ALKALOIDS IN CERTAIN SPECIES AND INTERSPECIFIC HYBRIDS OF NICOTIANA

By Harold H. Smith, assistant geneticist, Division of Tobacco Investigations, Bureau of Plant Industry, and Claude R. Smith, formerly chemist, Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

Although it has been known for many years that the main alkaloid in the cultivated species Nicotiana tabacum L. and N. rustica L. is nicotine (C_{10}H_{14}N_{2}), comparatively little analytical chemical work has been done on the wild species of Nicotiana. In 1934 Shmuk (15) stated that N. glauca contained an alkaloid other than nicotine that was not identified. The following year Smith (18) identified the principal alkaloid in this species as anabasine, beta-pyridyl-α'-piperidine. Concurrently, Shmuk and Khmura (17) reported that certain other species of Nicotiana contained alkaloids whose picrates had melting points different from that of nicotine, but again they were not identified. N. sylvestris and crosses of this species with N. tabacum were discussed in some detail.

In 1937 Smith (19) determined the main alkaloid in Nicotiana sylvestris to be nornicotine (C_{9}H_{12}N_{2}), and at approximately the same time Shmuk (16) reported:

It is known that alkaloids differing in chemical structure are contained in different species of Nicotiana; thus, N. tabacum, N. rustica, N. alata, N. Langsdorffii all contain nicotine; N. sylvestris and N. Rusbyi [N. tomentosiformis Goodsp.] contain an alkaloid belonging to the secondary bases—probably nornicotine, while N. glauca contains anabasine.

Nicotine has been found also in Nicotiana attenuata (1), N. suaveolens (18), and N. trigonophylla (10).

The investigations cited above constitute most of the relevant work that had been published on alkaloids in wild species of Nicotiana, not including hybrids, at the time this paper was written. The experiments hereafter described were begun in 1936. The object of the work was to determine the alkaloids in a large number of representative Nicotiana species and hybrids among different alkaloidal types; to perfect chemical methods for isolating and identifying alkaloids found in Nicotiana; and to consider the results obtained from the standpoint of developing new strains yielding larger amounts of alkaloids for possible use in insecticides.

Minute quantities of 11 or more secondary alkaloids, in addition to the principal one, nicotine, have been reported in the cultivated species Nicotiana tabacum (4); but of these only 8 have been confirmed by Späth and Keszttler (23). It has also been found that although most of the total alkaloidal content in N. sylvestris is nornicotine, there is a small percentage of l-nicotine (16, 19). The investigations reported here indicate that in many, if not all, species of Nicotiana the principal alkaloid is accompanied by 1 or more secondary forms, usually present in very small amounts.

1 Received for publication February 12, 1942.
2 Italic numbers in parentheses refer to Literature Cited, p. 358.
The three predominant alkaloids in *Nicotiana*, namely anabasine, nornicotine, and nicotine, are not peculiar to this genus. Anabasine was discovered in *Anabasis aphylla* L., of the Chenopodiaceae (12); nornicotine has been found in *Duboisia hopwoodii* F. Muell., of the Solanaceae (5); and nicotine has been reported to occur in *Asclepias syriaca* L., of the Asclepiadaceae (8), although in the last-named species only a minute quantity of the alkaloid was indicated.

**CULTURAL METHODS**

All plants in these experiments were grown at the Arlington Experiment Farm, Arlington, Va.3 The seedlings were planted in the field, 18 inches apart in rows 1 meter apart. Shortly before planting time, 4–8–4 fertilizer was applied uniformly in the drill at the rate of 800 pounds to the acre. The soil was Keyport silt loam, a heavy claylike type.

Many of the species were not topped and suckered as is usual in the culture of leaf tobacco, since their habit of growth was not adapted to this method of handling. Differences in cultural treatment are known to affect alkaloidal content, so that the total percentages reported for the 29 species are not considered to be strictly comparable. The identity of the alkaloids and their approximate relative amounts may be considered as characteristic.

All samples were air-dried and ground into a fine powder preparatory to chemical analysis. The samples comprised both leaves and stems in all species except the larger ones; namely, *Nicotiana benavidesii*, *N. glauca*, *N. otophora*, *N. raimondii*, *N. sylvestris*, *N. tomentosa*, *N. tomentosiformis*, and *N. wigandioides*, in which only the leaves were analyzed.

**CHEMICAL METHODS**

The determination and separation of the alkaloids were effected by the following procedures.

A 50-gm. sample was digested with water in a liter Erlenmeyer flask at the highest temperature of the steam bath for about 2 hours. The unextracted material was filtered from the solution and washed with several portions of boiling water. The extractions were combined, evaporated to a low volume on the steam bath, made strongly alkaline with sodium hydroxide, and then extracted with several portions of ether. The ether extractions were combined, and the alkaloid was washed out with dilute hydrochloric acid. The washed ether was used repeatedly to extract more alkaloidal material from the alkaline extract. Repeated extractions were made because persistent and incompletely separated emulsions usually formed. Their formation was reduced to a minimum by making the extractions in Erlenmeyer flasks, decanting after each extraction, and avoiding violent shaking. Careful manipulation usually permitted complete extraction and the final clearing of the emulsion.

The acid solution of the alkaloids was separated from the ether, made strongly alkaline, and extracted with several small portions of

---

3 Seeds of many of the species grown were made available through the kindness of Dr. T. H. Goodspeed, of the University of California. Some were obtained from Dr. R. E. Clausen, of the same institution. The remainder formed part of the collection maintained by the Division of Tobacco Investigations.
ether. The ether extractions were combined and dried for several hours or overnight with sticks of sodium hydroxide or preferably potassium hydroxide pellets. The dried ethereal solution was evaporated in a 50-cc. tared Erlenmeyer flask and subjected to the direct steam of the bath until all traces of alcohol and ether had been removed, the period of heating being no longer than necessary. The flask with residue was dried over sulfuric acid in a desiccator for 18 hours and was then weighed.

In the procedure just outlined, complete extraction by hot water may not be effected, but approximately 95 percent of the alkaloidal content is removed. Variations, such as the addition of oxalic acid or calcium carbonate (10 gm. to 700 cc. of water) followed by precipitation of the soluble lime by acidification with oxalic acid, did not appreciably affect the values found for total alkaloids.

In separating and identifying the alkaloids, nicotine was removed first by distillation through an efficient fractionating column such as the Widmer. The method is based on the discovery by one of the writers (20) that nicotine forms an azeotropic mixture with water at the boiling temperature when 2.5 gm. are contained in 100 cc. Neither anabasine nor nornicotine forms a similar mixture, nor does any other alkaloid so far encountered in the genus Nicotiana. The procedure is as follows.

The total alkaloids were washed into the distilling flask with 75 to 100 cc. of water and distilled slowly through the column until only about one-fifth or less of the volume remained. Sixty to eighty cubic centimeters of water was added to the mixture, which was then distilled again. The combined distillate represented all the nicotine together with some "nonvolatile" alkaloid and was titrated with standard acid to obtain the approximate amount of nicotine. The titrated distillate was made alkaline with standard alkali in slight excess of that required by the acid titration, and the distillations were repeated. The titration of this distillate gave a close check on the amount of nicotine contained and was somewhat less than the first titration if some nonvolatile alkaloid had distilled over with the nicotine. This highly purified second nicotine distillate was made appreciably acid to methyl red, evaporated to a low volume if necessary, and picric acid solution was added to form nicotine picrate.

In all species containing 0.2 percent or more of total alkaloids, melting-point determinations were made with mixtures of pure nicotine picrate. It is safe to conclude from the many melting points made that any alkaloid distilled under the conditions outlined will give the characteristic needles of nicotine picrate and that, when mixed with a known amount of nicotine picrate, the melting point will not be changed. Any residual nonvolatile alkaloid was precipitated as the picrate, which was recrystallized at least once. The picrate was recrystallized from water several times if sufficient material was available, and crops of crystals were formed of which the melting points were obtained. The presence of anabasine or nornicotine was indicated by the crystalline form and melting point. In no single species did both of these alkaloids occur together. With certain hybrids, however, both have been found along with nicotine, and careful fractionation was necessary to confirm their identification. Actual results with these hybrids are given below to show the basis
for the conclusions. All melting points given are corrected for stem exposure.

In the F1 hybrid *Nicotiana tabacum* × *N. glauca* (38114) we might expect only a mixture of anabasine and nicotine, but careful fractionation of the picrate of the nonvolatile alkaloids into (1) most soluble, (2) less soluble, and (3) least soluble yielded fractions with melting points of 188°, 194°, and 205° to 206° C. respectively. Recrystallized fraction 1 melted at 188° to 190° and was unchanged when mixed with nornicotine picrate. When fraction 3 was recrystallized it melted at 207° to 209°; this melting point remained unchanged when fraction 3 was mixed with racemic anabasine picrate. The specific rotation \([\alpha]\) of the nonvolatile alkaloid in acid solution was \(+7.8°\). It is probable that this optical activity is due to the presence of \(l\)-nicotine, which is dextrorotatory in its salts (\(l\)-nicotine and \(l\)-anabasine are also dextrorotatory in their salts). Anabasine in *N. glauca* has always been found to be essentially optically inactive.

The F1 hybrid *Nicotiana tabacum* × *N. glauca* (39115-1) contained a little nicotine; the picrate of the nonvolatile alkaloid melted at 201° to 202° C., which was raised on recrystallization to 205° to 206°; and the melting point of a mixture with racemic anabasine picrate was 205° to 209°. Optical rotation of the nonvolatile alkaloids was \([\alpha]_p = -20°\) to 20.8°. The conclusion was drawn that this hybrid contained essentially \(d\), \(l\)-anabasine with an excess of \(l\)-anabasine, since no crystals resembling nornicotine picrate could be obtained. It is evident that a quantitative method for separating anabasine and nornicotine is needed before final conclusions can be drawn regarding the composition of the hybrids of *N. glauca* with *N. tabacum* or any plant containing nicotine and nornicotine.

All samples of *Nicotiana glauca* that the writers have examined contained principally optically inactive anabasine, the picrate of which melts at 213° C. The optical activity is occasionally very slightly levorotatory, which probably indicates that a small excess of \(l\)-anabasine is present.

The sample of *Nicotiana glauca* shown in table 1, as well as others not reported here, was found to contain some nicotine. No nicotine has been found, however, in samples of *N. glauca* obtained from the wild. It is possible, but doubtful, that the nicotine in the former may have been introduced from accidental absorption during the drying or pulverizing process. This point may be settled easily by careful isolation of the writers' cultivated material.

The nornicotine that occurs in several species, such as *Nicotiana sylvestris* and *N. glutinosa*, has been found to be the nearly pure levorotatory form. In a number of other species the partly racemized form probably occurs, the picrates of which have melting points that vary from 186° to 192° C. When the content of nornicotine was less than 0.2 percent its presence was indicated only by the appearance and properties of the picrate. The picrates, even after several recrystallizations, usually appear in the colloidal form, but on long standing form hard crystals which repeat this behavior on further recrystallization.

Observations on the optical rotation of the alkaloids as found in the writers' material are admittedly incomplete, but it seems that nicotine occurs always in the \(l\)-form, anabasine principally in the
optically inactive form, and nornicotine largely in the L-form mixed with varying proportions of the optically inactive form.

In the progress of this work the possible presence of new alkaloids has been kept in mind, but none has been indicated. The new alkaloid anatabin, discovered by Späth and Kesztler, is closely related to anabasine and was expected to occur in *Nicotiana glauca* or *N. glauca* hybrids, but so far no indication of its presence has been obtained. Since this study was necessarily confined to the principal alkaloids it is quite probable that very small proportions of other alkaloids were present but could have been detected only by using much more material for separation.

**RESULTS**

A total of 29 wild species of *Nicotiana* were studied of which 4 apparently contained only nicotine, 5 only nornicotine, 2 had mixtures of anabasine and nicotine, and the rest mixtures of nicotine and nornicotine (table 1). Anabasine was the main alkaloid and nicotine the secondary one in *N. debneyi* and *N. glauca*, the only species in which these 2 alkaloids were found together as the chief alkaloidal constituents.

Table 1.—Alkaloids of some wild species of *Nicotiana*

<table>
<thead>
<tr>
<th>Species</th>
<th>Main alkaloid</th>
<th>Secondary alkaloid</th>
<th>Total alkaloidal content</th>
<th>Main alkaloid</th>
<th>Phylogenetic grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. alata</em> Lk. and Otto</td>
<td>Nicotine</td>
<td>None</td>
<td>0.06</td>
<td>0.06</td>
<td>100</td>
</tr>
<tr>
<td><em>N. benavidesii</em> Goodsp.</td>
<td>..do</td>
<td>Nornicotine</td>
<td>0.92</td>
<td>82</td>
<td>89</td>
</tr>
<tr>
<td><em>N. bigelovii</em> S. Wats.</td>
<td>..do</td>
<td>.?</td>
<td>0.18</td>
<td>16</td>
<td>89</td>
</tr>
<tr>
<td><em>N. cavanillesii</em> Dun.</td>
<td>Nornicotine</td>
<td>Nicotine</td>
<td>0.33</td>
<td>33</td>
<td>100</td>
</tr>
<tr>
<td><em>N. delneyi</em> Domin.</td>
<td>Anabasine</td>
<td>.do</td>
<td>0.46</td>
<td>39</td>
<td>85</td>
</tr>
<tr>
<td><em>N. erigxa Wheeler</em></td>
<td>Nornicotine</td>
<td>.do</td>
<td>0.26</td>
<td>24</td>
<td>92</td>
</tr>
<tr>
<td><em>N. glauca</em> Grah</td>
<td>Anabasine</td>
<td>None</td>
<td>0.64</td>
<td>62</td>
<td>97</td>
</tr>
<tr>
<td><em>N. glutinosa</em> L.</td>
<td>None</td>
<td>Nornicotine</td>
<td>1.80</td>
<td>1.80</td>
<td>100</td>
</tr>
<tr>
<td><em>N. goodspeedii</em> Wheeler</td>
<td>Nicotine</td>
<td>None</td>
<td>1.10</td>
<td>1.10</td>
<td>100</td>
</tr>
<tr>
<td><em>N. gossi Domin</em></td>
<td>None</td>
<td>Nicotine</td>
<td>0.22</td>
<td>0.22</td>
<td>73</td>
</tr>
<tr>
<td><em>N. langsdorffii</em> Weinm.</td>
<td>..do</td>
<td>Nornicotine</td>
<td>0.04</td>
<td>0.04</td>
<td>?</td>
</tr>
<tr>
<td><em>N. longiflora</em> Cav.</td>
<td>Nornicotine</td>
<td>None</td>
<td>0.28</td>
<td>0.28</td>
<td>100</td>
</tr>
<tr>
<td><em>N. maritima</em> Wheeler</td>
<td>None</td>
<td>Nicotine</td>
<td>0.22</td>
<td>0.22</td>
<td>100</td>
</tr>
<tr>
<td><em>N. megalosiphon</em> H. and M.</td>
<td>Nicotine</td>
<td>..do</td>
<td>0.18</td>
<td>0.18</td>
<td>94</td>
</tr>
<tr>
<td><em>N. nesophila</em> Johnst.</td>
<td>Nicotine</td>
<td>.do</td>
<td>0.10</td>
<td>0.10</td>
<td>100</td>
</tr>
<tr>
<td><em>N. nudicaulis</em> S. Wats.</td>
<td>Nicotine</td>
<td>.do</td>
<td>0.11</td>
<td>0.11</td>
<td>100</td>
</tr>
<tr>
<td><em>N. otophora</em> Griseb.</td>
<td>Nicotine</td>
<td>..do</td>
<td>0.28</td>
<td>0.28</td>
<td>71</td>
</tr>
<tr>
<td><em>N. paniculata</em> L.</td>
<td>Nicotine</td>
<td>..do</td>
<td>0.62</td>
<td>0.62</td>
<td>73</td>
</tr>
<tr>
<td><em>N. plumbaginifolia</em> Viv</td>
<td>Nicotine</td>
<td>..do</td>
<td>0.30</td>
<td>0.30</td>
<td>65</td>
</tr>
<tr>
<td><em>N. raimondii</em> Meabr.</td>
<td>.do</td>
<td>Nicotine</td>
<td>0.44</td>
<td>0.44</td>
<td>100</td>
</tr>
<tr>
<td><em>N. repandra</em> Lehm.</td>
<td>Nicotine</td>
<td>..do</td>
<td>0.37</td>
<td>0.37</td>
<td>93</td>
</tr>
<tr>
<td><em>N. stocktoni</em> Brandeg.</td>
<td>Nornicotine</td>
<td>None</td>
<td>0.65</td>
<td>0.65</td>
<td>70</td>
</tr>
<tr>
<td><em>N. suaveolens</em> Lehm.</td>
<td>Nornicotine</td>
<td>Nicotine</td>
<td>0.46</td>
<td>0.46</td>
<td>65</td>
</tr>
<tr>
<td><em>N. spinosatis</em> Spog. and Comes.</td>
<td>..do</td>
<td>Nicotine</td>
<td>0.44</td>
<td>0.44</td>
<td>100</td>
</tr>
<tr>
<td><em>N. tomentosa</em> Ruiz and Pav.</td>
<td>None</td>
<td>Trace</td>
<td>0.20</td>
<td>0.20</td>
<td>85</td>
</tr>
<tr>
<td><em>N. tomentosiformis</em> Goodsp.</td>
<td>Nicotine</td>
<td>..do</td>
<td>0.06</td>
<td>0.06</td>
<td>100</td>
</tr>
<tr>
<td><em>N. tripomophila</em> Dun.</td>
<td>None</td>
<td>Nicotine</td>
<td>0.08</td>
<td>0.08</td>
<td>100</td>
</tr>
<tr>
<td><em>N. undulata</em> Ruiz and Pav.</td>
<td>Nicotine</td>
<td>.do</td>
<td>0.17</td>
<td>0.17</td>
<td>85</td>
</tr>
<tr>
<td><em>N. wigandoides</em> Koch and Fint.</td>
<td>Nicotine</td>
<td>None</td>
<td>1.02</td>
<td>1.02</td>
<td>100</td>
</tr>
</tbody>
</table>

1 Only a trace of the secondary alkaloid was present.

*Nicotiana longiflora* and *N. plumbaginifolia* each contained both nicotine and nornicotine, but the amounts were so small that it was
not possible to determine which was present in the higher concentration. Among the 16 other species in which both nicotine and nor-
icotine were found, only *N. benavidesii*, *N. langsdorffii*, and *N. stocktoni* contained nicotine predominantly.

No wild species studied had as much as 2 percent alkaloidal content; whereas both the cultivated ones (*Nicotiana tabacum* and *N. rustica*) usually had at least this amount. Only 3 wild species gave analyses of over 1 percent alkaloid; and 22 had less than 0.5 percent alkaloid. In 23 species, 84 to 100 percent of the total alkaloidal content was taken up by the main alkaloid (table 1).

Hybrids were made between *Nicotiana* species in which different alkaloids were predominant so that crosses of nicotine types with nor-nicotine types, nicotine with anabasine, and anabasine with nor-
icotine could be investigated. Inheritance of alkaloids in *F*₁ hybrids of the first type was tested by crosses of *N. tabacum* (nicotine) with *N. sylvestris*, *N. tomentosa*, and *N. glutinosa* (all nor-nicotine species). The results are shown in table 2. In each hybrid the genetic factors controlling nor-nicotine formation were partly dominant over those producing nicotine. The total percentage of alkaloid in the *F*₁ plants was higher than in the nor-nicotine parents.

### Table 2.—Alkaloids of certain *Nicotiana* interspecific hybrids.¹

| *Nicotiana* interspecific cross | Family No. | Main alkaloid | Secondary alkaloid | Total alkaloidal content | Main alkaloid
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Percent</td>
<td>Percent</td>
<td>Percentage of total alkaloid</td>
<td></td>
</tr>
<tr>
<td><em>F</em>₁ (tabacum × sylvestris)</td>
<td>3790</td>
<td>Nornicotine</td>
<td>Nicotine</td>
<td>2.26</td>
<td>2.25</td>
</tr>
<tr>
<td>Do (tabacum × tomentosa)</td>
<td>39117</td>
<td>do</td>
<td>do</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td><em>F</em>₁ (tabacum × glutinosa)</td>
<td>39115</td>
<td>Anabasine</td>
<td>do</td>
<td>1.92</td>
<td>1.73</td>
</tr>
<tr>
<td><em>F</em>₁ (tabacum × glauca)</td>
<td>39116</td>
<td>do</td>
<td>do</td>
<td>0.96</td>
<td>0.90</td>
</tr>
<tr>
<td><em>F</em>₁ (glauca × tomentosa)</td>
<td>38287</td>
<td>?</td>
<td>?</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td><em>F</em>₁ (glutinosa × sylvestris)</td>
<td>38113</td>
<td>Nornicotine</td>
<td>Nicotine</td>
<td>1.36</td>
<td>1.26</td>
</tr>
</tbody>
</table>

¹ All data in this table are on leaves of topped plants.

The *F*₁ hybrid *Nicotiana tabacum × N. glauca* was used to investigate a cross between a nicotine and an anabasine type. The genetic factors for anabasine formation are partly dominant over those controlling nicotine formation in the *F*₁ (table 2). The total percentage of alkaloid and of anabasine was higher in the hybrid than in *N. glauca*. In a sample of the *N. tabacum × N. glauca* hybrid grown in 1938 (38114) the writers have noted the presence of nor-nicotine in addition to anabasine and nicotine.

The *F*₁ *Nicotiana glauca × N. tomentosa* was intended to be used to investigate a cross between an anabasine and a nor-nicotine type. It was found, however, that with the present chemical techniques it was not possible to separate these two alkaloids readily in a mixed sample having such a small total alkaloidal content (0.24 percent).

The dominance of nor-nicotine over nicotine was considered to be only partial since neither *Nicotiana glutinosa* nor *N. tomentosa* contained detectable amounts of nicotine, yet this alkaloid was found in hybrids between each of these species and *N. tabacum*. Similarly the
genetic factors controlling anabasine formation probably are not completely dominant over those for nicotine since the percentage of anabasine in the total alkaloid is lower in the \( N. \text{tabacum} \times N. \text{glauca} \) hybrid than in \( N. \text{glauca} \) itself.

\textit{Nicotiana glutinosa} and \( N. \text{sylvestris} \), two predominantly nornicotine species, were crossed, and, as expected, the alkaloidal content of the \( F_1 \) hybrid was mostly nornicotine with a small percentage of nicotine (table 2).

**GENETIC DISCUSSION**

**SPECIES**

Almost one-half of the definitely established species of the genus \textit{Nicotiana} were included in these investigations. Seven of the fifteen recognized Australasian species were analyzed; and some from each of the four centers of distribution in the Western Hemisphere; namely, Peru and Bolivia, Chile and Argentina, Uruguay and southeastern Brazil, Mexico and southwestern United States. There was no apparent relation between the geographical distribution of a species and the identity of its main alkaloid.

Each of the known chromosome numbers (2), from those with 9 to those with 24 pairs of chromosomes, was represented by 1 or more species; and most of the main phylogenetic groupings in the genus (3) were included in the types used.

With regard to chromosome number, an interesting relation was observed in the Australasian or \textit{suaveolens} group of \textit{Nicotiana}. The principal alkaloid was found to be nornicotine in species that had 16 and 20 pairs of chromosomes; namely, \textit{Nicotiana exigua}, \textit{N. maritima}, \textit{N. suareolens}, \textit{N. goodspeedii}, and \textit{N. megalosiphon}. Anabasine was the main alkaloid in \( N. \text{dehneyi} \), the only Australasian species so far determined to have 24 pairs of chromosomes; and nicotine was the main alkaloid in \( N. \text{gossei} \), the only species with 18 pairs of chromosomes.

Among American species, the cultivated \textit{Nicotiana tabacum} and \( N. \text{rustica} \) have 24 pairs of chromosomes and contain a large amount of alkaloid, which is predominantly nicotine. \( N. \text{sylvestris}, N. \text{tomentosa}, \) and \( N. \text{tomentosiformis} \), which are considered to be the probable wild progenitors of \( N. \text{tabacum} \) (3), have 12 pairs of chromosomes and contain a small amount of alkaloid, which is mainly or entirely nornicotine. Similarly, \( N. \text{paniculata} \) and \( N. \text{undulata} \), which are considered to be the probable wild progenitors of \( N. \text{rustica} \), have a small amount of alkaloid, which is predominantly nornicotine, and have 12 pairs of chromosomes.

The apparently inconsistent situation in which the hybridization of two species with low concentrations of alkaloid that is mainly nornicotine produces a new form having a high concentration of nicotine might be explained if evidence were available that the conditions under which the cultivated types were formed and are grown favor the production of large amounts of alkaloid and that high concentration of total alkaloid favors the formation of nicotine.

The following facts support the first part of this hypothesis: (1) There is experimental evidence that chromosome doubling (which is considered to have occurred in the formation of the cultivated species) causes an increase in alkaloidal content \((11, 24)\); (2) the cultural methods used in the growing of tobacco (topping, suckering, heavy
application of fertilizer) favor production of high alkaloidal content; (3) for centuries these species have been cultivated and used by man for the effect produced by the alkaloid that they contain. Therefore it seems not too speculative to assume that selection for high alkaloidal content (i.e., for genes that increase the production of alkaloid) has been practiced, and the fact that low-alkaloidal strains have been selected from some present-day varieties of *N. tabacum* supports this suggestion.

The following facts support the second part of the hypothesis; namely, that high concentrations of alkaloid favor the formation of nicotine (or low concentrations, the formation of nornicotine): (1) Large quantities of nornicotine have been found in low-alkaloidal strains of *Nicotiana tabacum* (6, 9); (2) the alkaloidal content of species in which nicotine predominated (table 1) was higher on an average than those in which nornicotine was the main alkaloid; (3) Shmuk (16) found that, out of 11 F₁ plants of *N. tomentosiformis* × *N. sylvestris*, 9 had a total alkaloidal content ranging from 0.129 to 0.510 percent, while the other 2 had 0.656 and 0.859 percent, respectively, and that the chief alkaloid in the first 9 F₁ plants was nornicotine, while in the remaining 2 it was nicotine, thus showing that a predominantly nicotine type, like *N. tabacum*, could have been formed from the crossing of 2 predominantly nornicotine types, provided the hybrid had a sufficiently high concentration of total alkaloid.

In regard to relations among species in the various phylogenetic groups, it was found that of those analyzed in the *paniculata* group (*Nicotiana benavidesii*, *N. glauca*, *N. paniculata*, *N. raimondii*, and *N. undulata*), all but *N. glauca* were of predominantly nicotine or nornicotine types. From cytological observations (3), *N. glauca* had already been considered aberrant in this group. The discovery that it is exceptional for a species to have anabasine as the main alkaloid is confirmatory evidence of the isolated position of *N. glauca*. The fact that *N. debneyi* of the *suaveolens* group was the only other species found to contain anabasine as the principal alkaloid is evidently a case of parallel evolution rather than any indication of homology between this species and *N. glauca*. Most species of the *alata* group (*N. alata*, *N. langsdorffii*, *N. longiflora*, and *N. plumbaginifolia*) tend to contain nicotine predominantly; and most of the *tomentosa* group (*N. glutinosa*, *N. otophora*, *N. tomentosa*, *N. tomentosiformis*, and *N. wigandioides*), with one exception, nornicotine predominantly.

### HYBRIDS

Russian investigators have reported in some detail on hybrids between different alkaloidal types, and with certain crosses the work has been continued to the F₂ and further inbred generations. Shmuk (16) has studied the following crosses: *Nicotiana tabacum* (nicotine) × *N. sylvestris* (nornicotine), *N. tomentosiformis* (nornicotine) × *N. sylvestris* (nornicotine), and *N. langsdorffii* (nicotine) × *N. alata* (nicotine). He also reported that anabasine dominated over nicotine in the F₁ hybrid *N. tabacum* × *N. glauca*; and that, in subsequent inbred generations, types with anabasine, nicotine, and mixtures of the two were produced.
A short time later Ternovsky, Khmura, and Žukov (24) published a paper on the alkaloids in various heteroploid types having extra chromosomal complements of *Nicotiana glauca* and of *N. tabacum*. All contained anabasine predominantly, even where there were two *N. tabacum* genomes to one of *N. glauca* (24tt+12g). This particular combination had a higher percentage of anabasine than others with proportionately more *N. glauca* chromosomes.

Ternovsky et al. (24) also investigated crosses between *Nicotiana rustica* and *N. glauca*. Only anabasine was found in the F₁, sesquidiploid (24r+12gg), and crosses of the sesquidiploid to *N. glauca* and *N. tabacum*. More recently Žukov (25) and Kostoff (7) have published results of their work on heteroploid *N. rustica* × *N. glauca* combinations. The amphidiploid (24rr+12gg) and the sesquidiploid (24rr+12g) had higher percentages of anabasine content than *N. glauca*. In backcrosses of the hybrid *N. rustica* × *N. glauca* to *N. rustica*, some plants were obtained containing only nicotine; some, only anabasine; and some, mixtures of nicotine and anabasine.

From their studies on the hybrids of *Nicotiana tabacum* with *N. sylvestris* and *N. glauca* the writers were able to confirm the general results reported by Shmuk and his coworkers, but their findings differ in certain respects from those of the latter. Shmuk (16) and Shmuk and Khmura (17) found no nicotine whatsoever in the cross of *N. tabacum* with *N. sylvestris*, whereas in all samples of this hybrid that the writers have analyzed some nicotine was present. It appears, however, that Shmuk was using the reciprocal cross, which conceivably might account for the different result. Shmuk and other Russian investigators found that in the F₁ hybrid *N. tabacum* × *N. glauca* nicotine was usually absent; but a few plants contained traces of this alkaloid. In analyses of this hybrid, the writers found that all samples contained some nicotine, and they consider it a characteristic secondary alkaloid of the F₁.

Results of the Russian investigations, which were continued beyond the first generation, may be summarized as follows: (1) Some segregants were produced that contained only one or the other of the alkaloids involved in the cross, while the remaining segregants contained mixtures of the two in different proportions. A secondary alkaloid that is present in small amounts in one parent may become the chief alkaloid in some individuals of a hybrid or inbred family. (2) No obvious Mendelian ratios were obtained in families segregating for different alkaloids, nor would they be expected, since the interspecific hybrids and extra chromosomal forms all show irregularities at meiosis.

**ECONOMIC POSSIBILITIES**

Since none of the wild species studied contained as much nicotine as the cultivated ones, *Nicotiana tabacum*, *N. rustica* and selections from crosses between these species are the most promising sources of this alkaloid. Nicotine is used widely as an insecticide; and although it is now obtained from scrap leaf material and stems of *N. tabacum* as a byproduct of the tobacco industry, new selections of
N. rustica (fig. 1, A) have been obtained that yield large quantities of this alkaloid and it may be possible to develop strains from them that can be grown profitably for the nicotine alone (22).

Anabasine is not used commercially as an insecticide in this country at present; but it has been shown experimentally to be more toxic than nicotine to Aphis rumicis L. (14). In Russia anabasine is evidently used extensively and is obtained from Anabasis aphylla, a shrub that grows wild in the region of the Caspian Sea (12). This

source is not enough to supply the demand (25), however, and consequently there has been interest in breeding forms of Nicotiana having a high anabasine content.

Nicotiana glauca appears to be the best species to use for this purpose, as the plant is larger and contains a higher concentration of alkaloid than N. debneyi, the only other species so far determined to have anabasine as the main alkaloid. The growth habit, yield, and alkaloidal content need to be improved, however. A fairly fertile strain (fig. 1, B) in which some of these improvements have been made (table 2) was produced by doubling the chromosomes of the F₁ hybrid N. tabacum × N. glauca with colchicine to form an amphidiploid (21). Further experiments are being carried out in this
laboratory in an attempt to combine the best qualities of *N. glauca*, *N. rustica*, *N. tabacum*, and possibly *N. debneyi* into strains that will yield still larger amounts of anabasine. It is expected that fertile combinations containing full chromosome complements of any two or three or all four species can be produced with the aid of colchicine as an agent for doubling chromosomes, provided multivalent associations at meiosis do not seriously interfere. The writers have obtained, but have not yet analyzed chemically, the following crosses and their double chromosome forms (diploid for each species): *N. debneyi* × *N. glauca*, *N. debneyi* × *N. tabacum*, *N. debneyi* × *N. glauca* × *N. rustica*, *N. debneyi* × *N. tabacum* × *N. glauca*, and *N. rustica* × *N. debneyi* × *N. tabacum*. Russian investigators have reported (7, 25) that the amphidiploid and sesquidiploid (24rr + 12g) of crosses between *N. rustica* and *N. glauca* contained a high percentage of anabasine.

Nornicotine, the most prevalent alkaloid among wild species of *Nicotiana*, also has been shown experimentally to be highly toxic to certain insects (14). The F1 hybrid *N. tabacum* × *N. tomentosa* was the largest predominantly nornicotine type that was obtained in the writers' investigations (fig. 1, C). Fourteen topped and suckered plants of this hybrid had an average of 1.18 percent nornicotine, an average dry leaf weight of 93.7 gm. per plant, and an estimated potential yield of 21.4 pounds of nornicotine per acre.

**SUMMARY AND CONCLUSIONS**

All 29 of the wild species of *Nicotiana* that were studied contained 1 or more alkaloids.

*Nicotiana alata*, *N. bigelovii*, *N. gossei*, and *N. wigandiiodes* apparently contained only nicotine.

*Nicotiana glutinosa*, *N. maritima*, *N. otophora*, *N. tomentosa*, and *N. trigonophylla* apparently contained only nornicotine.

*Nicotiana debneyi* and *N. glauca* contained largely d,l-anabasine. In the former about 15 percent of the total alkaloid was nicotine, whereas in the latter a much lower percentage of this alkaloid was found.

The remaining 18 species that were analyzed contained mixtures of nicotine and nornicotine. Of these, nicotine was predominant only in *N. benavidesii*, *N. langsdorffii*, and *N. stocktoni*; in *N. longiflora* and *N. plumbaginifolia* it was not possible to determine which of the two alkaloids was present in the higher concentration; and in the rest nornicotine predominated.

No wild species studied contained as much as 2 percent total alkaloidal content and most had less than 0.5 percent.

In 23 of the species, 84 to 100 percent of the total alkaloidal content was taken up by the main alkaloid.

In crosses between *Nicotiana tabacum*, which contains mostly nicotine, and species whose alkaloidal complex was made up largely or entirely of nornicotine, the hybrids contained mainly nornicotine together with small amounts of nicotine.

The F1 and amphidiploid *Nicotiana tabacum* (nicotine) × *N. glauca* (anabasine) contained mostly anabasine with some nicotine. In one sample of this F1 a trace of nornicotine was detected.
A quantitative method of separating anabasine from nornicotine is needed in order to study profitably the alkaloids of hybrids in which both are present.

Although the species considered as the probable wild progenitors of *Nicotiana tabacum* and *N. rustica* all have a low alkaloidal content that is predominantly nornicotine, the two cultivated species themselves have a high alkaloidal content that is mostly nicotine. A suggested explanation of this situation is that the manner of origin, selection, and culture of the cultivated species produced a high alkaloidal content, which in turn favored the formation of nicotine.

**LITERATURE CITED**


(15) SHMUK, A. A. 
1934. ON THE ALKALOIDS CONTAINED IN SOME NICOTIANA SPECIES. Kras- 

(16) 
1937. THE CHEMICAL COMPOSITION OF ALKALOIDS OF INTERSPECIES HY-
Ser. Biol., No. 6, pp. [1693]–1708. [In Russian. English sum-
mary, pp. 1707–08.]

(17) and KHMURA, M. I. 
1935. [VARIABILITY OF ALKALOID CONTENT OF THE HYBRIDS OF SOME 
Indus.), No. 15, 111–121. [In Russian.]

(18) SMITH, C. R. 
1935. OCCURRENCE OF ANABASINE IN NICOTIANA GLAUCAR GRAH. (SOLAN-

(19) 
1937. OCCURRENCE OF L-NORNICOTINE IN NICOTIANA SYLVESTRIS. Jour. 
Econ. Ent. 30: 724–727.

(20) 
1942. AZEOTROPISM IN THE SYSTEM NICOTINE-WATER. Indus. and Engin. 

(21) SMITH, H. H. 
1939. THE INDUCTION OF POLYPLOIDY IN NICOTIANA SPECIES AND SPECIES 
HYBRIDS BY TREATMENT WITH COLCHICINE. Jour. Hered. 30: 

(22) and BACON, C. W. 
1941. INCREASED SIZE AND NICOTINE PRODUCTION IN SELECTIONS FROM 

(23) SPÄTH, E., and KESZTLER, F. 
1937. ÜBER NEUE BASEN DES TABAKS (XIII. MITTEIL. ÜBER TABAK-ALKA-

(24) TERNOVSKY, M. F., KHMURA, M. I., and ŻUKOV, N. I. 
1937. INHERITANCE OF NICOTINE AND ANABASIN IN INTERSPECIFIC HYBRIDS 
Rend. (Dok.) 17: 43–45.

(25) ŻUKOV, N. I. 
1939. INHERITANCE OF NICOTINE AND ANABASINE IN INTERSPECIFIC 
HYBRIDS NICOTIANA RUSTICA L. X N. GLAUCAR GRAH. Acad. des Sci. 
INFORMATION IN REGARD TO THE POLICY OF THE JOURNAL OF AGRICULTURAL RESEARCH AND SUGGESTIONS TO AUTHORS

1. The Journal accepts articles only from the United States Department of Agriculture and the State agricultural experiment stations.

2. Each article submitted must bear the formal approval of the chief of the department bureau or the director of the experiment station from which it emanates. The letter of transmittal must state that the manuscript has been read and approved by one or more persons (named) familiar with the subject, that the data as represented by the tables, graphs, summaries, and conclusions have been approved from the statistical viewpoint by someone (named) competent to judge, and that the computations have been verified.

3. Manuscripts originating at the State agricultural experiment stations should be forwarded to the chairman of the committee acting for the Association of Land-Grant Colleges and Universities, and those originating in the Department should be transmitted to the Division of Publications, which will forward them for approval to the committee acting for the Department. Each manuscript is numbered and edited in the order received.


5. A recent copy of the Journal should be consulted and followed as to style, especially in regard to tables, illustrations, and literature citations.

6. Paper 8 x 10 1/2 or 8 1/2 x 11 inches, of good grade and medium weight, should be used.

7. All material except tables and quotations of more than three lines should be double-spaced. These may be single-spaced.

8. A table of contents properly indented to show the intended relationship between the different headings should accompany the manuscript.

9. Following the name of the author on the first page there should be given his official title and the name of the division, bureau, or station with which he is connected.

10. Each page of the manuscript should be numbered and should begin with a new paragraph; that is, no paragraph should carry over from one page to the next unless it is longer than one page.

11. Each footnote should be inserted in the text immediately after the line bearing the footnote reference.

12. Each table should be typed on a separate sheet, or on several if necessary. The page (or pages) carrying the table should immediately follow that containing the first reference to it. Each table should be referred to in the text and be numbered in the order of reference.

13. The illustrations in the Journal are usually shown as text figures, but to bring out fine detail plates may be used. Text figures and plates are each numbered in the order of reference. Each text-figure legend should be inserted in the text underneath the line carrying the first reference to it. Legends for plates should accompany the manuscript but should not be inserted in the text. All legends should be double-spaced and furnished in duplicate.

14. The major parts or units of illustrations are designated by capital italic letters; the subparts or subunits by lower-case italic letters. No final lettering on illustrations should be attempted, particularly on photographs. All lettering and necessary drafting will be done in the Section of Illustrations of the Division of Publications. Required letterings or markings should be indicated in the margins or lightly in pencil on the illustrations.

15. Graphs should be sent in final form, if possible, except for the lettering. If prepared in tentative form the curves and bars should be carefully indicated so that they may be accurately redrawn.

16. The plate or figure number and the title of the accompanying manuscript should be lightly written (not typed) on the back of each illustration. All photographs should be submitted unmounted, enclosed in an envelope.

17. Only references cited in the text should be listed in the literature citations. If there are seven or more they should be given at the end of the paper under the heading “Literature Cited.” If fewer than seven they should be given as footnotes. All numbers referring to literature citations should be enclosed in parentheses in the text. The footnote reference to the first citation in the manuscript should be worded as follows: “Italic numbers in parentheses refer to Literature Cited p.—.”

18. For further information consult Miscellaneous Publication No. 3 issued by the Joint Committee on Policy and Manuscripts. It may be obtained from the Division of Publications, United States Department of Agriculture.
CONSERVATION OF SCHOLARLY JOURNALS

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of a special committee of the American Library Association, headed by John R. Russell, the Librarian of the University of Rochester.

Because of the imminent paper shortage, attempts are being made to collect old periodicals for pulp. The Committee hopes to enlist the cooperation of subscribers to this Journal in preventing the sacrifice of this type of material to the pulp demand.

Questions concerning the project or concerning the value of particular periodicals should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.