

Chemical Composition of Tobacco Leaves Altered by Near-Ultraviolet and Intensity of Visible Light¹

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ABSTRACT

Low energies of near-ultraviolet radiation (300–400 nanometers), applied simultaneously with visible radiation to *Nicotiana tabacum* L. during daily illumination periods, increased levels of chlorogenic acid isomers, total soluble phenolics, alkaloids, and soluble sugars in expanding leaf lamina compared with controls that had near-ultraviolet filtered out. However, total nitrogen concentrations decreased. The responses to near-ultraviolet were interrelated with intensity of visible light. The presence of near-ultraviolet (which accounted for less than 4% of the total light energy) along with visible light resulted in component concentration differences similar to those caused by much greater increases of visible light without near-ultraviolet.

Plants in their natural habitats are exposed to relatively low intensities of near-ultraviolet radiation from sunlight, which includes wavelengths down to about 300 nm (20). The amount of this radiation varies with latitude, altitude, time of day, season, and atmospheric conditions.

Lower concentrations of soluble phenolics were found in lamina from tobacco (*Nicotiana tabacum* L.) plants grown in a greenhouse as compared with those in the field (11). Greenhouse plants received sunlight through glass, which filters out part of the near-UV radiation (20). In addition, the activity of polyphenoloxidase, an enzyme for which several plant phenolics serve as substrates, was lower in greenhouse-grown than in field-grown tobacco leaves (2). Low energies of near-UV (300–400 nm), along with one intensity of visible radiation, resulted in about twice the concentrations of total soluble phenolics and chlorogenic acid isomers in tobacco leaves compared with those that did not receive near-UV radiation (1).

The objectives of the present research were to test effects of near-UV on contents of several soluble chemical constituents of tobacco lamina important to tobacco quality and to ascertain whether effects of near-UV are independent of visible light intensity.

MATERIALS AND METHODS

Plant Materials. Tobacco (*Nicotiana tabacum* L.) seedlings were started and grown for about 8 weeks in expanded peat pellets at 28 C under 14-hr photoperiods from cool-white fluo-

rescent lamps. The visible light intensity was 1600 ft-c. All seedlings were subirrigated, as needed, with half-strength Hoagland's nutrient solution No. 1 (16) during the starting, conditioning, and treatment periods. Eight-week-old seedlings were transplanted to a field plot, or to soil in 2-liter pots and transferred to controlled environment chambers for conditioning and treatment.

Growth Conditions. Field-grown plants were 45 cm apart in east-west rows that were 100 cm apart at the South Farm of the Kentucky Agricultural Experiment Station at Lexington. Lower leaves from plants in the south end row received direct sunlight during most of the day, whereas those from midplot plants were shaded most of the time.

The controlled environment chambers were equipped with VHO cool-white fluorescent lamps. These lamps emit less than 4 % of their radiant energy in the 300 to 400 nm region and none below 300 nm (24). Plants in chamber I received wavelengths down to about 300 nm. Chamber II had a Mylar² barrier between the lamps and the plant growth compartment; thus, these plants did not receive wavelengths shorter than about 400 nm (see Fig. 1). The differences in near-UV energies in the 2400 ft-c growth areas of chambers I and II were determined with a model 585-11 EG and G spectroradiometer fitted with an ultraviolet S-5 filter. Plants in both chambers received 14-hr photoperiods at 25 C. White cheesecloth and position in the chamber were used to obtain three light intensities (1200, 1800, and 2400 ft-c) within each chamber.

Treatments and Sampling. When the plants in the field reached the floral bud stage, leaves were collected from 10 plants grown in end rows and from 10 others grown within the plots. Leaves were harvested from two stalk positions, designated as upper or lower. Leaves from the upper stalk position were less than fully expanded. Those from the lower position were fully expanded but not senescent. Lamina samples were pooled by stalk position within each plant location, frozen in Dry Ice, lyophilized, pulverized, and stored as described below for samples grown in chambers.

Ten plants were grown under each of three light intensities in each of the two chambers. After the plants had been conditioned to their controlled environments for 10 days, all leaves longer than 5 cm were removed and discarded. The plants were returned to their respective areas for another 10 days before first sampling. Thus, all leaves collected for analysis were developed under the indicated environments. At each of two sampling dates (10 and 20 days after the conditioning period), all leaves longer than 15 cm were collected from each of the 10

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² Trade names are given as part of the exact experimental conditions and do not constitute an endorsement of products by the United States Department of Agriculture or the Kentucky Agricultural Experiment Station.

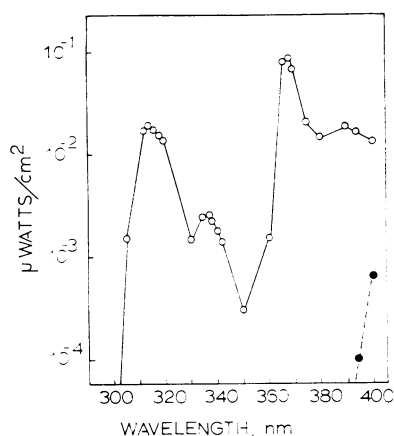


FIG. 1. Spectral distribution of near-ultraviolet radiant energy in areas receiving 2400 ft-c from VHO cool-white fluorescent lamps. ○: Values in nonfiltered chamber I; ●: those transmitted through the Mylar filter in chamber II.

plants from each of the six treatments. Samples were pooled by treatment, immediately frozen in Dry Ice, and lyophilized to prevent phenolic degradation during storage (17). Midveins were removed. The dried samples were pulverized and stored in the dark in desiccators *in vacuo* prior to chemical analysis. The entire experiment was repeated; thus, data presented in this report are means of two samplings from each of two experiments.

Analyses. Total soluble plant phenols were estimated by spectrophotometric measurement of the reduction of Folin's phenol reagent after the removal of interferences by polyvinylpyrrolidone (3). Total chlorogenic acid isomers were determined by a gas chromatographic method for caffeic acid moieties (5). Phenylalanine ammonia-lyase activity was measured by a gas-liquid chromatographic method (27). Chlorophyll was determined spectrophotometrically by a minor modification of the method of Comar (9), using absorption coefficients determined with chlorophyll standards obtained from the Sigma Chemical Co., St. Louis, Mo. Total nitrogen was determined by a Kjeldahl-volumetric method (6). Total sugars were determined with Technicon AutoAnalyzer spectrophotometric systems (8, 12). In one method (12) an extract of tobacco was treated with lead acetate to precipitate interfering phenolic compounds, hydrolyzed, and then allowed to react with ferricyanide to determine the glucose-reducing equivalent. The second method used for total and individual sugars involved the summation or determinations of individual sugars in a sample,

i.e., sucrose, glucose, fructose, maltose, and rhamnose. Extracts of tobacco were used to form sugar-borate complexes, they were chromatographed, and the sugar-orceinol color of eluted bands was measured (8). Total alkaloids (expressed as nicotine) were determined by the Griffith procedure (13).

RESULTS

FIELD STUDY

Results obtained with field-grown tobacco leaves suggested that shading and leaf maturity affected sugars, alkaloids, phenolics, and phenylalanine ammonia-lyase, one of the rate-limiting enzymes of plant phenolic biosynthesis (Table I). These findings and previously reported effects of temperature (1) and stalk position (4) on levels of phenolic compounds in tobacco leaves indicated the need for further experiments on the influence of near-UV applied simultaneously with different intensities of visible light.

CONTROLLED ENVIRONMENT STUDY

Substances Significantly Altered by Near-UV and Visible Light Intensity. Increased or decreased levels of chemical constituents associated with the light treatments were found for chlorogenic acid isomers, total soluble phenolics, total nitrogen, total alkaloids, and total soluble sugars (Table II).

Leaves from plants that received near-UV had higher levels of chlorogenic acid isomers at 2400 ft-c compared with those at 1200 ft-c. However, visible light intensity did not affect levels of these compounds in the absence of near-UV. The

Table I. *Effects of Leaf Maturity and Shading on Leaf Components in Field-grown Tobacco*

Values for all components are means of two or more replications expressed on a dry weight basis.

Plant and Stalk Position	Total Phenols	Total Sugars	Total Alkaloids	Phenylalanine Ammonia-Lyase Activity
				units/mg
	mg/g			
End row				
Upper (young) leaves	40.0	72.1	5.6	1.7
Lower (expanded) leaves	37.2	56.0	15.2	0.3
Mid-plot row				
Upper (young) leaves	47.8	74.5	8.4	1.4
Lower (expanded) leaves	27.6	60.5	17.4	0.1

Table II. *Effects of Near-Ultraviolet (300–400 nm) and Intensity of Visible Light on Components of Tobacco Lamina Grown at 25 C*

Values are expressed in mg/g of dry leaf lamina. They are means of four replications. Those in the same horizontal line (component) followed by the same letter do not differ significantly at $P < 0.10$.

Leaf Component	Daily 14-hr Light Periods					
	1200 ft-c		1800 ft-c		2400 ft-c	
	–Near UV	+Near UV	–Near UV	+Near UV	–Near UV	+Near UV
Total phenols	6.6 a	6.3 a	5.2 a	11.2 c	6.6 a	13.1 c
Total sugars	26.5 a	30.6 b	28.6 ab	33.9 c	29.5 b	35.6 c
Total nitrogen	57.1 a	56.0 ab	57.7 a	52.8 bc	54.7 abc	51.6 c
Chlorogenic acid isomers	5.0 a	7.1 ab	5.6 a	9.0 bc	7.4 ab	10.4 c
Total alkaloids (as nicotine)	8.1 a	8.9 a	10.7 b	12.1 c	8.8 a	11.6 c

presence of near-UV radiation resulted in more chlorogenic acids at the 1800 and 2400 ft-c intensities, respectively, compared with near-UV-filtered controls, but there was no difference at 1200 ft-c.

Total soluble phenol responses to visible and near-UV light were similar to those found for chlorogenic acids. When near-UV treatments were compared with UV-filtered controls, there were about 100 % increases at 1800 and 2400 ft-c, and no response at 1200 ft-c. Further, leaves had about 100 % more total phenols at 1800 than at 1200 ft-c when near-UV was present.

Total nitrogen was inversely proportional to increased intensity of visible light in leaves from plants that received near-UV. There was, however, no effect on total nitrogen for each of the three intensities of visible light that had near-UV filtered out. The effect for plants that received near-UV paralleled that for chlorogenic acid isomers and total phenols, except for the opposite direction of response of total nitrogen.

The presence of near-UV increased total alkaloids at 1800 and 2400, but not at 1200 ft-c compared with the respective controls. These effects paralleled those for chlorogenic acids and total phenols.

Total sugar concentrations were higher at each increased intensity increment of visible light when near-UV was present. Also, amounts of sugars were higher in leaves of plants grown without near-UV at 2400 ft-c than at 1200 ft-c. Sucrose and glucose accounted for 82 % of the total sugars averaged over all light treatments.

Substances Not Significantly Changed by Near-UV and Intensity of Visible Light. Chlorophyll, individual soluble sugars, and the activities of phenylalanine ammonia-lyase were not significantly ($P < 0.05$) affected by light intensity changes with or without near-UV, although differences in the means of some of these components indicated probable significance at $P > 0.10$. The quantities or activity of these constituents (on a dry weight basis, \pm standard deviations) in lamina averaged over all light treatments were: total chlorophyll, 28.3 ± 0.3 mg/g; sucrose, 15.9 ± 1.2 mg/g; glucose, 7.9 ± 3.2 mg/g; fructose, 3.6 ± 2.9 mg/g; maltose, 1.6 ± 0.5 mg/g; rhamnose, 0.9 ± 0.3 mg/g; and phenylalanine ammonia-lyase, 0.61 ± 0.02 unit/mg.

DISCUSSION

Our experiments showed effects of low levels of near-UV on certain aspects of tobacco leaf chemistry. The changes in phenolics, alkaloids, and sugars are of potential interest in near-UV energy regulation of plant growth and, obviously, to quality of tobacco. The effect of near-UV on certain phenolic constituents is not confined to tobacco. Near-UV radiation stimulated flavone glycoside synthesis in parsley suspension cultures (28) and anthocyanin formation in apple skin (7) and *Haplopopus gracillis* callus tissue (14). There is little information, however, concerning its influence on other constituents of higher plants, although physical effects were noted in potato plants (22). Our present experiments show that the intensity of visible light must be controlled to differentiate between its effects and those of near-UV. Further, the results point out potential problems when investigators attempt to compare data from plants grown in chambers that do not have barriers with data obtained from plants grown in chambers which have barriers that transmit most of the visible light but filter out near-UV.

Soluble organic non-nitrogenous compounds, such as total phenolics (*i.e.*, chlorogenic acid isomers, scopoletin, rutin, monohydroxyphenolic compounds, and soluble tannins) and

total sugars, had similar responses to near-UV light and intensity of visible light. Because the shikimic acid biosynthetic pathway between phenolic (phenylpropane) derivatives and carbohydrates is responsible for much of the synthesis and interconversion among these constituents in higher plants (21), the light parameters we used may have regulated enzyme systems in this pathway. L-Phenylalanine ammonia-lyase catalyzes the conversion of L-phenylalanine to *trans*-cinnamic acid, an important precursor of soluble plant phenolic compounds in higher plants (18), including tobacco (26). L-Phenylalanine ammonia-lyase activity in pea seedlings responds to white, blue, and far red light (23). Although we did not detect significant differences in L-phenylalanine ammonia-lyase activity in leaves from tobacco grown under the conditions of our chamber experiments, this may have been caused by such factors as time of sampling and the relatively low activity of PAL in tobacco leaves of the size we harvested.

The lower amounts of total nitrogen, along with higher total alkaloids caused by the presence of near-UV, needs clarification beyond the scope of this report. It was apparent, however, that biosynthesis of alkaloids (heterocyclic N-containing compounds) was stimulated by near-UV and higher intensities of visible light.

The increased amounts of phenolic precursors of lignin biosynthesis in the presence of near-UV light, and higher intensities of visible light (Table II) may affect secondary cell wall development and xylem differentiation (25).

The function of near-UV during the daily light period may be an extension of the effects attributed to the closely related wavelength range of blue light. Examples of the influence of prolonged irradiation with blue light and possible photoreceptor mechanisms are discussed elsewhere (10, 15, 19).

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