3.4	.07	T					
117	128	2.59	.54	7.35	2.2	T	T					
118	351	1.45	1.05	11.06	2.0	T	T					
119	326	1.73	.89	9.23	1.9	T	T					
120	272	.88	.36	3.71	.9	T	0					
121	35	3.11	.20	3.03	3.2	.11	0	2.29	1.2	.02	T	0
122	60	1.99	.24	5.99	2.5	.10	T	1.76	2.0	.03	T	0
123	101	2.99	.35	4.11	2.7	0	0					
124	113	2.54	.45	3.35	2.7	T	0					
125	31	2.89	.14	1.76	3.6	.13	0					
126	68	1.95	.21	4.24	2.3	T	0	1.58	1.0	T	0	0
128	80	2.69	.26	1.93	1.5	0	0					
129	35	2.44	.13	1.87	2.1	.07	0	2.00	1.9	T	0	T
130	96	3.83	.44	4.31	3.9	.17	0					
131	70	2.97	.25	2.01	3.4	.08	0	2.99	2.7	.02	T	T
132	89	3.42	.47	5.22	3.0	.19	0	3.37	2.1	.05	0	0
133	115	4.05	.57	6.68	3.6	.16	0					
134	36	2.41	.13	1.58	2.2	.07	0					
135	29	2.82	.13	1.91	1.8	0	0	2.19	1.2	.02	T	0
136	159	2.44	.63	5.58	2.9	.06	0					
138	25	2.94	.13	1.74	2.3	0	0	2.29	2.3	.03	T	T
140	26	2.64	.12	.93	2.7	0	0					
146	128	3.73	.67	4.93	2.2	.07	T	3.39	2.0	.02	0	0

Because of differences in the method for cured leaf, smaller quantities of minor alkaloids could be detected, but no significance can be attributed to differences in reported nornicotine values. Anabasine, though found in most samples, was smaller in amount than nornicotine in both fresh and cured samples. An unknown which has an R_f value about halfway between anabasine and nicotine was detected in most cured samples but was not found in samples at harvest. This unknown is found in cured N. tabacum but is not found in green N. tabacum, even when the methods are so modified as to obtain equal sensitivity on green and cured samples. Since the unknown also appears in pure nicotine or nornicotine solutions on standing a few months, it would appear to be formed either in solution or during curing.

There was a very wide range of total alkaloid contents in the different strains at time of harvest - from 4.55% to 13.13% - but, even though average fresh weight per plant ranged between 81 and 1,116 gm., there was no apparent relationship between plant size and percent total alkaloid content. Some of the strains bloomed early, while others remained vegetative for a long period. Since each strain was topped when it bloomed and was harvested about 4 weeks later, the size at harvest of the early-blooming strains was very much less than of the late-blooming strains. This is shown in Table 4 where it may be seen that the average plant weight for strains harvested in late July was 146 gm., rising steadily to 715 gm. for those harvested in September.

Table 4. Influence of Harvest Date on Yield.

Harvest Date	Strains	Fresh Weight of Leaf per Plant			Total Alkaloid Concentration of Leaf (Dry Basis)			Total Alkaloid Content of Leaves per Plant		
		Min Gm.	Max Gm.	Av Gm.	Min %	Max %	Av %	Min Gm.	Max Gm.	Av Gm.
7/25-30	7	81	330	146	8.22	12.16	10.15	0.81	3.76	1.68
8/6-9	5	259	456	363	4.55	7.60	6.39	1.67	4.03	2.85
8/13-16	7	176	611	415	7.38	13.13	9.73	2.83	6.81	5.06
8/30-31	11	385	1116	689	4.98	10.56	7.60	3.35	10.72	8.40
9/5-6	8	571	853	715	4.67	8.28	6.36	6.07	10.84	7.98

There was no consistent relationship of harvest date to total alkaloid percentage on the weight basis. Since the average alkaloid percentage of the early-blooming plants was as high or higher than those blooming later, it is evident that these plants were just as mature from the standpoint of alkaloid production. Since they were so much smaller, however, the weight of alkaloid formed per plant was much less than in the larger, later-maturing strains.

A study of the total alkaloid content before and after curing indicates that the samples of relatively low alkaloid concentration (below 7%) did not lose large quantities of alkaloid during curing. Though the results are not consistent on all strains, probably for the most part because of the small number of plants per sample, there appears to be a tendency for the high alkaloid samples to lose a considerable proportion of their alkaloid during curing. It is not known whether or not this is due to the greater amount of volatilization which would be expected to occur from high concentrations.

Summary

Thirty-eight strains of Nicotiana rustica were grown at Beltsville, Md., under normal practices for the growing of tobacco. Each was topped at blooming time, kept suckered, harvested about 4 weeks later, and analyzed for the amount and composition of the alkaloid at harvest and after air curing.

All strains contained predominantly nicotine, though traces of nornicotine and anabasine were found. After curing, an unknown substance, apparently a decomposition product of nicotine and nornicotine, was also found.

The strains which bloomed early, when the plants were relatively small, had a similar range of total alkaloid content expressed on a dry-weight basis, but contained much less alkaloid per plant than the later-blooming, larger strains.

The strains which had very high alkaloid concentrations at harvest lost a larger proportion of their alkaloid during curing than the strains having alkaloid concentrations below about 7%.

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