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EFFECTS OF NEAR-ULTRAVIOLET RADIATION AND TEMPERATURE ON SOLUBLE PHENOLS IN *NICOTIANA TABACUM**

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Abstract—Low levels of near-ultraviolet radiation (300–400 nm) along with visible radiation during the daily illumination periods resulted in tobacco (*Nicotiana tabacum* L.) plants with about twice the concentrations of total soluble phenolics and chlorogenic acid isomers present in leaves of tobacco that did not receive near-u v radiation. Plants grown at 24° had about 150 per cent of the total soluble phenolic concentrations contained in plants that received the same quality of light, but were maintained at 32°.

INTRODUCTION

SUNLIGHT at the earth's surface includes radiation down to about 300 nm. Plants in their natural habitats are, therefore, exposed to low intensity near-u.v. radiation (300–400 nm), which varies with latitude, altitude, time of day, season and atmospheric conditions.[1] Plants that receive sunlight through glass or plastic receive less u.v. because of the filtering characteristics of these materials.

Several investigators have reported differences in concentrations of soluble phenolics in tobacco grown under greenhouse and field conditions.[2–4] Levels of phenolic compounds and an associated enzyme, polyphenoloxidase, were higher in field-grown than in greenhouse-grown tobacco plants.[2] These results may have been associated with differences in the spectral composition of light, differences in temperature, or differences in both light and temperature received by plants grown in the field and in the greenhouse.

The objective of the research reported herein was to test, under controlled-environments, the effects of low levels of near-u.v. radiation and of temperature upon the soluble phenol content of tobacco leaves.

RESULTS AND DISCUSSION

Preliminary results showed that contents of total soluble phenolic compounds and chlorogenic acid isomers did not differ significantly among the two stalk positions or three sampling dates. Therefore, the results presented are averaged over stalk positions and sampling dates.

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[1] J LOCKHART and U. BRODFUHRER-FRANZGROTE, in *Encyclopedia of Plant Physiology XVI* (edited by W RUHLAND), pp 532–54, Springer-Verlag, Berlin (1961).
[2] R A. ANDERSEN, R LOWE and T. H VAUGHN, *Phytochem* **8**, 2139 (1969)
[3] T P. FERGUSON and A S WEAVING, *Nature* **183**, 64 (1959)
[4] H. V. LOTT, *Planta* **55**, 480 (1960).

Both the 300–320 nm and the 320–400 nm regions of the spectrum appeared to increase accumulations of total soluble phenolics and chlorogenic acid isomers in tobacco leaves (Table 1). However, the 300–320 nm wavelength band was relatively more effective than the remainder of the near-u.v. region. Leaves from plants that received wavelengths down to 300 nm contained 172 and 193 per cent of the levels of total soluble phenolics present in those grown under the 320 and 400 nm cut-off filters, respectively. Similarly, the amounts of the chlorogenic acid isomers in plants that received some radiation down to 300 nm were 144 per cent those in plants grown under the 320 nm cut-off filter and 195 per cent those in plants grown under the 400 nm cut-off filter. The total soluble phenols measured include all phenolic compounds which hydrogen bond to polyvinylpyrrolidone at pH 3·5 (e.g. chlorogenic acid isomers, scopoletin, monohydroxyphenols and soluble tannins); chlorogenic acid isomers are usually regarded as the principal soluble phenols of *Nicotiana tabacum*.[5]

TABLE 1. EFFECTS OF NEAR-U.V. RADIATION ON TOTAL SOLUBLE PHENOL AND CHLOROGENIC ACID ISOMER CONTENTS OF TOBACCO LEAF LAMINA GROWN AT 24°

	Soluble phenols (% of dry wt)*		
	300 nm cut-off (no filter)	320 nm cut-off (glass filter)	400 nm cut-off (Mylar filter)
Total soluble phenols	2 37 ± 0 36	1 38 ± 0 27	1·23 ± 0 29
Chlorogenic acids	1 05 ± 0 15	0 73 ± 0 14	0·54 ± 0 14

* Data are means (± standard deviations) for 12 observations (i e. duplicate determinations for two stalk positions from each of three sampling dates)

In the second experiment, we tested the combined effects of temperature and near-u.v. radiation on the accumulation of soluble phenolic compounds in tobacco (Table 2). Within each light quality group, higher levels of total soluble phenolic compounds were present in leaves that developed at 24° than in leaves that developed at 32°. Leaves from plants grown at 24° had 131 and 154 per cent [in the 300 nm (unfiltered) and the 400 nm cut-off groups, respectively] of the amounts of total soluble phenolics present in leaves grown at 32°.

TABLE 2 EFFECTS OF NEAR-U.V RADIATION AND TEMPERATURE ON TOTAL SOLUBLE PHENOL AND CHLOROGENIC ACID ISOMER CONTENTS OF TOBACCO LEAF LAMINA GROWN AT 24° AND 32°

		Soluble phenolics (% of dry wt)*	
	Temperature, (°)	300 nm cut-off (no filter)	400 nm cut-off (Mylar filter)
Total soluble phenols	24	1 61 ± 0 22	1 05 ± 0 07
	32	1 23 ± 0 24	0 68 ± 0 21
Chlorogenic acids	24	0 83 ± 0 33	0 64 ± 0 11
	32	0 71 ± 0 13	0 56 ± 0 09

* Data are means (± standard deviations) for 12 observations (i e duplicate determinations for two stalk positions from each of three sampling dates).

[5] R. A ANDERSEN, J. F. CHAPLIN, R. E. CURRIN and Z. T. FORD, *Agronomy J.* **62**, 415 (1970)

The presence of low levels of near-u.v. during the daily light periods resulted in higher levels of total soluble phenolics in leaves from plants grown at either 24° or 32° when compared with the contents in leaves from plants that did not receive near-u.v. At 24° and 32°, leaves that received near-u.v. radiation contained 153 and 180 per cent, respectively, of the total soluble phenolics present in leaves grown without u.v. The sums of chlorogenic acid isomers tended to follow a similar pattern.

Low levels of near-u.v light, and temperature variations within a normal biological range influenced the accumulation of total soluble phenolics and chlorogenic acid isomers in tobacco leaves (Tables 1 and 2). The amount of reduction in total soluble phenolic levels in leaves from u.v.-filtered 'FC 402' tobacco reported in this paper (Table 1) was about the same as the reduction previously noted in 'FC 402' greenhouse-grown tobacco as compared with 'FC 402' field-grown tobacco.[2] This comparability of results is consistent, because the proportions of u v. to visible radiation in sunlight and in radiation from cool-white fluorescent lamps are very similar.[1]

EXPERIMENTAL

Plant Materials and Growth Conditions

Seedlings of 2 high-phenol varieties of tobacco (*Nicotiana tabacum* L. cv. 'FC-402' and 'Ky 151')[6] were started in expanded peat pellets at 28° under 14 hr, 16,000 lx photoperiods from cool-white fluorescent lamps. Seedlings were subirrigated, as needed, with half-strength Hoagland's nutrient solution No. 1[7] during the starting, conditioning and treatment periods. Six-week-old seedlings were selected for uniformity and transplanted to a soil–perlite (2:1, v/v) mixture in 2-l pots, and transferred to controlled-environment chambers for conditioning and treatment All chambers were equipped with VHO cool-white fluorescent lamps. These lamps emit about 3 per cent of their radiation in the 320–400 nm region, less than 1 per cent in the 300–320 nm region and no energy below about 300 nm.[8] Thus, the shortest wavelengths emitted by these lamps were similar to those received from sunlight.[1] Light intensities were about 20,000 lx in each chamber during daily 14-hr photoperiods. Conditions and materials used in individual experiments were as follows:

First experiment. Plants of 'FC 402' were used. The growth room was divided with black curtains into three subchambers, each maintained at 24° Plants in one subchamber received unfiltered radiation from the VHO cool-white fluorescent lamps (i.e. wavelengths of light longer than 300 nm). Another subchamber received radiation filtered through greenhouse glass which removed wavelengths shorter than 320 nm. The third subchamber received radiation filtered through a Mylar barrier that filtered out wavelengths shorter than 400 nm. Transmission characteristics of the glass and Mylar were determined against an air blank in a Beckman DB recording spectrophotometer. The filters were placed 30 cm below the lamps and 150 cm above the plants to avoid heat build-up around the plants. The curtains were arranged to allow adequate air circulation.

Second experiment. 'Ky 151' plants were used in two growth rooms, one maintained at 24° and the second at 32°. Each room was divided with a black curtain into two subchambers. One subchamber received unfiltered radiation from the lamps and the other received radiation filtered through a Mylar barrier. As in the first experiment, the filters were placed 30 cm below the lamps and 150 cm above the plants.

Both experiments Plants of both experiments were conditioned to their respective controlled environments for 2 weeks, after which 15 uniformly sized plants were retained in each subchamber. All leaves longer than 8 cm were removed and discarded to insure that leaves collected later for analysis had developed under the various light and temperature programs

Sampling and Sample Preparation

At each of the three sampling dates (i e. 10, 20 and 30 days after the conditioning period) three leaves were collected from the upper and three from the middle stalk positions from each of five plants per subchamber. Samples were pooled by stalk position within each treatment, frozen with dry ice, and freeze-dried.[9] Midveins were removed. The dried samples were pulverized and stored in evacuated desiccators prior to chemical analysis.

[6] S J. Sheen and J. Calvert, *Tobacco Sci* 13, 10 (1969).
[7] D. R. Hoagland and D. I Arnon, *California Agri.* 347 (1950).
[8] Sylvania Electric Products Inc , Danvers, Massachusetts, Engineering Bulletin 0.283.
[9] C. J. Keller and M. J. Kasperbauer, *J. Agri. Food Chem.* 17, 327 (1969).

Analyses

Total phenols were determined by the method of Andersen and Todd,[10] while total chlorogenic acid isomers were determined by a paper chromatographic-spectrophotometric method as described by Sheen and Calvert [6] All analyses were run in duplicate Since phenolic contents did not differ with stalk position or sampling date, data presented in this report are the means (± standard deviation) for 12 determinations within each light and temperature combination (i e. duplicate determinations for two stalk positions at each of three sampling dates)

Note Mention of trade names anywhere in this paper is as part of the exact experimental conditions and not as an endorsement by the U.S. Department of Agriculture.

[10] R A. ANDERSEN and J. R. TODD, *Tobacco Sci.* **12,** 107 (1968)