

NITROGEN FERTILIZATION, NITROGEN AND
NICOTINE CONTENT OF BURLEY TOBACCO

by

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TABLE OF CONTENTS

	Page
I INTRODUCTION	1
II LITERATURE REVIEW	2
A. Effects of Nitrogen Rate	3
1. Nicotine and nitrogen contents	3
2. Growth, yield and quality of tobacco	6
B. Effect of Time of Nitrogen Application	8
1. Rate of growth	9
2. Nitrogen uptake during the growing season	9
3. Effect of time of nitrogen application	10
C. Fertilizer Placement	14
III MATERIALS AND METHODS	17
A. Fertilizer Level and Time of Application	17
B. Experimental Design	17
C. Plant Preparation	21
D. Agricultural Practices During the Growing Season	21
E. Sampling Procedures and Preparation	23
1. During growing season	23
2. Final harvest	23
F. Curing and Grading	25
G. Chemical Analyses	25
1. Total nitrogen	25
2. Total alkaloids	28
IV RESULTS AND DISCUSSION	29
A. Plant Growth	29
1. During the growing season	29
2. Effect of split applications of nitrogen	35
3. Effect of rate of nitrogen	39
B. Yield of Cured Leaves	39
1. Effect of split applications of nitrogen	39
2. Effect of nitrogen rates	41
C. Leaf Quality and Acre-Value	45
1. Average price	45
2. Acre-value	45
D. Flowering at Time of Topping	47
E. Nitrogen Uptake	47
1. Nitrogen percent	48
2. Total nitrogen uptake	61

	Page
F. Nicotine Synthesis	70
1. Nicotine concentrations	70
2. Amount of synthesized nicotine	77
G. Nicotine-Nitrogen Relationship	82
H. Effect of Nitrogen Placement	85
V SUMMARY AND CONCLUSION	87
VI LITERATURE CITED	92
VII VITA	96

TABLES

	Page
Table 1: Chemical analyses of soil samples of a Dunmore silt loam, Southwest Virginia Research Station Glade Spring, Virginia	18
Table 2: Rate and time of nitrogen applications to Burley 21 during two growing seasons	20
Table 3: Dry weight of tops removed at topping at different levels of nitrogen in 1966 and 1967 . .	22
Table 4: Time of transplanting, sampling, topping, and final harvest during two growing seasons . . .	24
Table 5: Percentage composition of farm grades of the total weight of cured leaves in 1966 and 1967 . .	26
Table 6: Relative weight (%) of leaves, stalks and shoots at the time indicated in relation to their total weight at harvest in 1966 and 1967	30
Table 7: Effect of topping on yield of dry matter of non-cured plant parts of Burley 21 in 1966	36
Table 8: Effect of split applications of rates of nitrogen on dry weight of shoots of Burley 21 at time of harvest in 1966 and 1967	38
Table 9: Effect of rates of nitrogen on dry weight of plant parts of Burley 21 at time of harvest in 1966 and 1967	40
Table 10: Effect of split applications of various nitrogen rates on acre-yield of Burley 21 in 1966 and 1967	42
Table 11: Effect of rates of nitrogen on yield, average price and acre-value of cured leaves of Burley 21 in 1966 and 1967	43
Table 12: Effect of split applications of rates of nitrogen on average price of cured leaves of Burley 21 in 1966 and 1967	46
Table 13: Percent nicotine and nitrogen, and nicotine-N/total-N ratio of Burley 21 tobacco during 1966 and 1967	50

TABLES (Cont.)

	Page
Table 14: Effect of topping on percent nicotine and nitrogen, and nicotine-N/total-N ratio of Burley 21 in 1966	53
Table 15: Effect of topping on the amount of nitrogen of plant parts of Burley 21 in 1966	54
Table 16: Effect of split applications of rates of nitrogen on nitrogen percent of non-cured leaves of Burley 21 at the time of harvest in 1966 and 1967	55
Table 17: Effect of split applications of nitrogen rates of nitrogen percent of stalks of Burley 21 at time of harvest in 1966 and 1967	56
Table 18: Effect of split application of rates of nitrogen on nitrogen percent of cured leaves of Burley 21 in 1966 and 1967	57
Table 19: Effect of nitrogen rates upon nicotine and nitrogen percentages and nicotine-N/total-N ratio of leaves and stalks of the last green sample and cured leaves of Burley 21 tobacco in 1966 and 1967	60
Table 20: Relative weight (%) of nitrogen, nicotine and shoots at the time indicated in relation to their total weight at harvest in 1966 and 1967	65
Table 21: Effect of nitrogen rates on amounts of nitrogen and nicotine in leaves, stalks and shoots of Burley 21 at the time of harvest in 1966 and 1967	69
Table 22: Effect of split application of nitrogen rates on nicotine percent of non-cured leaves of Burley 21 at the time of harvest in 1966 and 1967	73
Table 23: Effect of split application of nitrogen rates of nicotine percent of stalks of Burley 21 in 1966 and 1967	74

TABLES (cont.)

	Page
Table 24: Effect of split applications of rates of nitrogen on nicotine percent of cured leaves of Burley 21 in 1966 and 1967	75
Table 25: Amount of dry matter, nicotine and nitrogen synthesized by Burley 21 during 1966 and 1967 . . .	80
Table 26: Effect of methods of nitrogen fertilizer application on yield and chemical composition of leaves, stalks and shoots of Burley 21 in 1966	86

FIGURES

	Page
Figure 1: Accumulation curves of precipitation at the Southwest Virginia Research Station, Glade Spring and Saltville, Virginia	19
Figure 2: Rate of growth of leaves, stalks and shoots of Burley 21 during 1966 and 1967	31
Figure 3: Ratio of weight of dry stalks to weight of dry shoots during 1966 and 1967	33
Figure 4: Amount of dry matter synthesized per day by one pound of leaves of Burley 21 in 1966 and 1967	34
Figure 5: Effect of topping on rate of growth of leaves, stalks and shoots of Burley 21 in 1966	37
Figure 6: Effect of rates of nitrogen on weight and value of cured leaves and dry weight of green leaves and stalks in 1967	44
Figure 7: Nitrogen percent of leaves, stalks and shoots of Burley 21 during the 1966 and 1967 growing season	49
Figure 8: Effect of topping on nicotine and nitrogen percent of leaves and stalks of Burley 21 in 1966	51
Figure 9: Effect of nitrogen rates on nicotine and nitrogen contents of cured leaves, and non-cured leaves and stalks in 1967	58
Figure 10: Dry weight of leaves, stalks and shoots during 1966 and 1967	62
Figure 11: Weight of nitrogen in leaves, stalks and shoots during 1966 and 1967	63
Figure 12: Rate of nitrogen uptake in leaves, stalks and shoots in Burley 21 during 1966 and 1967	64
Figure 13: Effect of topping on rate of nicotine and nitrogen accumulation in leaves and stalks of Burley 21 during the period after topping in 1966	67

FIGURES (cont.)

	Page
Figure 14: Nicotine percent of leaves, stalks and shoots of Burley 21 during 1966 and 1967	71
Figure 15: Weight of nicotine in leaves, stalks and shoots of Burley 21 during 1966 and 1967	78
Figure 16: Rate of nicotine accumulation in leaves, stalks and shoots of Burley 21 in 1966 and 1967	79
Figure 17: Relationship between nitrogen and nicotine content of Burley 21	83
Figure 18: Percent nitrogen incorporated into nicotine at various rates of nitrogen in 1967	83
Figure 19: Percent nitrogen incorporated into nicotine in 1966 and 1967	84

I. INTRODUCTION

Due to increases in nicotine content of burley tobacco, the tobacco companies are encountering difficulties in incorporating this tobacco into cigarette blends.

The introduction of burley tobacco varieties resistant to black root rot and the existence of a support program conducive to the production of high yields are contributing factors to the increase in nicotine content (Kentucky Agr. Exp. Sta. Farm and Home Sci., 1955). However, the growing of tobacco in continuous culture and in association with heavy and often split applications of fertilizer, especially nitrogen, has also been suggested as a major factor in this increase.

It was postulated that time and rate of nitrogen application would be a practical and efficient method of regulating the nicotine content of burley tobacco.

This investigation was conducted to study the effects of (1) split applications and (2) rates of nitrogen upon nitrogen uptake, rate of growth and nicotine synthesis in association with acre-yield and acre-value.

II. LITERATURE REVIEW

There are wide differences among kinds of tobacco and their related cultural and management practices. However, in order to gain a broad perspective of some aspects of the problem studied, references pertaining to the effects of nitrogen fertilization on composition, yield and quality of tobaccos other than burley are included in this review.

Nicotine content in tobacco is determined by heredity and environmental factors. Tso and Jeffrey (1956) found nicotine contents of Maryland tobacco to be associated with varietal differences. McMurtrey et al. (1942) reported that the species Nicotiana rustica contained considerably more nicotine than Nicotiana tabacum. Topping, suckering or soil moisture contents also influenced the nicotine level in plants.

Soil nitrogen is another factor which influences nicotine content. The nicotine molecule contains two atoms of nitrogen making up 17.2 percent of its molecular weight. Nitrogen is also necessary for shoot and root development as well as for other physiological processes. Wolf and Bates (1964) showed that nicotine synthesis was almost wholly localized in tissues near the root tips, and plants having the largest root system synthesized the largest total amount of nicotine. Tso et al. (1960) found nicotine content to increase when calcium and boron were deficient.

A. Effects of Nitrogen Rates

1. Nicotine and nitrogen contents

Interrelationships between nicotine and nitrogen contents are by no means clear. Some reports showed such relationships to exist while others found no effect of nitrogen on nicotine. Merker (1964) reported that the alkaloid content in the leaves of Nicotiana tabacum and thus the level of alkaloid production was mostly influenced by the level of nitrogen.

Nichols et al. (1958) showed that increasing the nitrogen supply to burley tobacco increased the nicotine and calcium and decreased the phosphorus and potassium percentages in the cured leaf. Whitty et al. (1966) found total alkaloid concentration in burley tobacco leaves to increase with increasing rates of nitrogen up to 240 pounds of nitrogen per acre. At the higher rates, however, the increase was not so consistent or pronounced. Nitrogen content of leaves generally increased as the rate of applied nitrogen was increased. However, the increase was quadratic. Hamilton and Bortner (1966) confirmed the findings of Whitty. They also found little difference in the concentration of total alkaloids when nitrogen rates were increased from 60 to 240 pounds per acre. However, total nitrogen in the leaves increased as the amount of nitrogen in the soil increased. But a 300 percent increase in nitrogen application changed the nitrogen concentration in the leaf only 26 percent. Schipfer (1966) grew burley tobacco on a soil containing adequate amounts of phosphorus

and potassium. He found that increasing the rate of nitrogen increased yield without influencing the nicotine content.

White (1965) treated cigar tobacco with 0, 24, and 60 pounds of nitrogen per acre and found little difference in nicotine, non-nicotine or total alkaloid content of cured leaf. Anitla et al. (1961) working with Baragan 230 Cultivar tobacco found nicotine content to increase with additional nitrogen up to an intermediate nitrogen level; however, beyond that level nicotine percent decreased. When nitrogen was applied in small quantities the nicotine content was greatest in the lower leaves and least in the upper leaves; with the higher rates of nitrogen the nicotine was less abundant in the lower than in the upper leaves.

Takahashi (1964) studied nicotine synthesis in Turkish tobacco in relation to levels of nitrogen. He found nicotine content in leaves of plants receiving various levels of nitrogenous fertilizer to be low and indicated no influence of nitrogen level until topping time. At time of topping, higher rates of nitrogen resulted in higher percentages of nitrogen in the plant. After topping there was a sharp increase in nicotine content, and the effects of nitrogen levels became more apparent. Higher rates of applied nitrogen resulted in higher nicotine percentages in the leaves. Furthermore, at high levels of nitrogen the rate of nicotine synthesis continued to increase for 45 days after topping while at the low level of nitrogen the maximum rate appeared between 16 and 30 days after topping after which synthesis decreased rapidly. He also found that at the

high level of nitrogen the nitrogen expressed as grams per plant initially increased after topping and then decreased, while at the low rate of nitrogen there was no initial increase in nitrogen content and subsequently less reduction. Salmon (1967) found that applying 30 pounds of nitrogen per acre to flue-cured tobacco raised the nicotine level from 2.55 to 3.11 percent.

Pritchett et al. (1959) applied 45 pounds of nitrogen to field grown flue-cured tobacco at time of planting. Two weeks after planting 0, 45, 90, and 135 pounds of nitrogen were applied as side-dressings. They found that nitrogen content in the leaves increased by 0.09 percent for each 15 pounds of nitrogen applied. Nicotine and total alkaloid percentages significantly increased with increasing rates of nitrogen. The quadratic regression of nicotine on nitrogen rates was highly significant indicating that the nicotine content did not increase as rapidly at the higher rates as at the lower rates, but on the average for each one percent increase in nitrogen in the leaves, the nicotine content increased by about 0.7 percent.

Mashtovtsev and Sirotenko (1961) applied 0, 0.45, 0.90, and 1.80 g of nitrogen to individual tobacco plants. They found the dry weight per mature plant to be 68.7, 181.7, 170.1, and 149.6 g while the nicotine percent was 0.18, 0.62, 0.62, and 0.58, respectively. The maximum nicotine percent and dry weights were obtained with an application of 0.45 g nitrogen per plant; beyond this level no further increase was noticed. The percentage of nitrogen, however, was 1.0, 1.5, 2.5, and 3.1, respectively, indicating that nitrogen percent

continued to increase at the higher rates of nitrogen. Thus, the ratio of nicotine-N over total-N was 2.9, 6.6, 4.3, and 4.0, respectively, and non-linear.

2. Growth, yield and quality

A positive relationship between acre-yield and the amount of available nitrogen in the soil has been established. Because of the difficulties involved in the estimation of the amount of available nitrogen in the soil, most investigations have dealt with the relationship between acre-yield and the amount of nitrogen applied to a specific soil. Whitty et al. (1966) grew burley tobacco on Hiwassee clay loam. They found a significant increase in yield with nitrogen up to 160 pounds per acre in 1961 and up to 180 pounds per acre in 1962. The average price decreased with nitrogen rates greater than 160 pounds per acre in 1961 and greater than 180 pounds per acre in 1962. Increasing nitrogen applications to 80 pounds per acre increased plant height very slightly. Very high rates of nitrogen decreased plant height; the decrease was 12 inches at 670 pounds.

Adding 80 pounds of nitrogen per acre shortened the time period to flowering to 68 days; it increased to 80 days at 640 pounds of nitrogen. Wallace and Rock (1966) found that applications of 120 to 240 pounds of nitrogen per acre to burley tobacco gave the highest acre-value, while the lowest acre-value was obtained with an application of 720 pounds of nitrogen per acre. The leaves had a harsh irritating flavor at 480 to 720 pounds of nitrogen per acre. Atkinson (1966) reported that the addition of nitrogen without irrigation did

not increase the yield of burley tobacco. With irrigation increasing nitrogen from 100 to 200 and from 100 to 300 pounds per acre increased the acre-yield 13 and 21 percent, respectively. The average price of cured leaves was not influenced by nitrogen application, while irrigation increased the average price. Leaf density (weight per unit area) increased with each addition of nitrogen and decreased with irrigation and population increase.

Garner et al. (1934) studied the effect of nitrogen levels on plant growth of Maryland cigarette type tobacco. They found that plant height was low at the low level of nitrogen (20 pounds per acre) while moderate levels of nitrogen (40 pounds per acre) produced the most rapid increase in plant height and caused early flowering and early ripening of leaves to occur. Increasing nitrogen levels up to 80 pounds per acre decreased plant height and leaf density and increased leaf size, however, yield of leaves and stalks did not increase significantly over the moderate levels of nitrogen. Peterson (1964) grew cigar type tobacco on a Waupun silt loam. There was a positive yield response to nitrogen applications up to 150 pounds per acre, while the quality of cured leaves was improved with increasing levels of nitrogen up to 200 pounds per acre.

Wedin (1960) grew cigar tobacco on two different soils to study the response of yield and quality to nitrogen levels. The maximum significant yield was obtained with nitrogen rates of 75 pounds on a Parr silt loam and of 150 pounds per acre on a Stronghurst silt loam. The yield response was quadratic, and differed between soils.

He also found a highly significant increase in the quality of leaves on both soils with nitrogen application up to 150 pounds per acre. White (1965) grew cigar tobacco on a sandy loam and studied the interaction between nitrogen levels and years as well as between nitrogen levels and varieties. He found that the levels of nitrogen had greater effect in certain years on all agronomic characteristics except leaf number and percentage of midrib. His experiment revealed that increasing nitrogen levels from 24 to 60 pounds per acre had little effect on Resistant Havana 211 other than to increase the weight per leaf. However, increasing nitrogen rates for Pennsylvania Broadleaf increased the yield, weight per leaf and the percentage of filler leaf, but also tended to decrease the percentage of midrib and the percentage of yellow and trashy leaf. Corbett (1945) found that the optimum grade of Jamaica wrapper leaves was obtained from plants treated with 40 pounds of nitrogen per acre, while applying 53 pounds of nitrogen per acre significantly reduced leaf grade. Volodarskii (1954) found that an optimum amount of nitrogen had a favorable effect on color, size, anatomy and shape of tobacco leaves. Nitrogen hastened stem growth and formation of inflorescences and increased yield and quality of seeds.

B. Effect of Time of Nitrogen Application

It is important to have nitrogen available to the plant at the time most needed. Therefore, the relationship between growth rate and nitrogen uptake during the growing season is of importance.

1. Rate of growth

Grizzard et al. (1942) found that flue-cured tobacco made only 2.5 percent of its total growth during the first three weeks of a 9-week growth period. During the last 28 days of a 63-day growing season 80 percent of the growth was made. The rate of growth increased from 1.8 pounds per acre per day on the 21st day after transplanting to 19.1 pounds per acre per day on the 35th day, and reached a maximum of 43.2 pounds per acre per day when the plants were 63 days old. Davies (1940) reported for flue-cured tobacco that only 2 percent of its total growth was made during the first 3 weeks. During the periods from 4 to 5, 6 to 7, and 8 to 9 weeks for the growing season 17, 49.4, and 31.8 percent of the total growth was made, respectively. These data indicate that the highest rate of growth was made between the 6th and 7th weeks. Botha (1965), however, found the greatest rate of growth of flue-cured tobacco grown in pots to occur between the 8th and 9th weeks of growth.

2. Nitrogen uptake during the growing season

Grizzard et al. (1942) showed that flue-cured tobacco absorption of nitrogen increased from 0.08 pounds per acre per day when the plant was 21 days old to 0.84 pounds on the 35th day and reached a maximum of 1.02 pounds per acre per day on the 49th day. Absorption of nitrogen then declined to 0.17 pounds per acre per day on the 63rd day of growth. The rate of nitrogen uptake was higher than the rate of growth early in the season, but it was lower late in the season.

Davis (1940) found the greatest utilization of nitrogen by flue-cured tobacco to occur during the 6th and 7th week of the growing season.

Takahashi (1964) studied the nitrogen absorption and nicotine synthesis of Turkish tobacco during the growing season. He found that most nitrogen was absorbed between the 30th and 60th day of the growing season while most nicotine was synthesized between the 60th and 90th day. Botha (1965) grew the F_1 of the cross Vamorr x Yellow Mammoth in pots. He found that more than two-thirds of the total utilized nitrogen was taken up by the plants before they reached a height of 12 inches. The nitrogen uptake rate was greatest between the 6th and 7th week of the growing season. Maximum nitrogen uptake was two weeks earlier than the maximum growth rate.

3. Effect of time of nitrogen application

a. Nitrogen and nicotine contents

Pearse (1960) grew flue-cured tobacco in pots containing a sand and vermiculite mixture. Nutrient solution was applied by a constant drip technique. Replacing the nutrient solution with distilled water at the early flowering stage produced leaves containing 1.19 percent nitrogen and 0.66 percent nicotine. A continuous supply of full strength nutrient solution to early flowering raised the nitrogen level to 1.36 percent and nicotine to 1.15 percent, respectively. Continued feeding of nitrogen until topping time raised the nitrogen level to 1.79 percent and nicotine to 2.76 percent. A continuous supply of nitrogen until one week after

topping raised the nitrogen concentration very slightly to 1.87 percent, while nicotine increased greatly to 3.37 percent. The percent of nicotine-N over total-N was 3.2, 4.9, 8.8, and 10.4 percent with respect to the duration of the nutrient solution application. It is clear that the longer the supply was continued the greater was the amount of nicotine produced per unit of nitrogen in the leaves. The importance of the time factor is evident when comparing these data with results obtained when various nitrogen rates were applied at the same time. Pritchett et al. (1959) applied different amounts of nitrogen to flue-cured tobacco at a specific time. It was calculated from his data that the ratio of nicotine-N over total-N was 3.9, 4.7, 4.5, and 4.25 at 45, 90, 135, and 180 pounds of nitrogen available per acre, respectively. The ratios increased with nitrogen rates up to 90 pounds per acre followed by a decrease, while in Pearse's (1960) data the ratio continued to increase with a continuation of nitrogen feeding. It is suggested that the increase in the ratio of nicotine-N to total-N was due to the effect of late nitrogen supply.

Yoshida and Takahashi (1961) grew Nicotiana tabacum var. Hicks in water culture and in the field. Labeled nitrogen (N^{15}) was applied in the field at planting time and after topping in the water culture. The water culture experiment revealed that 8.7 percent of nitrogen absorbed before topping was incorporated into nicotine, while 15.1 percent of nitrogen absorbed after topping was incorporated into nicotine. Most of the nicotine was synthesized after topping,

while most the nitrogen used in nicotine synthesis was absorbed before topping. At topping time a considerable amount of protein-nitrogen in the leaves started to change to non-protein forms and to migrate to the stalk and root to be incorporated into nicotine. About 1/2 of the protein-nitrogen in the leaves of plants grown in the field decomposed to non-protein forms after topping. He also found that nitrogen absorbed after topping was distributed evenly among various parts of the plant. The field experiment showed that 90 percent of the total nitrogen absorbed before topping was derived from fertilizer. After topping, the plant absorbed only soil-nitrogen.

Takahashi (1964) found that nitrogen absorbed by Turkish tobacco after topping was more effectively incorporated into nicotine than the nitrogen absorbed before topping. That is, 15.1 percent of nitrogen absorbed after topping and 8.9 percent of nitrogen absorbed before topping were incorporated into nicotine. Since the greater increases of nicotine resulted when nitrogen was absorbed after topping, he concluded that to obtain low-nicotine tobacco, nitrogen should neither be applied late nor in excessive amounts to prevent its availability to the plant after topping.

b. Yield and leaf quality

After a uniform application of 10 tons of farm manure per acre, Shaw (1963) found no difference in yield and average price of burley tobacco when he applied 48 pounds of nitrogen from sodium nitrate, ammonium nitrate or ammonium sulfate either before or 10-14 days after transplanting. Parks and Safley (1961) found that yield

and grade index of a dark fire-cured tobacco (broadleaf Madole variety) were significantly higher in only one out of three years when all of the 120 pounds of nitrogen was applied at planting time or half at planting time and half as a sidedressing than when all of the nitrogen was applied as a sidedressing. The three year averages indicated that applying all of the nitrogen at planting or half at planting and half as sidedressing resulted in essentially the same acre value. However, applying all the nitrogen as a sidedressing resulted in a significantly lower acre value.

Kangas (1941) found that sidedressing flue-cured tobacco 21 days after transplanting with one-third (9 pounds) of the total nitrogen applied coming from sodium nitrate increased the yield very slightly, but had no effect on the utilization of nitrogen. Davies (1940) found that sidedressing flue-cured tobacco with part of the 27 pounds of nitrogen per acre 21 days after transplanting decreased the total amount of nitrogen utilized.

Wierzba (1962) studying the influence of date of nitrogen top-dressing on yield and leaf quality of Virginia Skroniowska tobacco, reported that nitrogen greatly increased the yield. The highest yield of leaves was obtained with an application of nitrogen, phosphorus, potassium, and farm yard manure applied 10-20 days after transplanting. Nitrogen applied at a late date (30 days after transplanting) prolonged the growth period and thus caused difficulties in drying of the leaves.

Corbett (1945) studied split applications of 26, 40, and 53 pounds per acre of nitrogen on Jamaica Wrapper Cigar tobacco in Mauritius. He found split applications of sodium nitrate to result in better yields than one application. One application, however, produced the higher grades. One application of ammonium sulfate produced the higher yields, while two applications produced better grades. Khemchandani et al. (1958) studied the effect of time of application of ammonium sulfate on cigar tobacco. They found that 100 pounds of N per acre, one-half applied in bands before planting and one-half applied as topdressing about seven weeks after planting, gave the highest acre-value.

Volodarskii (1954) found that young plants require a low but constant nitrogen supply. Increasing nitrogen at this stage depressed growth. With increasing growth the demand for nitrogen increased until the flowering stage. During the early and final stages of growth, nitrogen inhibited the development of leaves. Positive effects of increased nitrogen levels were observed during rapid growth of leaves and were most pronounced on central and upper leaves.

C. Fertilizer Placement

Most of the studies dealing with methods of fertilizer placement were carried out using mixed fertilizer. However, Whitty et al. (1966) applied ammonium nitrate in band widths of 8 and 32 inches to burley tobacco grown on Hiwassee clay loam. He found that responses to the volume of N enriched soil were more evident soon after transplanting than at the time of harvest. Yield and chemical

composition of the cured leaf (including nicotine and nitrogen content) were usually influenced more by the rate of nitrogen than by the differences in volume of soil fertilized. The lack of differences in yield between the two placement procedures is believed to be due to the mobility of nitrogen in the soil, the rapid uptake and translocation of nitrogen and the lack of differences in root growth. At a given rate of nitrogen, mortality was greater in the narrow band than in the wide band of fertilized soil. Application of nitrogen in the 8 inch band resulted in slower growth than did an equal rate in the 32 inch band. The time from transplanting to flowering was longer when nitrogen was placed in the narrow band than when the same rate of nitrogen was placed in the wide band. Only in one out of two years did the placement in narrow band result in a lower average price than in wide band. The mentioned differences became greater as the rate of nitrogen increased. Scot (1951) applied 1000 pounds of 4-8-10 fertilizer per acre to burley tobacco grown on a sandy loam. He concluded that band application was superior to broadcast.

McKee and Street (1963) grew Maryland tobacco on Mommoth fine sandy loam. They made a comparison between broadcast and band application of a 4-8-12 fertilizer and found no difference between band and broadcast application in yield, acre value, and rate of seedling mortality. However, leaf-burn was higher with band application than with broadcast. Bowling and Wilson (1964) grew Maryland tobacco on a loamy fine sand. They applied 800, 1600 and 2400 pounds per acre of 3-9-15 fertilizer. The fertilizer was applied broadcast

or under the row two weeks before planting. The data revealed no significant difference in yield, average price, acre value, fire holding capacity and flowering date between broadcast and under the row treatments at all rates of fertilizer. However, the nitrate nitrogen of fresh plant tissue at the 2400 pound per acre rate was significantly higher in the under the row treatment. The percent of plant mortality at the 1600 and 2400 pound per acre fertilizer rate was also higher in the under the row treatment.

DeRoo (1958) summarizing some of the results obtained in fertilizer placement studies concluded that:

(1) No evidence favored the usual practice of applying fertilizer early (1 to 2 weeks before planting time), and there might be some gain in applying it when the plants are set, probably through avoiding early leaching of some of the soluble nutrients.

(2) Band application of all of the fertilizer on both sides of the row during planting (only 4 inches from the plant stalk and about 4 inches deep) increased quality but not yield.

(3) Band placement of the fertilizer mixture gave a better return than the broadcast method, indicating that by using the band method, the grower could reduce appreciably the amount of fertilizer needed.

(4) Unfortunately, in dry seasons the band applications caused considerable root injury from excessive concentrations of fertilizer in the soil if the entire amount, 1900 to 2400 pounds per acre of 8-4-8, was within 4 inches of the plants.

III. MATERIALS AND METHODS

This study was conducted on a Dunmore silt loam at the Southwest Virginia Research Station, Glade Spring, Virginia, during 1966 and 1967. Soil samples were taken and analyzed prior to planting (Table 1). Rainfall measurements were made during the growing season (Figure 1).

A. Fertilizer Levels and Time of Application

In 1966, acre-rates of 150 pounds of nitrogen, and in 1967, 75, 150, and 225 pounds of nitrogen from NH_4NO_3 (33.5% N) were applied prior to planting or in split applications after transplanting (Table 2). All plots received a uniform broadcast application of 200 pounds of P_2O_5 from 48 percent $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and 400 pounds of K_2O from 50 percent K_2SO_4 , per acre, respectively.

B. Experimental Design

A randomized block design with four replications was used. In 1966 plants were grown in three-row plots (10.5 feet x 105 feet) with a spacing of 3.5 feet between the rows and 1.5 feet within the row.

In 1967 plot size varied. Plot size for treatments also used in 1966 was 90 x 7 feet, each row containing 60 plants, some of which were used to supply plants for sampling during the growing season. Plot size of the new treatments was 42 x 7 feet, each row containing 23 plants. In this case no samples were taken during the growing season. Each plot consisted of three rows with two rows serving as a common border with adjacent plots. Spacing between rows was 3.5 feet with 1.5 feet within the row.

Table 1. Chemical analyses of soil samples of a Dunmore silt loam
 Southwest Virginia Research Station
 Glade Spring, Virginia.

Year	Statistical Description	pH	% OM	Nutrients in lb /acre and level			
				CaO	MgO	P ₂ O ₅	K ₂ O
1966	Avg of 4 reps	5.7	1.4	1261 high	371 high	166 high	275 high
1967	Avg of 4 reps	5.7	1.4	666 medium	383 high	119 high	132 medium

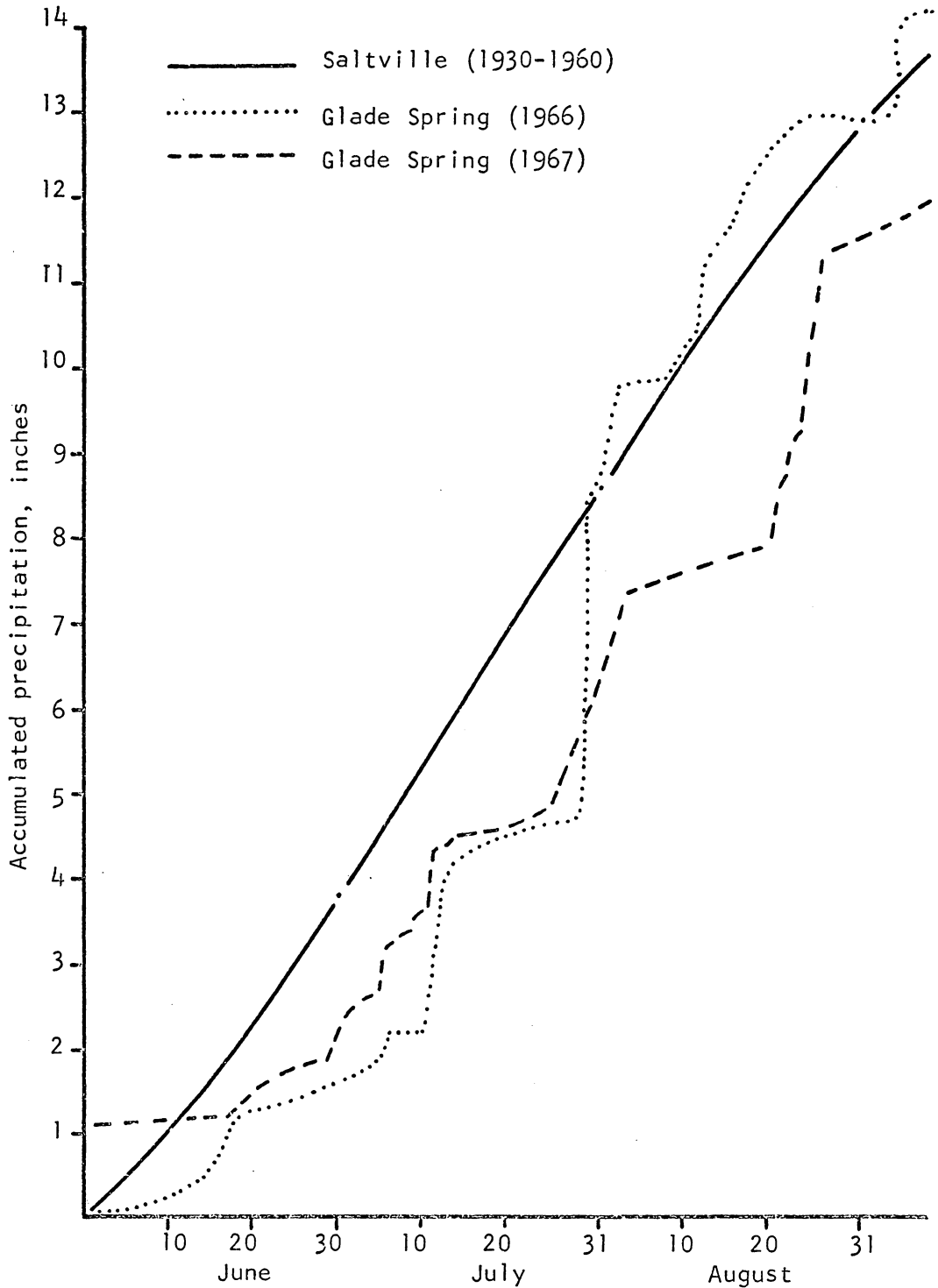


Figure 1: Accumulation curves of precipitation at the Southwest Virginia Research Station, Glade Spring, and Saltville, Virginia. (Saltville is located 9 miles from the station).

Table 2. Rate and time of nitrogen applications to Burley 21 during two growing seasons.

Rate lb/acre	Nitrogen application		
	0	21	36
- 1966 -			
150	150	0	0
150	100	50	0
150	50	100	0
150	50	50	50
150	0	150	0
150*	0	150	0
- 1967 -			
0	0	0	0
75	75	0	0
75	50	25	0
75	25	50	0
75	25	25	25
75	0	50	25
75	0	25	50
150	150	0	0
150	100	50	0
150	50	100	0
150	50	50	50
150	0	100	50
150	0	50	100
225	225	0	0
225	150	75	0
225	75	150	0
225	75	75	75
225	0	150	75
225	0	75	150

* The nitrogen fertilizer was applied in a band, while in others as side-dressing.

C. Plant Preparation

The following procedure was used to obtain uniform plants for transplanting. Flats were filled with a mixture of equal volumes of soil, sand and peat. Also, 50 grams of a 4-9-3 fertilizer were mixed with the soil in each flat. All the soil was sterilized by steam for three hours to kill weed seeds. Seeds of Burley 21 were sown on the surface at the rate of about 0.1 gram of seed per square meter, and covered with a very thin layer of fine gravel to prevent washing of seeds during watering. The flats were covered with a cheese cloth to reduce evaporation and prevent seedling burn.

After germination the plants were watered once a day. The seedlings were transplanted into 2" x 2" peat pots when the leaves were about 2.5 cm in diameter (approximately 40 days after sowing). The seedlings were hardened by exposing them to the weather outside of the greenhouse prior to being transplanted into the peatmoss pots. Fertilizer was applied as the need arose. After attaining a height of 10-15 cm the plants and pots were transplanted into the field.

D. Agricultural Practices during the Growing Season

The plants were transplanted to the field on May 31 in 1966 and on June 6 in 1967. The plants were topped after 70 days in 1966 and 72 days in 1967 to the best commercial leaf. However, it was found that the dry weight of tops removed was higher in 1967 than in 1966 (Table 3) indicating that topping level was lower in 1967 than in 1966. It was also found that the weight of flowers

Table 3. Dry weight of tops removed at topping at different levels of nitrogen in 1966 and 1967.

Part of Tops	Level of Nitrogen, lb/acre				
	150	0	75	150	225
	1966	1967**			
		lb/acre			
Flowers	24.6	63.6ab*	65.1a	58.5ab	54.1b
Leaves	42.9	79.3b	146.5a	143.6a	130.9a
Stalks	29.5	182.4a	185.2a	176.6a	178.6a
Total	97.0	325.3	396.8	378.7	363.6

* Values within rows not followed by common letters differ significantly ($p = 0.05$, Duncan's Multiple Range test).

** Levels of significance apply to 1967 data only.

removed at the time of topping was higher in 1967 than in 1966 implying that plants were topped at an earlier stage in 1966 than in 1967. Suckers were removed by hand after topping when the need arose.

E. Sampling Procedures and Preparation

1. During growing season

Plants were harvested for chemical analysis at intervals throughout the growing season. In 1966 twelve samples were taken from each treatment. On the basis of the data obtained in 1966, the number of samplings was reduced to 6 in 1967 (Table 4). Although the 1967 investigation contained more treatments, only those treatments were repeatedly sampled which were also used in 1966.

At each sampling time two plants were harvested, one from each end of the sampling row. As the plant grew larger, every other plant was harvested in order to reduce border effects.

Leaves were separated from the stalks. Compressed air was used to remove the adhering dust particles. Leaves as well as stalks were chopped, oven dried in cheese cloth bags at 140° F in a forced-draft oven and their dry weight determined. Samples were ground and subsamples taken for chemical analysis.

2. Final harvest

In 1966 twenty-five plants were harvested from each plot on September 9, 101 days after transplanting. In 1967, twenty-five plants were harvested by September 13, 99 days after transplanting from those treatments which were also used in the 1966 investigation.

Table 4. Time of transplanting, sampling, topping, and final harvest during two growing seasons.

Sample (1966)			Sample (1967)		
Description	Date	Days after planting	Description	Date	Days after planting
<u>Transplanting</u>	May 31	0	<u>Transplanting</u>	June 6	0
Sample No. 1	June 14	14	Sample No. 1	June 27	21
Sample No. 2	June 21	21	Sample No. 2	July 6	30
Sample No. 3	June 28	28	Sample No. 3	July 17	41
Sample No. 4	July 1	31	Sample No. 4	Aug. 17	72
Sample No. 5	July 6	36	<u>Topping</u>	Aug. 17	72
Sample No. 6	July 11	41	Sample No. 5	Aug. 31	86
Sample No. 7	July 18	48	Sample No. 6	Sept.13	99
Sample No. 8	July 28	58	<u>Final Harvest</u>	Sept.13	99
Sample No. 9	Aug. 8	69			
<u>Topping</u>	Aug. 10	70			
Sample No. 10	Aug. 18	79			
Sample No. 11	Aug. 25	86			
Sample No. 12	Sept. 9	101			
<u>Final Harvest</u>	Sept. 9	101			

Twenty plants were taken from all other treatments. After cutting, the plants were left in the field for a few hours to wilt, and subsequently, taken to the barn for curing.

After curing, the leaves were separated from the stalks, divided into farm grades, weighed, and subsamples taken. The midrib was removed from each leaf, and the lamina dried at 140° F. All grades were ground separately and composited on the basis of their percentage of the total weight of the cured leaf.

F. Curing and Grading

After curing, the tobacco was stripped according to farm grades by five men, each one pulling a grade. The weight of each grade was determined (Table 5) and a subsample representing each grade taken for final chemical analyses. For the rest of the sample U. S. grades were obtained. The average season price for each grade was used to calculate the acre-value.

G. Chemical Analyses

1. Total nitrogen

The standard Kjeldahl method was used with some modifications where indicated. Samples were digested. The ammonia was separated by steam distillation, collected in boric acid-indicator and determined by titration with standard acid. The following procedure was used.

(1) Reagents:

(a) Concentrated sulfuric acid

(b) Sodium hydroxide solution - 10N

Table 5. Percentage composition of farm grades of the total weight of cured leaves in 1966 and 1967.

No.	Description	Grade Leaf position	Percent of total cured weight	
			1966	1967
1	Flyings	Scrap trash and Trash lugs	6.57	11.93
2	Lugs	Sand lugs	29.62	19.37
3	Leaves	Good lugs and Leaves	32.52	33.27
4	Reds	Red leaves	24.53	20.46
5	Tips	Tips and Green leaves	6.57	14.12

- (c) Hydrochloric acid solution - 0.1N
- (d) Digestion mixture: 200 parts of anhydrous Na_2SO_4 , 4 parts of anhydrous CuSO_4 and 1 part of Se metal (Peterson and Chesters 1964).
- (d) Boric acid-indicator solution: 20 g of reagent grade boric acid were dissolved in about 700 ml hot water, cooled and transferred to a liter volumetric flask containing 200 ml of 95% ethanol and 20 ml of mixed indicator solution prepared by dissolving 0.66 g of bromocresol green and 0.330 g of methyl red in 1,000 ml of 95% ethanol. After mixing the contents of the flask, 0.05 N NaOH was added cautiously until a color change from pink to pale green was just detectable when 1 ml of the solution was treated with 1 ml of water. Then the solution was diluted with water to one liter and mixed thoroughly (Bremner and Edwards 1965).
- (2) Procedure: One half gram oven dry sample was placed in a dry Kjeldahl flask and approximately 5 g of the digestion mixture and 10 ml of concentrate sulfuric acid were added in the sequence indicated. The flasks were shaken to mix the sample with the sulfuric acid. Samples were digested at low temperature until foaming was reduced, after which the digestion proceeded at high temperature for 1 1/4 to 1 1/2 hours following the appearance of a green color.

Samples were cooled and diluted with distilled water. About 50 ml of 10N NaOH were added and the ammonia was distilled into 50 ml of 2% boric acid (each 1 ml of 2% boric acid will effectively absorb about 1 mg of ammonia - N). More than 250 ml of distillate were collected within 20 minutes, and the ammonia was titrated with 0.1 N HCl. Percent nitrogen was calculated according to the formula:

$$\% \text{ of N} = \frac{\text{ml of HCl} \times \text{normality of HCl} \times 14}{\text{weight of sample (g)} \times 10}$$

2. Total alkaloids

According to the method by Cundiff and Markunas (1955) an alkaline tobacco mixture was extracted with 9 : 1 benzene-chloroform solution and aliquots of the extract titrated with standard perchloric acid. The alkaloid percentage was calculated as percent nicotine. Ammonia did not interfere in this procedure.

IV. RESULTS AND DISCUSSION

A. Plant Growth

1. During the growing season

The rates of growth of shoots, leaves, and stalks during the 1966 and 1967 growing seasons are presented in Figure 2. The rate of shoot (sum of leaves and stalks) growth was relatively low for the first month after transplanting. It was followed by a rapid increase, up to a maximum of approximately 125 and 150 pounds of dry matter per day per acre, 50 to 65 days after transplanting in 1966 and 1967, respectively. After the maximum had been reached the rate of growth decreased more rapidly in 1967 than in 1966. The rapid decrease in rate of growth in 1967 was probably due to the lower height of topping in 1967. The dry weight (for the 150 lb nitrogen rate) of the topped portion was 97.0 lb/acre in 1966 and 378.7 lb/acre in 1967 (Table 3). With the higher height of topping more of the young leaves were left thus contributing to growth.

It was found (for the 150 lb nitrogen rate) that during the three time intervals studied, 0-41, 41-70, and from 70-100 days respectively, the weight of shoots constituted 14.9, 58.7, and 26.3 percent of the final total weight (Table 6). Most of the growth in 1967 was made earlier than in 1966. Rate of growth was more rapid in 1967 than in 1966 (Figure 2).

The rate of leaf development during the 70 days after transplanting was higher than the rate of stalk growth. However, after

Table 6. Relative weight (%)* of leaves, stalks and shoots at the time indicated in relation to their total weight at harvest in 1966 and 1967.**

Plant Parts	Season	Approximate time intervals, days		
		0-41	41-70	70-100
Leaves	1966	19.1	55.2	25.7
	1967	26.4	70.9	2.7
	Avg.	22.7	63.0	14.2
Stalks	1966	5.0	44.0	51.0
	1967	6.4	68.4	25.2
	Avg.	5.7	56.2	38.1
Shoots	1966	12.8	47.7	39.5
	1967	17.1	69.8	13.1
	Avg.	14.9	58.7	26.3

* (Weight at interval/total weight at harvest) x 100.

** Fertilization per acre: 150 lb of N, 200 lb of P_2O_5 , and 400 lb of K_2O .

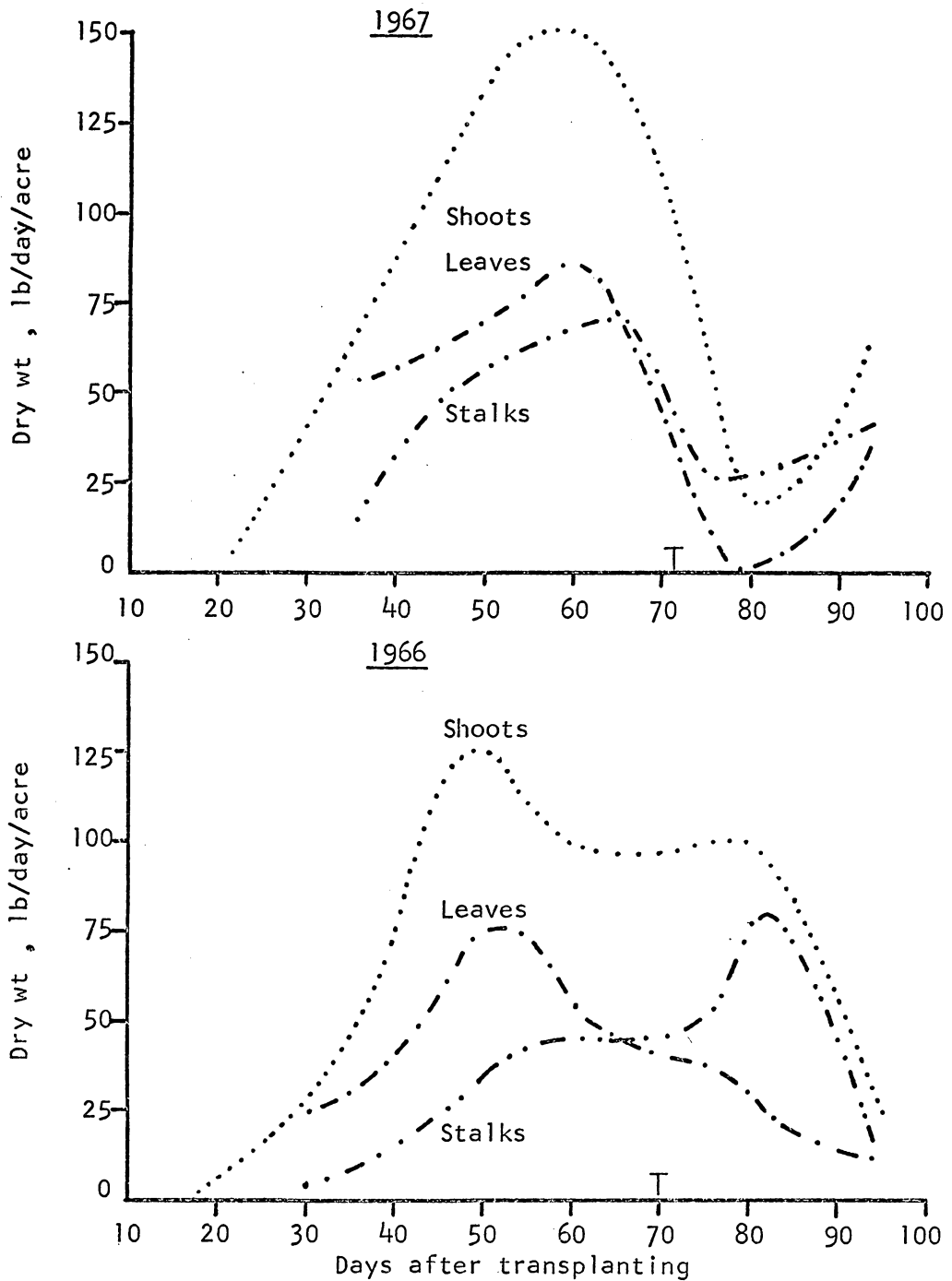


Figure 2: Rate of growth of leaves, stalks and shoots of Burley 21 during 1966 and 1967. (T indicates time of topping).

this period the rate of stalk growth was higher than leaf growth (Figure 2). During the last 30 days of the growing season about 38.1 percent of the final dry weight of the stalk was made while only 14.2 percent of the final dry weight of leaves was produced (Table 6). It appears that stalk growth tends to increase late in the growing season while most of the leaf growth occurs earlier. Hence, stalk weight expressed as a percent of the total shoot weight increased with time (Figure 3).

Since leaves are the site of dry matter production the rate of growth per day is determined by the average activity of one unit-weight of leaves multiplied by the number of unit-weights which are contributing to dry matter production. The number of leaf unit-weights were considered to be the total dry weight of leaves expressed in pounds. The activity of leaves expressed as the total weight of shoots produced per unit-weight of leaves per day during the growing season is illustrated in Figure 4. The data shows that leaf activity was low at time of transplanting. However, when plant roots became established the activity of the leaves increased rapidly and reached its maximum 20 days after transplanting followed by a steady decrease until final harvest. The weight of shoots at any time (t) during the growing season is equal to $\int_0^t W(x)A(x)dx$ where (W) is the weight of leaves contributing to the dry matter production and (A) is the average activity of leaves in dry matter production.

The effect of topping on growth was studied in 1966 by making comparisons between topped and untopped plants when 150 lb of

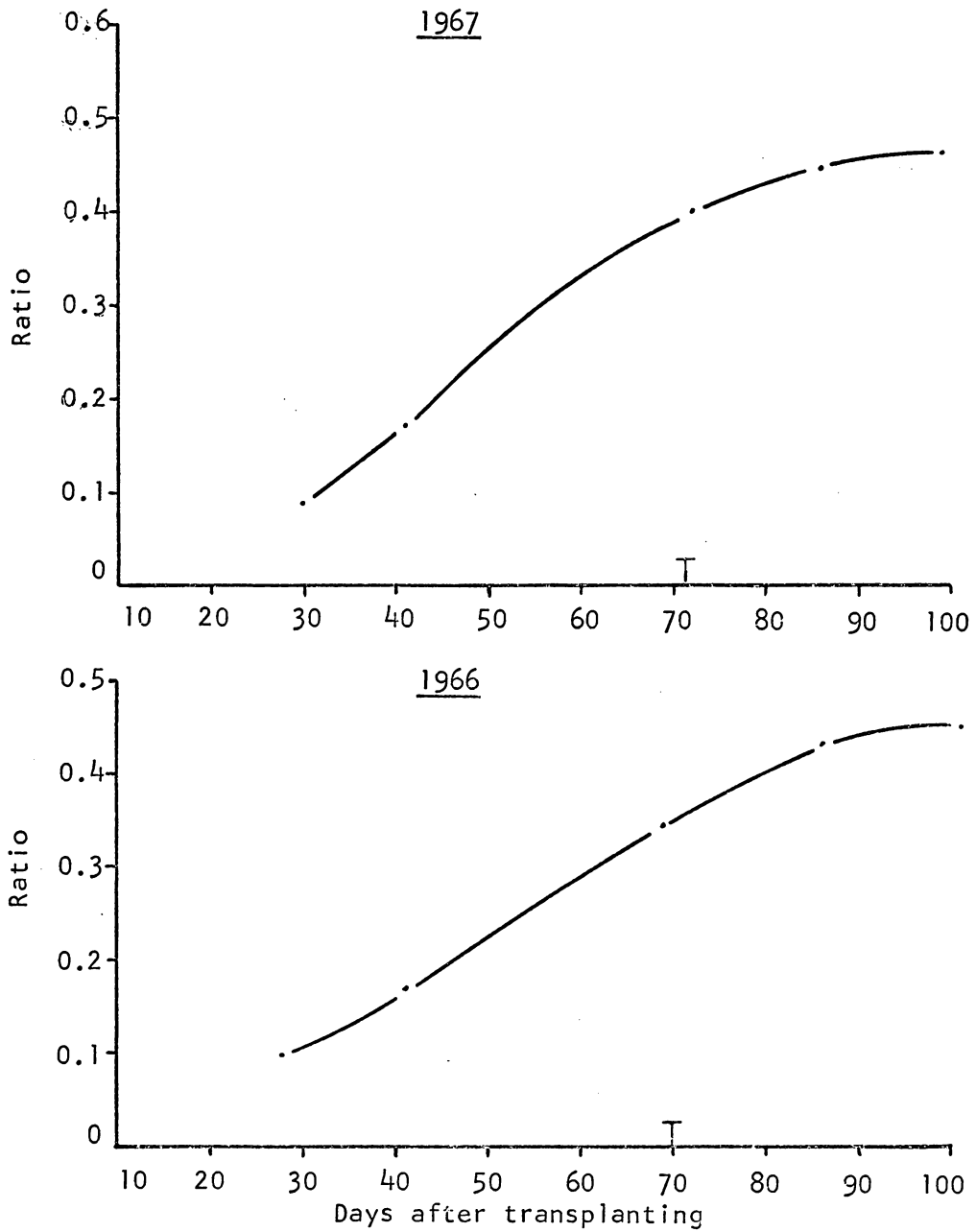


Figure 3: Ratio of weight of dry stalks to weight of dry shoots during 1966 and 1967. (T indicates time of topping).

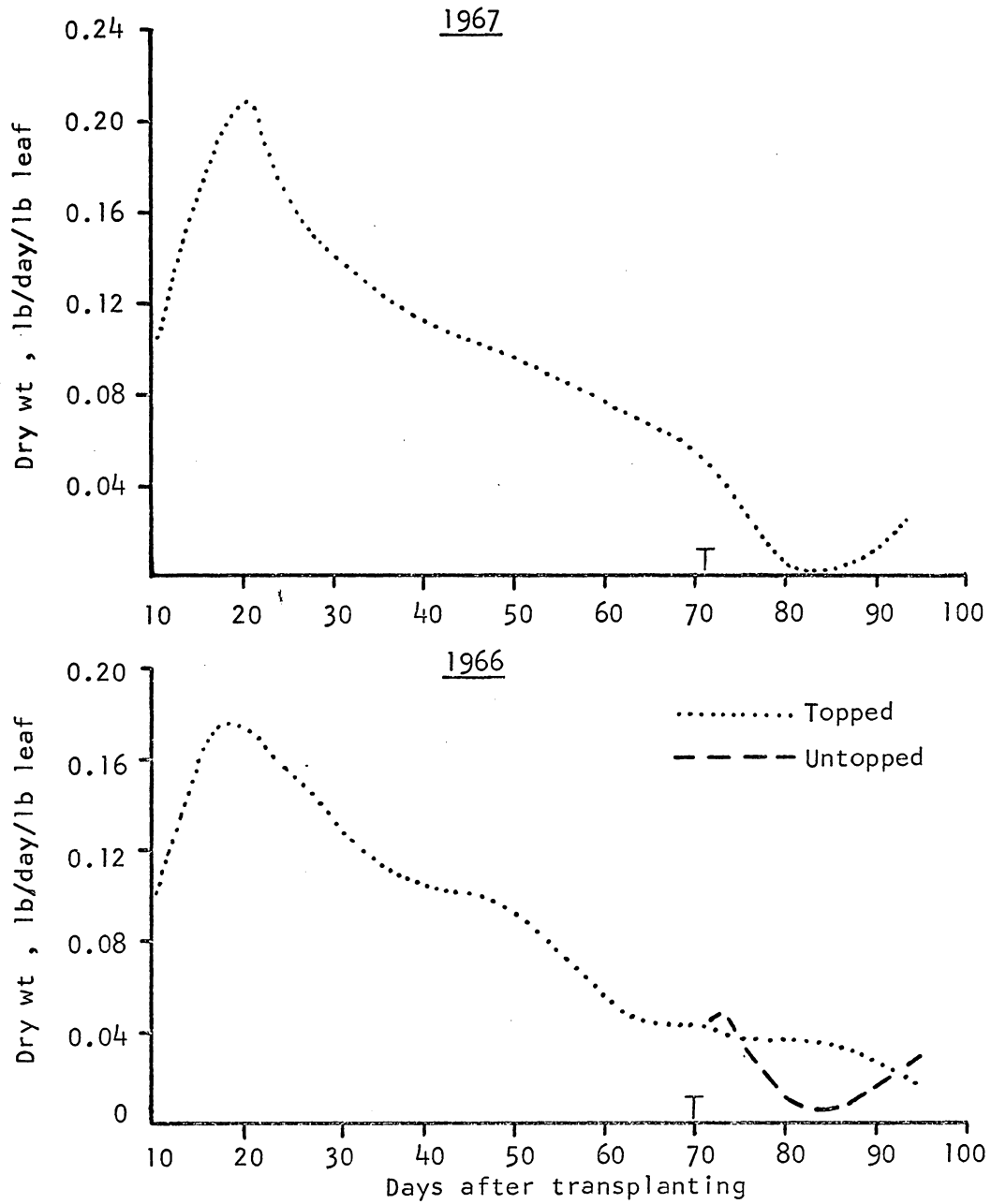


Figure 4: Amount of dry matter synthesized per day by one pound of leaves of Burley 21 in 1966 and 1967. (T indicates time of topping).

nitrogen was applied prior to planting. At the end of the growing season the weight of shoots as well as that of stalks was higher for untopped plants than topped ones, while the weight of leaves was higher in topped plants (Table 7). The rate of stalk growth was higher in topped plants (Table 7). The rate of stalk growth was higher than the rate of leaf growth after time of topping for both topped and untopped plants. The rate of leaf growth was higher with the topped plants than with the untopped ones. The rate of stalk growth for topped plants increased after topping to a point higher than untopped plants. This was followed by a rapid decrease (to almost zero) while the rate of growth for untopped plants continued to be high to the end of the growing season (Figure 5). This implied that dry matter accumulation occurred immediately after topping. There was a slight decrease in the rate of stalk growth of untopped plants in the last 20 days before harvesting. The activity of the leaves in dry matter production was stimulated after topping (Figure 4).

2. Effect of split applications of nitrogen

Differences in dry weight of shoots resulting from split applications of 150 pounds of nitrogen per acre in 1966 and 75, 150, and 225 pounds per acre in 1967 were not significant (Table 8). The following possibilities would appear to explain the results obtained:

- (1) Split applications of nitrogen were made at a time when rate of growth and hence, demand for nitrogen was low.

Table 7. Effect of topping on yield of dry matter of
non-cured plant parts of Burley 21 in 1966.*

Days after topping	Leaves		Stalks		Flower	Total	
	Untopped	Topped	Untopped	Topped	Untopped	Untopped	Topped
				lb/acre			
0	2290	-----	1177	-----	24	3491	-----
9	2994	2679	1828	1533	72	4894	4212
16	2642	2891	2255	2255	92	4989	5146
31	2789	2910	2965	2272	323	6077	5182

* All treatments received a preplant application of 150 lb of N per acre.

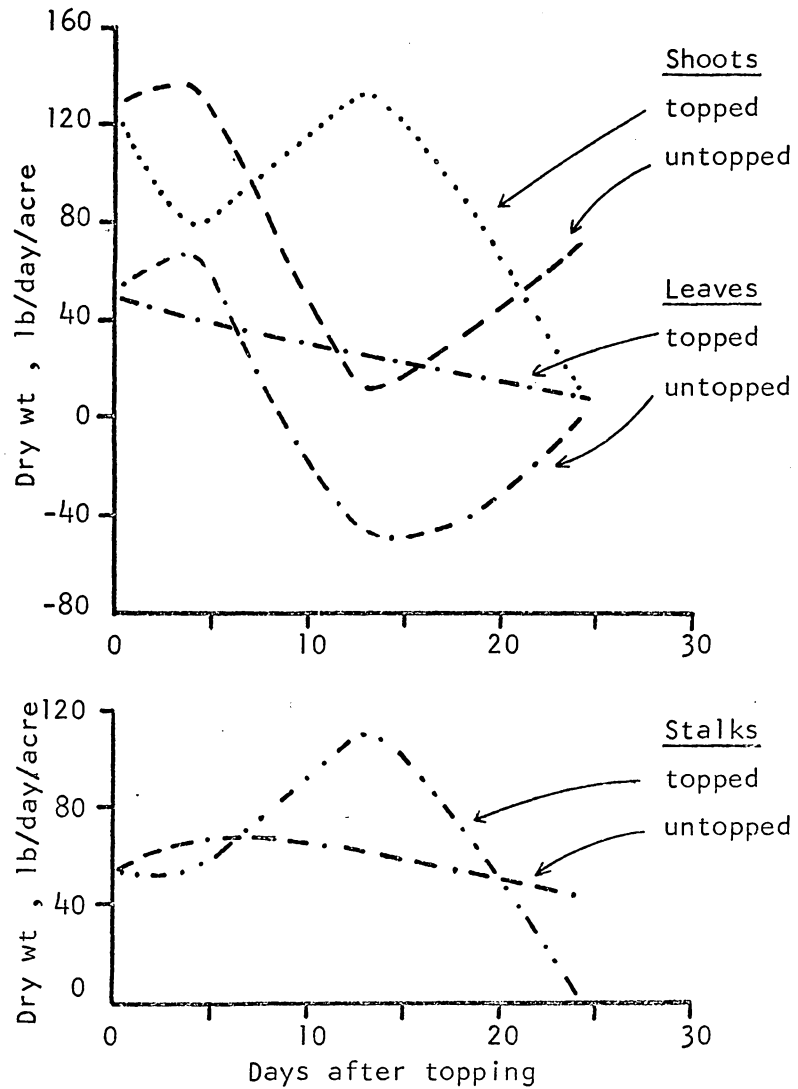


Figure 5: Effect of topping on rate of growth of leaves, stalks, and shoots of Burley 21 in 1966.

Table 8. Effect of split applications of rates of nitrogen on dry weight of shoots of Burley 21 at time of harvest in 1966 and 1967.

Treatments		Rates of nitrogen, lb/acre			
No.	Fraction* and time	150	75	150	225
		1966**	1967**		
		lb/acre			
1 :	1 - 0 - 0	5181	4906	5781	5963
2 :	2/3-1/3- 0	5642	5867	5645	5273
3 :	1/3-2/3- 0	5269	5405	5544	5629
4 :	1/3-1/3-1/3	5705	5243	5994	5414
5 :	0 - 1 - 0	5444	-----	-----	-----
6 :	0 -2/3-1/3	-----	6174	5798	5467
7 :	0 -1/3-2/3	-----	5065	5738	5470

* In the order given, the figures indicate the fractions of the total nitrogen which were applied at preplant, 21 days and 36 days (1966) and 41 days (1967) after transplanting, respectively.

** The differences between means within columns were not significant ($p = 0.05$, Duncan's Multiple Range test).

- (2) Rainfall was low during the time when split applications were made, hence no nitrogen was lost due to leaching and there was still enough nitrogen available either from that applied at transplanting or resulting from nitrification.
- (3) The size of samples taken was too small to detect a small difference if it existed.

3. Effect of rate of nitrogen

The effect of rates of nitrogen on the dry weight of shoots was determined at time of harvest in 1967. Nitrogen applications significantly increased the weight of dried leaves when compared with the no nitrogen treatment (Table 9). However, there was no significant difference in dried leaf weight between treatments receiving 75, 150 and 225 pounds of nitrogen per acre, respectively. Probably the size of sample, two plants from each plot, was too small to show the effect of treatments. Rates of nitrogen did not affect the dry weight of stalks, hence the no nitrogen treatments showed a higher percentage of stalk.

The weight of leaves removed at topping time from the no nitrogen treatment was significantly lower than those obtained from the treated plots. However, the weight of the removed stalks was not affected by nitrogen treatments (Table 3).

B. Yield of Cured Leaves

1. Effect of split applications

Split application of 150 pounds of nitrogen in 1966 did not affect acre-yields. However, in 1967 split application of only

Table 9. Effect of rates of nitrogen on dry weight of plant parts of Burley 21 at time of harvest in 1966 and 1967.

Plant Parts	Rates of nitrogen, lb/acre				
	150	0	75	150	225
	1966	1967			
		lb/acre			
Leaves	3025	2310b*	2873a	3093a	2992a
Stalks	2453	2393a	2558a	2662a	2541a
Shoots	5478	4703	5432	5755	5533

* Values within rows not followed by common letters differ significantly ($p = 0.05$, Duncan's Multiple Range test). Tests of significance apply to 1967 data only.

75 pounds resulted in significant differences. In treatments 3 (1/3-2/3-0) and 6 (0-2/3-1/3) yields of tobacco were significantly higher than yields obtained from treatments 2 (2/3-1/3-0) and 7 (0-1/3-2/3) for 75 pounds of nitrogen applied (Table 10). The higher yields were obtained when 2/3 of the nitrogen was applied three weeks after transplanting. Lower yields were obtained when all or 2/3 of the nitrogen was applied at time of transplanting or when 2/3 of nitrogen was applied 41 days after transplanting. Split applications of higher nitrogen rates did not increase yields because the higher amounts of nitrogen available for plants obscured any decrease in the efficiency of the nitrogen applied.

2. Effect of nitrogen rates

Increasing nitrogen rates to 75, 150, and 225 pounds per acre resulted in step-wise increases in yield (Table 11). The increase did not form a straight line relationship (Figure 6). The use of 75 pounds of nitrogen per acre gave a yield increase of 4.4 pounds of cured leaf for each pound of nitrogen. By increasing the rate of nitrogen from 75 to 225 pounds per acre, yields increased only 2 pounds of cured leaf for each pound of nitrogen applied. The average yields in 1966 and 1967 resulting from 150 pounds of nitrogen per acre were 3081 and 3348 pounds per acre, respectively.

Others found that the amount of nitrogen needed to produce the highest acre-yield varied for different varieties during different years and on different soils. To obtain the highest acre-yield of burley tobacco, Whitty et al. (1966) applied 180

Table 10. Effect of split applications of various nitrogen rates on acre-yield of cured leaves of Burley 21 in 1966 and 1967.

Treatments		Rates of nitrogen, lb/acre			
No.	Fraction* and time	150	75	150	225
		1966	1967		
1	1 - 0 - 0	2965a**	3122ab	3443a	3580a
2	2/3-1/3- 0	3213a	3060b	3299a	3473a
3	1/3-2/3- 0	3102a	3325a	3278a	3354a
4	1/3-1/3-1/3	3140a	3277ab	3302a	3531a
5	0 - 1 - 0	2988a	-----	-----	-----
6	0 -2/3-1/3	-----	3326a	3398a	3375a
7	0 -1/3-2/3	-----	3061b	3372a	3635a

* The nitrogen was applied as either a preplant (first figure) and/or at 21 and 36 days after transplanting in 1966, (second figure) as preplant and/or 21 and 41 days after transplanting in 1967 (third figure) at the ratios indicated.

** Values within columns not followed by common letters differ significantly ($p = 0.05$, Duncan's Multiple Range test).

Table 11. Effect of rates of nitrogen on yield, average price and acre-value of cured leaves of Burley 21 in 1966 and 1967.

Units	Rates of nitrogen, lb/acre				
	150	0	75	150	225
	1966	1967			
Acre-Yield (lb/acre)	3062	2866d*	3195c	3348b	3491a
Average Price (Cents/lb)	68.13	72.09a	71.80a	71.54a	70.69b
Acre-Value (\$/acre)	2098	2067d	2295c	2396b	2468a

* Values within rows not followed by common letters differ significantly ($p = 0.05$, Duncan's Multiple Range test). Tests of significance apply only to 1967 data.

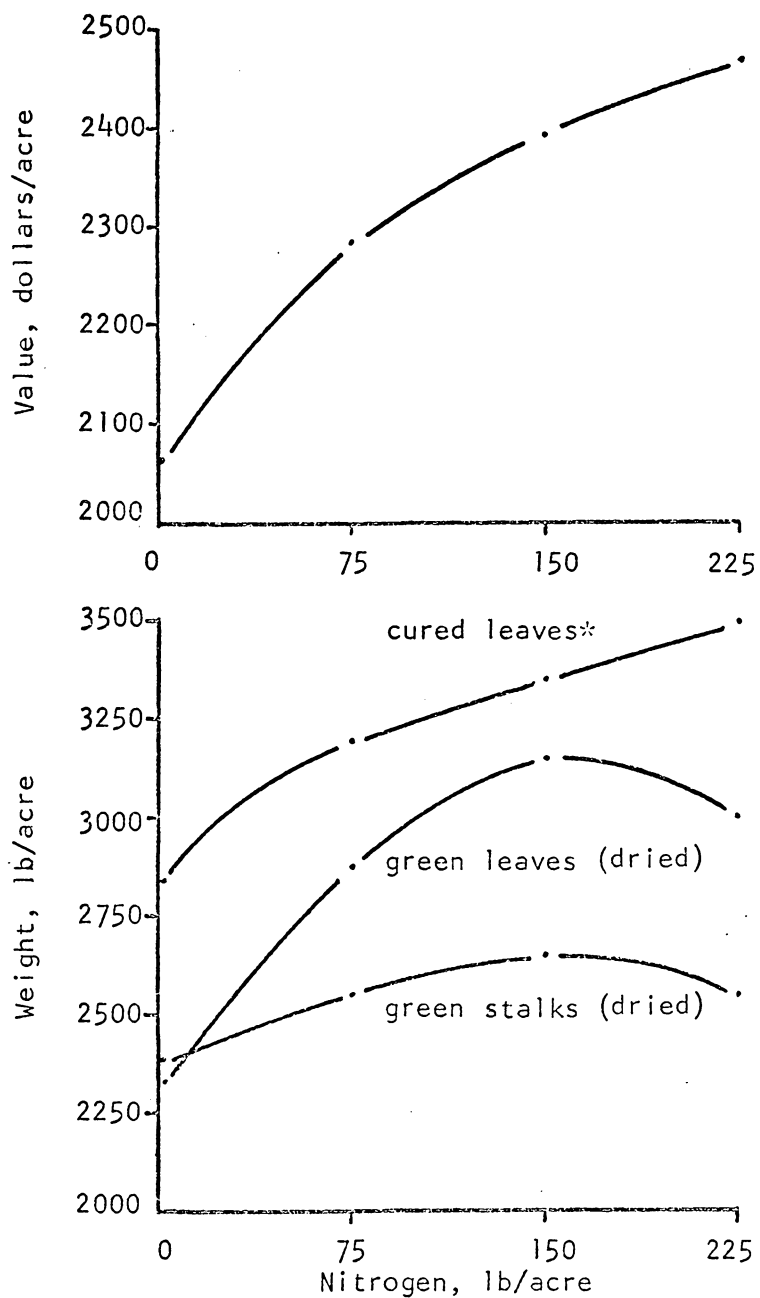


Figure 6: Effect of rates of nitrogen on weight and value of cured leaves and dry weight of green leaves and stalks in 1967.

* Contain Moisture

pounds of nitrogen while Wallace and Rock (1966) used 240 pounds of nitrogen. On the other hand, Atkinson (1966) found that nitrogen did not increase the yield of burley tobacco without irrigation.

C. Leaf Quality and Acre-Value

1. Average price

The average price is considered to be indicative of the quality of cured leaves.

a. *Effect of split application of nitrogen*

Split applications of nitrogen did not influence the average price in 1966 and 1967 (Table 12).

b. *Effect of nitrogen rates*

The average price at 0, 75, 150 and 225 pounds of nitrogen per acre was 72.09, 71.80, 71.54, and 70.69 cents per pound, respectively. It is obvious that increasing nitrogen rates resulted in decreasing leaf quality; however, the average price was significantly lower only for the 225 pound per acre nitrogen rate. The average price at the 150 pound rate of nitrogen in 1966 and 1967 was 68.13 and 71.54 cents per pound, respectively (Table 11).

2. Acre-value

Acre-value is a function of yield and average price. Since the average price decreased very slowly with increasing nitrogen levels, yield became the most important factor in the determination of acre-value.

a. *Effect of split application of nitrogen*

Yield differences resulting from split applications of

Table 12. Effect of split applications of rates of nitrogen on average price of cured leaves of Burley 21 in 1966 and 1967.

Treatments		Rates of nitrogen, lb/acre			
No.	Fraction* and time	150	75	150	225
		1966**	1967**		
		Cents per pound			
1	: 1 - 0 - 0	67.71	71.79	71.52	70.43
2	: 2/3-1/3- 0	67.39	72.24	71.58	70.75
3	: 1/3-2/3- 0	69.16	71.40	71.51	70.61
4	: 1/3-1/3-1/3	68.68	71.57	70.99	70.84
5	: 0 - 1 - 0	68.04	-----	-----	-----
6	: 0 -2/3-1/3	-----	71.91	71.85	70.85
7	: 0 -1/3-2/3	-----	71.89	71.74	70.65

* In the order given, the figures indicate the fractions of the total nitrogen which were applied at preplant, 21 days and 36 days (1966) and 41 days (1967) after transplanting, respectively.

** The differences between means within columns were not significant ($p = 0.05$, Duncan's Multiple Range test).

nitrogen were not significant; therefore, differences in acre-value were also not significant.

b. Effect of nitrogen rates

Increasing nitrogen rates resulted in a significant increase in acre-values. At 0, 75, 150 and 225 pounds of nitrogen applied the acre-values were 2067, 2294, 2396 and 2468 dollars per acre, respectively. The increases in acre-value did not follow a straight line relationship (Figure 6). Increasing nitrogen rates from 0 to 75, from 75 to 150 and from 150 to 225 pounds per acre, respectively, resulted in increases in acre-value of 3.00, 1.36 and 0.94 dollars for each pound of nitrogen applied.

D. Flowering at Time of Topping

The stage of flowering was expressed as the weight of flowers at time of topping. Split applications did not affect the flowering stage. The weight of flowers at the 0, 75, 150 and 225 levels of nitrogen was 63.5, 65.1, 58.5 and 54.1 pounds per acre, respectively. Only the weight of flowers at the 225 pound per acre rate of nitrogen was lower than flower weight obtained at the 75 pounds of nitrogen rate. It is obvious that the higher rates of nitrogen caused delay in flowering. Similar results were obtained by Whitty et al. (1966). The weight of flowers at time of topping in 1966 was 24.6 pounds per acre implying that plants were topped at an earlier stage of growth in 1966 than in 1967 (Table 3).

E. Nitrogen Uptake

Nitrogen uptake is a function of nitrogen percent and weight of dry matter. The weight of dry matter was discussed in section (A).

1. Nitrogen percent

a. *During the growing season*

The nitrogen concentration was determined on plant samples obtained from plots which received 150 pounds per acre of nitrogen in 1966 and 1967. In 1967 the nitrogen level in leaves and stalks decreased up to topping time (Figure 7). After topping there was no significant change in the nitrogen percentage in leaves, while the nitrogen level in stalks increased significantly (Table 13). In 1966 there was little change in nitrogen level in leaves 41 days after transplanting, while nitrogen concentration in stalks continued to decrease up to 9 days after topping; beyond that there was no significant change. It is evident that in 1966 and 1967 prior to topping, the percent nitrogen in stalks decreased more rapidly in the leaves (Figure 7). The higher rate of plant growth in 1967 during the period prior to topping was associated with a more rapid decrease in nitrogen level in the shoots than in 1966 (Figures 2 and 7). The rapid increase in stalk growth in 1966 between 60 and 86 days after transplanting was associated with a 0.28 percent reduction in nitrogen level, while the rapid decrease in stalk growth for the same period in 1967 was associated with a 0.11 percent increase in nitrogen.

A comparison was made between nitrogen present in topped and untopped plants in 1966. Percent nitrogen in leaves and stalks of untopped plants continued to decrease steadily until final harvest (Figure 8). The nitrogen level in plants harvested at the end of

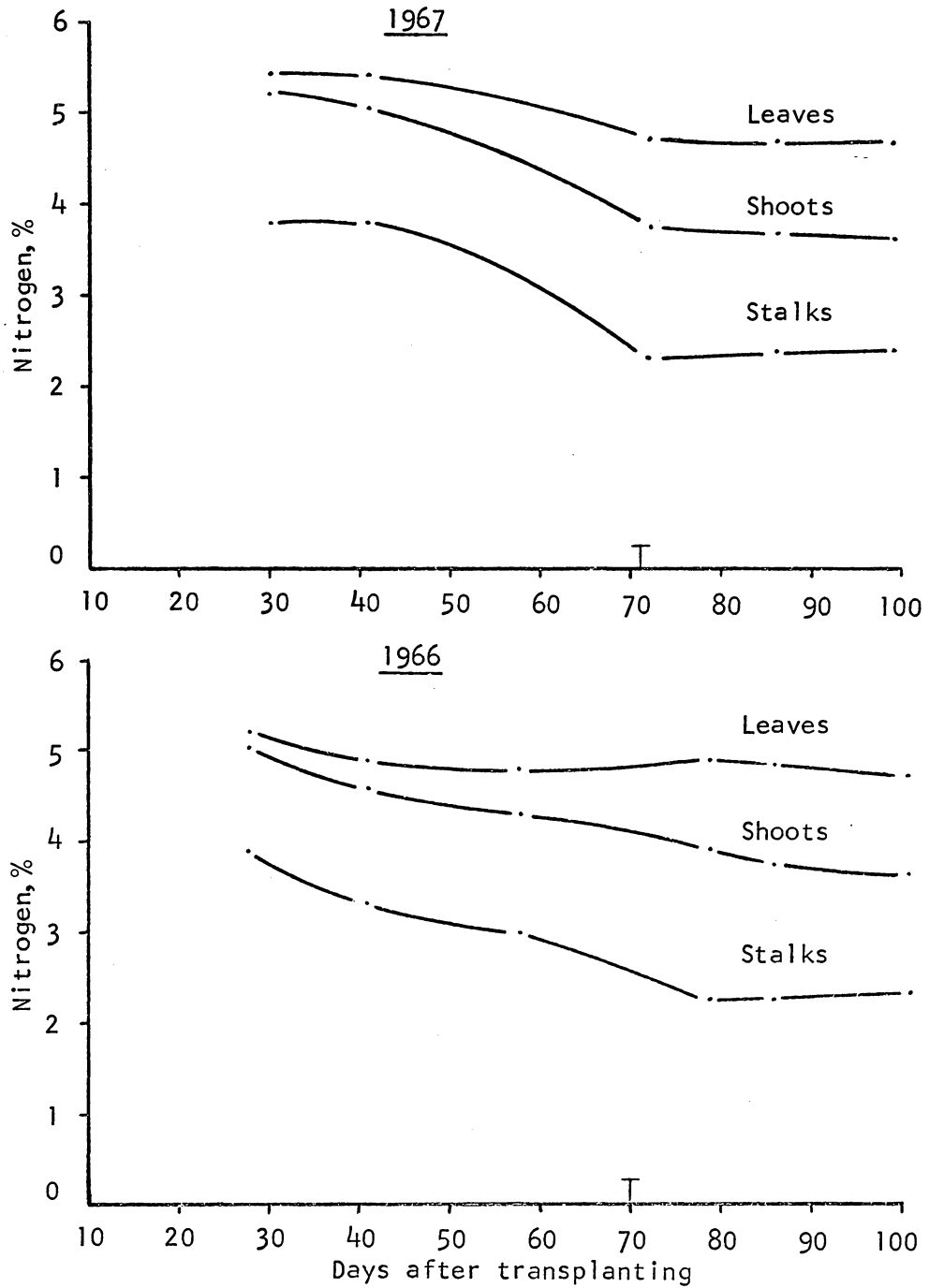


Figure 7: Nitrogen percent of leaves, stalks and shoots of Burley 21 during the 1966 and 1967 growing season. (T indicates time of topping).

Table 13. Percent nicotine and nitrogen, and nicotine-N/total-N ratio of Burley 21 tobacco during 1966 and 1967.

Plant Parts	Year	Days after transplanting			
		41	69-72*	86	99-101**
<u>Nicotine %</u>					
Leaves	1966	1.21d***	1.47c	2.20b	3.20a
	1967	.84d	1.36c	2.73b	3.72a
Stalk	1966	.41b	.24d	.37c	.50a
	1967	.33bc	.21c	.63a	.60ab
Shoots	1966	1.07	1.06	1.40	1.99
	1967	.75	.90	1.80	2.28
<u>Nitrogen %</u>					
Leaves	1966	4.88a	4.85a	4.86a	4.73ab
	1967	5.40a	4.70b	4.73b	4.63bc
Stalks	1966	3.33a	2.55b	2.27c	2.33c
	1967	3.76a	2.32c	2.43b	2.44b
Shoots	1966	4.61	4.05	3.73	3.65
	1967	5.12	3.75	3.71	3.62
<u>(Nicotine-N/Total-N) x 100</u>					
Leaves	1966	4.28	5.22	7.82	11.72
	1967	2.68	5.00	9.97	13.88
Stalks	1966	2.12	1.60	2.81	3.70
	1967	1.52	1.56	4.48	4.24
Shoots	1966	4.01	4.52	6.37	9.50
	1967	2.54	4.15	8.39	10.87

* Sampling time was 69 and 72 days after transplanting in 1966 and 1967, respectively. (Topping occurred one day after sampling in 1966 and on day of sampling in 1967).

** Harvesting time was 101 and 99 days in 1966 and 1967, respectively.

*** Values within rows not followed by common letter differ significantly ($p = 0.05$, Duncan's Multiple Range test).

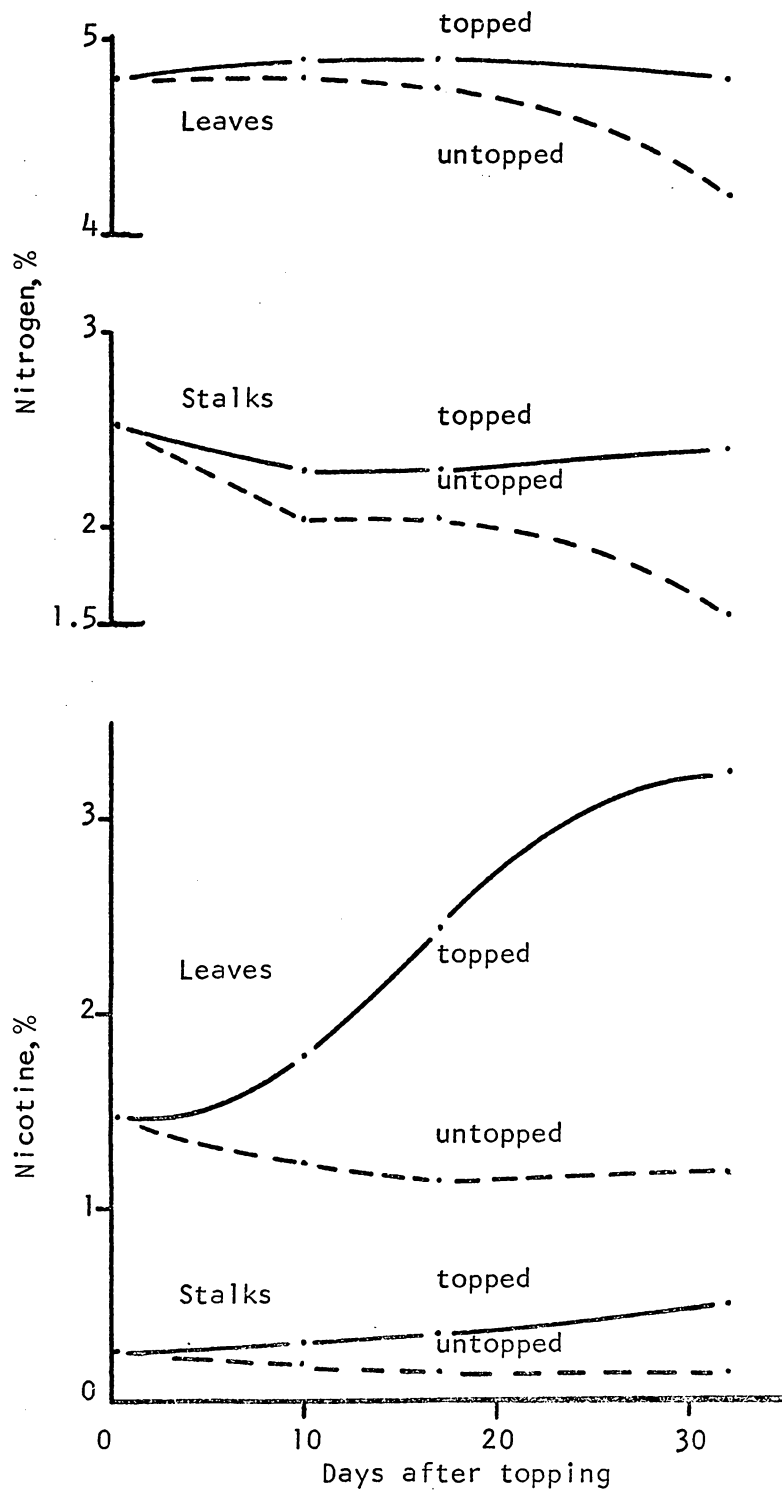


Figure 8: Effect of topping on nicotine and nitrogen percent of leaves and stalks of Burley 21 in 1966.

the growing season was higher in topped plants than in the untopped ones. The difference in percent nitrogen was 0.83 (Table 14). Two factors were believed to have caused this difference. Firstly, the amount of nitrogen was more diluted in the untopped plants than the topped ones. The total dry matter per acre of untopped plants was 895 pounds more than that of topped plants (Table 7). The difference in nitrogen percent caused by dilution was 0.50 percent. Secondly, the amount of absorbed nitrogen per acre was 16.55 pounds more in topped plants than in the untopped ones (Table 15), resulting in a 0.33 percent increase in nitrogen of topped plants over the untopped ones.

b. Effect of split application of nitrogen

Split applications of nitrogen in 1966 and 1967 did not affect the nitrogen level in leaves (Table 16) and stalks (Table 17) of plants harvested during the growing season. Cured leaves also showed no differences in percent nitrogen (Table 18).

c. Effect of nitrogen rates

Increasing nitrogen rates significantly increased nitrogen concentration in leaves and stalks of plants harvested at the end of the growing season as well as in cured leaves (Figure 9). Increasing the nitrogen rates from 0 to 75, from 75 to 150 and from 150 to 225 pounds per acre significantly increased the percent nitrogen in non-cured leaves. The increases were 0.10, 0.06 and 0.04 percent for each 10 pounds of nitrogen within the respective rates given above. The percent nitrogen in stalks increased 0.09,

Table 14. Effect of topping on percent nicotine and nitrogen, and nicotine-N/total-N ratio of Burley 21 in 1966.*

Plant Parts	Topping	Days after topping			
		0	9	16	31
<u>Nicotine %</u>					
Leaves	Topped	----	1.74	2.43	3.25
	Untopped	1.47	1.34	1.14	1.20
Stalks	Topped	----	.28	.36	.49
	Untopped	.23	.18	.17	.16
Shoots	Topped	----	1.21	1.52	2.04
	Untopped	1.05	.91	.70	.65
<u>Nitrogen %</u>					
Leaves	Topped	----	4.93	4.86	4.81
	Untopped	4.80	4.88	4.75	4.21
Stalks	Topped	----	2.28	2.23	2.37
	Untopped	2.54	2.03	2.05	1.56
Shoots	Topped	----	3.96	3.70	3.74
	Untopped	4.04	3.81	3.53	2.91
<u>(Nicotine-N/Total N) x 100</u>					
Leaves	Topped	----	7.65	8.63	11.67
	Untopped	5.30	4.75	4.14	4.92
Stalks	Topped	----	2.12	2.78	3.57
	Untopped	1.57	1.54	1.43	1.77
Shoots	Topped	----	5.30	7.07	9.42
	Untopped	4.50	4.14	3.43	3.98

* All treatments received a preplant application of 150 lb of N per acre.

Table 15. Effect of topping on the amount of nitrogen in plant parts
of Burley 21 in 1966.**

Days after topping	Leaves		Stalks		Total	
	Untopped	Topped	Untopped	Topped	Untopped	Topped
	Lb/acre					
0	111.10	-----	29.92	-----	141.02	-----
9	149.65	132.10	37.11	34.96	186.76	167.06
16	129.89	140.49	46.22	50.28	176.11	190.77
31	131.05	140.00	46.26	53.86	177.31	193.86

** All treatments received a preplant application of 150 lb of N per acre.

Table 16. Effect of split applications of rates of nitrogen on nitrogen percent of non-cured leaves of Burley 21 at the time of harvest in 1966 and 1967.

No.	Treatments Fraction* and time	Rates of nitrogen, lb/acre			
		150	75	150	225
		1966**	1967**		
1	: 1 - 0 - 0	4.81	4.21	4.67	4.95
2	: 2/3-1/3- 0	4.72	4.33	4.56	5.02
3	: 1/3-2/3- 0	4.66	4.12	4.56	4.91
4	: 1/3-1/3-1/3	4.65	4.28	4.63	4.93
5	: 0 - 1 - 0	4.75	----	----	----
6	: 0 -2/3-1/3	----	4.19	4.74	4.97
7	: 0 -1/3-2/3	----	4.17	4.72	5.01

* In the order given, the figures indicate the fractions of the total nitrogen which were applied at preplant, 21 days and 36 days (1966) and 41 days (1967) after transplanting, respectively.

** The differences between means within columns were not significant ($p = 0.05$, Duncan's Multiple Range test).

Table 17. Effect of split applications of nitrogen rates on nitrogen percent of stalks of Burley 21 at time of harvest in 1966 and 1967.

Treatments		Rates of nitrogen, lb/acre			
No.	Fraction* and time	150	75	150	225
		1966**	1967**		
1	: 1 - 0 - 0	2.37	2.21	2.48	2.61
2	: 2/3-1/3- 0	2.26	2.31	2.41	2.72
3	: 1/3-2/3- 0	2.30	2.28	2.45	2.61
4	: 1/3-1/3-1/3	2.28	2.26	2.36	2.62
5	: 0 - 1 - 0	2.33	----	----	----
6	: 0 -2/3-1/3	----	2.27	2.50	2.39
7	: 0 -1/3-2/3	----	2.39	2.51	2.54

* In the order given, the figures indicate the fractions of the total nitrogen which were applied at preplant, 21 days and 36 days (1966) and 41 days (1967) after transplanting, respectively.

** The differences between means within columns were not significant ($p = 0.05$, Duncan's Multiple Range test).

Table 18. Effect of split application of rates of nitrogen on nitrogen percent of cured leaves of Burley 21 in 1966 and 1967.

Treatments		Rates of nitrogen, lb/acre			
No.	Fraction* and time	150	75	150	225
		1966**	1967**		
1	: 1 - 0 - 0	4.70	4.29	4.87	5.29
2	: 2/3-1/3- 0	4.57	4.40	4.80	5.25
3	: 1/3-2/3- 0	4.55	4.30	4.86	5.12
4	: 1/3-1/3-1/3	4.51	4.22	4.75	5.17
5	: 0 - 1 - 0	4.47	----	----	----
6	: 0 -2/3-1/3	----	4.30	4.78	5.20
7	: 0 -1/3-2/3	----	4.21	4.90	5.41

* In the order given, the figures indicate the fractions of the total nitrogen which were applied at preplant, 21 days and 36 days (1966) and 41 days (1967) after transplanting, respectively.

** The differences between means within columns were not significant ($p = 0.05$, Duncan's Multiple Range test).

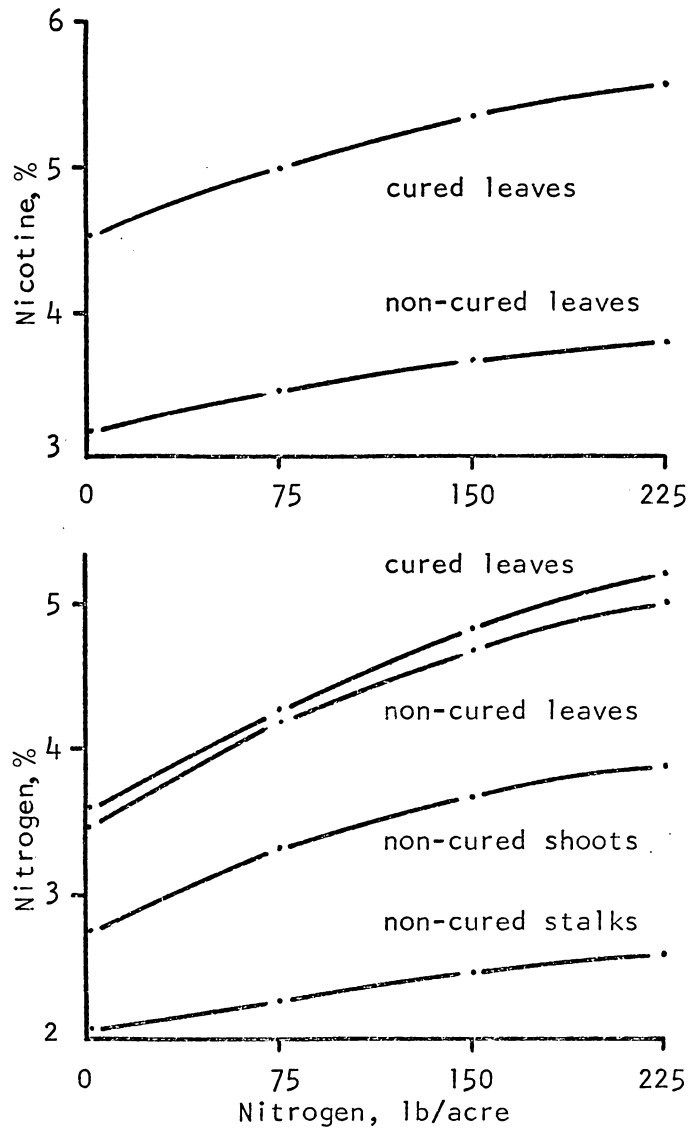


Figure 9: Effect of nitrogen rates on nicotine and nitrogen contents of cured leaves, and non-cured leaves and stalks in 1967.

0.07 and 0.06 percent, respectively. These results indicate that the rate of increase in nitrogen level declined with increasing rates of nitrogen. In 1967 the nitrogen level in cured leaves was slightly higher than in the green leaves, while in 1966 it was lower (Table 19). The percent nitrogen in cured leaves can be influenced by the presence or absence of the midrib as well as by the curing process. Garner et al. (1934) working with Maryland tobacco found that nitrogen concentration in the midrib to be one-half that of the lamina. Hence, removal of midribs would increase the percent nitrogen of leaf samples. On the other hand, Palmer (1963) reported that during the burley curing process a migration of products of the protein hydrolysis into the stalk occurred. He found a 30 percent reduction of nitrogen in the cured leaves. Burton and Wright (1961) showed that nitrogen in the leaves of burley tobacco decreased 22 percent during the curing process. Taking these factors into consideration it appears that under certain conditions nitrogen losses due to the curing process might be offset by gains resulting from the removal of the midrib.

Compositing ground lamina of all farm grades on the basis of their percentage of the total weight of cured leaf may introduce an error. It is frequently assumed that all grades have the same midrib and moisture percent. Preliminary investigations showed that the percent of midrib of dried cured leaves of flyings, lugs, leaves, reds and tips was 29.94, 33.46, 33.52, 29.10 and 26.10 percent, respectively, while the moisture percent of cured leaf

Table 19. Effect of nitrogen rates upon nicotine and nitrogen percentages and nicotine-N/total-N ratio of leaves and stalks of the last green sample* and cured leaves of Burley 21 tobacco in 1966 and 1967.

Plant Part	Rates of nitrogen, lb/acre				
	150	0	75	150	225
	<u>1966</u>	<u>1967</u>			
			<u>Nicotine %</u>		
<u>Cured leaves</u>	3.85	4.53d	5.00c	5.34b	5.57a
<u>Non-cured</u>					
Leaves	3.20	3.20c	3.46b	3.71a	3.77a
Stalks	.50	.54b	.60a	.60a	.61a
Shoots	2.00	1.85	2.11	2.28	2.32
			<u>Nitrogen %</u>		
<u>Cured leaves</u>	4.59	3.60d	4.28c	4.82b	5.24a
<u>Non-cured</u>					
Leaves	4.73	3.48d	4.21c	4.63b	4.96a
Stalks	2.33	2.08d	2.28c	2.44b	2.58a
Shoots	3.65	2.76	3.30	3.62	3.86
			<u>(Nicotine-N/Total-N) x 100</u>		
<u>Cured leaves</u>	14.50	21.74	20.18	19.14	18.36
<u>Non-cured</u>					
Leaves	11.72	15.89	14.20	13.88	13.13
Stalks	3.70	4.48	4.54	4.24	4.08
Shoots	9.50	11.69	11.06	10.87	10.36

* Samples obtained at the time of harvesting.

was 12.76, 18.76, 20.49, 16.56 and 12.00 percent, respectively. The data indicate that the midrib and moisture percent of cured leaves was the highest in the middle leaves and decreased in tips and bottom leaves. Therefore, the leaves from the various plant positions were disproportionately represented in the composite samples. Bowman et al. (1958) and Bowman and Nichols (1968) showed that the chemical composition of individual leaves of burley tobacco vary according to their position on the stalk, hence some alteration in the result of chemical analysis of composited samples is to be expected. More research is needed to evaluate the importance of this phenomena.

2. Total nitrogen uptake

a. *During the growing season*

The amount of nitrogen taken up by the leaves and stalks harvested during the growing season was determined. The close relationship between total dry weight and nitrogen uptake is illustrated in Figures 10 and 11. The rate of nitrogen absorption was relatively low for the first 30 days and reached a maximum between 45 and 55 days after transplanting in 1966 and between 45 and 65 days in 1967 (Figure 12). However, the rate of nitrogen uptake was higher than the rate of growth early in the season while it was lower late in the growing season (Table 20). Grizzard et al. (1942) obtained the same result with flue-cured tobacco. Botha (1965) found the maximum rate of nitrogen uptake to occur two weeks earlier than the maximum rate of plant growth. After the

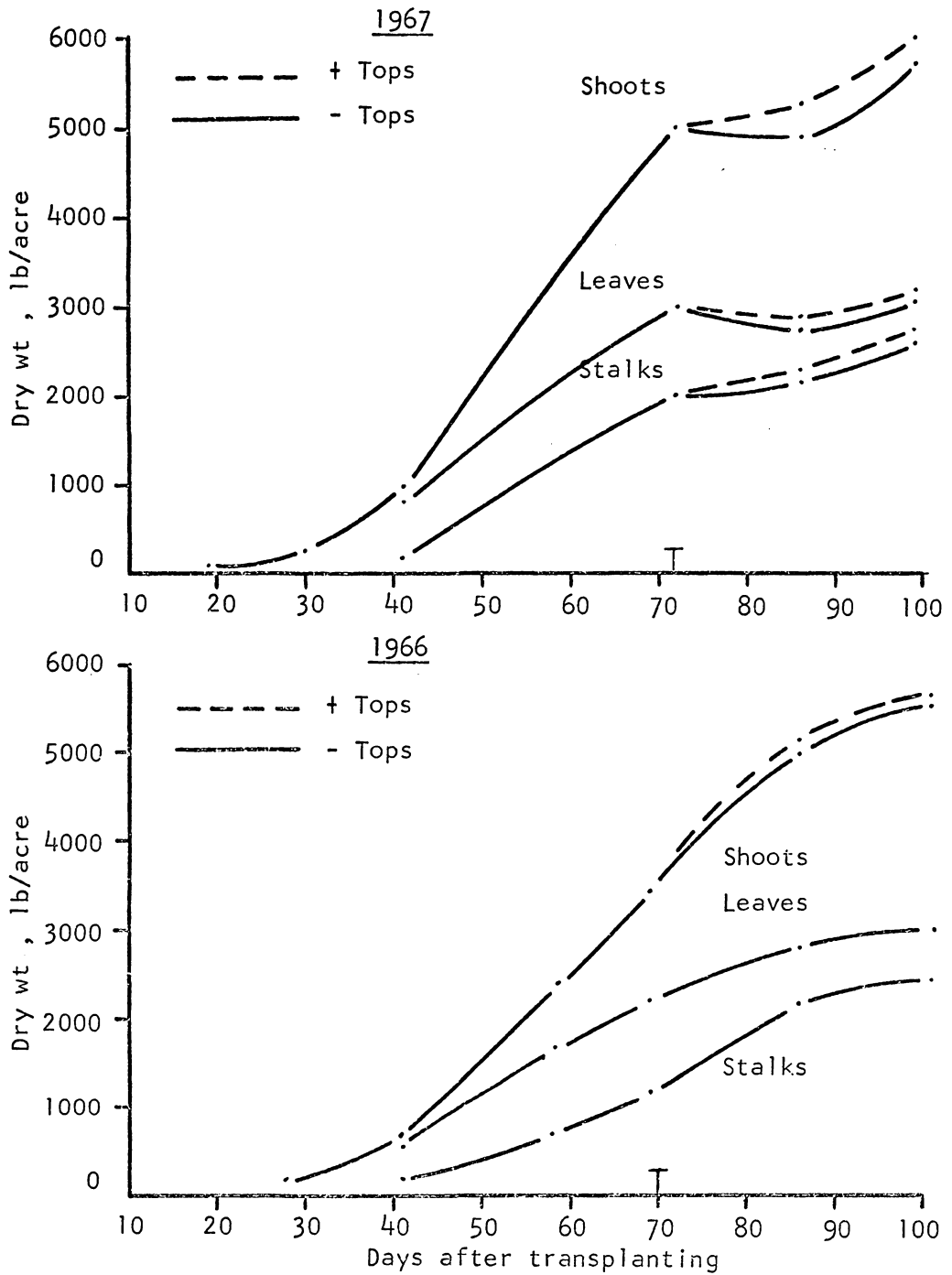


Figure 10: Dry weight of leaves, stalks and shoots during 1966 and 1967. (T indicates time of topping).

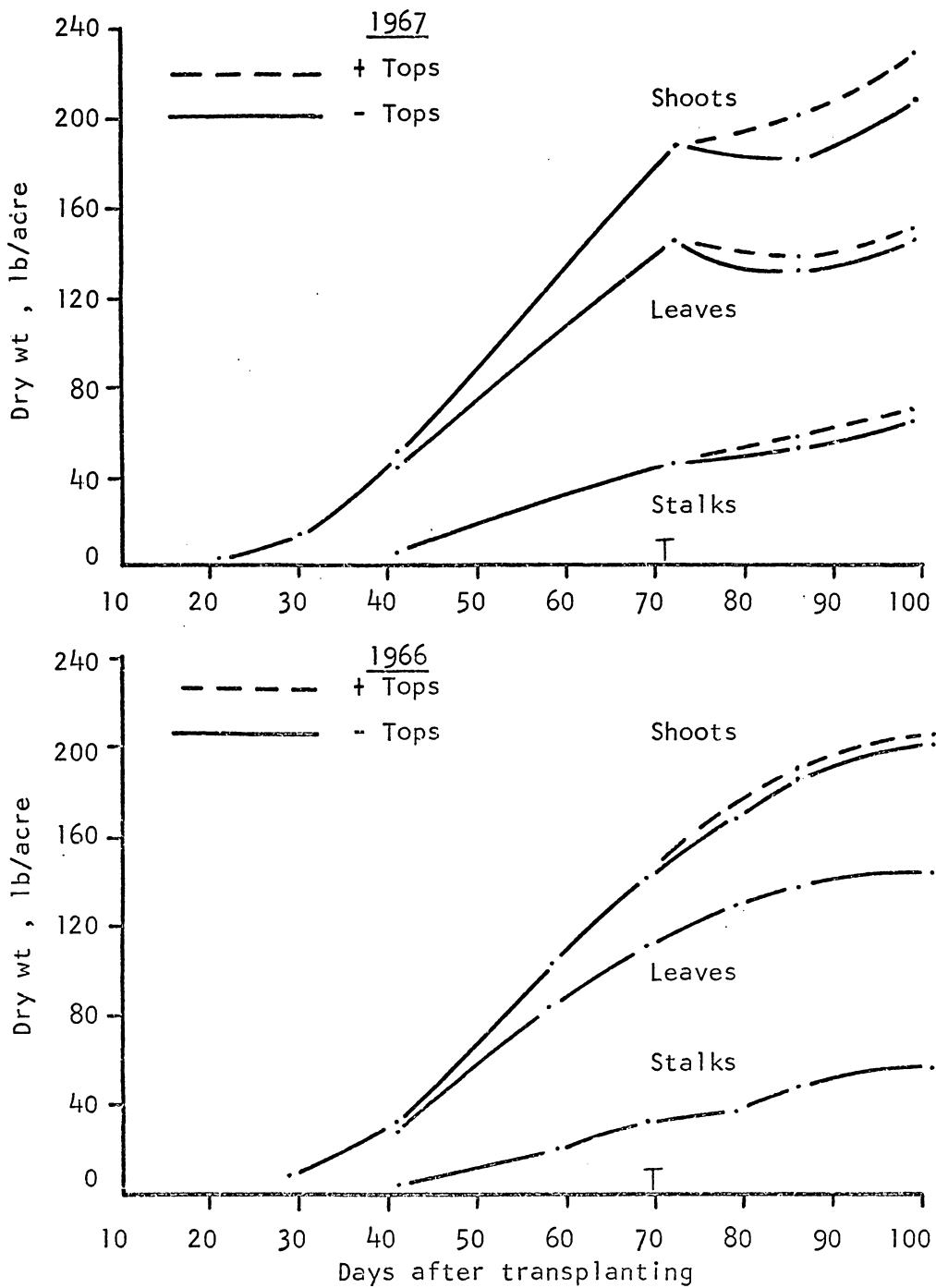


Figure 11: Weight of nitrogen in leaves, stalks, and shoots during 1966 and 1967. (T indicates time of topping).

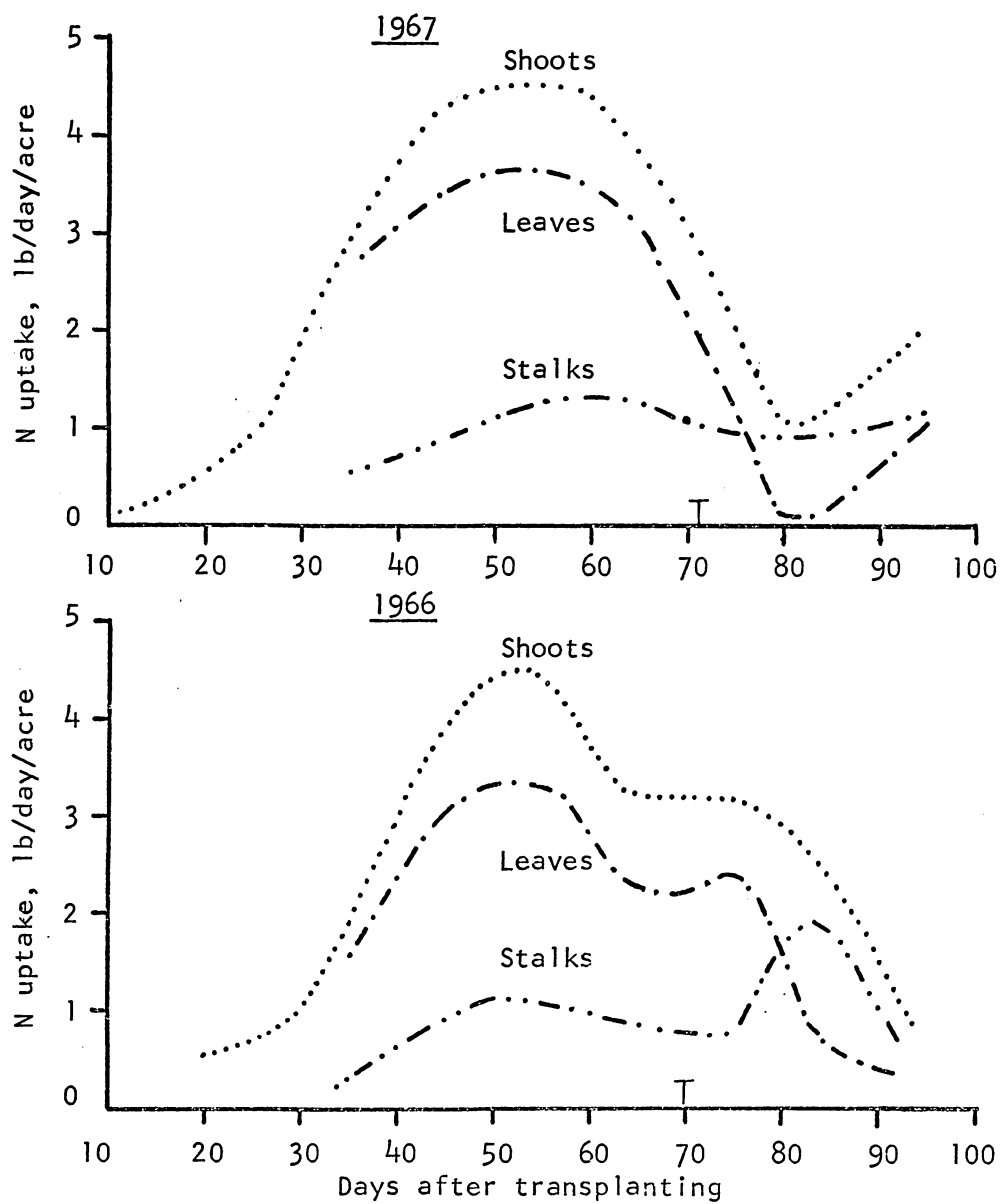


Figure 12: Rate of nitrogen uptake in leaves, stalks and shoots in Burley 21 during 1966 and 1967. (T indicates time of topping).

Table 20. Relative weight (%)* of nitrogen, nicotine and shoots at the time indicated in relation to their total weight at harvest in 1966 and 1967.**

Year	Approximate time intervals, days		
	0-41	41-70	70-100
	<u>Relative nitrogen weight (%)</u>		
1966	16.1	53.9	30.0
1967	24.1	65.9	10.0
Avg.	20.1	59.9	20.0
	<u>Relative nicotine weight (%)</u>		
1966	6.8	26.8	66.4
1967	5.6	28.8	65.6
Avg.	6.2	27.8	66.0
	<u>Relative shoots weight (%)</u>		
1966	12.8	47.7	39.5
1967	17.1	69.8	13.1
Avg.	14.9	58.7	26.3

* (Weight at interval/total weight at harvest) x 100.

** Fertilization per acre; 150 lb of N, 200 lb of P_2O_5 and 400 lb of K_2O .

maximum had been reached, the rate of nitrogen uptake decreased moderately in 1966 and rapidly in 1967. The data reveals that 59.9 percent of the nitrogen was taken up in the month prior to topping (Table 20).

Prior to topping the rate of nitrogen accumulation was higher in the leaves than in the stalks. Ten days after topping the rate of nitrogen accumulation in topped and untopped plants was higher in the stalks than in the leaves (Figure 13). It is obvious that nitrogen accumulation continued to occur in the stalks late in the growing season because stalks continued to grow at a high rate late in the growing season. The rate of nitrogen accumulation was higher in the stalks and leaves of topped plants than of the untopped ones, which indicated that topping stimulated nitrogen uptake in the plant. There was a loss in the nitrogen from leaves of untopped plants in the last 20 days before harvest; the loss was probably due to the migration of nitrogen from the leaves to stalks and flowers which continued to grow.

The amount of nitrogen increased in leaves of untopped plants until 79 days after transplanting followed by a slight decrease, while nitrogen content of stalks continued to increase until 86 days after transplanting followed by no change up to the end of the growing season (Table 13).

b. Effect of split applications of nitrogen

The dry weights and nitrogen contents of green plants were not affected by split applications. Therefore, split applications

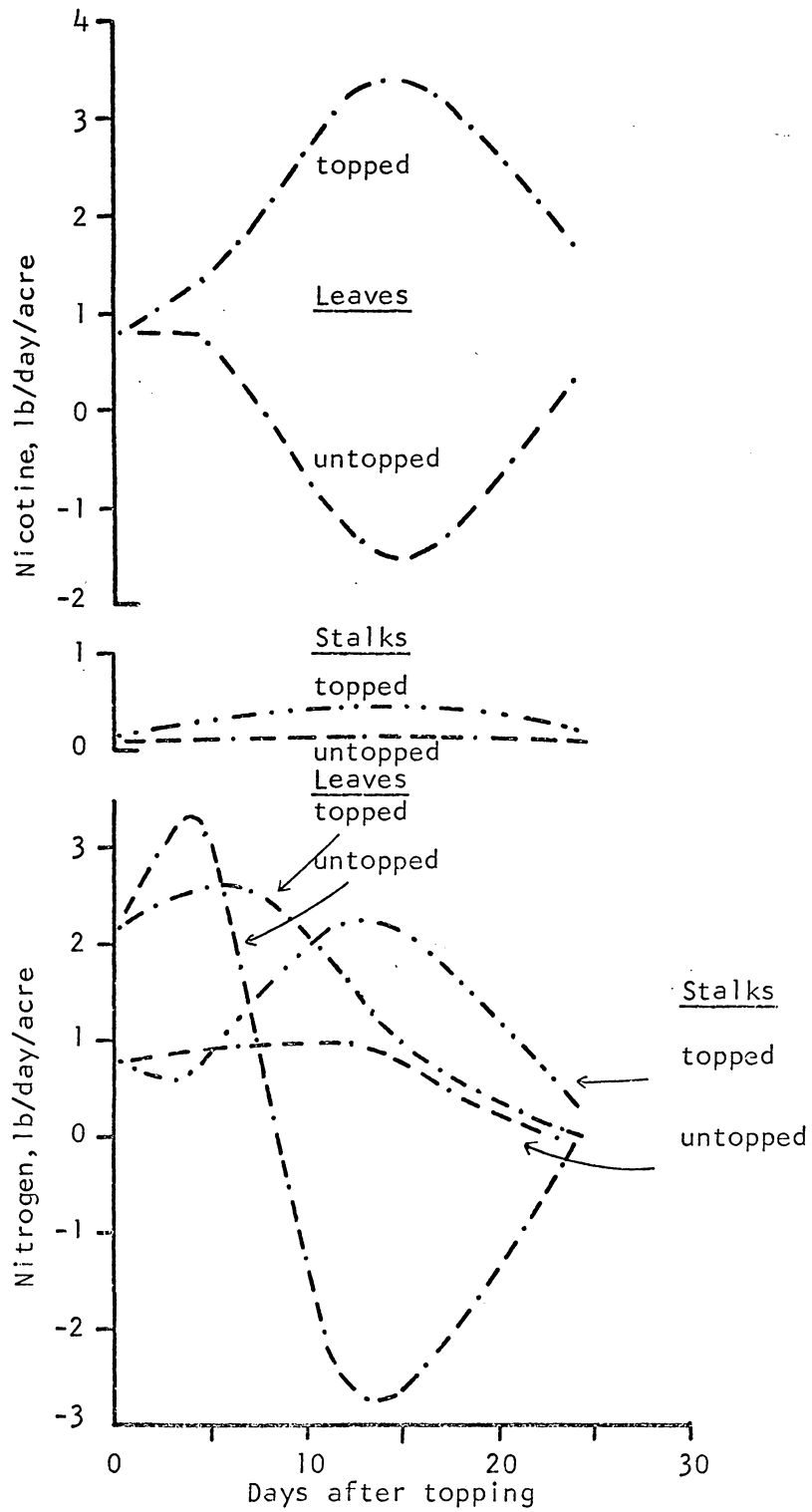


Figure 13: Effect of topping on rate of nicotine and nitrogen accumulation in leaves and stalks of Burley 21 during the period after topping in 1966.

did not affect the total amount of nitrogen in the leaves and stalks. However, applying 2/3 of the 75 pounds of nitrogen 21 days after transplanting increased the yield of cured leaves (Table 10), without any increase in percent nitrogen (Table 18). Since the dry weight of cured leaves was not available, nothing can be said about the total amount of nitrogen in the cured leaves.

c. Effect of nitrogen rates

The effect of nitrogen rates on nitrogen uptake was determined on green plants harvested at the end of the growing season. A significant increase in nitrogen uptake was obtained with nitrogen applications up to 150 pounds (Table 21). Higher rates of nitrogen did not increase nitrogen uptake because there was no increase in dry weight. However, nitrogen concentrations as well as yields of cured leaves showed significant increases up to 225 pounds of nitrogen per acre (Tables 19 and 11). When compared to the no nitrogen treatment, increasing nitrogen rates from 0 to 75, from 75 to 150 and from 150 to 225 pounds per acre increased the nitrogen in cured leaves 18.8, 15.0 and 11.6 percent; while the increase in the yield of cured leaves was 11.6, 5.3, and 5.0 percent. The response of yield and percent nitrogen to nitrogen rates is quadratic; therefore, the increase in nitrogen uptake with applied nitrogen rates is also quadratic. However, it is clear from the above data that increasing nitrogen rates raised the nitrogen level in the leaves or shoots more than the yield.

Table 21. Effect of nitrogen rates on amounts of nitrogen and nicotine in the leaves, stalks, and shoots of Burley 21 at the time of harvest in 1966 and 1967.

Plant Parts	Rates of nitrogen, lb/acre				
	150	0	75	150	225
	<u>1966</u>	<u>1967</u>			
	<u>Nitrogen, lb/acre</u>				
Leaves	65.1	36.5c*	55.0b	66.2a	67.4a
Stalks	25.9	22.6c	26.5ab	29.4a	29.8a
Shoots	91.0	59.1c	81.5b	95.6a	97.2a
	<u>Nicotine lb/acre</u>				
Leaves	44.0	33.6c	45.2b	53.0a	51.2a
Stalks	5.6	5.8b	6.9a	7.2a	7.0a
Shoots	49.6	39.4c	52.1b	60.2a	58.3a

* Values within rows not followed by common letters differ significantly ($p = 0.05$, Duncan's Multiple Range test). Tests of significance apply only to 1967 data.

F. Nicotine Synthesis

1. Nicotine concentrations

a. *During the growing season*

The nicotine level in leaves was low at the beginning of the growing season. In 1966, nicotine in leaves increased slowly and steadily until topping time. In 1967, nicotine in the leaves increased up to 30 days after transplanting, and then gradually decreased for the next 20 to 30 days. After this decline there was again an increase in percent nicotine until topping time. The slight and steady increase of nicotine in the leaves in 1966 was associated with a moderate rate of growth, while the decrease in nicotine after 30 days from transplanting in 1967 was associated with a high rate of growth.

Nicotine concentrations in stalks decreased steadily and slowly in 1966 and 1967 until topping time (Figure 14). At topping time the nicotine level in leaves as well as in stalks was slightly higher in 1966 than in 1967 (Table 13).

After topping, the percent nicotine of the leaves increased very rapidly to the end of the growing season, while there was a moderate increase in the stalks (Figure 14). The rate of the increase in nicotine was higher in 1967 than in 1966 and was associated with the lower rate of growth after topping in 1967 than in 1966 which was thought to be caused by the more severe topping in 1967. The lower the rate of growth after topping the greater the amount of substrate available for migration to the roots for

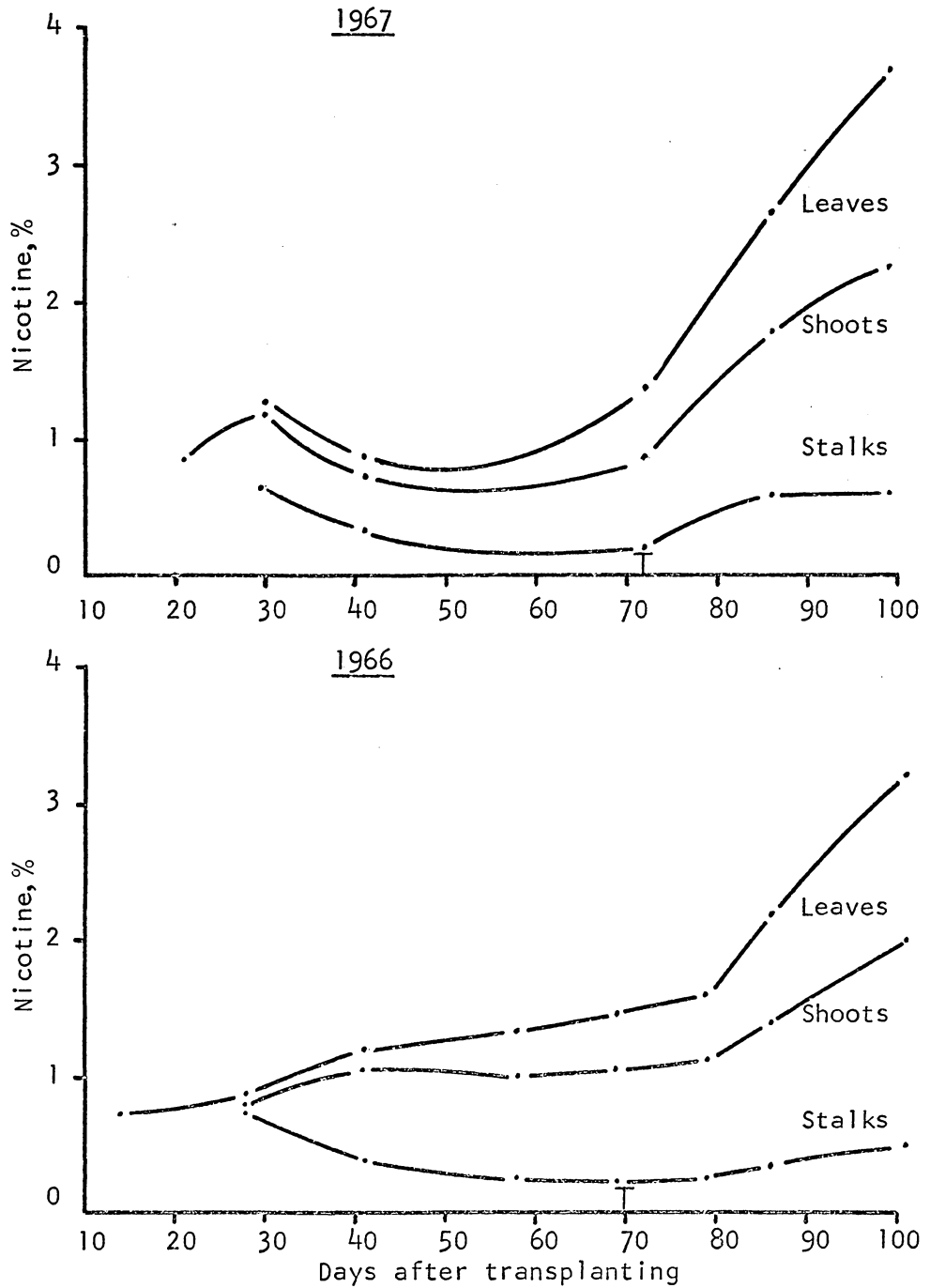


Figure 14: Nicotine percent of leaves, stalks and shoots of Burley 21 during 1966 and 1967. (T indicates time of topping).

nicotine synthesis. Therefore, 85.93 pounds of nicotine were synthesized per acre after topping in 1967, while only 72.39 pounds of nicotine were synthesized in 1966. The nicotine levels in green leaves of the last sample harvested and in cured leaves were higher in 1967 than in 1966 (Table 19).

The percent nicotine in leaves and stalks of untopped plants started to decrease slowly 70 days after transplanting (Figure 8). The reason for this decrease in nicotine percent was due to the increase in dry weight of shoots without any concomitant increase in nicotine in the shoot; rather a decrease in nicotine took place.

b. Effect of split application of nitrogen

Split applications did not affect the nicotine levels of non-cured leaves (Table 22) and stalks (Table 23) or of cured leaves (Table 24). Adding nitrogen earlier or later for some of the treatments did not affect the percent nicotine of leaves and stalks during the period following the nitrogen application. Takahashi (1964) also found percent nicotine of leaves prior to topping to be unaffected by nitrogen application. However, he concluded that adding nitrogen late enough to be available after topping increased the nicotine level of leaves. Although some of the nitrogen used in this study was applied as late as 41 days after transplanting, it did not affect the percent nicotine. It was found that about 80 percent of the nitrogen in the shoots was taken up before topping. It is therefore possible that most of the fertilizer nitrogen had been utilized and was not available to plants after topping. The nicotine content of

Table 22. Effect of split applications of nitrogen rates on nicotine percent of non-cured leaves of Burley 21 at the time of harvest in 1966 and 1967.

Treatments		Rates of nitrogen, lb/acre			
No.	Fraction* and time	150	75	150	225
		1966**	1967**		
1	: 1 - 0 - 0	3.25	3.46	3.71	3.98
2	: 2/3-1/3- 0	3.34	3.50	3.73	4.02
3	: 1/3-2/3- 0	2.91	3.44	3.59	3.71
4	: 1/3-1/3-1/3	3.30	3.48	3.81	3.48
5	: 0 - 1 - 0	3.18	----	----	----
6	: 0 -2/3-1/3	----	3.41	3.74	3.67
7	: 0 -1/3-2/3	----	3.49	3.67	3.77

* In the order given, the figures indicate the fractions of the total nitrogen which were applied at preplant, 21 days and 36 days (1966) and 41 days (1967) after transplanting, respectively.

** The differences between means within columns were not significant ($p = 0.04$, Duncan's Multiple Range test).

Table 23. Effect of split applications of nitrogen rates on nicotine percent of stalks of Burley 21 in 1966 and 1967.

No.	Treatments Fraction* and time	Rates of nitrogen, lb/acre			
		150	75	150	225
		1966**	1967**		
1	: 1 - 0 - 0	.49	.60	.58	.61
2	: 2/3-1/3- 0	.51	.58	.65	.65
3	: 1/3-2/3- 0	.46	.63	.61	.60
4	: 1/3-1/3-1/3	.52	.62	.58	.66
5	: 0 - 1 - 0	.46	---	---	---
6	: 0 -2/3-1/3	---	.55	.61	.55
7	: 0 -1/3-2/3	---	.62	.58	.62

* In the order given, the figures indicate the fractions of the total nitrogen which were applied at preplant, 21 days and 36 days (1966) and 41 days (1967) after transplanting, respectively.

** The differences between means within columns were not significant ($p = 0.05$, Duncan's Multiple Range test).

Table 24. Effect of split applications of rates of nitrogen on nicotine percent of cured leaves of Burley 21 in 1966 and 1967.

Treatments		Rates of nitrogen, lb/acre			
No.	Fraction* and time	150	75	150	225
		1966**	1967**		
1	: 1 - 0 - 0	3.77	4.89	5.45	5.62
2	: 2/3-1/3- 0	3.96	5.17	5.46	5.74
3	: 1/3-2/3- 0	3.91	4.95	4.38	5.61
4	: 1/3-1/3-1/3	3.84	4.95	5.19	5.54
5	: 0 - 1 - 0	3.69	----	----	----
6	: 0 -2/3-1/3	----	4.94	5.30	5.28
7	: 0 -1/3-2/3	----	5.09	5.29	5.63

* In the order given, the figures indicate the fractions of the total nitrogen which were applied at preplant, 21 days and 36 days (1966) and 41 days (1967) after transplanting, respectively.

** The differences between means within columns were not significant ($p = 0.05$, Duncan's Multiple Range test).

leaves and stalks was not affected by split applications because the nitrogen content of the shoots was not affected and the other factors affecting the ratio of nitrogen incorporation into nicotine were the same for all treatments.

c. *Effect of nitrogen rates*

Nicotine level was determined on leaves and stalks of plants sampled at the end of the growing season. The data showed that the nicotine in the leaves increased with increasing rates of nitrogen up to 150 pounds per acre. Beyond that rate the increase in nicotine percent was not significant. Nicotine in the stalks increased with the addition of 75 pounds of nitrogen per acre. Beyond 75 pounds of nitrogen there was no significant increase (Table 19). However, the nicotine in stemmed cured leaves continued to increase with increasing rates of nitrogen up to 225 pounds per acre. Increasing nitrogen rates from 0 to 75, from 75 to 150 and from 150 to 225 increased nicotine of cured leaves 0.062, 0.045 and 0.030 percent for each 10 pounds of nitrogen, respectively. The increase in nicotine was not a straight line relationship (Figure 9). The rate of increase in percent nicotine of cured leaves declined with increasing rate of nitrogen.

The average percent nicotine of cured leaves was $1 \frac{1}{3}$ of that of non-cured leaves. The reason for the higher nicotine in cured leaves is suggested to be due to (a) the removal of the midrib [Darkis et al. (1952) found nicotine percent of flue-cured midrib to be about one-fifth that of nicotine percent of the lamina], and

(b) the small loss of nicotine during curing processes. Palmer (1963) found no reduction in percent nicotine of leaves after 85 days of curing on the stalk, while Burton and Wright (1961) reported a 17 percent reduction in nicotine in leaves during the curing processes.

2. Amount of synthesized nicotine

The amount of nicotine found in the shoots is a function of nicotine concentration and weight of shoots.

a. *During the growing season*

The total amount of nicotine in the shoots was determined during the growing season. The amount of nicotine synthesized during the first 41 days after transplanting was low in 1966 and 1967 (Figure 15). Subsequently, up to time of topping there was a moderate increase in rate of nicotine synthesis in 1966, while there was a more rapid increase in 1967 (Figure 16). It was found that during the 70 days before topping only 33.6 and 34.4 percent of the final amount of nicotine was accumulated in the shoots in 1966 and 1967, respectively. More than 65 percent of the total nicotine was synthesized in the plant during the 30 days after topping. The rate of nicotine synthesis increased rapidly after topping until 85 days after transplanting followed by no change in 1967 and a slight decrease in 1966. The amount of synthesized nicotine was higher in 1967 than in 1966 (Table 25).

The rate of nicotine accumulation was steady in the untopped plants up to 79 days after transplanting. Beyond that the rate of

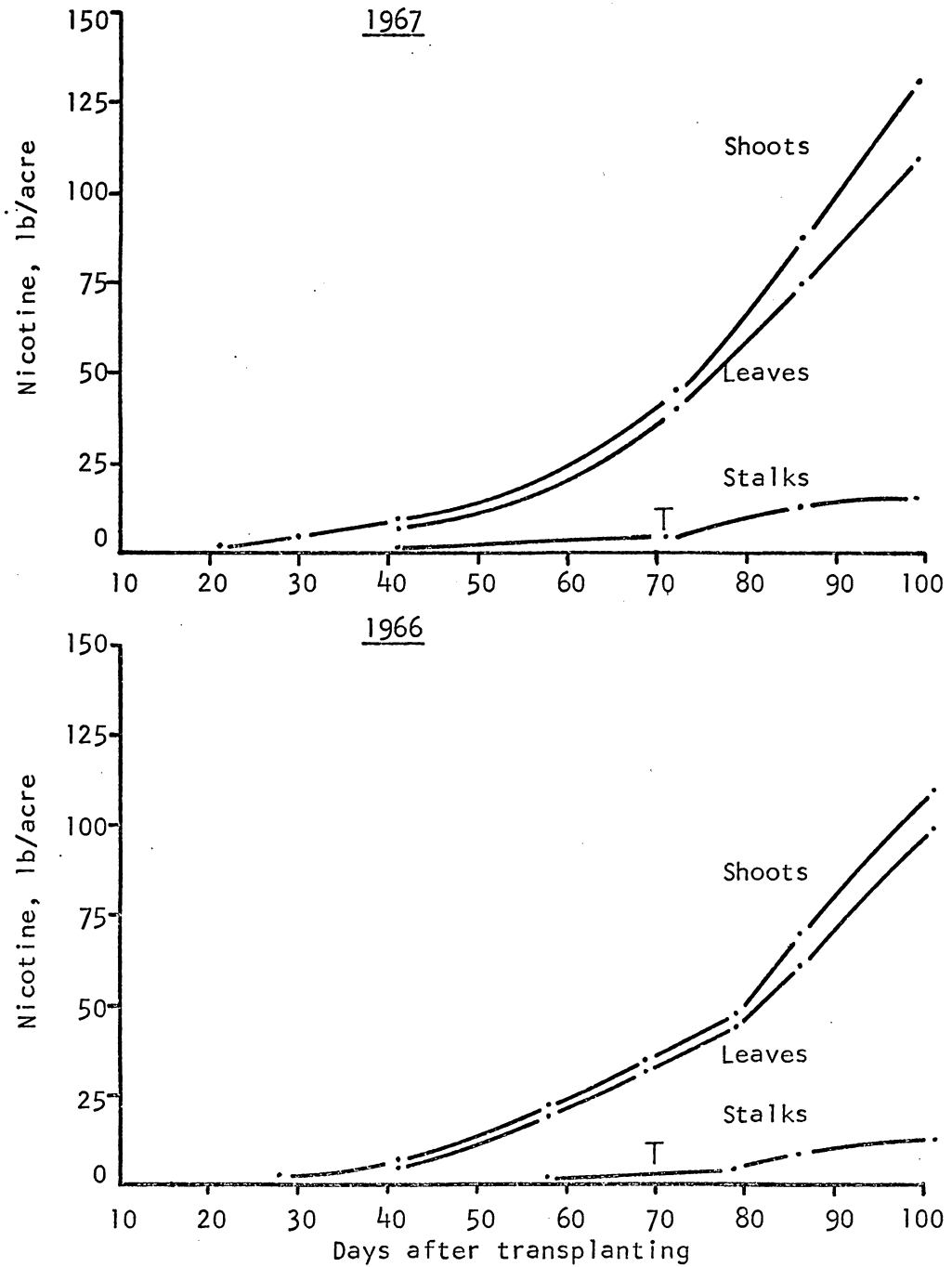


Figure 15: Weight of nicotine in leaves, stalks and shoots of Burley 21 during 1966 and 1967. (T indicates time of topping).

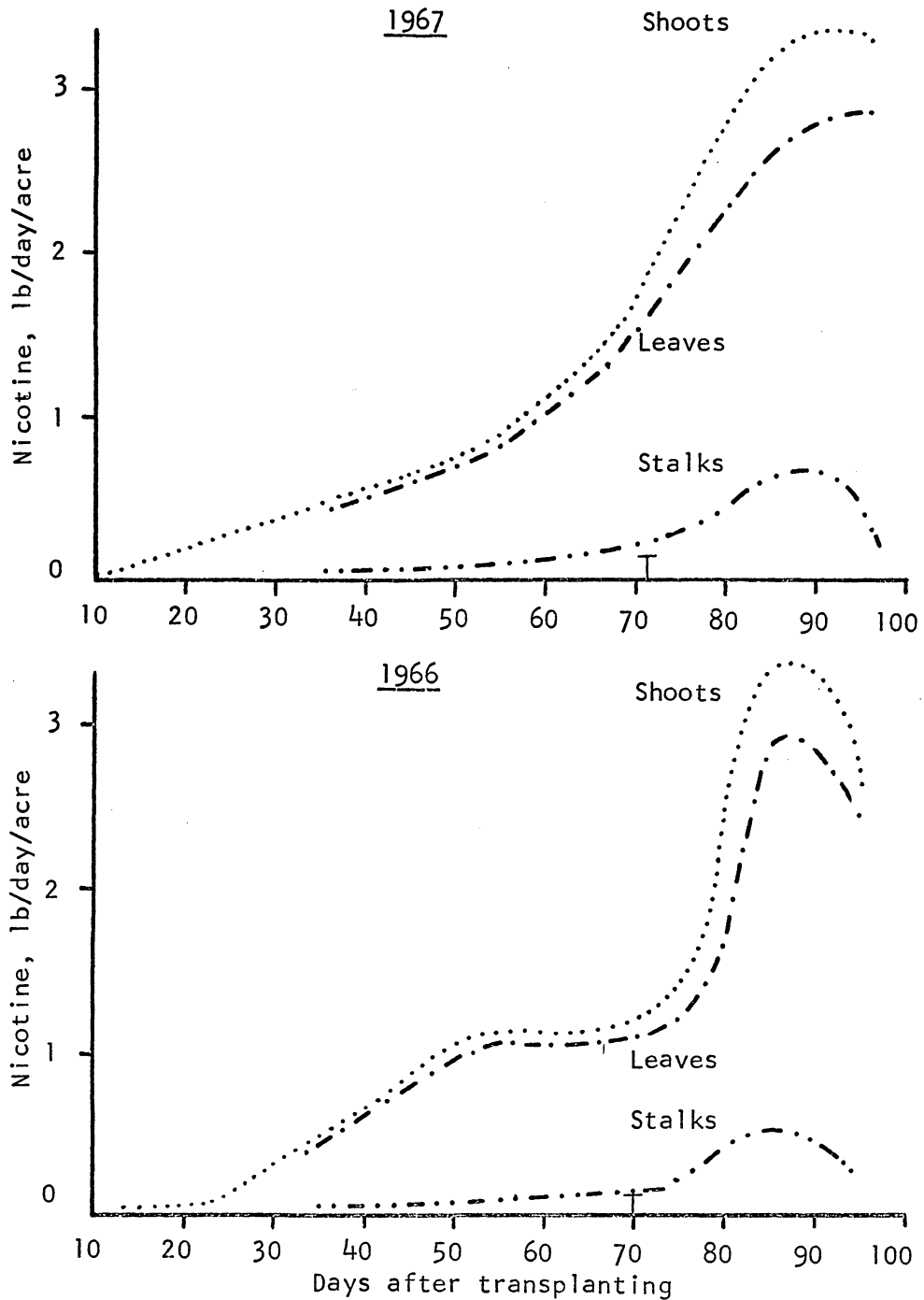


Figure 16: Rate of nicotine accumulation in leaves, stalks and shoots of Burley 21 in 1966 and 1967. (T indicates time of topping).

Table 25. Amount of dry matter, nicotine and nitrogen synthesized by Burley 21 during 1966 and 1967.

Plant Parts	Year	Days after transplanting			
		41	69-72*	86	99-101**
<u>Dry matter lb/acre</u>					
Leaves	1966	578	2248	2818	3027
	1967	816	3009	2743	3093
Stalks	1966	121	1201	2184	2450
	1967	169	1991	2162	2662
Shoots	1966	699	3449	5002	5477
	1967	985	5000	4905	5755
<u>Nicotine lb/acre</u>					
Leaves	1966	7.0	34.0	62.0	96.9
	1967	6.8	40.9	74.9	115.0
Stalks	1966	0.5	2.7	8.1	12.2
	1967	0.6	4.2	13.6	16.0
Shoots	1966	7.5	36.7	70.1	109.1
	1967	7.4	45.1	88.5	131.0
<u>Nitrogen lb/acre</u>					
Leaves	1966	28.3	109.5	137.0	143.2
	1967	44.1	141.4	129.7	143.2
Stalks	1966	4.1	30.5	49.6	57.1
	1967	6.4	46.2	52.5	64.9
Shoots	1966	32.4	140.0	186.6	200.3
	1967	50.5	187.6	182.2	208.1

* Sampling time after 69 days in 1966 and 72 days in 1967. (Topping occurred one day after sampling in 1966 and on day of sampling in 1967).

** Harvesting time after 101 days in 1966 and 99 days in 1967.

nicotine accumulation declined to a negative value, indicating that some of the nicotine had degenerated and migrated to the stalks and flowers. Following this decline the nicotine accumulation in the leaves was raised to a point slightly higher than zero during the last week of the growing season. The rate of nicotine accumulation in the stalks of untopped plants continued to be low to the end of the growing season (Figure 13).

b. Effect of split application of nitrogen

Split applications of nitrogen did not affect the total amount of nicotine synthesized because split applications did not affect nicotine percent (Tables 22 and 23) nor the dry weight of shoots (Table 8).

c. Effect of nitrogen rates

The amount of nicotine in non-cured plants increased with increasing rates of nitrogen up to 150 pounds per acre (Table 21). However, yield and percent nicotine of cured leaves significantly increased with increasing rates of nitrogen up to 225 pounds per acre. The discrepancy between nicotine in non-cured and cured leaves might possibly be due to the small sample taken of the non-cured plants. It was concluded that nicotine synthesis increased with increasing rates of nitrogen up to 225 pounds per acre. Most of the nicotine was stored in the leaves; only 14.88, 13.37, 11.97 and 12.08 percent of total nicotine was found to be in the stalks at 0, 75, 150 and 225 pounds of nitrogen applied, respectively.

G. Nicotine - Nitrogen Relationship

An increase in percent nitrogen of the leaves was associated with an increase in nicotine percent (Figure 17). For each one percent increase in nitrogen percent in the leaves, nicotine increased by 0.64 percent in cured leaves and 0.40 percent in non-cured leaves.

The ratio of nicotine-N over total-N is an indication of nitrogen incorporation into nicotine. An increase in the amount of nitrogen applied resulted in a decrease in the percent of nitrogen incorporated into nicotine (Figure 18). This phenomenon was consistent in leaves and stalks of non-cured plants as well as cured leaves (Table 19).

The nitrogen incorporation into nicotine was low prior to topping; however, the incorporation was lower in 1967 than in 1966 due to the more rapid rate of growth in 1967 than in 1966 (Figure 19).

At topping time only 4.52 and 4.15 percent of the total nitrogen was incorporated into nicotine in 1966 and 1967, respectively (Table 13). The incorporation increased rapidly after topping.

After topping, 6.1 and 7.1 percent of the total nitrogen in the shoots was incorporated into nicotine in 1966 and 1967, respectively, while only 0.54 percent of the nitrogen was incorporated into nicotine in untopped plants during the period after topping. The incorporation of nitrogen into nicotine after topping was higher in 1967 than in 1966 (Table 13) [perhaps because the growth rate after topping was lower in 1967 than in 1966 (Figure 2)] and thus the final

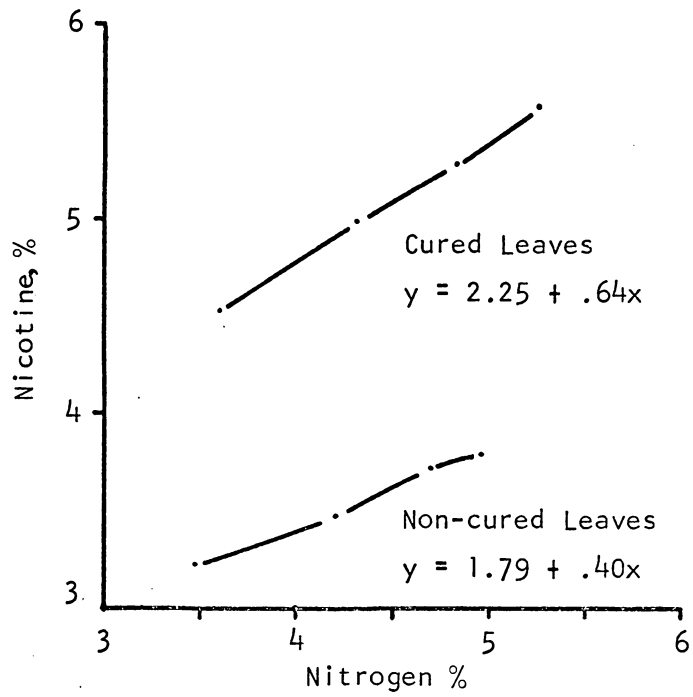


Figure 17: Relationship between nitrogen and nicotine content of Burley 21.

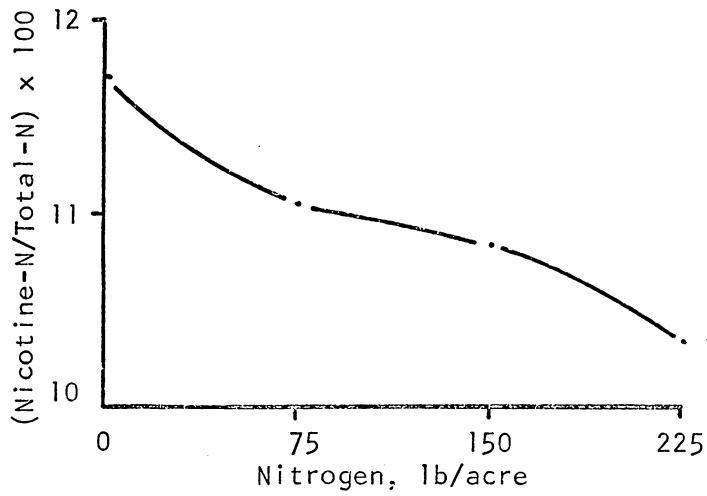


Figure 18: Percent nitrogen incorporated into nicotine at various rates of nitrogen in 1967.

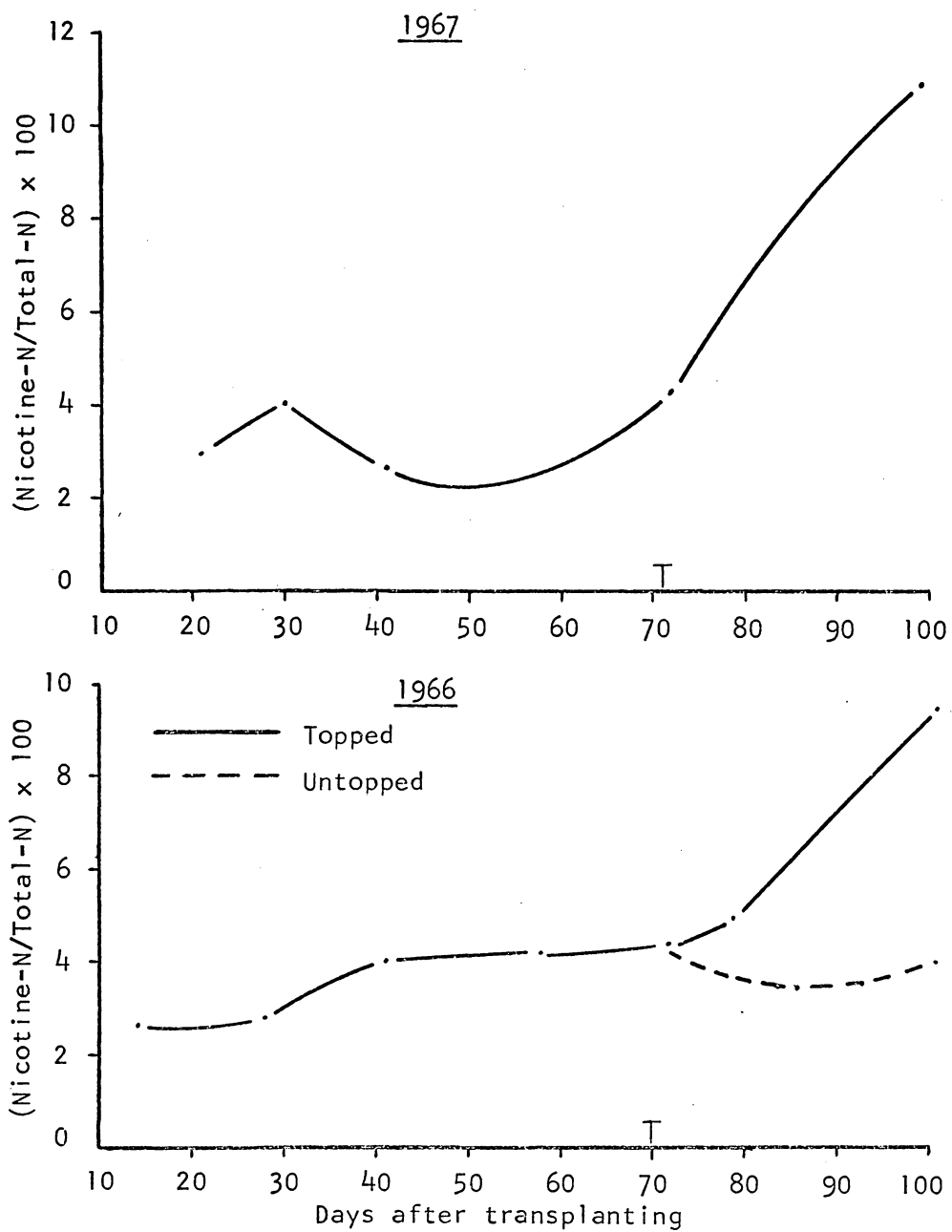


Figure 19: Percent nitrogen incorporated into nicotine in 1966 and 1967. (T indicates time of topping).

nicotine percent of shoots was higher in 1967 than in 1966 (Table 13) even though the nitrogen percent of shoots was almost the same in both years.

In conclusion the percent nicotine in the plant is a function of percent nitrogen and the fraction of this nitrogen incorporation into nicotine. There are many factors affecting the ratio of nitrogen incorporation into nicotine, which warrant further investigation.

H. Effect of Nitrogen Placement

In 1966 a comparison was made between band and broadcast application. Nitrogen at the rate of 150 pounds per acre was applied 21 days after transplanting. The data obtained from these two treatments revealed that there was no difference in growth, final yield, and percent nicotine or nitrogen of leaves and stalks (Table 26). There was no difference in nitrogen uptake resulting from either method of application. A possible explanation might be that the level of nitrogen applied together with the nitrogen resulting from nitrification was in excess of plant requirements. Any loss due to leaching would, therefore, not be expressed in yield. Furthermore, nitrogen is highly mobile and moves readily into the root zone irrespective of the method of application.

Table 26. Effect of methods of nitrogen fertilizer application on yield and chemical composition of leaves, stalks and shoots of Burley 21 in 1966.*

Items	Broadcast**	Band
<u>Dry weight, lb/acre</u>		
	<u>Non-cured</u>	
Leaves	2975	3113
Stalks	2467	2522
Shoots	5442	5635
<u>Nitrogen %</u>		
Leaves	4.75	4.80
Stalks	2.33	2.45
Shoots	3.65	3.75
<u>Nicotine %</u>		
Leaves	3.18	3.22
Stalks	.46	.55
Shoots	1.96	2.20
	<u>Cured leaves</u>	
Yield, lb/acre***	2988	3069
Nitrogen %	4.47	4.74
Nicotine %	3.69	3.94

* Fertilization per acre: 150 lb of N, 200 lb. of P_2O_5 and 400 lb of K_2O .

** The differences between means within rows were not significant ($p = 0.05$, Duncan's Multiple Range test).

*** Contains moisture.

V. SUMMARY AND CONCLUSIONS

Field experiments with burley 21 tobacco were conducted on a Dunmore silt loam at the Southwest Virginia Research Station, Glade Spring, Virginia. The effect of rates and times of nitrogen application on rate of growth, yield, value and nicotine and nitrogen contents of leaves and stalks were determined. The nitrogen rate was 150 pounds per acre in 1966 and 0, 75, 150 and 225 pounds per acre in 1967, respectively. Nitrogen was applied just prior to planting and/or at 21 and 36 to 41 days after transplanting.

A. Plant Growth

Rate of growth was relatively low for the first 30 days. The maximum rate of growth occurred 50 to 60 days after transplanting. Of the total dry matter of shoots, 58.7 percent was accumulated in the month before topping. The rate of leaf growth was higher than stalk growth early in the season, while it was lower late in the season. The ratio of stalk-weight to shoot weight increased with time. The average activity of one pound of leaves in dry matter production was maximum 21 days after transplanting and then decreased with time.

B. Nitrogen Content of Shoots

Nitrogen concentrations in leaves and stalks decreased with time; a high rate of growth enhanced the decrease in nitrogen level; the decrease in nitrogen in stalks was more rapid than that of leaves.

The rate of nitrogen uptake generally paralleled the rate of growth. However, early in the growing season the rate of nitrogen uptake was slightly higher than the rate of growth, while it was lower late in the season. About 80 percent of the total nitrogen of shoots was absorbed prior to topping, 60 percent of the total nitrogen was absorbed during the month prior to topping.

C. Nicotine Content of Shoots

Nicotine levels in leaves was constant or increased slowly with the time in the period prior to topping. However, a high rate of growth tended to reduce the nicotine level. Percent nicotine in stalks decreased with time up to topping. The nicotine in leaves and stalks increased rapidly after topping; this increase was enhanced when rate of growth was low. About 2/3 of the total nicotine content of shoots was accumulated during the period after topping.

D. Effect of Topping

Topping resulted in a subsequent increase in dry weight of leaves, while that of stalks and shoots decreased. Nitrogen uptake and the activity of one pound of leaves in dry matter production increased after topping. Reduction in nitrogen percent of leaves and stalks with time ceased after topping. Nicotine level of the shoots of topped plants was almost three times that of the untopped ones. The increase in nicotine was greater when the topping was lower.

E. Effect of Split Applications of Nitrogen

Adding 2/3 of the 75 pounds of nitrogen per acre 21 days after transplanting increased the yield over the treatments that received

most of the nitrogen later or earlier. The differences in acre-value were not significant at the 5 percent level of probability. Split application of 150 and 225 pounds of nitrogen per acre did not affect the yield or acre-value. Split applications of any nitrogen rate did not affect the price; nitrogen and nicotine contents of leaves and stalks. All treatments reached the same flowering stage at the time of topping.

F. Effect of Nitrogen Rates

Increasing nitrogen rates up to 225 pounds per acre significantly increased the yield, acre-value, nitrogen uptake and nitrogen concentration of non-cured leaves and stalks as well as of cured leaves. The rate of increase was lower at the higher rates of nitrogen. Increasing nitrogen rates decreased the average price of cured leaves; however, the only significant difference was at the 225 pounds of nitrogen rate. Flowering stage at the time of topping was significantly less advanced at the 225 pound nitrogen rate than at the 75 pound rate. Increasing nitrogen rates up to 75, 150 and 225 pounds per acre significantly increased the percent nicotine of stalks, non-cured leaves and the lamina of cured leaves, respectively. The rate of the increase slowed down at the higher rates of nitrogen. The ratio of nitrogen incorporated into nicotine over total nitrogen decreased with increasing nitrogen rates. The increase in percent nicotine was associated with an increase in nitrogen percent. For each one percent increase in nitrogen of cured leaves, nicotine increased 0.6 percent.

G. Effect of Nitrogen Placement

There was no significant difference between band and broadcast application of 150 pounds of nitrogen in yield, average price and nicotine and nitrogen contents of leaves and stalks when applied 21 days after transplanting.

Conclusion: Nicotine in burley tobacco is a function of the plant nitrogen content and the fraction of this nitrogen incorporated into nicotine. Increasing nitrogen rates increased nitrogen levels in plants and slightly decreased the rate of nitrogen incorporated into nicotine. The increase of nicotine in the plant with increasing nitrogen rates did not, therefore, exhibit a straight line relationship.

Split applications of nitrogen did not affect the percent nitrogen nor the fraction of this nitrogen incorporated into nicotine, hence nicotine content was not affected by split applications.

In order to control nicotine content in tobacco, consideration should be given to the following:

1. Nitrogen levels in the plant can be controlled by controlling the amount of available nitrogen through population density and/or level of fertilization.

2. The fraction of total plant nitrogen incorporated into nicotine can be controlled by adjusting time and level of topping at each level of plant nitrogen content. In regard to the latter, it is suggested that the nitrogen content be determined 70 days

after transplanting since 80 percent of the total nitrogen in shoots was taken up during this period.

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Nitrogen Fertilization, Nitrogen and Nicotine Content
of Burley Tobacco

by

Abdullatif Badr

ABSTRACT

Field experiments were conducted to study the effect of rates and time of nitrogen application on growth, nitrogen and nicotine contents of leaves and stalks of Burley 21. Rates of nitrogen used were 0, 75, 150 and 225 pounds per acre. Nitrogen was applied as preplant and/or as post plant applications.

The maximum rate of growth and nitrogen uptake occurred during the month prior to topping. During the period prior to topping 74 percent of the total dry matter, 80 percent of the total nitrogen and 34 percent of the total nicotine accumulated in the shoots.

Applying 2/3 of the 75 pounds of nitrogen per acre 21 days after transplanting increased the yield over treatments in which nitrogen was applied earlier or later. However, split application of 150 and 225 pounds of nitrogen per acre did not affect the yield. Split applications in general did not affect average price, nitrogen and nicotine contents of leaves and stalks. Increasing nitrogen rates up to 225 pounds per acre significantly increased the nitrogen and nicotine levels of cured leaves as well as the nitrogen levels in non-cured leaves and stalks. The increase in percent nicotine of non-cured stalks and leaves was not significant beyond applications

of 75 and 150 pounds of nitrogen per acre, respectively. Increasing nitrogen rates lowered the ratio of nitrogen incorporated into nicotine in relation to total shoot nitrogen. The average increase in percent nicotine was 0.6 percent for each 1 percent increase in percent nitrogen of cured leaves. Yield and acre-value significantly increased with nitrogen rates up to 225 pounds per acre. Applying 225 pounds of nitrogen per acre significantly decreased the average price and delayed the flowering.