"Ripening" Tobacco with the Ethylene-Releasing Agent 2-Chloroethylphosphonic Acid¹⁾

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INTRODUCTION

Ethylene is a plant growth regulator, found in many plants and plant parts (1). Applied ethylene produces many physiological responses depending upon plant species, stage of growth, concentration, and other such factors. It stimulates flowering in some plants and causes leaf abscission. In stored potatoes it causes a rise in respiration rate and an increase in sugar content. Mature but unripe fruits can be ripened with ethylene. Changes induced by ethylene treatment are normal or natural changes which would take place in due time, as fruits ripen or as other plant parts senesce.

Recent intensive work with 2-chloroethylphosphonic acid (CEPA), an ethylene releasing agent, has shown that it can have effects attributable to ethylene. CEPA is water soluble and releases free ethylene above about pH 5 (2). With increasing pH, there is an increasing evolution of ethylene per unit of time.

Reports at the 23rd Tobacco Chemists' Research Conference, University of Maryland, College Park, Maryland, January 1970⁴⁾, indicated that spray treatments of CEPA produced the external appearance of ripening when it was applied to mature leaves of greenhouse-

grown, flue-cured tobacco plants. The extent of ripening or leaf "yellowing" was more pronounced and uniform on topped plants. When applied to mature field-grown, flue-cured plants, CEPA caused yellowing of all leaves in four days. These leaves (65 to 70% of the crop) were harvested on the 4th day after treatment and cured in 3 days. The number of harvestings were, therefore, reduced from 5 to 3; and the curing time was reduced from 5 to 3 days. These reports suggested that the leaves of treated flue-cured plants may go through a partial "yellowing" or "coloring" phase in the field since curing time could be markedly reduced.

The present experiment was designed to determine effects of length of "yellowing" or "coloring" on CEPA-treated and untreated flue-cured tobacco leaves.

EXPERIMENTAL

This experiment was conducted at the Pee Dee Experiment Station, Florence, South Carolina, USA, during the 1969 growing season. The results presented are to be considered preliminary since only one year's data are available. The experiment was repeated in 1970 and the combined data will be summarized elsewhere. Table 1 presents details of the experimental methods.

After the leaves yellowed for the specified length of time, 26 sq. cm sections of leaf lamina were removed, frozen, and then freeze-dried. Leaves which had gone through the complete curing process were used for quality evaluations and analytical samples. Samples were composited over stalk positions, and midribs were removed prior to analysis.

RESULTS AND DISCUSSION

The CEPA treatment had a marked effect on leaf color when applied to mature flue-cured tobacco. Leaves on treated plants turned yellow 3 to 4 days after treatment.

The relative values of the cured leaf are shown in Table 2. Based on U.S. Federal grades, values per unit weight for treated leaf were higher than for untreated leaf. Untreated leaf of 0 yellowing time gave the lowest value.

¹⁾ Presented at the 5th International Tobacco Scientific Congress, Hamburg, September 1970.

This is a report on the current status of research involving the use of certain chemicals that require registration in the United States under the Federal Insecticide, Fungicide, and Rodenticide Act. It does not contain recommendations for the use of such chemicals, nor does it imply that the uses discussed have been registered in the USA. All uses of these chemicals must be registered by the appropriate State and Federal agencies before they can be recommended for any use whatsoever in the United States.

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Table 1. Effect of 2-chloroethylphosphonic acid on flue-cured tobacco.

(Florence, South Carolina, USA, 1969)

Variety — Flue-cured *Nicotiana tabacum* L. cv. Coker 258.

Fertilization — 700 pounds 4-12-12 per acre.

Topping height — "Low profile" — topped down to 60 cm when plants were 75–90 cm tall June 23. Approximately 12–14 leaves remained per plant.

Sucker control — Maleic hydrazide at the rate of about 135 mg/plant June 25.

Chemical treatment — 2-Chloroethylphosphonic acid (2 pounds/gallon, propylene glycol base, technical acid minimum 90%) — 100 mg active chemical was applied in 20 ml of solution per plant as a fine spray 45 days after topping (August 7).

Harvesting — Four days after treatment all leaves were removed from both CEPA treated plants and untreated plants.

Curing — Five "yellowing" or "coloring" times were involved — 0, 12, 24, 36, and 48 hours. During yellowing, the temperature was 35° C and the relative humidity 90%. After the specified length of yellowing, the leaves were cured under standard conditions as follows: The temperature was increased from 35° C to 55° C an average of 0.5° C/hour with vents open and kept there until lamina dried; and, finally, the temperature was increased from 55° C to 72° C an average of 1° C/hour and kept there until midribs dried.

Treated leaf not yellowed or else yellowed for only 12 hours, gave the highest values. Weight of treated cured leaf was significantly lower than that of untreated leaf (Table 2). There seemed to be a trend toward lower leaf weights as the yellowing times increased; this proved true for both the treated and untreated leaf. Table 3 shows that the relative acre value of the treated cured leaf was lower than that of the untreated leaf. This is because treated leaves weighed less than untreated leaves (Table 2). Filling value of the treated cured leaf was higher than for the untreated leaf (Table 3); but the average values were not significantly different. Filling values of only the treated leaf tended to increase as yellowing times increased.

Based on calcium values of freshly harvested tissue vs. fully cured leaf, dry weight losses during curing amounted to 10 to 15%. Data for the chemical composition of tissue after various periods of yellowing and after curing were not corrected for loss of dry weight. The freeze-dried samples were not exactly comparable to the cured leaf samples; the freeze-dried samples were only portions of the lamina, whereas the cured leaf samples were of the entire leaf lamina.

Total nitrogen of freeze-dried tissue from treated leaves was slightly lower than from untreated leaves (Table 4). This tended to also be true of the cured leaf, but the averages were not statistically different. No trend was established as to length of yellowing, with either the freeze-dried tissue or cured leaf. The overall differences in total nitrogen between the freeze-dried tissue and the cured leaf tissue can be accounted for by the 10 to 15% loss of dry weight during the cure.

Table 2. Effect of 2-chloroethylphosphonic acid on flue-cured tobacco (cured leaf)*.

Yellowing time**	Value/100 pounds		Average weight/leaf	
	Untreated \$	Treated \$	Untreated g	Treated g
0	57.97	e 74.60 a	13.89 a	11.99 cd
12	67.03	d 74.66 a	13.12 ab	11.52 cd
24	65.99	d 73.57 ab	13.68 a	11.07 d
36	69.22	cd 73.38 ab	13.12 ab	11.29 d
48	68.42	cd 70.51 bc	12.52 bc	11.17 d
	65.69 b	73.30 a	13.27 a	11.41 b

Table 3. Effect of 2-chloroethylphosphonic acid on flue-cured tobacco (cured leaf)*.

Yellowing time**	Value/acre (relative)		Filling value	
	Untreated \$	Treated \$	Untreated cc/g	Treated cc/g
0	805 de	894 ab	3.42 c	3.68 c
12	880 abc	859 abcd	3.44 c	3.70 c
24	901 a	814 cde	3.48 c	4.01 ab
36	907 a	828 bcde	3.57 c	3.99 b
48	855 abcd	787 e	3.53 c	4.26 a
	870 a	837 b	3.49 a	3.93 a

Table 4. Effect of 2-chloroethylphosphonic acid on flue-cured tobacco (total nitrogen)*.

Yellowing time**	Freeze-dried lamina		Cured leaf	
	Untreated %	Treated %	Untreated %	Treated %
0	2.19 a	1.97 bc	2.67 abc	2.49 c
12	2.21 a	1.97 bc	2.54 abc	2.55 abc
24	2.16 a	1.85 c	2.67 abc	2.60 abc
36	2.18 a	1.97 bc	2.75 a	2.53 bc
48	2.24 a	1.99 b	2.73 ab	2.58 abc
	2.20 a	1.95 b	2.67 a	2.55 a

Table 5. Effect of 2-chloroethylphosphonic acid on flue-cured tobacco (protein nitrogen)*.

Yellowing time**	Freeze-dried lamina		Cured leaf	
	Untreated %	Treated %	Untreated %	Treated %
0	1.20 a	.95 d	1.18 a	1.00 bc
12	1.20 a	.89 e	1.13 a	.99 bc
24	1.13 b	.80 f	1.12 a	.99 bc
36	1.01 c	.76 fg	1.04 b	.93 c
48	.92 de	.74 g	1.04 b	.97 c
	1.10 a	.83 b	1.10 a	.97 b

* Values with common letters are not significantly different (5% level).
** Hours.

Table 6. Effect of 2-chloroethylphosphonic acid on flue-cured tobacco (total alkaloids)*.

Yellowing time**	Freeze-dried lamina		Cured leaf	
	Untreated %	Treated %	Untreated %	Treated %
0	3.42 c	3.44 c	5.00 b	4.98 b
12	3.54 bc	3.79 a	5.07 ab	5.12 ab
24	3.80 ab	3.83 a	5.12 ab	5.00 b
36	3.97 a	3.99 a	5.33 a	5.14 ab
48	3.97 a	4.02 a	5.34 a	5.09 ab
	3.74 a	3.81 a	5.17 a	5.07 a

Table 7. Effect of 2-chloroethylphosphonic acid on flue-cured tobacco (starch)*.

Yellowing time**	Freeze-dried lamina		Cured leaf	
	Untreated %	Treated %	Untreated %	Treated %
0	17.6 a	12.5 b	2.1 a	1.8 ab
12	13.8 b	8.7 c	1.4 c	1.3 c
24	9.4 c	6.2 d	1.0 cd	1.2 c
36	6.7 d	4.2 e	0.8 d	1.4 bc
48	4.6 e	2.7 f	0.7 d	1.3 c
	10.4 a	6.8 b	1.2 a	1.4 a

Table 8. Effect of 2-chloroethylphosphonic acid on flue-cured tobacco (reducing sugars)*.

Yellowing time**	Freeze-dried lamina		Cured leaf	
	Untreated %	Treated %	Untreated %	Treated %
0	2.8 e	7.0 d	13.0 ab	11.6 ab
12	6.4 d	10.1 c	13.7 a	12.7 ab
24	9.0 c	12.1 ab	10.9 b	12.4 ab
36	10.9 bc	13.1 a	11.8 ab	11.7 ab
48	12.8 a	13.2 a	11.7 ab	10.7 b
	8.4 a	11.1 a	12.2 a	11.8 a

* Values with common letters are not significantly different (5% level).
** Hours.

Protein nitrogen was lower in both treated freeze-dried tissue and cured leaf (Table 5), compared to untreated material. A trend for lower protein nitrogen as yellowing times increased is observed with freeze-dried tissue but was not evident with cured leaf. On the basis of the weight loss correction of 10 to 15%, protein nitrogen of cured leaf was lower than that of the freeze-dried tissue.

Total alkaloids were not significantly changed by CEPA treatment (Table 6). Total alkaloid values of freeze-dried tissue and cured leaf subjected to shorter yellowing times were lower than for tissue subjected to longer yellowing times. This trend was particularly true with the untreated leaf. The higher total alkaloid values of cured leaf, as compared to the freeze-dried tissue, cannot be explained by the 10 to 15% loss in dry weight

during the curing process. Bacon et al. (3) found that nicotine levels changed very little from harvest to final cure, after dry weight loss corrections were made. In our case, the sampling technique mentioned earlier may account for the differences.

The starch content of freeze-dried tissue at the end of each yellowing time was significantly lower for treated tissue compared with untreated (Table 7). Increased yellowing time reduced the level of starch in the freeze-dried tissue in a relatively uniform manner. After completion of the cure, the gradient which had existed for the freeze-dried tissue was essentially lost, although those leaves subjected to shorter yellowing times usually contained more starch.

As would be expected, reducing sugars were lower in freeze-dried tissue yellowed for shorter periods of time (Table 8). There was a tendency for CEPA treated, freeze-dried tissue to contain higher levels of reducing sugars than corresponding untreated material. After the completion of the cure, the gradient which existed with the freeze-dried tissue was lost. Also, both the treated and untreated cured leaf contained about the same amount of reducing sugars, irrespective of length of yellowing time.

SUMMARY

Treatment of flue-cured tobacco with the ethylene releasing agent 2-chloroethylphosphonic acid (CEPA) caused mature leaves to lose their green color and turn yellow. The treated leaves appeared to go through a partial "yellowing" or "coloring" phase prior to harvest. Treated leaves at harvest contained greater amounts of reducing sugars and lower levels of starch, relative to comparable untreated leaves. At the end of the curing process, comparisons between treated and untreated leaf showed that only small differences existed in total nitrogen, total alkaloids, starch, and reducing sugars. Statistically, protein of leaf treated with CEPA was significantly lower as compared to untreated leaf.

For all yellowing times, dollar values per hundred weight of cured leaf from treated plants were higher than from untreated plants, but the average weight per leaf from treated plants was lower. During the curing process, leaf treated with CEPA can probably be subjected to shorter periods of yellowing or coloring than untreated leaf. If further work with "ripening" chemicals such as CEPA show that they can be used to hasten yellowing or ripen tobacco successfully, as well as to reduce the time required for curing the crop, economic advantages are likely to accrue. It is to be hoped that such chemicals may help reduce the labor and investment required for crop production, and at the same time improve crop quality.

ZUSAMMENFASSUNG

Die Behandlung von röhrengetrocknetem Tabak (flue-cured) mit Äthylen freisetzender 2-Chloräthylphosphorsäure (CEPA) führte bei reifen Blättern zum Verlust

ihrer grünen Farbe und zum Vergilben. Die behandelten Blätter schienen eine Teil-Vergilbungs- bzw. Teil-Verfärbungsphase vor der Ernte zu durchlaufen. Die behandelten Blätter enthielten zum Zeitpunkt der Ernte im Vergleich zu entsprechenden unbehandelten Blättern größere Mengen reduzierender Zucker und hatten einen geringeren Gehalt an Stärke. Der Vergleich behandelter und unbehandelter Blätter am Ende des Trocknungsprozesses zeigte nur geringe Unterschiede hinsichtlich des Gesamtstickstoff- und Gesamtalkaloidgehaltes sowie hinsichtlich des Gehaltes an Stärke und reduzierenden Zuckern. Der Eiweißgehalt der Blätter, die mit CEPA behandelt worden waren, war im Vergleich zu unbehandelten Blättern statistisch signifikant niedriger.

Während aller Vergilbungsphasen lagen die Dollarerlöse je 100 Pfund trockenen Blattgutes behandelter Pflanzen höher als die des Blattgutes unbehandelter Pflanzen; das durchschnittliche Blattgewicht war jedoch bei behandelten Pflanzen niedriger. Während des Trocknungsvorganges kann bei Blättern, die mit CEPA behandelt wurden, wahrscheinlich eine stärkere Verkürzung der Vergilbungs- oder Verfärbungsphase bewirkt werden als bei unbehandelten Blättern. Sollten weitere Untersuchungen mit „reifefördernden“ Mitteln wie zum Beispiel CEPA zeigen, daß diese chemischen Verbindungen geeignet sind, sowohl das Vergilben oder Reifen des Tabaks zu beschleunigen als auch die Trocknungszeit des Erntegutes zu verkürzen, würden sich damit wahrscheinlich wirtschaftliche Vorteile ergeben. Es besteht die Hoffnung, daß chemische Verbindungen dieser Art dazu beitragen können, den Arbeitsaufwand und die Investitionen, die die Erzeugung der Ernte erfordert, zu vermindern und gleichzeitig die Qualität des Erntegutes zu verbessern.

RESUME

Le traitement de tabac flue-cured par un agent dégazeant de l'éthylène, l'acide 2-chloro-éthylphosphonique (CEPA), provoque chez les feuilles mûres un virement de couleur du vert au jaune. On a observé qu'avant la récolte les feuilles traitées subissent un «jaunissement» ou une «prise de couleur» partielle. A la récolte les feuilles traitées contiennent relativement plus de sucres réducteurs et moins d'amidon que les feuilles non traitées. A la fin du séchage des comparaisons entre feuilles traitées et non traitées ont démontré que, pris globalement, les taux de nitrogène, d'alcaloïdes, d'amidon

et de sucres réducteurs diffèrent très peu. Le taux de protéines des feuilles traitées au CEPA est inférieur à celui des feuilles non traitées, et cela de façon significative.

Pour l'ensemble des durées de jaunissement, la valeur en dollar par cent livres des feuilles séchées de plantes traitées était supérieure à celle des plantes non traitées, mais la moyenne de poids par feuille était inférieure chez les plantes traitées. Il est probable que pendant le séchage, on peut soumettre les feuilles traitées au CEPA à des durées de jaunissement et de prises de couleur inférieures aux feuilles non traitées. Des avantages économiques accrus pourraient être obtenus, si d'autres travaux, concernant des substances chimiques accélérant le mûrissement telles que le CEPA, démontrent que ces produits peuvent être employés avec succès, d'une part pour l'accélération du jaunissement ou le mûrissement du tabac, d'autre part pour la réduction du temps nécessaire pour le séchage de la récolte. On peut espérer que de telles substances chimiques puissent réduire le travail et l'investissement nécessaire à la récolte, et en même temps en augmenter la qualité.

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