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Use of Growth Regulators to Control Senescence of Wheat at Different Temperatures during Grain Development

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Wheat (*Triticum aestivum* L.) was grown under greenhouse and field conditions to study efficacy of growth regulators in mitigating senescence and increasing grain yields. L-Serine, 6-benzyladenine, cycloheximide, and terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) were applied on plants grown under 21 °C/16 °C, 26 °C/21 °C, and 31 °C/26 °C day/night regimes during grain development and under field conditions. Low concentrations of growth regulators had little effect on senescence processes, whereas high concentrations accentuated high temperature induced senescence. Benzyladenine and terbacil increased protease activity and decreased leaf area duration, protein concentration, kernel size, and yields. None of the growth regulators increased yields under field conditions; serine had no effect, and rates above 5 g ha^{-1} of benzyladenine, 50 g ha^{-1} of cycloheximide, and 125 g ha^{-1} of terbacil decreased yields. We concluded that plant growth regulators probably affect some senescence processes differently under induced and natural conditions and that use of the materials tested to control senescence under field conditions may not be feasible.

INTRODUCTION

The positive relationship between photosynthetic leaf area during grain filling and wheat (*Triticum aestivum* L.) yield is well documented (Fischer and Kohn, 1966; Spiertz et al., 1971). It suggests that plant photosynthetic activity during grain filling limits yields and that photosynthesis might be increased by delaying the rapid senescence that occurs after anthesis (Fischer and Kohn, 1966). Senescence is characterized by de novo synthesis of protease enzymes, degradation of photosynthetic processes, loss of leaf viability, and eventual cessation of grain development (Thomas and Stoddart, 1980).

Environmental stresses, particularly drought and high temperatures, accelerate most leaf senescence processes (Thomas and Stoddart, 1980). In wheat, for instance, high temperatures after anthesis accentuate the normal increase in protease enzyme activity and the decrease in ribulose-1,5-biphosphate carboxylase and Hill reaction activities (Al-Khatib and Paulsen, 1984). Leaf area and grain filling durations are also shortened, causing grain yields to decline markedly.

Cytokinins, cycloheximide, and other growth regulators delay induced senescence of many plant species (Martin and Thimann, 1972a, 1972b; Peterson and Huffaker, 1975; Ries, 1976). In detached leaves and seedlings placed in the dark, these chemicals inhibit de novo protease synthesis (Martin and Thimann, 1972a), retard chlorophyll loss (Peterson and Huffaker, 1975), increase nitrate reductase activity (Ries, 1976), maintain synthesis of fraction I protein (Butler and Simon, 1971), and preserve membrane function (Beutelmann and Kende, 1977). L-Serine, on the other hand, specifically enhances leaf senescence in darkness, possibly via its incorporation into the active site of protease enzyme (Martin and Thimann, 1972a, 1972b; Shibaoka and Thimann, 1970).

The importance of senescence to grain yield of wheat and the known ability of specific growth regulators to delay induced senescence prompted investigations of efficacy of the chemicals on intact plants. Experiments were conducted under controlled conditions with three temperature

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regimes and with field plots at two locations to ascertain the feasibility of regulating senescence to increase wheat productivity.

EXPERIMENTAL SECTION

Greenhouse Studies. Vernalized hard red winter wheat (cultivar "Triumph 64") seedlings from a foundation seed production field were transplanted into 3 kg of soil in 20-cm diameter containers. Each container held six seedlings. The soil, Tully silt clay loam, was fertilized with 35 ppm N as ammonium nitrate and 50 ppm P as triple superphosphate.

Plants were grown under greenhouse conditions at 21 °C day/16 °C ± 3 °C night temperatures until ten days after first anther extrusion. Foliar treatments of four chemicals at two rates each with 5 g kg^{-1} of Tween 20 then were applied to saturate the foliage prior to temperature treatments. Chemicals used were 6-benzyladenine and cycloheximide at 0.14 and 1.4 mM, L-serine at 31.5 and 315 mM, and terbacil at 0.75 and 7.5 mM. Control plants received no treatment. The containers were divided randomly in three groups and the plants were grown to maturity under temperature regimes of 21 °C/16 °C, 26 °C/21 °C, and 31 °C/26 °C day/night. A 3 × 4 × 3 factorial experiment with the three temperatures, four chemicals, and three chemical concentrations was arranged in a completely randomized block design with three replications.

Assay Procedures. Plants were sampled for leaf area duration and metabolic activity 0, 7, 14, and 21 days after initiation of the temperature treatments and for grain yield at maturity. Maturity—considered to be when the grain was hard and contained ca. 140 g kg^{-1} moisture—varied among temperature treatments, occurring 45, 42, and 35 days after initiation of treatments of 21 °C/16 °C, 26 °C/21 °C, and 31 °C/26 °C, respectively.

Leaf area duration measurements used three plants from each treatment at each sampling date. Viable leaf area (estimated visually on basis of color) was measured with a photoelectric meter. Leaf area duration (LAD) was calculated according to formula of Hunt (1978).

Leaf blades of three plants from each replication of each treatment were cut into 1-cm sections each date. In vivo nitrate reductase activity was measured by the method of Heuer and Plaut (1978), nonspecific protease activity was measured with azocasein substrate at 50 °C (Al-Khatib and Paulsen, 1984), total chlorophyll was determined by the method of Arnon (1949), and soluble protein was assayed by a modified Lowry method (Miller, 1959).

Six plants were harvested for grain yield at maturity. Samples were dried at 70 °C for 72 h and grain dry weight was recorded. Grain nitrogen content was determined by micro-Kjeldahl (AOAC, 1950).

Field Studies. Field experiments were conducted at Manhattan and Hesston, KS. The Tully silt clay loam at Manhattan had pH 6.8 and contained 47 kg ha^{-1} of available N ($NH_4^+ + NO_3^-$), 62 kg ha^{-1} of available P, 370 kg ha^{-1} of exchangeable K, and 15 g kg^{-1} of organic matter. The Ladysmith clay loam at Hesston had pH 5.2 and contained 50 kg ha^{-1} of available N, 25 kg ha^{-1} of available P, 300 kg ha^{-1} of exchangeable K, and 10 g kg^{-1} of organic matter. Phosphorus (0–20–0) fertilizer as triple superphosphate was applied at 35 kg ha^{-1} before planting and ammonium nitrate was top dressed at 45 kg of N ha^{-1} during early spring. "Triumph 64" wheat was seeded at 85 kg ha^{-1} during Oct at both locations. The experimental design was a split plot with three replications. Chemicals were main plots and chemical rates were subplots.

Chemicals were selected for their purported effects on senescence processes. 6-Benzyladenine, cycloheximide, L-serine, and terbacil were applied in total volume of 300 L ha^{-1} by using a knapsack sprayer when wheat was approximately ten days past first another extrusion. Rates used were 1, 5, 25, and 50 g ha^{-1} of 6-benzyladenine and cycloheximide, 100, 500, 2500, and 5000 g ha^{-1} of L-serine, and 5, 25, 125, and 250 g ha^{-1} of terbacil. Tween 20 at 5 g kg^{-1} was used as a surfactant. Control plots received no treatment. Individual plots were 1.5 × 10 m with six rows spaced 25 cm apart.

Plants from two 0.25-m long rows were cut at ground level from the center of each plot at Manhattan at weekly intervals for 5 weeks from approximately 10 days after first anther extrusion until grain maturity. Total leaf area of each sample was determined by a photoelectric meter and leaf area duration was calculated by a formula given by Hunt (1978). A 0.25-m^2 area was harvested from each subplot at maturity for measuring yield components (kernels per spike and 1000-kernel weight).

A 10.8-m^2 area in each subplot was harvested by plot combine at maturity at Manhattan and Hesston. Grain moisture content was determined with an electronic moisture meter and test weight was measured on a 0.473-L sample. Grain protein concentration was determined by infrared reflectance. All values were expressed on the basis of 140 g kg^{-1} of grain moisture.

Source of Chemicals. Benzyladenine, cycloheximide, and serine were from Sigma Chemical Co., St. Louis, MO. Technical grade terbacil (80% a.i.) wettable powder was a gift from E. I. duPont de Nemours and Co., Wilmington, DE.

Statistical Analysis. Statistical analysis of data was by standard analysis of variance procedures. Least significant differences (LSD's) among means were tested at the $P = 0.05$ level of probability.

Safety Considerations. Cycloheximide, a plant growth regulator (Windholz, 1983), is highly toxic and teratogenic (Snow, 1982). Terbacil also exhibits mammalian toxicity (Windholz, 1983). For safety, protective clothing and respirators were worn when applying all chemicals. Excess chemicals and plant materials left after chemical analysis were disposed by burial.

RESULTS

Distinctive phytotoxicity symptoms on wheat were observed from different chemicals in both greenhouse and field experiments (data not shown). High concentrations of benzyladenine caused black necrotic lesions on floral parts, particularly glumes, and produced shriveled grain. Cycloheximide caused similar but less detrimental effects. Terbacil caused chlorosis of leaves and awns, which later became white and desiccated; loss of color and senescence of stems, however, was delayed 3 to 4 days compared with control plants. Terbacil treatment also induced small, dark, hard kernels, some of which were shriveled. No phytotoxicity symptoms except early senescence at high concentrations were observed after L-serine treatment.

Effects of applying L-serine, benzyladenine, cycloheximide, and terbacil on mean chlorophyll content and leaf area duration over sampling dates under three temperature regimes are shown in Table I. Both chlorophyll content and leaf area duration of untreated control plants decreased as the temperature increased. Low concentrations of all chemicals had little effect on the mean total chlorophyll content and leaf area duration. High concentrations of all chemicals, on the other hand, significantly decreased mean chlorophyll content and leaf area duration under all temperature treatments. The greatest reduction in mean chlorophyll content was with benzyladenine at 21

Table I. Mean Total Chlorophyll and Leaf Area Duration of "Triumph 64" Wheat Leaves as Affected by Nine Chemical Treatments, Three Temperature Regimes, and Three Sampling Dates under Controlled Environmental Conditions

chemical	concn, mM	total chlorophyll at C, mg g fw^{-1}			leaf area duration at °C, dm^2 days		
		21/16	26/21	31/26	21/16	26/21	31/26
check		4.6	4.1	3.7	48.1	31.2	22.1
serine	31.50	4.6	4.2	3.6	45.1	28.6	19.5
serine	315.00	3.9	3.6	3.2	42.9	22.1	15.0
benzyladenine	0.14	4.7	4.1	3.7	46.8	28.3	20.8
benzyladenine	1.40	3.6	3.2	3.0	36.4	17.0	14.1
cycloheximide	0.14	4.5	4.2	3.5	44.2	31.2	22.7
cycloheximide	1.40	3.8	3.2	3.0	39.0	26.0	18.2
terbacil	0.75	4.5	4.2	3.6	44.2	30.0	22.1
terbacil	7.50	3.8	3.5	2.8	35.1	23.4	14.3
LSD (0.05) between checks		0.1	0.1	0.1	2.6	2.6	2.6
LSD (0.05) between checks and chemicals		0.2	0.2	0.2	3.7	3.7	3.7
LSD (0.05) between chemicals		0.2	0.2	0.2	5.2	5.2	5.2

Table II. Mean Nitrate Reductase Activity, Soluble Protein Concentration, and Protease Activity of "Triumph 64" Wheat as Affected by Nine Chemical Treatments, Three Temperature Regimes, and Three Sampling Dates under Controlled Environmental Conditions

chemical	concn, mM	nitrate reductase activity at °C, μmole NO_2^- gfw^{-1} h^{-1}			soluble protein at °C, mg gfw^{-1}			protease activity at °C, Δ A unit gfw^{-1} h^{-1}		
		21/16	26/21	31/26	21/16	26/21	31/26	21/16	26/21	31/26
check		5.8	4.3	3.3	6.1	4.6	4.0	4.1	4.3	4.4
serine	31.50	4.2	4.0	3.0	5.9	4.4	4.0	4.0	4.2	4.4
serine	315.00	4.7	4.1	2.5	5.2	3.9	3.0	4.7	4.8	5.2
benzyladenine	0.14	5.7	3.4	3.7	5.7	4.5	3.8	4.1	4.3	4.5
benzyladenine	1.40	5.3	3.4	3.3	5.0	3.7	2.8	4.8	5.4	5.6
cycloheximide	0.14	6.1	4.7	3.7	5.8	4.4	3.8	4.2	4.3	4.6
cycloheximide	1.40	3.7	2.4	1.4	5.1	3.4	2.6	4.4	4.8	4.9
terbacil	0.75	6.2	3.9	3.1	6.1	4.8	3.3	4.2	4.2	4.5
terbacil	7.50	6.8	3.8	2.9	5.4	3.3	2.4	5.1	5.4	5.4
LSD (0.05) between checks		0.7	0.7	0.7	0.2	0.2	0.2	0.1	0.1	0.1
LSD (0.05) between checks and chemical		0.9	0.9	0.9	0.3	0.3	0.3	0.1	0.1	0.1
LSD (0.05) between chemicals		1.1	1.1	1.1	0.4	0.4	0.4	0.2	0.2	0.2

Table III. Mean Grain Yield, Grain Weight, and Grain Nitrogen Concentration as Affected by Chemical Treatments and Three Temperature Regimes under Controlled Environmental Conditions

chemical	concn, mM	grain yield at °C, g $plant^{-1}$			grain weight at °C, g 1000^{-1}			grain nitrogen at °C, g kg^{-1}		
		21/16	26/21	31/26	21/16	26/21	31/26	21/16	26/21	31/26
check		2.6	1.9	1.5	30.0	21.5	17.0	26.1	30.5	32.9
serine	31.50	2.7	1.7	1.4	30.0	19.7	16.0	26.8	31.6	32.3
serine	315.00	2.3	1.5	1.1	26.6	17.4	12.4	29.7	33.4	35.1
benzyladenine	0.14	2.7	1.7	1.5	30.1	19.1	17.1	27.7	31.2	32.7
benzyladenine	1.40	2.0	1.2	1.1	23.1	13.6	16.0	28.5	33.2	36.8
cycloheximide	0.14	2.4	1.8	1.6	27.3	20.3	17.6	26.1	31.6	33.8
cycloheximide	1.40	2.2	1.4	1.3	24.5	16.1	15.0	28.6	33.6	36.7
terbacil	0.75	2.4	1.8	1.6	27.2	20.3	17.5	25.8	29.2	33.5
terbacil	7.50	2.1	1.3	1.0	23.4	14.8	11.4	28.4	33.1	36.1
LSD (0.05) between checks		0.2	0.2	0.2	0.2	0.2	0.2	1.1	1.1	1.1
LSD (0.05) between checks and chemicals		0.3	0.3	0.3	0.3	0.3	0.3	1.6	1.6	1.6
LSD (0.05) between chemicals		0.3	0.3	0.3	0.4	0.4	0.4	2.1	2.1	2.1

°C/16 °C and 26 °C/21 °C and terbacil at 31 °C/26 °C and the highest reduction in leaf area duration was with terbacil at 21 °C/16 °C and benzyladenine at 26 °C/21 °C and 31 °C/26 °C. Leaf area duration was more sensitive than chlorophyll content to high chemical concentrations under the 31 °C/26 °C regime.

High temperature treatments significantly decreased leaf nitrate reductase activity and soluble protein content and increased protease activity (Table II). Low chemical concentrations had little effect on any constituents; high chemical concentrations, however, increased proteolytic activity, particularly in the case of terbacil and benzyladenine. Increased mean protease activity coincided with decreased mean soluble protein concentration. Mean nitrate reductase activity also was affected by high concentration of cycloheximide.

Grain yield and grain size were decreased and grain nitrogen concentration was increased by high temperatures after anthesis (Table III). Low concentrations of all chemicals had little effect, but high concentrations significantly reduced grain yield and grain size. The greatest reduction was with benzyladenine and terbacil under all temperature regimes. Grain nitrogen concentration was increased by high concentration of all treatments. However, no significant differences were measured between low and high concentrations of benzyladenine at 21 °C/16 °C and serine at 26 °C/21 °C.

Mean grain yields, grain weights, grain protein concentrations, test weights, harvest indexes, and leaf area durations of field-grown "Triumph 64" winter wheat are summarized in Table IV. None of the chemical treatments increased grain yields. Serine had no effect, and

Table IV. Mean Grain Yield, Grain Weight, Test Weight, Grain Protein Concentration, Harvest Index, and Leaf Area Duration of "Triumph 64" Wheat as Affected by Four Chemicals at Five Rates at Manhattan and Hesston, KS

chemical	rate, g ha^{-1}	yield, kg ha^{-1}	grain weight, g 1000^{-1}	test weight, kg hl^{-1}	protein, g kg^{-1}	harvest index	leaf area duration, dm^2 days
serine	0	3615	35.4	75.1	106	0.38	75.5
	100	3594	35.0	75.1	112	0.40	74.9
	500	3492	34.1	75.1	112	0.37	72.9
	2500	3435	33.5	74.9	115	0.37	71.5
	5000	3375	32.5	74.5	117	0.38	69.6
benzyladenine	0	3621	34.6	75.2	106	0.40	74.4
	1	3278	32.9	73.9	116	0.40	67.4
	5	3040	29.8	72.9	116	0.38	66.5
	25	2710	27.3	69.2	122	0.37	60.9
	50	2310	23.0	67.8	126	0.33	48.5
cycloheximide	0	3553	34.5	75.3	110	0.40	74.0
	1	3568	34.9	74.8	112	0.40	69.1
	5	3446	33.5	74.8	113	0.41	66.0
	25	3353	31.6	74.1	117	0.41	65.1
	50	2926	27.2	73.0	119	0.38	48.9
terbacil	0	3543	34.7	75.0	106	0.40	74.2
	5	3399	33.5	74.0	109	0.40	70.1
	25	3208	31.4	73.9	114	0.41	67.0
	125	2954	29.9	72.9	116	0.39	65.7
	250	2436	24.3	66.8	125	0.33	49.9
LSD (0.05) for different chemicals at same rate		376	0.1	0.4	2	0.03	4.5
LSD (0.05) for same chemical at different rates		488	0.1	0.4	2	0.03	4.7

rates above 5 g ha^{-1} of benzyladenine, 50 g ha^{-1} of cycloheximide, and 125 g ha^{-1} of terbacil decreased grain yields. All chemical treatments except 1 g ha^{-1} of cycloheximide decreased 1000-grain weight and, except for lower rates of serine, also decreased grain test weight. Grain protein concentration, on the other hand, was increased by the four chemicals. Mean harvest index only was affected by high concentration of benzyladenine and terbacil. Mean leaf area duration, however, was decreased by 5000 g ha^{-1} of serine, by all rates of benzyladenine and cycloheximide, and by 25 g ha^{-1} and higher of terbacil.

DISCUSSION

Delaying senescence would be expected to increase grain yield of wheat because of its positive relationship with leaf area duration (Fischer and Kohn, 1966; Spiertz et al., 1971). Benefits should be particularly pronounced under stress conditions, such as high temperatures, that accelerate senescence processes (Al-Khatib and Paulsen, 1984; Thomas and Stoddart, 1980). Purported regulation of senescence by plant growth substances (Martin and Thimann, 1972a, 1972b; Shibaoka and Thimann, 1970) and known effects of several compounds on induced senescence (Martin and Thimann, 1972a, 1972b; Peterson and Huffaker, 1975; Ries, 1976; Shibaoka and Thimann, 1970) suggest the use of chemicals to prolong leaf viability and increase grain yields. Our results, however, show that chemical treatments that alter senescence processes under induced conditions have different, even contrary effects, under natural conditions.

Benzyladenine and cycloheximide inhibit protease synthesis to delay senescence induced by detaching or darkening of leaves (Martin and Thimann, 1972b). Reasons for increased loss of leaf area and reduced grain yields of treated whole plants under high temperatures or in the field are not clear. Senescence is slower and responds less to exogenous cytokinin in attached leaves than in detached leaves (Hall et al., 1978; Muller and Leopold, 1966). Growth substances from other plant parts might influence differences between intact plants and detached leaves (Kende, 1965). Effects of light on cytokinin (Mishra and Pradham, 1973; Singh and Mishra, 1965) also differ between natural senescence and senescence induced by darkness.

The multiplicity of processes during reproductive ontogeny—metabolism of vegetative constituents, translocation, and reconstitution during grain development—undoubtedly are more complex than those during induced senescence. Inhibition of synthesis of non-protease proteins by benzyladenine and cycloheximide during natural senescence, for instance, might outweigh any beneficial effect of the chemicals on protease synthesis. During induced senescence, as in darkened seedlings, on the other hand, decreased protease synthesis could well be more important than any adverse effects of the chemicals on non-protease proteins.

Serine is implicated in senescence by promotion of protease activity by exogenous serine and by high levels of endogenous serine during induced dark senescence (Malik, 1982). Protease activity might be promoted by incorporation of serine into the active site of serine protease (Martin and Thimann, 1972a). Exogenous serine probably was less effective on intact maturing plants during natural or high-temperature senescence than on plants in which senescence is induced for several reasons. The appreciable quantities of serine that are always present in leaves (Malik, 1982) might dilute the effect of exogenous serine. Continued supplies of serine also can come from mitochondria (Wallsgrove et al., 1980) for synthesis of serine protease in the cytoplasm (Thimann, 1978).

Our results and those of others (Cuello and Sabater, 1982; Dybing and Lay, 1981; Hall et al., 1978; Wittenbach, 1977) indicate possible fundamental differences between natural senescence and senescence induced by darkening or detaching leaves. Reasons for these differences are unclear, but they might come from the greater complexity of processes and the more numerous interactions among constituents during natural senescence than during induced senescence. Consequently, compounds that affect induced senescence may have limited potential for controlling natural senescence of wheat.

Registry No. Protease, 9001-92-7; L-serine, 56-45-1; 6-benzyladenine, 1214-39-7; cycloheximide, 66-81-9; terbacil, 5902-51-2.

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Isolation and Characterization of the Sucrose Esters of the Cuticular Waxes of Green Tobacco Leaf

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The cuticular waxes of a tobacco budworm resistant tobacco, TI-165, contain a series of polar, high molecular weight compounds, which were separated from other components by solvent partitioning and Sephadex LH-20 gel chromatography. Glass capillary gas chromatography (GC-2) of these constituents as trimethylsilyl ethers and GC-2/mass spectrometry indicated that there were six groupings of isomers differing in mass, each from the next by 14 amu. Saponification of the total mixture of compounds yielded sucrose and a series of C_2- to C_8-aliphatic acids. The major acids were acetic, 2-methylbutyric, and 3-methylvaleric acids. Repeated gel chromatography resulted in the isolation of 6-*O*-acetyl 2,3,4-tri-*O*-[3-methylvaleryl]-α-D-glucopyranosyl-β-D-fructofuranoside, the major isomer, as defined by NMR and MS data. Other sucrose esters with similar molecular weights were isolated by preparative gas chromatography and saponified, and their acid compositions were determined. Partial hydrolysis of the SE yielded known tetraacylglucopyranosides.

INTRODUCTION

During our investigation of the cuticular leaf waxes of green tobacco from budworm-resistant and budworm-susceptible genotypes, several tobacco introductions (TI) were found to produce a series of polar, high molecular weight (MW) components. The observed field resistance of these tobaccos was postulated to be due to an antibiosis factor (Johnson and Severson, 1984). These high MW components were first detected when the glass capillary gas chromatographic (GC-2) profile of the cuticular waxes from the green leaf of a budworm-susceptible, flue-cured tobacco, NC 2326, was compared to that of the waxes from a resistant tobacco, TI-165 (Figure 1). Major components common to both tobaccos were the diterpenes, α- and β-4,8,13-duvatriene-1,3-diols (diols), docosanol ($C_{22}OH$), and the C_{25}–C_{36} aliphatic hydrocarbons. The high MW components were very apparent in the GC-2 chromatogram of the TI-165 cuticular waxes. A crude isolate of the high MW components from TI-165 was obtained by column chromatography on basic alumina (Severson et al., 1981). NMR analysis of the isolate indicated the presence of a sucrose ring system with the glucose moiety fully esterified and with an acetate group on the C_6 position, while the fructose portion showed four free hydroxy groups (Figure 2). Alkaline hydrolysis of the isolate confirmed the presence of sucrose. GC-2 analysis of the hydrolyzate, after acidification, confirmed the presence of C_2–C_8 acids, with the major acids being acetic, 3-methylbutyric, and 3-methylvaleric acids. We postulated that these green leaf sucrose esters (SE) were the precursors of the 6-*O*-acetyl triacylglucopyranosides (glucose esters, GE), previously isolated from a hexane-soluble fraction of cured Turkish tobaccos (Schumacher, 1970; Rivers, 1981). In addition, we have found SE in the cuticular extracts of the green leaf of Turkish cultivars (Severson et al., 1984). These SE and GE are believed to be the precursors of the important Turkish tobacco smoke flavor components, 3-methylbutyric and 3-methylvaleric acids (Kallianos, 1976; Chu-

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