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EFFECT OF VARIETY AND HARVEST TREATMENTS ON PROTEIN YIELD OF CLOSE-GROWN TOBACCO[1]

By WILLIAM G. WOODLIEF[2], JAMES F. CHAPLIN[2], C. RAY CAMPBELL[3], AND DONALD W. DE JONG[2]

Extractable protein yield of NC PY-10, LAFC 53, Speight G-28, and SC 58 tobacco (*Nicotiana tabacum* L.), planted as "close-grown," was compared by utilizing four harvest treatments: (A) harvest during the button stage, (B) harvest of ratoon regrowth following cutting and discarding of the mature tobacco, (C) harvest of mature tobacco, and (D) harvest of three successive mid-growth crops by utilizing ratooning. Results show no significant differences among the tobacco cultivars tested. Treatment D, with three successive mid-growth harvests, produced a significantly greater amount of extractable protein/ha than the other harvest treatments tested.

INTRODUCTION

A system of growing flue-cured tobacco, known as "close-grown," has been investigated by Campbell *et.al.* (1). Close-grown tobacco is grown with plant populations of 85,000-100,000/ha in contrast to conventional flue-cured tobacco with plant populations of 14,000-15,000/ha. Close-grown tobacco produces large amounts of fresh tissue which might be suitable for the protein extraction and recovery process described by De Jong and Lam (3).

About half of the total protein in green tobacco can be removed in aqueous extraction. The remainder is water insoluble, presumably being bound to membranes in the leaf. In aqueous extraction of tobacco leaf, green lamellar proteins appear as a fine green suspension (2). In this paper, extractable protein refers to the soluble protein precipitable from aqueous solution by the addition of $KHSO_4$, after the removal of the green chloroplastic debris. Soluble proteins in green leaves are composed of fraction I and II proteins. In small tobacco leaves, the ratio of fraction I proteins to fraction II proteins is about 1:10. Just before maximum leaf elongation, the ratio is 1:1. Thereafter, the ratio decreases to 1:10 (5). As the leaf becomes larger, the chloroplasts also enlarge and protein levels tend to increase to the point of maximum elongation of the leaf. During leaf growth and elongation, protein synthesis surpasses protein degradation until the leaf matures and senescence begins, at which time protein degradation surpasses protein synthesis within the leaf (2). Unfortunately, all the leaves on the tobacco plant do not senescence or reach maximum elongation simultaneously. Thus, when researchers investigate harvesting the entire tobacco plant for extractable

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[2]Biological Research Technician, Research Agronomist, previous Research Chemist, respectively, Tobacco Research Laboratory, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Oxford, N.C. 27565.
[3]Station Superintendent, Oxford Tobacco Research Station, N.C. Department of Agriculture, Oxford, N.C. 27565. Contribution received December 29, 1980 Tob. Sci. XXV: 83-86, 1981.

Table 1. Comparison of tobacco varieties and harvest treatments[a] on the yield of fresh weight and extractable protein from "close-grown" tobacco.

	1978			1979		
	Extractable protein yield[b]	Fresh weight harvested	Extractable protein[b,c]	Extractable protein yield[b]	Fresh weight harvested	Extractable protein[b,c]
	kg/ha	kg/ha	%	kg/ha	kg/ha	%
Varieties						
SC 58	-	-	-	222	48567	2.8
NC PY-10	172	57464	2.3	213	50732	2.8
LAFC 53	147	56083	2.2	233	52319	2.9
Speight G-28	147	51294	2.5	243	52862	2.8
Average	155	54947	2.3	228	51120	2.8
LSD .01	NS	5836	NS	NS	NS	NS
Treatments[a]						
A	153	47128	2.5	218	58942	2.1
B	-	-	-	36	10436	2.2
C	109	65661	1.4	242	60734	2.3
D	205	52053	3.3	415	74369	4.6
Average	155	54947	2.3	228	51120	2.8
LSD .01	42	5460	1.0	64	7329	0.8

[a]All varieties: A = Harvest at button stage; B = Harvest of ratoon regrowth following cutting and discarding of mature tobacco; C = Harvest at maturity; D = Harvest 3 successive times at mid-growth (40 days after transplanting, then 26 and 17 days later). [b]Represents 100% protein - Kjeldahl N x 6.25. [c]Percent dry weight of tobacco.

protein, they must consider the stage of plant growth at harvest (4).

The experiment reported here was conducted to determine the effect of varieties and harvest treatments on extractable protein yield of close-grown tobacco.

MATERIALS AND METHODS

The experiment was conducted at Oxford, North Carolina in 1978 and 1979. The tobacco was produced in a close-grown system with approximately 100,000 plants per hectare (1). Plants were grown in a randomized split-plot design (3 reps) with varieties as the whole plots and harvest treatments as subplots. Each subplot measured 1.63 x 2.74 m. Nitrogen was applied at 184.5 kg/ha. In 1978, three varieties were grown: NC PY-10, LAFC 53, and Speight G-28. In 1979, a fourth variety, SC 58 was grown. The varieties were subjected to the following harvest treatments: (A) entire plants were harvested at the button stage (6), (B) plants were cut at maturity and discarded 84 days after transplanting, and the ratooned crop was harvested 70 days later (1979 only), (C) entire plants were harvested about 84 days after transplanting when they were judged to be mature for the close-grown system, and (D) three successive harvests were made; each harvest consisted of entire mid-growth plants with the initial harvest 40 days after transplanting followed by the 2nd and 3rd harvests (from ratooning), 26 and 17 days, respectively, after the previous harvest. Plants were harvested by cutting the entire plant about 5-7 cm above ground level. No additional fertilizer was applied after any of the harvests.

Two plants per plot were selected at random for protein analysis and dry weight determination. Protein extraction was accomplished by homogenizing the entire plant with 2 parts (w/v) of deionized water containing 1.0 g sodium metabisulfite per liter. The homogenate was filtered and 1-2 mg of magnesium carbonate was added to each 100 ml of green filtrate. After the filtrate was centrifuged to remove the green chloroplastic debris, $KHSO_4$ (7 mg/ml) was added to the clear supernatant to precipitate protein. After freeze-drying the precipitate, we determined the protein content by the micro-Kjeldahl method described by De Jong and Lam (3). Because an additional variety was grown in 1979, analyses of variance were run separately for each year.

RESULTS AND DISCUSSION

Generally, there were no significant differences among varieties for extractable protein yield, fresh weight harvested, and extractable protein percent in either year **(Table 1).** Speight G-28, however, produced significantly less fresh weight than NC PY-10 in 1978. Significant variety x treatment interaction occurred for fresh weight harvested/ha. Speight G-28, with Treatment A, produced less fresh weight/ha than NC PY-10 and LAFC 53 with the same treatment.

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Table 2. Comparison of tobacco varieties and Treatment D harvests[a] (3 successive times at mid-growth) on yield of fresh weight and extractable protein from "close-grown" tobacco.

	1978			1979		
	Extractable protein yield[b]	Fresh weight harvested	Extractable protein[b,c]	Extractable protein yield[b]	Fresh weight harvested	Extractable protein[b,c]
	kg/ha	kg/ha	%	kg/ha	kg/ha	%
Varieties						
SC 58	-	-	-	130	24261	4.2
NC PY-10	79	18251	3.6	131	23469	4.7
LAFC 53	70	17876	3.1	134	24691	5.3
Speight G-28	56	15917	3.2	159	26740	4.2
Average	68	17351	3.3	138	24790	4.6
LSD .01	NS	NS	NS	NS	NS	NS
Treatment D harvests[a]						
1	69	12487	4.0	145	24623	3.9
2	99	21114	4.6	134	26813	4.5
3	37	18453	1.3	137	22933	5.4
Average	68	17351	3.3	138	24790	4.6
LSD .01	31	4822	1.1	NS	NS	NS

[a]All varieties: 1 = first harvest (40 days after transplanting); 2 = second harvest (26 days after 1st harvest); 3 = third harvest (17 days after 2nd harvest) of Treatment D plots. [b]Represents 100% protein - Kjeldahl N x 6.25. [c]Percent dry weight of tobacco.

Treatment C, of all varieties for both years, produced more fresh weight/ha than Treatment A or D; however, Treatment D produced the largest amount of extractable protein/ha and the highest percentage of extractable protein.

In 1979, there were significant differences among harvest treatments for all parameters studied. No significant variety x treatment interactions occurred; however, SC 58 and LAFC 53, with Treatment A, tended to produce less fresh weight/ha than Speight G-28 and NC PY-10 with the same treatment. Treatment D produced the most extractable protein/ha from the largest amount of fresh weight. It also produced the highest percentage of extractable protein of all treatments tested. In contrast to Treatment D, Treatment B produced the least amount of extractable protein/ha. The percent extractable protein of Treatment B; however, was not significantly different from that of Treatments A or C.

In 1978 and 1979, data for the three harvests of Treatment D show no significant differences between varieties for the parameters studied **(Table 2).** In 1978, harvests were significantly different for all the parameters studied. Harvest 2 produced the largest amount of extractable protein/ha; however, Harvest 2 was not significantly different from Harvest 1. Harvest 3 produced less extractable protein/ha and the lowest percentage extractable protein, probably due to seasonal conditions. Harvest 1 produced significantly less fresh weight/ha because of smaller plants. No significant variety x harvest interactions occurred; however, NC PY-10 and LAFC 53 with Harvests 1 and 3 tended to produce more extractable protein/ha than Speight G-28 with the same harvests. In 1979, harvests were not significantly different for the parameters studied.

In 1978, extractable protein production averaged 155 kg/ha and in 1979, it averaged 228 kg/ha for an increase of 46% over 1978 **(Table 1).** The increase was probably due to a combination of factors. Observational data indicate that more lower leaves deteriorated in 1978 than in 1979, especially in Treatment C. The close plant proximity of close-grown tobacco promotes overshadowing of the lower leaves by the uppermost leaves. The lower leaves tend to deteriorate prematurely owing to insufficient sunlight, especially as the plant grows larger. Consequently, close plant proximity results in a loss of protein-containing leaves. In addition to close plant proximity, seasonal variations and plant senescence can influence protein production and dry weight of the tobacco.

In general, the more fresh weight harvested, the more extractable protein obtainable provided the protein has not been degraded due to senescence processes. In 1978, Treatment C, which was harvested at maturity, produced a large amount of fresh weight with a low extractable protein yield. Although plant proximity resulting in a loss of lower leaves might be responsible, senescence or growth conditions, or both, could have been equally responsible for the low extractable protein yield.

Treatment B was the harvest of ratooned regrowth following the harvesting and discarding of mature tobacco. In 1979, the ratooned regrowth of the plants in this treatment was very poor, producing only a small yield of extractable protein (36 kg/ha) due primarily to the low amount of fresh weight produced. The poor ratooning of Treatment B was probably due to seasonal conditions, lack of available N, and/or the absence of viable leaves remaining on the plant after harvesting and discarding of mature tobacco.

Although close plant proximity of the close-grown tobacco may influence the protein yield due to deterioration of the

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lower leaves, it may not be an influential factor when plants are harvested at the mid-growth stage. Shading of the lowermost leaves was not apparent in the three mid-growth harvests of Treatment D.

De Jong and Lam reported 8% extractable protein yield from tobacco leaf (2). In the present experiment, where the whole plant was utilized, Treatment D produced 4.6% extractable protein in 1979, the highest yield of all treatments studied. Because the stalk is responsible for about half of the dry weight and contains negligible amounts of extractable protein (unpublished data), the percentage extractable protein yield would have been about 9.2% if the stalk had been excluded. Likewise, the third harvest of Treatment D producing 5.4% extractable protein from the entire plant **(Table 2)**, would have produced 10.8% extractable protein if the stalk had been excluded. Processing the entire plant may be more energy-consuming than processing the leaf alone. The inclusion of the stalk; however, may facilitate cell rupture during homogenization.

CONCLUSIONS

For both years, there was no significant difference among varieties for extractable protein yield/ha. Treatment D, which utilized three harvests of mid-growth tobacco (two were from ratooning), produced significantly greater amounts of extractable protein/ha than any of the other treatments because of the large amount of fresh weight harvested and its higher protein content. Treatment D produced 34% more extractable protein than Treatment A and 87% more than Treatment C in 1978. In 1979, Treatment D produced 91% more than Treatment A, 72% more than Treatment C, and 1058% more than Treatment B. The extractable protein yield for all treatments was greater in 1979.

In 1979, there was no difference between the extractable protein yield/ha of each harvest of Treatment D. The three harvests averaged 138 kg extractable protein/ha. In 1978, all three harvests were different with respect to extractable protein yield, with Harvest 2 producing 43% more protein than Harvest 1, and 167% more than Harvest 3.

Close plant proximity seems to be important in obtaining maximum extractable protein yield from close-grown tobacco in Treatments A and C because the lower leaves are lost. This effect is not evident if plants are harvested at mid-growth. Seasonal conditions and senescence seem to be crucial factors influencing extractable protein production.

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