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THE EFFECT OF NITROGEN DEFICIENCY ON THE CONCENTRATION OF CAFFEOYLQUINIC ACIDS AND SCOPOLIN IN TOBACCO

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Abstract—Roots, stems, and leaves of tobacco plants grown on complete and on nitrogen-deficient nutrient solutions were each analyzed quantitatively for content of scopolin and of chlorogenic, neochlorogenic, and 4-*O*-caffeoylquinic acids. Increases in scopolin and chlorogenic acid concentrations were found in the nitrogen-deficient leaves, stems, and roots as compared to corresponding parts of control plants. These increases correlated approximately with the time of first observable deficiency symptoms.

INTRODUCTION

ONLY a few quantitative studies have been reported concerning effects in tobacco of mineral deficiencies on the concentration of scopolin (7-glucoside of scopoletin) and chlorogenic acid (3-*O*-caffeoylquinic acid, abbreviated CGA). We were unable to find literature describing such deficiency conditions which also included quantitative analyses of neochlorogenic acid (5-*O*-caffeoylquinic acid, neoCGA) and 4-*O*-caffeoylquinic acid (4-CQA, sometimes called "Band 510"). Watanabe and coworkers[1,2] reported increases in scopolin in boron-deficient tobacco and sunflower leaves, Fowler[3] showed that increased chloride in tobacco leaves of the same physiological age was associated with increased scopoletin (6-methoxy-7-hydroxycoumarin). He also reported that other factors affecting the nutrient balance, such as low potash content, also appeared to favor the accumulation of scopoletin. Chouteau and Loche[4,5] found increases in scopolin and decreases in chlorogenic acid in tobacco leaves deficient in magnesium, calcium, or phosphorus. They found a decreased concentration of CGA in potassium-deficient tobacco leaves, but an increased concentration of CGA in nitrogen-deficient tobacco leaves. Tso *et al.*[6] reported increases in CGA and scopolin in the leaves of three varieties of tobacco with increases in nitrogen fertilization. For a fourth tobacco variety in their studies, however, the concentrations of scopolin and CGA were found to be related inversely to the levels of nitrogen fertilization.

The study reported here was undertaken to determine quantitatively—not only in leaves, but also in roots and stems of nitrogen-deficient as well as control tobacco plants—the changes

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occurring in the concentration of scopolin and the several caffeoylquinic acids over a period of 5 weeks.

RESULTS AND DISCUSSION

The concentrations of scopolin, CGA, neoCGA, and 4-CQA obtained by quantitative analysis of leaves from control and nitrogen-deficient tobacco plants are shown in Table 1. Results of similar analyses of stems and roots of the same plants are shown in Table 2. In control leaves and roots, concentrations of the caffeoylquinic acids and scopolin generally increased with age through the 5-week treatment period, whereas the concentrations of these compounds in stems decreased during the 5 weeks under the conditions of these experiments.

It can readily be seen from Table 1 that in leaves from nitrogen-deficient tobacco, pronounced accumulation of CGA had occurred. By the end of the first week, the total concen-

TABLE 1. CONCENTRATIONS OF CAFFEOYLQUINIC ACIDS AND SCOPOLIN IN NITROGEN-DEFICIENT AND CONTROL TOBACCO LEAVES

Age of plant and treatment	μg/g fr. wt.				
	CGA	4-CQA	neoCGA	Total of these three caffeoylquinic acids	Scopolin
1-Week					
control	456	280	219	955	5·4
deficient	1668	353	157	2178	8·0
3-Week					
control	645	371	194	1210	6·0
deficient	3742	544	236	4522	10·0
5-Week					
control	653	367	305	1325	7·2
deficient	5629	572	209	6410	13·6

CGA—Chlorogenic acid; 4-CQA—4-*O*-Caffeoylquinic acid; neoCGA—Neochlorogenic acid; light intensity used was 100 lx.

tration of the caffeoylquinic acids studied was more than twice that of the controls. After 5 weeks, this total concentration in the nitrogen-deficient leaves had increased to almost five times that in the control. These increases resulted mainly from increases in CGA concentration. Concentrations of neoCGA were actually lower in the leaf analyses for weeks 1 and 5 than in controls. Expressed as a per cent of the total of the caffeoylquinic acids studied, CGA comprised in control leaves approximately 50 per cent, but in the treated leaves, after 5 weeks, CGA comprised almost 88 per cent of these acids.

Concentration of CGA decreased with age in stems of control tobacco plants, but in the stems from deficient plants, CGA concentration first decreased, and then went back up again with age and time after treatment. The CGA concentration in deficient stems was approximately five times that of control stems after 5 weeks on treatment. Roots from deficient plants were found to have greater concentrations of CGA than controls throughout the treatment period.

If any neoCGA or 4-CQA were present in the analyzed samples of roots or stems, they

were there in concentrations below those capable of being quantitated by the procedure used. None was detected in the roots or stems.

As shown in Table 1, scopolin concentrations were greater in the leaves of nitrogen-deficient tobacco plants than in those of the controls. These increases approximated the time of appearance of deficiency symptoms, with increases evident after 1 week on nitrogen deficiency. Increases were also found in the roots of the deficient plants, with the increases especially apparent after 1 week. In roots from nitrogen-deficient plants, the level reached by

TABLE 2. CONCENTRATIONS OF CHLOROGENIC ACID AND SCOPOLIN IN NITROGEN-DEFICIENT AND CONTROL TOBACCO STEMS AND ROOTS

Age of plant and treatment	μg/g fr. wt.	
	CGA	Scopolin
Stems		
1-Week		
control	449	22·9
deficient	1034	55·2
3-Week		
control	317	18·4
deficient	693	46·6
5-Week		
control	192	14·8
deficient	932	70·8
Roots		
1-Week		
control	251	69·6
deficient	489	116·4
3-Week		
control	417	133·0
deficient	597	162·8
5-Week		
control	479	140·6
deficient	923	289·9

Concentrations present, if any, of neochlorogenic and 4-*O*-caffeoylquinic acids in roots or stems were below those capable of being quantitated by the procedure used.
Light intensity used was 100 lx.

week 5 was about twice that of controls. In nitrogen-deficient stems, scopolin generally increased with age, reaching a level by the fifth week of about five times that of controls.

Numerous different inhibitory and/or injurious conditions may produce an accumulation of scopolin, as well as of scopoletin, in tobacco. These include subjecting the tobacco to growth-inhibiting amounts of u.v. irradiation,[7] 2,4-dichlorophenoxyacetic acid (2,4-D),[8]

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maleic hydrazide,[9] chilling temperatures,[10] and growing the tobacco with deficiency of boron.[2] Accumulation of scopolin and/or scopoletin has also been reported by other workers after infection of the tobacco plant with certain bacteria, fungi, or viruses.[11,12] Thus the increases in scopolin found in the present study are consistent with these previous reports concerning scopolin and "stress" conditions.

On the basis of both external structural characteristics and phenolic concentration, it would appear that the nitrogen-deficiency symptoms found in the present study are essentially similar to those recently reported for tobacco exposed to chilling temperature.[10] Mineral deficiencies are not always due to low mineral levels in the medium or the soil, but often are related to the ability of the plant to take up available minerals. Low temperature is a factor that can limit mineral uptake.[13] Usual suggested roles for chlorogenic acid and for scopolin, involving possible lignification and/or indoleacetic acid oxidase functions in control tobacco and in tobacco subjected to chilling temperatures, have been previously discussed by Koeppe *et al.*[10]

Allelopathic effects of certain plant species which are important in old-field succession may be accentuated due to increases in concentrations of scopolin and chlorogenic acid resulting from very low levels of nitrogen in such areas. Rice and his colleagues have found that chlorogenic acid[14] and scopolin[15] have significant phytotoxic effects.

EXPERIMENTAL

Tobacco plants (*Nicotiana tabacum*, cv. One-Sucker) were grown in pure quartz sand in Percival growth chambers on a daily cycle with a 16-hr light period at 28·8° and an 8-hr dark period at 16·6°. Plants were watered with Fe-EDTA double strength Hoagland's nutrient solution[16] until treatment began. Approximately 70 days after germination, plants were selected for uniformity, and the pots were leached thoroughly with distilled water. Control plants were watered thereafter with the double strength nutrient solution, whereas the treated plants were made deficient for nitrogen by watering with similar nutrient solutions made deficient for these minerals by a procedure similar to that outlined by Machlis and Torrey.[17] Soil jars were leached every 7 days with 3000 ml of distilled water. Illumination in this study was 100 lx. Deficient plants were raised on blocks so that apices of deficient and control plants were kept at the same level.

A control plant and treated plant were harvested at the end of 1, 3, and 5 weeks. Leaves at various developmental stages were harvested from each plant, with sampling kept uniform. The top 1 cm of the plant was removed before the stem was harvested, while the entire root system was harvested. These separate harvests were ground and extracted by the procedure used by Wilson *et al.*[18]

Quantitative analysis of scopolin and the three caffeoylquinic acids was performed by the method described by Koeppe *et al.*[7]

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