GROWING FLUE-CURED TOBACCO TO PRESPECIFIED LEAF CHEMISTRIES THROUGH CULTURAL MANIPULATIONS[1]

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Field studies were conducted at the Central Crops Research Station, Clayton, NC. during 1977 and 1978 to determine whether prespecified leaf chemistries could be achieved through cultural manipulation.

The flue-cured variety, NC 2326, was grown under five treatments: Irrigated (IRR), Rainfed (RAIN), Leached (LEACH), Stressed (STRESS), and in 1978 only, Irrigated plus 2X-Nitrogen (IRR 2X-N). The IRR treatment was fertilized and cultured normally; soil moisture was maintained above 15% available water by irrigation. The RAIN treatment received normal N fertility and the levels of nicotine and reducing sugars were allowed to be determined by the response to rainfall. The LEACH treatment was fertilized normally, soil moisture was maintained above 30% available water by irrigation, and residual N was leached from the root zone at about topping time to induce an early transition to starch accumulation. In the STRESS treatment, N fertility was increased by 25% and transparent plastic shelters were erected to impose an 18-day moisture stress beginning at Crop Day 42 (38-day moisture stress beginning at Crop Day 49 in 1978) in order to prolong N uptake and metabolism and delay starch accumulation. In 1978, a treatment (IRR 2X-N) was added in which N fertility was increased two-fold (relative to IRR) and soil moisture was maintained above 15% available water by irrigation.

These treatments were successful both years in moving nicotine levels in the desired directions relative to the IRR treatment. In 1978, the alkaloid concentrations in the IRR 2X-N and STRESS tobaccos were significantly higher, and that in the LEACH tobacco was lower, than in the IRR tobacco. However, judicious irrigation in 1977 did not overcome the deleterious effects of the hot, dry growing season. The nicotine levels of all treatments were higher than the projected levels but, again, the nicotine level in the STRESS treatment was increased and in the LEACH treatment was decreased significantly relative to the IRR treatment. Sugar levels were lower in all treatments than had been expected. The anticipated changes were, however, in the desired directions in all cases. Thus it appears that sugar levels are much less amenable to manipulation than are nicotine levels. Yields of the various treatments generally were not significantly different. Usability of the tobaccos was decreased in response to higher N fertility and/or low soil moisture. Smoke evaluation of the 1978 tobaccos by a trained panel indicated that only the IRR 2X-N tobacco was unacceptable.

These results suggest that it is possible to manipulate leaf chemistries but only within limits that are relative to the IRR treatment whose actual chemistry levels will be determined by the season.

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INTRODUCTION

In flue-cured tobacco production, the availability of N in the soil must be controlled within relatively narrow limits (1). To obtain well-matured tobacco the available N in the soil must be depleted by the time maximum growth is obtained (4).

N fertility and nicotine accumulation are directly related (4, 16, 19). In addition to N fertilization, the amount and distribution of rainfall throughout the growing season are of critical importance and greatly affect the yield and quality of cured leaf (2,3,9,17,18). The associations of low nicotine and high sugars in wet weather tobacco and, conversely, of high nicotine and low sugars in dry weather tobacco, are well established (2,3,11-14).

The rate of water use varies during plant development. With most tobacco types, a relatively dry soil condition shortly after transplanting is considered desirable for development of the root system (6,9,10). During shoot development there must be a supply of water sufficiently continuous to prevent interruptions to growth. During maturation and harvest, the water requirement is less. Nonetheless, water stress during this period can prolong maturation (6,10), thereby increasing yield and thickness of the leaf, but quality factors such as color and texture usually deteriorate, particularly when the stress is excessive (10). In general, it appears that relatively rapid maturation produces the best quality leaf (8).

Recent management experiments in North Carolina have helped to explain the sugar/nicotine imbalances in dry season tobaccos (7,17,18). Heavy rainfalls, especially early in the season, leach the nitrogen reserves from the root zone, prematurely terminating nitrate uptake and reduction (via nitrate reductase) by the plant. This apparently triggers the onset of starch accumulation, and the prolonged period for starch accumulation results in a high sugar/nicotine ratio in the cured leaf. However, under inadequate moisture conditions, nitrate uptake is prolonged and the onset of starch accumulation is delayed. Therefore, less starch accumulates in dry season tobaccos, giving rise to lower sugar/nicotine ratios.

The experiments reported here represent an attempt to use our understanding of biological quality control to manipulate, in a predictable fashion, leaf chemistry (specifically alkaloids and reducing sugars) of a standard flue-cured variety through modification of soil moisture and N fertility in the field.

Table 1. Precipitation and mean soil moisture, 1977.

Treatment	Crop days 0-41	42-60	61-123
	Precipitation[1/] (cm)		
IRR	18.3	2.9	23.0
LEACH	18.3	13.0	23.0
STRESS[2/]	18.3	0	23.0
RAIN	18.3	2.9	19.3
	Mean soil moisture (%)		
IRR	7.2	4.7	4.5
LEACH	7.2	5.5	5.9
STRESS[2/]	6.8	3.0	4.0
RAIN	7.1	4.6	3.4

1/ Rainfall + irrigation

2/ Sheltered, Crop Days 42-60

MATERIALS AND METHODS

The experiment was conducted on Norfolk sandy loam at the Central Crops Research Station at Clayton, N.C. Field capacity and wilting point were determined to be about 11% and 2%, respectively. In 1977, a completely random design with four replications was used; two replications in a randomized complete block design was used in 1978. Four-row plots were used with the middle two harvested. Flue-cured tobacco (cv.NC 2326) was planted at 15,650 plants per hectare using normal station practices.

Climatological data from the Central Crops Research Station, Clayton, N.C., reveals that in a year with "normal" amount and distribution of rainfall during the tobacco growing season, approximately 2.5 cm of rainfall per week is received. Under these conditions and with normal fertility and cultural practices, the plant average for a standard flue-cured variety (NC 2326) was 3.45% total alkaloids and 16.8% reducing sugars in 1974 (Long and Weybrew, unpublished data) and 3.34% total alkaloids and 16.7% reducing sugars in 1975 (Seltmann, unpublished data). These data, in addition to observations at other research stations over the years, formed the basis for the projected chemistry levels of the various treatments detailed as follows:

Irrigated (IRR) treatment-

To simulate a normal year, the tobacco was fertilized at 72 kg N/ha in 1977 (69 kg N/ha in 1978), cultured normally, and soil moisture maintained above 15% available water by irrigation. Tobacco grade mineral fertilizers were used which supplied approximately 50% of the total N in the nitrate form. Under these conditions, leaf chemistry was targeted at approximately 3% alkaloids and 18% reducing sugars.

Rainfed (RAIN) treatment-

The tobacco was fertilized and cultured as in the IRR treatment. However, due to severity of drought in 1977, two irrigations were made (1.2 cm on Crop Day 15 and 2.5 cm on Crop Day 50) to insure plant survival. Leaf chemistry was dependent upon rainfall and, hence, unpredictable.

Leached (LEACH) treatment-

The tobacco was fertilized and cultured as in the IRR treatment. Soil moisture was maintained above 30% available water by irrigation. In 1977, the plots were irrigated excessively with 5.1, 2.5, and 2.5 cm on Crop Days 52, 58, and 59, respectively, to leach the residual N from the root zone. In 1978, the plots were irrigated excessively with 3.8 cm (two irrigations of 1.9 cm each spaced 4 hours apart) on Crop Day 60 following irrigations of 2.5 cm each on Crop Days 49 and 59. Alkaloids were expected to be lower, and reducing sugars higher, than in the IRR treatment and were targeted at about 2.5% and 24%, respectively.

Stressed (STRESS) treatment-

N fertility was increased to 90 kg/ha (84 kg/ha in 1978) by adding the additional N above the IRR treatment rate as ammonium nitrate. A dry season was simulated by erecting plastic shelters (described below) over the tobacco plants from Crop Days 42 to 60 (Crop Days 49 to 87 in 1978). Alkaloids were expected to be higher, and reducing sugars lower, than in the IRR treatment and were targeted at 3.75% and 13%, respectively.

Irrigated plus 2X-nitrogen (IRR 2X-N; 1978 only) treatment-

N fertility was increased above the IRR treatment rate to 134 kg/ha (with supplemental ammonium nitrate) and soil moisture was maintained above 15% available water by irrigation as in the IRR treatment. Because of the high N level, leaf chemistry was targeted at 4% alkaloids and 10.5% reducing sugars.

Rectangular A-shaped wooden frame shelters (covered with clear, 4 mil polyethylene film) which covered an area 3.7 m wide (to cover four rows) by 12.2 m long were used. These shelters were supported by posts at the corners and raised periodically as plant height increased. Photosynthetically active radiation was reduced 19% under the shelters at full sunlight, an amount determined not sufficient to reduce photosynthesis of tobacco leaves (C. D. Raper, personal communication). Ventilation was provided through vents at the top of the frame. Air temperature was increased about 1.5 C at mid-afternoon in a full crop canopy (at mid-canopy height). Acceleration of the loss of soil moisture was thought to be the major effect of the slightly elevated temperature.

Soil samples were taken at semi-weekly intervals to determine soil moisture. Six samples from the top 30 cm of the soil were taken from each plot and combined to form one analytical sample and these samples were oven-dried at 105 C overnight to determine soil moisture.

Table 2. Effect of cultural manipulation on tobacco yield, quality index, and usability, 1977.

Treatment	Yield (kg/ha)	Quality index	Usability Co. A (%)	Usability Co. B (%)
IRR	2777	19.0	41	28
RAIN	2559	27.2	53	40
STRESS	2766	15.6	18	14
LEACH	2973	31.2	41	48
CV (%)	14	37		
LSD .05	ns	ns		

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Except for limited hand suckering, all other management practices, including chemical sucker control, harvesting, and curing, were those normally used on the station. The cured tobaccos, by plot and primings, were weighed for yield, assigned government grades, and evaluated for usability by leaf personnel of two domestic manufacturers. Quality index values were assigned according to the procedures of Wernsman and Price (15). Reducing sugars and total alkaloids as nicotine were determined (5) on cured leaf lamina, dried under standard analytical conditions. Data are reported as weighted means.

Table 3. Effect of cultural manipulation on total alkaloids and reducing sugars, 1977.

Treatment	Total alkaloids (%)		Reducing sugars (%)	
	Expected	Observed	Expected	Observed
IRR	3.00	4.27	18.0	5.5
RAIN	1/	4.14	1/	7.0
STRESS	3.75	4.53	13.0	4.5
LEACH	2.50	3.73	24.0	10.4
CV (%)		5.0		27.0
LSD .05		0.34		3.0
LSD .01		0.50		4.3

1/ Variable, depending on seasonal rainfall.

RESULTS

1977: The 1977 growing season at Clayton was hot and dry and was not conducive to the production of quality tobacco. Precipitation (rainfall plus irrigation) during different periods of growth for the treatments is given in **Table 1.** Maximum and minimum temperatures were unusually high. Most notable was the fact that the maximum temperature during July averaged 36 C; the latter half of June and the first half of August were all above 32 C (data not shown). Because of these unusually high temperatures, rainfall received during those times was insufficient to support unrestricted growth. Four irrigations totaling 7.7 cm were made to the IRR treatment; two irrigations were made to the RAIN treatment to insure plant survival.

The plastic shelters successfully diverted precipitation from the STRESS treatment; the plants were subjected to a mean soil moisture level of 3.0% during the sheltered period in contrast to 4.7% soil moisture for the IRR treatment during the same time (**Table 1**). Conversely, the LEACH treatment resulted in higher soil moisture levels than the IRR treatment. The effect of leaching was evident during harvesting; tobacco ripened and was harvested faster than the other treatments. Similarly, the effect of increased N fertility and/or low soil moisture on the STRESS treatment was evident during harvesting; that tobacco was the last to ripen.

Although the differences were not significant, the LEACH treatment exhibited the highest yield and quality index (**Table 2**). Most surprising of all, however, was the relatively high quality tobacco from the RAIN treatment. The tobaccos from the STRESS treatment were given relatively poor usability ratings by both companies (**Table 2**). The tobaccos from the RAIN and LEACH treatments were more usable than that from the IRR treatment although the ratings of the three treatments revealed preferences in company buying patterns.

Total alkaloids (nicotine) and reducing sugars of the cured tobacco were changed as expected with respect to the IRR treatment (**Table 3**). The nicotine level in the STRESS treatment was increased, but not significantly, in relation to the check (IRR). However, the nicotine concentration in the LEACH treatment was about one-half percent lower than the IRR treatment. Although the values for reducing sugars for the various treatments changed as anticipated, the levels were much lower than predicted.

1978: The cold, wet spring retarded seedling development so that transplanting was delayed. In addition, these weather conditions induced premature flowering. The first plant in the study flowered on Crop Day 40; plants that flowered so early were topped and the second sucker "turned out" to make the full complement of leaves.

The 36 cm precipitation that fell during the growing season might have been sufficient had the distribution been more uniform. However, three irrigations of 2.5 cm each were necessary to provide sufficient moisture for growth. Soil moisture in the STRESS treatment averaged 3.0% during the sheltered period as opposed to 5.6% for the IRR treatment **Table 4).** However, differences in soil moisture between the IRR and LEACH treatments were obscured because of the distribution of rainfall in relation to the time of leaching.

As in 1977, the effect of leaching was evident during harvesting; tobacco ripened and was harvested much earlier

Table 4. Precipitation and mean soil moisture, 1978.

Treatment	Crop days		
	0-48	49-87	88-115
	Precipitation 1/ (cm)		
IRR	15.2	24.3	4.4
LEACH	15.2	28.1	4.4
STRESS	15.2	0	4.4
IRR 2X-N	15.2	24.3	4.4
RAIN	15.2	19.3	1.9
	Mean soil moisture (%)		
IRR	5.8	5.6	5.6
LEACH	5.6	5.8	5.6
STRESS 2/	5.9	3.0	3.7
IRR 2X-N	5.6	5.0	5.2
RAIN	5.9	4.0	4.6

1/ Rainfall + irrigation

2/ Sheltered, Crop Days 49-87

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Table 5. Effect of cultural manipulation on tobacco yield, quality index, and usability, 1978.

Treatment	Yield (kg/ha)	Quality index	Usability Co. A (%)	Usability Co. B (%)
IRR	2450	39.9	95	65
RAIN	2138	36.4	98	27
LEACH	2230	45.5	100	56
STRESS	2481	27.4	51	14
IRR 2X-N	2955	29.5	98	38
CV (%)	15	23		
	364	8.2		
	489	11.0		

than other treatments, while tobacco from the IRR 2X-N treatment was the last to ripen and to be harvested. Only the IRR 2X-N treatment significantly outyielded the IRR treatment (**Table 5**).

The LEACH treatment produced the highest quality tobacco while the IRR 2X-N and STRESS treatments resulted in low quality indices (**Table 5**). As for usability, company preferences were again evident (**Table 5**). The IRR and LEACH tobaccos were much more usable than the STRESS tobacco. Surprisingly, the IRR 2X-N treatment tobacco had greater usability than the STRESS tobacco.

Total alkaloids in the cured tobaccos moved in the expected directions and their concentration in the IRR, STRESS, and LEACH treatments rather closely approximated the predicted concentrations (**Table 6**). However, reducing sugar levels consistently were in the desired directions and below expected concentrations but were much higher than in the 1977 tobaccos.

Cigarettes were made from tobaccos of each treatment and evaluated by a smoking panel. Cigarettes from the RAIN, STRESS, and LEACH treatments were acceptable compared to the IRR treatment. However, the IRR 2X-N tobacco was unacceptable because the smoke was judged bitter, strong, and less flavorful than that of the IRR treatment.

Table 6. Effect of cultural manipulation on total alkaloids and reducing sugars, 1978.

Treatment	Total Alkaloids (%) Expected	Total Alkaloids (%) Observed	Reducing sugars (%) Expected	Reducing sugars (%) Observed
IRR	3.00	3.00	18.0	14.5
RAIN	1/	3.57	1/	10.7
LEACH	2.50	2.53	24.0	15.7
STRESS	3.75	3.92	13.0	9.2
IRR 2X-N	4.00	3.83	10.5	9.8
CV (%)		13		18
LSD .05		0.44		2.2
LSD .01		0.60		2.9

1/ Variable, depending on seasonal rainfall.

DISCUSSION

Although the precipitation data for the different plant growth phases given in **Tables 1** and **4** obscure the fact, distribution of rainfall in 1978 was more uniform than in 1977. As a result of high temperatures and unfavorable rainfall distribution in 1977, the tobacco was subjected to moisture deficits more often than in 1978. These conditions resulted in cured leaf with high alkaloids and low reducing sugars in 1977 and lower alkaloids and higher sugars in 1978, confirming earlier observations.

Short periods of imposed low soil moisture prolong soil nitrate availability and plant nitrate reductase activity (7). Similar conditions prevailed during the dry growing season in 1977 (from observations in adjacent studies) and thus were responsible, in part, for the observed delay in maturation and ripening. Support for this proposition is provided by the fact that the final harvest in 1977 occurred, on the average, 63 days after the first harvest compared to the normal harvest period of 42-49 days. Additionally, it has been suggested that starch accumulation in tobacco begins when nitrate reduction terminates (17). Thus, a prolonged period of nitrate uptake and reduction would delay the onset of starch accumulation, and when this occurs, as in the 1977 crop, the quantity of starch accumulated would be severely restricted. In the present study, sugars in the cured leaf were very much lower than expected (**Table 3**). In contrast, sugars in the 1978 crop were higher in response to the more normal seasonal rainfall.

There is a positive correlation between N fertilization and total N or nicotine concentration (4,16,19) and a negative correlation between sugar concentration and total N in the flue-cured leaf (4,16). Results from this study agree with these observations. The increased N fertilization under the STRESS and IRR 2X-N treatments produced significant increases in the nicotine concentration of the cured leaf. The LEACH treatment received normal fertilization but was subjected to leaching irrigation at topping time. These irrigations were designed to leach the residual N from the root zone, thereby forcing the transition from nitrate reduction to starch accumulation prematurely. The high sugar and low nicotine concentrations in the cured leaf attest to the success of this maneuver.

The use of shelters in the STRESS treatment produced moderate soil moisture stress and apparently slowed, and thus extended the period of, nitrate uptake; the period of prolonged nitrate reduction in the plant allowed for extra nicotine accumulation and shortened the period of starch accumulation. The result was high nicotine and low sugars in the cured leaf.

The sugar concentrations in both seasons were consistently lower than projected. This was attributed to the persistent uptake and reduction of N beyond Crop Day 70. For example, in 1978 this situation limited starch concentration to only 24% in the IRR treatment at harvest (Weybrew, unpublished data) instead of the more usual 30%. Had starch accumulation reached 30%, sugars would have approximated 18% (each 1% starch at harvest equating to 0.6% sugar after curing), yield would have been approximately 360 kg/ha more, and quality index and usability would likely have been better. Presumably other treatments were affected proportionately. We speculate that similar explanations apply to the low sugar concentrations in the 1977 crop as well.

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As far as leaf quality and usability are concerned, the LEACH treatment tended to produce tobacco superior to the other treatments. These results confirm the importance of irrigation in supplying water necessary for unrestricted plant growth and/or in terminating prolonged nitrogen uptake and metabolism.

Although it is tempting to do so, equating the effects of low soil moisture to those of over-fertilization may not be strictly valid. In this study, the IRR 2X-N treatment failed to produce tobacco with an alkaloid concentration higher than that of the STRESS treatment (which included 25% additional N). This suggests that low soil moisture is of greater importance in determining leaf chemistry than is over-fertilization.

Clearly, under a "normal" growing season and through adjustments in N fertilization and irrigation schedules, one can, without significant decrease in yield, move alkaloid and reducing sugar concentrations in prespecified directions as the prevailing market demands. However, the extent to which one can manipulate the nicotine and reducing sugar levels will be influenced by weather conditions

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