# EFFECTS OF 2-CHLOROETHYLPHOSPHONIC ACID ON THE DEVELOPMENT AND MATURATION OF FLUE-CURED TOBACCO[1]

By R. C. LONG, J. A. WEYBREW, W. G. WOLTZ, and C. A. DUNN[2]

Field studies were conducted during a two-year period to investigate the effects of CEPA (2-chloroethylphosphonic acid) on flue-cured tobacco. A quick (3-4 days) yellowing response of physiologically responsive leaves (midway between maturity and ripeness) was noted. Initially unresponsive leaves were observed to yellow 10-14 days post-treatment only if the chemical had contacted the leaves directly. Low rates of CEPA stimulated starch accumulation in mature, green leaves; higher rates resulted in decreased starch concentration and some sugar accumulation. CEPA dosages above 60-90 mg/plant were not additionally beneficial with respect to whole-plant yellowing. Seasonal effects on the yellowing response were observed.

## INTRODUCTION

The accelerating trend toward mechanical harvesting of flue-cured tobacco has led to modification of cultural practices designed to increase the efficiency of harvesting operations. Concepts such as "low-profile tobacco", "once-over harvesting", and "ripening agents" were first put forward in the late 1960's. Recently researchers have begun to ponder the consequences of these practices.

Cutler and Gaines (1) reported the use of a ripening agent to accelerate the *in situ* yellowing of tobacco under greenhouse conditions. Field studies conducted at the Georgia Coastal Plain Experiment Station, Tifton, Georgia (5) revealed that, in general, the application of 2-chloroethylphosphonic acid (up to 150 mg/plant) to flue-cured tobacco after the second priming resulted in rapid yellowing of the remaining leaves allowing them to be harvested 3 to 4 days after application. The present paper reports the effects of 2-chloroethylphosphonic acid on the development and maturation of tobacco plants in North Carolina.

## MATERIALS AND METHODS

Plants of *Nicotiana tabacum* L. cv. Coker 254 were spaced either 14⅔ in. or 22 in. in rows 3 ft. 9 in. apart to provide populations of 9504 or 6336 plants/acre, respectively, in 1970 and 1971 on the Oxford Tobacco Research Station, Oxford, North Carolina. Each treatment consisted of at least four rows 40 ft. 3 in. long. In most cases, two replications were used. The plants of the 9504 population were topped at 12 leaves and the 6336 populations at 18 leaves, thus keeping the leaf population constant at 114,048 leaves/acre. Normal cultural practices were followed (2) and axillary suckers were removed by hand when necessary (4 to 6 in. long).

The ripening agent 2-chloroethylphosphonic acid (CEPA, Ethrel)[3] - formulation 68-240 - was sprayed onto the upper leaves of the plants as follows—

$CEPA_{1970}$: 6 mg/ml concentration in 0.01% Triton X-100, applied at the rate of 180 mg/12 leaf plant or 240 mg/18 leaf plant in 1970 at about the time of ripeness of the lower (first harvested) 2 or 3 leaves;

$CEPA_{1971}$: Variable concentrations applied in 20 ml 0.01% Triton X-100 at various stages of plant development in 1971.

Counting from the bottom, leaves were removed from node positions 3, 7, and 11 (9504 population) from each of 5 plants selected at random within a plot-row unless otherwise noted. These leaves were removed at intervals prior and subsequent to treatment. The leaf samples were analyzed chemically either fresh or after freeze-drying.

Nitrate reductase (NRase) activity and starch were determined by the procedures of Long and Woltz (3) and Rosa (6), respectively.

## RESULTS AND DISCUSSION

Visually, the most dramatic consequence of the treatment with CEPA is the disappearance of chlorophyll from responsive leaves. By the third day following treatment of 12-leaf plants with $CEPA_{1970}$, most of

[1]Paper number 4220 of the North Carolina State University Agricultural Experiment Station, Raleigh, N.C.

[2]Associate Professor and Professor, Department of Crop Science; Professor, Department of Soil Science; former Graduate Research Assistant in Crop Science, (now Assistant Professor, Texas A & M University, Yoakum, Texas), North Carolina State University, Raleigh, North Carolina, 27607.

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[3]The mention of a trade name does not constitute a guarantee or warranty of the product by the North Carolina State University and does not imply its approval to the exclusion of other products that may also be suitable.

the chlorophyll had been destroyed in the lower 5 or 6 leaves, i.e., the leaves were a bright yellow color. By the seventh day those yellowed leaves were moribund.

From our observations, it appears as if a leaf, to be responsive to CEPA, must have attained a particular physiological age which we estimate to be about midway between physiological maturity (maximum leaf dry weight) and ripeness. Evidence to support this conclusion was obtained as follows: a row of topped (18-leaf plants) and a row of not topped plants were treated with 360 mg CEPA over the entire plant in graduated dosages—60 mg to the bottom-third, 120 mg to the middle-third, and 180 mg to the top-third. Only the lower 6-8 leaves yellowed rapidly (in about four days time).

In contrast to the quick-yellowing effect, delayed-yellowing of the upper leaves was observed 10-14 days post-treatment with $CEPA_{1970}$. The delayed-yellowing was evident only in those upper leaves that had received the CEPA spray directly. We observed that the portion of an upper leaf that had been "shaded" by an adjacent leaf during CEPA application remained green. We postulated that delayed-yellowing from CEPA resulted from the interdiction of chlorophyll synthesis, whereas quick-yellowing was probably due to accelerated degradation via ethylene-stimulated chlorophyllase activity (7).

Further proof that the quick-yellowing effect is translocated downward to physiologically responsive leaves and that delayed-yellowing occurs only in those leaves contacted by the spray was developed by applying $CEPA_{1970}$ to 12-leaf plants when the 5th leaf was ripe. During the actual spraying with CEPA, leaves below the 9th node were protected with plastic-bag "rain coats". Five days post-treatment, the leaves below the 8th node had yellowed via the translocatable effect. By the 12th day post-treatment those lower leaves were dead and the remaining upper leaves, with the exception of the 8th leaf, were yellowed. Only the 8th leaf remained green; it apparently had been physiologically immature and did not respond to the translocated effect and it had been shielded from the spray solution (no delayed yellowing).

To test whether the CEPA effect could be translocated upward into physiologically responsive leaves, 120 mg $CEPA_{1970}$ per plant was applied to the lower-third, or middle-third, of 18-leaf plants. Response to the lower-third application was limited to about the 7th leaf and below. By contrast, all leaves below the 11th quick-yellowed from the middle-third application. Thus, the quick-yellowing effect was only translocated basipetally.

Starch concentration in green leaves was markedly affected by 180 mg/plant $CEPA_{1970}$ treatment **(Fig. 1)**. Three days after treatment, starch concentration in the fresh leaves was <50% that of untreated leaves. CEPA-treated samples contained nearly twice the reducing sugars of the control. However, the absolute increase in sugars accounted for <30% of the starch decrease. In the absence of definitive evidence, one may, therefore, postulate that the rapid loss of starch was due to accelerated respiration, decreased photosynthesis, translocation of starch-originated materials from the senescing leaf, or a combination. It has been reported that whereas differences in light-dependent $CO_2$ fixation and starch concentration in detached ethylene-treated and untreated leaves were not observed, reducing sugars and respiration were higher in the treated leaves (7). Hence, the acceleration of respiration as the major factor involved in the CEPA-induced depletion of starch appears the most attractive explanation. CEPA may also interdict NRase activity. Although the NRase activity in the untreated plants was low (0, 0.17, and 0.88 umols $NO_2$ hr/g. F.Wt. for leaves 3, 7, and 11 respectively), activity was not detected in the CEPA treated plants at four days post-treatment.

The application of CEPA to overfertilized, mature but green tobacco resulted in both starch accumulation and depletion at five days post-treatment **(Fig. 2)**. Although little yellowing occurred in the lower 5 or 6 leaves, a response gradient was noted as the CEPA rate increased to 60 mg/plant; higher rates did not result in more yellowing. Lower rates are thought to result in the accumulation of starch following cessation of nitrate reduction in the plant. Higher rates may have resulted in accelerated respiration which quickly over-

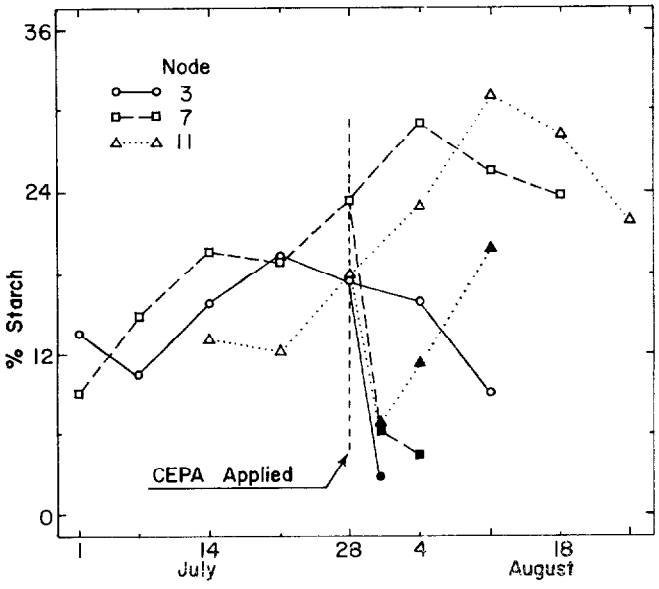


**Figure 1. Effect of CEPA on starch concentration. Closed symbols indicate CEPA treatments (Oxford, 1970).**

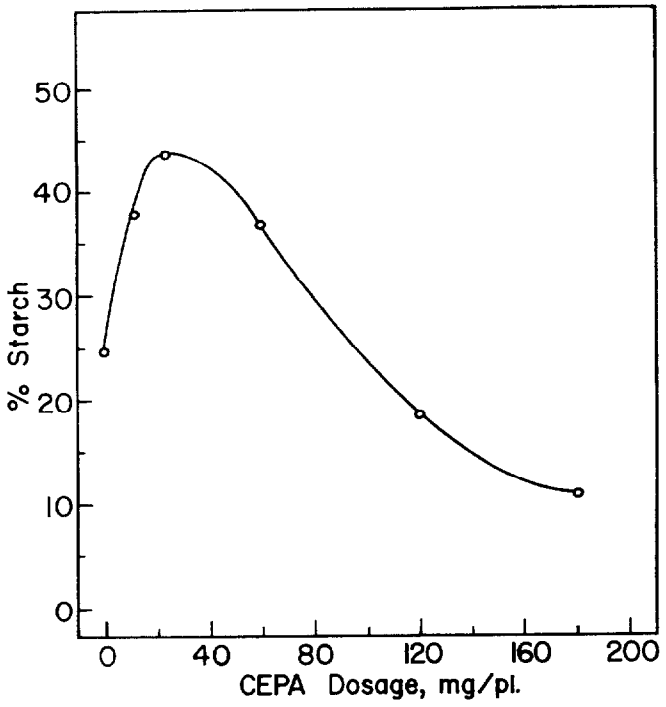


**Figure 2. The effect of CEPA dosage on the starch concentration of leaves from the 9th node position of mature, green tobacco (NC-2326) five days post-treatment (Oxford, 1970).**

came, in part, any effect of starch accumulation.

Ethylene is produced by the decomposition of CEPA in aqueous solution (4, 8) and its action in accelerating the yellowing of fruits and leaves is well known. It was assumed, therefore, that the CEPA effect resulted from, directly or indirectly, release of ethylene. To test this hypothesis, 240 mg $CEPA_{15\%}$ were applied to each of four plants which were immediately encased in large plastic bags supported with 4- 1" x 1" x 48" tobacco sticks. Each of four similar plants was exposed to 250 cc (312 mg) ethylene. Bagged plants, without either treatment, served as controls. The bags were removed after 20 hours to prevent leaf injury (scorch). The leaves on the lower half of the plants treated with either CEPA (equivalent to 47 mg ethylene) or ethylene were a bright yellow-color two days later; no visual difference between the two treatments were obvious. The higher starch concentration in the ethylene treated plants two days after treatment may have been the result of more rapid absorption, metabolic regulation, or unequal dosage effect **(Table 1)**.

**Table 1. The effect of CEPA and ETHYLENE on starch concentration 2 days post-treatment (Oxford, 1970).**

| Treatment | Node 16 | Node 10 | Node 4 |
|---|---|---|---|
| Check | 10.2 | 13.7 | 14.9 |
| Ethylene | 20.4 | 22.0 | 17.3 |
| CEPA | 13.9 | 14.3 | 16.4 |

In 1970, various concentrations of benzyladenine (BA) were applied to the lower third of a group of plants about 10 days before the first priming was ripe in an attempt to delay ripening of that portion of the plant. No visual difference in rate of ripening was evident between controls and BA-treated plants. However, the BA-treated leaves remained viable nearly a week longer than the control leaves when permitted to remain on the plant past ripeness. When CEPA treatment was super-imposed on plants one week after application of BA (100 mg plant) it required 90 mg plant CEPA to produce the same degree of yellowing of lower leaves as 60 mg plant CEPA applied alone. When kinetin was substituted for BA, no delayed ripening or observable metabolic effect was found.

Extreme shortening of internodes resulted from application of 60 mg $CEPA_{15\%}$ to plants six to ten weeks after transplanting in 1971; the plants displayed a distinct rosette appearance. Leaf yellowing was not observed. Ultimately, bolting occurred and flowering was delayed slightly. Subsequent CEPA application to these, and control plants, resulted in yellowing of the lower leaves which were physiologically responsive.

In general, no additional benefit of CEPA rates above 60-90 mg/plant was observed with respect to whole-plant yellowing during the two years. However, seasonal effects were quite evident. The application of CEPA to 12-leaf plants in 1970 when the lower three leaves were ripe resulted in quick yellowing of the next higher five or six leaves. In 1971, plants treated at the same stage of ripeness resulted in nearly all remaining leaves yellowing quickly. The application of 90 mg $CEPA_{15\%}$ to 18-leaf plants after the lower nine leaves had been primed normally resulted in rapid yellowing of the lower half of the remaining leaves. At no time was uniformity of leaf yellowing, or intensity of yellowing, comparable to results obtained at the Georgia Coastal Plain Experiment Station (personal observation by J. A. W. and W. G. W.) in 1971. Loss of chlorophyll in the upper leaves resulted in distinct yellowing in 1970, whereas bronzing of the upper leaves was observed in 1971. No explanation is put forward to explain these results.

Our experience demonstrates that CEPA pre-yellowed leaves fail to cure fast enough in conventional barns to prevent some darkening of color in the cured leaf. Raising the barn temperature immediately after wilting, e.g., decreasing the yellowing period, generally resulted in green areas throughout the leaf. This problem was particularly prominent along the midribs.


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## REFERENCES


1. Cutler, H. G., and T. Powell Gaines. Some preliminary observations on greenhouse-grown tobacco treated with 2-chloroethylphosphonic acid at varying pH's. **Tob Sci.** 15:100-102. 1971.
2. Hawks, S. N., Jr. **Principles of flue-cured tobacco production.** S. N. Hawks, Jr., Raleigh, N.C. 1970. 239 p.
3. Long, R. C. and W. G. Woltz. Depletion of nitrate reductase activity in response to soil leaching. **Agron. J.** 64:789-792. 1972.
4. Maynard, J. A., and J. M. Swan. Organophosphorus compounds. I. 2-chloroethylphosphonic acid as phosphorylating agents. **Australian J. Chem.** 16:596-608. 1963.
5. Miles, J. D., G. L. Steffens, T. P. Gaines, and M. G. Stephenson. Flue-cured tobacco yellowed with an ethylene releasing agent prior to harvest. **Tob. Sci.** 16:71-74. 1972.
6. Rosa, Nestor. An automated method for analyzing starch in green and mature leaf lamina. **Tob. Sci.** 15:58-61. 1971.
7. Sisler, E. C., and A. Pian. Effect of ethylene and cyclic olefins on tobacco leaves. **Tob. Sci.** 17:68-72. 1973.
8. Yang, S. F. Ethylene evolution from 2-chloroethylphosphonic acid. **Plant Physiol.** 44:1203-1204. 1969.