show the great effect of the nyctotemperature upon SD induction. The optimum nyctotemperature was approximately 20° and the minimum between 10 and 14°; within the range of temperatures employed there was no maximum.

Parker and Borthwick (5) working with Biloxi soybean, a SDP, have also shown that the effect of temperature was almost completely confined to the nyctoperiod. The differences between Cestrum and soybean are of a quantitative nature; there was a broad optimum nyctotemperature range for soybean between 21 and 32° (for Cestrum the optimum was 20°) and the minimum was below 7° (for Cestrum the minimum was between 10 and 14°). If the photosensitized reactions controlling floral initiation are the same in Cestrum and Biloxi soybean, these experiments show that there are differences in the associated thermochemical reactions which have a great effect upon the nyctotemperature requirements of SD induction. The results for Cestrum and soybean are in accord with one of the implications of the theory of Borthwick et al (1), discussed previously; namely, the nyctotemperature should have the greatest effect upon SD induction, for it is during the nyctoperiod that the pigment should be in the proper state (absorbing red radiation) for coupling with thermochemical reactions involved in the synthesis of the floral stimulus or its precursors.

Summary

The effect of temperature upon LD and SD induction in *Cestrum nocturnum*, a long-short day plant, has been investigated. It has been shown that the phototemperature has the greatest effect upon LD induction and that the nyctotemperature has the greatest effect upon SD induction. The implications of these results, particularly with regard to the "pigment" theory of Borthwick, Hendricks, and Parker, was discussed, and it was concluded that the facts for Cestrum are in accord with this theory.

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STUDIES ON TOBACCO ALKALOIDS. I. CHANGES IN NICOTINE AND NORNICOTINE CONTENT IN NICOTIANA[1,2]

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Since the publication of Nath's reports on the tobacco-tomato grafting (13, 14), numerous workers have employed similar techniques to study the biogenesis of tobacco alkaloids. However, these investigations did not result in any generally accepted interpretations until the publication of Dawson's work in 1941 and 1942 (2, 3, 4). At that time he stated "of all the organs of the tobacco plant, only the root possesses an appreciable capacity for synthesizing nicotine" (4). As a result of subsequent work (5) Dawson stated that "nornicotine is produced only in the plant leaf and at the expense of nicotine." In Mothes (11) recent review, evidence from a number of workers is cited which indicates that the synthesis, or breakdown, of tobacco alkaloids may not be as simple as these generalizations indicated.

Recent advances in experimental methods, particularly the use of paper chromatography (16), make possible the detection and separation of pyridine alkaloids when present in much smaller quantities than could be handled previously. It seemed desirable to re-examine the changes in the alkaloids during the development of the tobacco plant using these tech-

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niques. The results of this study as it concerns the major alkaloids are reported here, together with certain other experiments which may assist in the interpretation of the results obtained. The following paper, part II (17), reports on the formation of nicotine and nornicotine employing N^{15} in the solution culture of intact and grafted plants and of detached leaves.

Materials and Methods

Nicotiana glutinosa and *N. Tabacum* var. Robinson Medium Broadleaf Maryland tobacco were used in these experiments because previous studies had shown that they sometimes contained considerable quantities of nornicotine. Ten gm of seed of each species were ground in 2-gm portions to avoid difficulties due to the oiliness of the seeds. Each entire sample was then extracted successively with different selective solvents and the pyridine compounds in the extracts were separated by paper chromatography and estimated by ultraviolet absorption as described by Tso and Jeffrey (16) to determine the initial content of these substances.

Weighed quantities of seeds were germinated in Petri dishes on filter paper in the presence of distilled water and daylight. Seed and seedling lots were extracted at selected intervals with 50 % aqueous acetone (v/v) to follow the changes during germination and early growth. The results were calculated on the basis of weight of alkaloid present in the seeds or seedlings derived from a given initial weight of seed.

Plants for analysis at later stages were germinated in a sterilized sand flat, starting on September 24, 1953, and grown in the greenhouse in aerated solution culture, using McMurtrey's C_1 solution (10). Samples were taken at different time intervals until January 18, 1954. These plants were also extracted with 50 % acetone, taking into consideration the water content in the plant tissue; and the alkaloids were separated and estimated as before (16). In certain cases the values are reported as zero. This means that the substance was not detected on the paper by a technique capable of detecting 0.2 μgm, which with the usual size sample corresponded to about 1 ppm in the fresh tissue. Thus the upper limit possibly present per plant would depend on plant size.

Table I

Alkaloid Content in Seed of Nicotiana Tabacum Var. Robinson Medium Broadleaf and N. glutinosa

Extracts (16)	N. Tabacum		N. glutinosa	
	Substance	Quantity	Substance	Quantity
		mg/gm		*mg/gm*
Petroleum ether	None	0*	Nicotine	0.012
Chloroform	None	0	Unknown	0.023
Alcohol	None	0	Nicotinic acid	0.114
Water	None	0	Nicotinic acid	0.024
Total		0		0.175

* Substance not detected by technique used; less than 0.2 μgm.

Table II

Alkaloid Contents During Various Stages of Germination of Seed of Nicotiana Tabacum Var. Robinson Medium Broadleaf and N. glutinosa

Time interval	N. Tabacum		N. glutinosa	
	Substance	Quantity	Substance	Quantity
hrs		*mg/gm*		*mg/gm*
64	.....	...	Nicotine	0.45
72	None	0*	.....	...
88	.....	...	Nicotine	1.50
103	Nicotine	0.29	.....	...
168	Nicotine	10.76	.....	...

* Substance not detected by technique used; less than 0.2 μgm.

Results and Discussion

Alkaloids in the Seed and During Germination: Table I shows the results obtained from seeds of *N. glutinosa* and Robinson tobacco. No alkaloids or related compounds were detected in the seed of Robinson, but nicotinic acid, an unknown compound, and a very small amount of nicotine were found in the seed of *N. glutinosa*. The unknown had an R_f of 0.24 in the 1 : 1 *tert* amyl alcohol–pH 5.6 aqueous acetate buffer system used. Its absorption spectrum showed a minimum at 246 mμ and a maximum at 260 mμ. A yellowish-brown color was produced with PABA-CNBr (16). On the basis of these results it appeared to be a pyridine derivative. Since it appeared in the chloroform fraction it was evaluated as oxynicotine. It is generally accepted that commercial tobacco seed does not contain nicotine (6), though contrary views have been expressed (18). However, nicotine has been found in *N. rustica* seed by Schmid and Serrano (15) and by Mothes and Romeike (12). The different results of previous workers and in the present data may be due to (a) different species or varieties, (b) different stages of maturity of the seeds at harvest, (c) different storage conditions, and (d) different analytical methods, in the case of previous workers.

The increase in nicotine and related compounds during germination of the seeds in shown in table II. The results are expressed on the basis of one gram of air-dry seed. This seemed to be the only practical basis for estimating synthesis, as the weights of the seeds and seedlings were changing constantly.

The formation of alkaloids in *N. glutinosa* during germination was much more rapid than in Robinson tobacco. *N. glutinosa* seeds contained nicotinic acid which disappeared during germination and may have been used in nicotine synthesis or metabolized in other ways. Dawson (8) did not find any conversion of nicotinic acid–carboxyl C^{14} or of its ethyl ester into nicotine in tobacco but this does not exclude the possi-

bilities that such conversion might occur in a very young *N. glutinosa* seedling or that the carboxyl group from the nicotinic acid may have been replaced by a pyrrolidine group.

ALKALOIDS IN N. GLUTINOSA FROM SEEDLING TO MATURE PLANT: *N. glutinosa* seedlings were grown under two different conditions in the greenhouse. One test was grown using a sterilized white sand, while the other was grown using sterilized greenhouse soil. Table III shows the results obtained. Though dilute nutrient solution (one-half of the concentration used later in solution culture) was supplied to both lots of seedlings, growth conditions were evidently more favorable in soil than in sand. The fresh weight increased more rapidly in the plants growing in soil than in those growing in sand but the fresh weight at which a given alkaloid content was found was similar in the two groups. Therefore the alkaloid synthesis was more rapid in plants growing in soil, but this was an indirect result. Significantly nornicotine appeared either earlier or about the same time in the root as in the shoot. It seems very unlikely that nicotine was formed in the root, translocated to the leaf, converted to nornicotine, and then translocated back to the root at such an early stage of plant growth. In four experiments with Robinson tobacco grown in soil, nornicotine was either undeterminable in the shoots or present in lower concentration than in the root at first analysis. The percentages of nornicotine in the root on green-weight basis were 0.095, 0.074, 0.090, and 0.132, while in the shoot they were 0.032, 0, 0.016, and 0, respectively. In some instances the lower concentration in the shoot than in the root persisted for more than half of the growing period in this variety. Thus it seems highly probable that nornicotine can be formed in the root—at least in strains known to contain high proportions of nornicotine in the leaf at later stages. Nornicotine has not been detected in the roots of young plants of strains containing predominantly nicotine in the leaves throughout the season.

Since the majority of the alkaloid synthesis undoubtedly occurs in the finer roots, which cannot be recovered quantitatively in the case of plants grown in sand or soil, *N. glutinosa* seedlings were transplanted from the sand flat to solution culture on the thirty-third day after planting of the seed. This was the same day as the second sampling from the sand flat shown in table III, but the first harvest of material from solution culture was not made until a week after the last sampling from the sand flat. The solution used was half the concentration of McMurtrey's C_1 solution (10) used for the older plants. The results obtained with these plants are shown in table IV. It should be noted that the results, both as to fresh weight in grams and the milligrams of alkaloid, are expressed on a per plant basis rather than the per 100 plant basis used in table III. The nornicotine content of the shoot exceeds the nicotine content by the 61st day from seeding. By the 68th day the shoot had become large enough to be separated into stem and leaf samples and it is shown that the nornicotine was principally in the leaf portion.

After the plants had grown in the starter solution for two weeks some of them were transferred to full-strength culture solution, one plant per 2-gallon crock. The week following the last sampling of the starter-solution plants, or four weeks after the plants had been transferred to the full-strength solution, sampling of these plants was begun. They had been

TABLE III

DEVELOPMENT OF ALKALOIDS AND FRESH WEIGHT OF YOUNG SEEDLINGS OF NICOTIANA GLUTINOSA

DAYS AFTER SEEDING	FRESH WT (GM)	ALKALOID CONTENT (MG/100 PLANTS)*					
		WHOLE PLANT		ROOT		SHOOT	
		NIC.	NOR.	NIC.	NOR.	NIC.	NOR.
		Sterilized White Sand					
12	2	0.26	0**	....	....	....	....
33	20	3.36	0**	....	....	....	....
40	44	2.92	0.04	1.47	0.02	1.45	0.02
47	100	36.89	0.58	3.09	0.08	33.80	0.50
		Sterilized Soil †					
12	10	1.44	0**	....	....	....	....
19	20	3.38	0**	0.02	Trace††	3.36	0**
26	42	4.99	Trace††	0.12	Trace††	4.87	0**
33	83	11.22	0.44	1.82	0.03	9.40	0.41
40	116	31.39	2.43	4.18	0.08	27.21	2.35
47	170	62.68	20.17	5.56	0.09	57.12	15.08

* Analysis was made by using from 6 to 100 plants, depending on size.

** No color on paper.

† Roots from sterilized soil cannot be recovered completely.

†† Colored spots located in the nornicotine area by paper chromatography but not in sufficient quantity for accurate ultraviolet determination. Less than 0.01 mg.

TABLE IV

DEVELOPMENT OF ALKALOIDS AND FRESH WEIGHT PER PLANT OF NICOTIANA GLUTINOSA GROWN IN STARTER SOLUTION

TIME AFTER SEEDING	FRESH WT/ PLANT	ALKALOID CONTENT/PLANT PART							
		ROOT		SHOOT		STEM		LEAVES	
		NIC.	NOR.	NIC.	NOR.	NIC.	NOR.	NIC.	NOR.
days	*gm*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
54	3.35	0.05	0.01	0.67	0.41	...	...	...	...
61	13.6	0.36	0.13	0.88	1.72	...	...	...	...
68	17.8	0.98	0.13	1.74	2.67	0.77	0.31	0.97	2.36

Plants transplanted into solution 33 days after seeding.

topped to 6 to 7 leaves for two weeks at that time and and were suckered frequently thereafter. The results are shown in table V.

In the case of plants at the stage of development represented in this table the major increase in alkaloid content is in the form of nornicotine in the leaves. There was also some increase in nicotine in the leaves at first, but this was reversed later, resulting in an amount too small to be determined by the techniques used. These techniques certainly would have detected 1 mg of nicotine in the approximately 150 gm of leaf material per plant, had this much been present, and probably would have detected considerably less.

There was also a noticeable increase in nicotine in the root and a much smaller increase in nornicotine there. A slow increase in nicotine occurred in the stem, but the nornicotine in the stem, always low, seems to have passed through a maximum at about the same time as the leaf nicotine reached a maximum.

It is very difficult to explain these data on the assumption that all of the nornicotine was formed in the leaf and translocated to the other parts. For example, in the week between these last two samplings, when nicotine was not determinable in the leaves, the amount of nornicotine in the leaves increased by more than 100 mg per plant. It would have to be assumed that twice as much nicotine was synthesized in the root during the last week as it contained at any one time, and that this occurred in a small fraction of the total root tissue. It would also have to be assumed that this nicotine passed through the stem without appreciable effect on the quantity there, though that quantity was only one-fourth as great, and the concentration was steadily falling due to growth of the stem. Most difficult of all, it must be assumed that this nicotine was immediately and quantitatively converted to nornicotine as it reached the leaf—even that portion which was in the conductive tissue, as the midribs of the leaves were included in the leaf samples analyzed. It is admitted that no incontrovertible proof has been demonstrated here of the synthesis of nornicotine without passing through nicotine. Nevertheless, in view of the data obtained on the young roots and the mature leaf an explanation based on the hypothesis of such a synthesis seems less taxing to the imagination than the older alternative.

On the other hand, if these data are examined while keeping in mind the limitations of the analytical methods available at the time the previous hypotheses were developed it is very easy to see how an investigator would conclude that the root and stem contained nicotine and the leaf contained nornicotine. Using picrate precipitation methods it is difficult to perceive, and impossible to estimate accurately, a minor alkaloid in the presence of a major one. Advances in understanding of physiological behavior can be made only as the tools of measurement become available.

No mention is made in the preceding five tables of compounds other than nicotine or nornicotine, though extensive evidence was obtained of the presence of other substances capable of forming colored spots on the paper with PABA-CNBr (16) and of absorbing ultraviolet light of the wave lengths absorbed most completely by pyridine compounds. Most of these compounds have not been identified and so cannot be assigned quantitative values, though the available evidence indicates that most of them are 3-pyridines. If their specific extinctions are of the same order of magnitude as those of nicotine and nornicotine the combined amount of these unknowns, in some cases,

TABLE V

DEVELOPMENT OF ALKALOIDS AND FRESH WEIGHT PER PLANT OF NICOTIANA GLUTINOSA GROWN IN CULTURE SOLUTION

TIME AFTER SEEDING	FRESH WT/ PLANT	ALKALOID CONTENT/PLANT PART					
		ROOT		STEM		LEAF	
		NIC.	NOR.	NIC.	NOR.	NIC.	NOR.
days	*gm*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
75	100	8.2	1.6	9.8	1.1	13.3	49
82	149	14.0	2.1	14.9	1.7	21.4	145
89	142	12.3	3.0	16.8	1.5	36.2	156
96	176	22.0	3.1	14.1	2.3	38.3	224
103	242	52.1	2.6	14.8	1.9	19.1	262
110	245	45.4	3.1	23.6	1.1	0.0	327
117	299	57.0	4.4	24.2	0.9	0.0	430

Plants transplanted from starter solutions 46 days after seeding.

was equal to or even greater than the combined quantity of nicotine and nornicotine. The quantity of unknowns relative to the knowns was greatest in the root, intermediate in the stalk, and least in the leaf and tended to reach a maximum in the middle of the growth period and decrease in all three areas as maturity approached. More than ten spots were observed from certain samples. At the present time it is not known whether any of these compounds are precursors or decomposition products of the known alkaloids or were derived independently. Estimates of them could not be included in the preceding tables without confusing the results with respect to known compounds.

The results obtained on mature field-grown *N. glutinosa* plants, some of which had been allowed to flower and some of which had been topped and suckered, are shown in table VI expressed on the fresh-weight basis. In addition to nicotine and nornicotine, anabasine and myosmine could be identified and determined. Certain other substances which gave color with PABA-CNBr (16) and had an absorption maximum in the ultraviolet near that of the other 3-pyridine compounds could not be positively identified, and so an estimate of the sum of such substances is given using assumptions previously outlined (9). In this instance, also, nicotine was the principal alkaloid in the root and stem and nornicotine was the principal alkaloid in the mature leaves. Myosmine was found only in those samples which contained considerable amounts of nornicotine, which is in line with results on cured tobacco samples (9), some of which contained principally nornicotine with some myosmine and some principally nicotine and practically no myosmine.

ALKALOID COMPOSITION OF ROBINSON TOBACCO: A number of different experiments have been performed which provide contributory evidence to that presented above.

The Transformation of Nicotine to Nornicotine in Different Parts of Plants: There is little doubt that nicotine demethylation is one of the principal sources of nornicotine formation. This experiment was designed to determine in which part of the plant this process takes place. This can be done more unequivocally when the parts of the plant are detached from each other.

TABLE VI

ALKALOIDS IN MATURE GREEN PLANT OF NICOTIANA GLUTINOSA

ALKALOIDS	NOT TOPPED OR SUCKERED			TOPPED AND SUCKERED		
	ROOT	STEM	LEAF	ROOT	STEM	LEAF
	% alkaloid on green wt basis					
Nicotine	0.165	0.038	0.031	0.191	0.133	0.017
Nornicotine	0.006	0.008	0.095	0.013	0.017	0.853
Anabasine	0.005	0.003	0.006	0.006	0.003	0.036
Myosmine	0*	0*	0.002	0*	0*	0.036
Others	0.016	0.007	0.017	0.026	0.007	0.121
Total	0.192	0.056	0.151	0.236	0.160	1.063

* Less than 0.001 %.

TABLE VII

THE TRANSFORMATION OF NICOTINE TO NORNICOTINE DURING AIR-DRYING IN ROBINSON MEDIUM BROADLEAF TOBACCO

PLANT PART	ALKALOID CONTENT/PLANT PART					
	ORIGINAL			AFTER 1-WK AIR-DRYING		
	TOTAL*	NICOTINE	NORNICOTINE	TOTAL*	NICOTINE	NORNICOTINE
	mg	*mg*	*mg*	*mg*	*mg*	*mg*
Root	56	45	9	45	35	8
Stem	43	34	6	32	29	3
Leaf	230	179	47	183	26	142

* Including alkaloids other than nicotine and nornicotine.

Previous studies in this laboratory have shown that, unlike *N. glutinosa,* the transformation of nicotine to nornicotine in Robinson tobacco occurs principally during senescence. Starting from the bottom of two mature plants, even-numbered leaves from plant A and odd-numbered leaves from plant B were removed and a composite of these leaves was analyzed immediately to determine the original alkaloid content. The other leaves, odd-numbered from plant A and even-numbered from plant B, were hung in the greenhouse after tying each leaf to a stick with string. These leaves were analyzed one week later. Stems and roots were split in half longitudinally and one-half of similar parts from each plant was used for immediate analysis, the other halves were hung in the greenhouse. The results in table VII show an apparent decrease of total alkaloid, which is usual during air-curing. The observed decrease in nornicotine was slightly less proportionally in the root and considerably greater in the stem than the decrease in nicotine. However, the only safe conclusion from this experiment is that no positive evidence was found of the conversion of nicotine to nornicotine in the root or stem. On the other hand, evidence of the increase of nornicotine at the expense of nicotine in the leaf is clear. This agrees with Dawson's statement (7) that only intact green leaves were able to conduct this transformation. Thus it appears that the nornicotine found in the root was probably produced by synthesis rather than by degradation. The sum of the nicotine and nornicotine in the dried leaf was 15 mg less than the total alkaloid value, indicating a much larger quantity of degradation products in this sample than in any other.

The Content of Nicotine and Nornicotine at Different Leaf Positions: The Robinson tobacco plant used in this experiment was grown in the greenhouse in solution culture. Six days after topping the leaves were carefully removed in order, starting from the

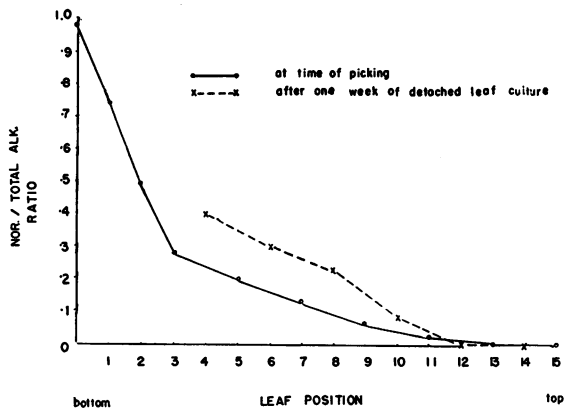

FIG. 1. Nornicotine/total alkaloid ratio at different leaf positions.

bottom of the plant. Leaves number 4, 6, 8, 10, 12, and 14 were subjected to one week of detached leaf culture, using the methods described by Dawson (1). The remaining leaves were immediately analyzed individually for total alkaloid, nicotine, and nornicotine. The detached leaf culture leaves were analyzed similarly one week later. The concentration of total alkaloid on an initial fresh-weight basis was essentially constant from the bottom to the top of the plant and was unchanged by a week of leaf culture. Thus the results can be shown most clearly when expresesd as the proportion of the total alkaloid which is nornicotine. These results, shown in figure 1, indicate that the mature basal leaves, when analyzed at the same time, contain more nornicotine and less nicotine than the immature upper leaves. The nornicotine content in the lowest leaf reached 98 percent of the total alkaloid content, while the top one contained no detectable nornicotine. The detached leaves, which had an additional period of one week for nicotine transformation, show an increase of nornicotine content up through the tenth leaf but still have a higher proportion of nornicotine in the older leaves than in the younger. Evidently the system, presumably enzymatic, by which nicotine is converted to nornicotine had not become active in the most immature leaves.

The Effect of Topping and Suckering on the Conversion of Nicotine to Nornicotine: The nornicotine content in Robinson tobacco differs greatly from season to season. Since the great majority of the nornicotine is derived from nicotine in strains such as this in which large quantities of nornicotine do not appear until late in the season, it is evident that the nornicotine content is influenced by the amount of nicotine synthesized, as well as by the proportion which is converted to nornicotine. Experiments have been conducted in which the influence of moisture, temperature, and topping practices on the conversion of nicotine to nornicotine have been observed. Of these variables, only topping appears to have a consistent influence. The results of some of these experiments are shown in table VIII. Plants were grown in pots during two seasons of the year and in two greenhouse sections each time. Attempts were made to keep one of these sections at 75° F (24° C) night and day and the other at 50 to 65° F (10 to 18° C) at night and 65 to 75° F (18 to 24° C) in the daytime. These conditions could be maintained fairly well during the experiment extending from October to February, but during the previous experiment ex-

TABLE VIII

THE EFFECT OF TOPPING AND SUCKERING AND OF TEMPERATURE ON THE ALKALOID COMPOSITION AND PLANT SIZE OF ROBINSON MEDIUM BROADLEAF TOBACCO

TRANSPLANTING DATE	GREENHOUSE SECTION	PLANT PART	NOT TOPPED OR SUCKERED					TOPPED AND SUCKERED				
			HARVEST FRESH WT	NICOTINE	NORNICOTINE	TOTAL ALKALOID	NORNIC. ×100/ TOTAL ALK.	HARVEST FRESH WT	NICOTINE	NORNICOTINE	TOTAL ALKALOID	NORNIC. ×100/ TOTAL ALK.
			gm	*mg*	*mg*	*mg*		*gm*	*mg*	*mg*	*mg*	
Mar. 22, 1954	Warm*	Root	83	53	8	64	13	90	156	22	182	12
		Shoot	859	100	236	499	47	675	1188	259	1586	16
	Cool**	Root	95	59	16	75	22	148	128	28	168	17
		Shoot	657	161	215	426	50	482	742	553	1438	38
Oct. 20, 1954	Warm†	Root	32	27	7	35	19	73	94	15	114	13
		Shoot	399	190	65	270	24	450	838	140	989	14
	Cool††	Root	52	37	9	47	18	78	154	24	190	13
		Shoot	380	76	30	110	27	313	800	251	1130	22

* Intended temperature 75° F (24° C) day and night, but often warmer in daytime during the latter part of the growing period. Harvested May 18, 1954.

** Intended temperature 65 to 75° F (18 to 24° C) daytime, 50 to 65° F (10 to 18° C) at night but often warmer in daytime during the latter part of the growing period. Harvested June 3, 1954.

† Intended temperature same as warm section of spring experiment but more nearly maintained. Harvested Jan. 19, 1955.

†† Intended temperature same as in cool section of spring experiment but more nearly maintained. Harvested Feb. 14, 1955.

tending from March to June the temperature of both sections rose above the intended levels at times during the latter part of the experiment and the difference between warm and cool sections was not maintained.

The growth of the plants was more rapid in the warm section, necessitating the topping of these plants on April 19 and November 29, whereas the plants in the cool section were not ready to top until May 6 and December 12. Similar differences in harvest dates occurred, as shown in the table, but the fresh weight of plant obtained and the amount of alkaloid found in both root and shoot was not significantly different due to temperature in the two sections so long as extra growth time was given to the plants in the cool section. On the other hand, the average amount of total alkaloid in the root of the topped plants is over three times as high as in the corresponding untopped plants and in the shoot over five times as high. The amount of nornicotine found in the topped plants is also higher than in the corresponding part of the untopped plants, but the difference in nornicotine content is not as great as the difference in total alkaloid content. Thus, the ratio of nornicotine to total alkaloid content is significantly lower in the topped plants than in the untopped plants.

Completely satisfactory evidence does not exist at present as to the chemical mechanism of the conversion of nicotine to nornicotine, i.e., whether a demethylation or a transmethylation is involved. Similarly, unequivocal evidence is not available concerning the possibility of one or more enzyme systems being involved. However, if the concersion is enzymatic and the activity of the enzyme system is limiting the rate of nornicotine formation, one would expect to find a relation of substrate quantity to degree of conversion such as here observed.

It should be noted, however, that different species or strains have different ability for nicotine-nornicotine conversion. *N. glutinosa* shows a much higher power of such conversion than does Robinson tobacco. As shown in table VI, the topped and suckered *N. glutinosa* plant has seven times more total alkaloid than that not topped or suckered, while the nornicotine to total alkaloid ratio is even higher in the leaf of the topped than the untopped plants. These results indicate that the activity of the enzyme system which is responsible for nicotine–nornicotine conversion in *N. glutinosa* is much greater than in Robinson tobacco, and therefore an increase in substrate would not affect appreciably the amount converted. Also, since topped plants had a higher proportion of mature leaves than did the untopped plants, a higher nornicotine to total alkaloid ratio is to be expected in the former than the latter if the enzyme is not a limiting factor.

Summary

The content of nicotine and of nornicotine in two selections from the genus Nicotiana, known to contain more nornicotine than most commercial varieties of tobacco, was observed at different stages from seed to mature plant.

No pyridine alkaloids were found in the seeds of Robinson tobacco, while nicotinic acid and nicotine were detected in the seeds of *N. glutinosa*. The initial rate of nicotine synthesis was much higher in *N. glutinosa* than in Robinson tobacco. In young seedlings of both strains nornicotine was detected in the root as early or earlier than in the shoot. From *N. glutinosa* seedlings to mature plants the nornicotine content per plant increased continuously, but nicotine increased and then decreased. No nicotine was detected in the leaves of over-mature, greenhouse-grown *N. glutinosa* during the last two weeks when the nornicotine increased 62 and 103 mg per plant weekly. There was little change of nicotine and nornicotine content in the root or stem at this time. All these results indicate that some nornicotine found in the root or in the shoot may not be formed from nicotine.

In Robinson tobacco the conversion of nicotine to nornicotine is not accelerated to the same degree as a result of topping as is nicotine synthesis, thus the ratio of nornicotine to total alkaloid is lower in topped plants. In *N. glutinosa*, where the nicotine to nornicotine conversion system is strongly active even in immature plants, this system is not limiting even when the alkaloid content is increased greatly due to topping and suckering.

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UTILIZATION OF GLYCEROL IN THE TISSUES OF THE CASTOR BEAN SEEDLING [1, 2]

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Although it has been recognized for some time that glycerol, as one of the products of lipase action, is produced during the germination of fatty seeds, no definitive work seems to have been done on its subsequent metabolism. The present experiments with glycerol-1-C^{14} were begun in the hope that the use of labeled material might allow a successful approach to this problem.

While this work was in progress Stumpf reported an investigation of glycerol oxidation by cell-free preparations from peanut seedlings (11). He succeeded in providing evidence for the existence of enzyme systems which cooperated in converting glycerol to CO_2 by a pathway including α-glycerol phosphate and pyruvate and subsequent oxidation in the Krebs cycle. Although the amounts of glycerol oxidized by the cell free system were very small, the results gave firm support for the conclusion that an oxidation pathway which might have appeared the most likely from a knowledge of comparative biochemistry did in fact exist in the tissue concerned.

All of the experiments in the present report were carried out on intact tissues. It will be shown, however, that there is evidence for the participation of a sequence of reactions, analogous to that described by Stumpf, in the very active oxidation of glycerol by castor bean cotyledons. In addition, it becomes clear that oxidation to CO_2 represents only one of the metabolic fates of glycerol in the tissues; a considerably larger fraction of the supplied glycerol is rapidly incorporated into sucrose. Preliminary reports of this work have appeared elsewhere (2, 4).

MATERIALS AND METHODS

Castor bean seeds (*Ricinus communis* L.) var. U.S. 70 were germinated as described elsewhere (5). Under these conditions the seedling produces a primary root of about 8 cm in 4 days, when the hypocotyl is just beginning to elongate. After 7 to 8 days the hypocotyl has reached a height of about 10 cm and the endosperm tissue has by then been almost completely absorbed by the enlarging cotyledons. It has been shown (4, 10) that active hydrolysis of the fats in the seed begins after 3 to 4 days of germination and free sugars, particularly sucrose appear in quantity in the seedling. Most of the experimental work in the present paper was done with cotyledons taken from 5- to 6-day seedlings; i.e., at a stage when an active breakdown and utilization of fats in the endosperm was occurring. Some experiments with other tissues of the seedling are also included, and for comparison the ability of some other tissues to oxidize glycerol was also examined.

Glycerol-1-C^{14} with a rated specific activity of 1 millicurie per millimole was supplied by California Foundation for Biochemical Research. For use it was diluted with an unlabeled glycerol solution so that 0.1 ml contained 10 micromoles and from 1,000 to 6,000 cpm (counts per minute). The actual activities of the glycerol solutions used in the individual experiments were determined by oxidation in the apparatus of Stutz and Burris (12).

The methods used are similar to those which have been described previously in another connection (6). The intact cotyledons (or thin slices of the other tissues) were placed in 100-ml Warburg flasks with 3 to 4 ml solution containing 0.5 ml 0.1 M potassium phosphate at pH 5.0. The CO_2 was collected in KOH, converted to $BaCO_3$ and assayed for radioactivity in a windowless gas flow counter. The figures for radioactivity are recorded in cpm, corrected for background and self-absorption.

At the end of the experimental period the tissues were extracted in hot 80% ethanol. After evaporation of the water and alcohol on a steam bath the residue was washed with ether and then dissolved in water. The solution was treated with Amberlite MB-1 exchange resin (Rohm and Haas) to remove ionic materials, and concentrated to a small volume for application as a band on Whatman No. 3 paper. It was chromatographed for 24 hours with *n*-butanol-ethanol-water (52.5:32:15.5 v/v) as the solvent. The solvent was allowed to drip off the paper. After drying, the paper was cut crosswise into ½″ strips.

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