sponsible for sample extractions. R. W. Biro performed resin component quantitations.

Registry No. Guayulin A, 31685-97-9; guayulin B, 31685-98-0; argentatin A, 31324-30-8.

LITERATURE CITED

Arreguin, B.; Bonner, J.; Wood, B. J. *Arch. Biochem. Biophys.* **1951**, *31*, 234.
Banigan, T. F.; Verbiscar, A. J.; Weber, C. W. *J. Agric. Food Chem.* **1982**, *30*, 427.
Banthorpe, D. V.; Charlwood, B. V.; Francis, M. J. O. *Chem. Rev.* **1972**, *72*, 115.
Belmares, H.; Jimenez, L. L.; Ortega, M. *Ind. Eng. Chem. Prod. Res. Dev.* **1980**, *19*, 107.
Benedict, H. M.; McRary, W. L.; Slattery, M. C. *Bot. Gaz. (Chicago)* **1947**, *108*, 535.
Bhowmick, A. K.; Rampalli, S.; McIntyre, D. *J. Appl. Polym. Sci.* **1985**, *30*, 2367.
Bonner, J. *Bot. Gaz. (Chicago)* **1943**, *105*, 233.
Bonner, J. *Bot. Gaz. (Chicago)* **1946**, *107*, 343.
Bonner, J.; Galston, A. W. *Bot. Gaz. (Chicago)* **1944**, *106*, 185.
Bonner, J.; Galston, A. W. *Bot. Rev.* **1947**, *13*, 543.
Buchanan, R. A.; Otey, F. H.; Russell, C. R.; Cull, I. M. *J. Am. Oil Chem. Soc.* **1978**, *55*, 657.
Budiman, S.; McIntyre, D. *Rubber Chem. Technol.* **1981**, *54*, 871.
Cedarleaf, J. D.; Welch, B. L.; Brotherson, J. D. *J. Range Manage.* **1983**, *36*, 492.
Curtis, O. F., Jr. *Plant Physiol.* **1947**, *22*, 333.
Eagle, F. A. *Rubber Chem. Technol.* **1981**, *54*, 662.
Engler, C. R.; McIntyre, D. Department of Commerce Report EDA/RED-84-43, Feb 1984; pp 7–12.
Garrot, D. J., Jr.; Johnson, D. L.; Rubis, D. D.; Dill, G. M. *Anal. Chem.* **1981**, *53*, 543.
Hammond, B. L.; Polhamus, L. G. "Research on Guayule (*Parthenium argentatum*): 1942–1959"; U.S. Dept. of Agriculture: Washington, DC, 1965; pp 11–114, 130–131.
Keller, R. W.; Winkler, D. S.; Stephens, H. L. *Rubber Chem. Technol.* **1981**, *54*, 115.
Kruempelman, M.; Danielson, N. D. *J. Liq. Chromatogr.* **1982**, *5*, 1679.
Lloyd, F. E. "Guayule, a Rubber-Plant of the Chihuahuan Desert"; Carnegie Institute: Washington, DC, 1911; pp 183–188.
McFadden, K.; Nelson, S. H. Department of Energy Report DOE/ER/30006-T1, Sept 1981; pp 78–79.
Mears, J. A.; Larson, R. A. *J. Arid Environ.* **1982**, *5*, 169.
Meeks, J. W.; Banigan, T. F., Jr.; Planck, R. M. *India Rubber World* **1950**, *122*, 301.
Nivert, J. J.; Glymph, E. M.; Snyder, C. E. In "Guayule: Reencuerto en el Desierto"; CONACYT (National Council for Science and Technology): Mexico, D.F., 1978; pp 357–374.
Prokof'ev, A. A. *Izv. Akad. Nauk SSSR, Ser. Biol.* **1939**, 908.
Proksch, P.; Behl, H. M.; Rodriguez, E. *J. Chromatogr.* **1981**, *213*, 345.
Rodriguez, E.; Reynolds, G. W.; Thompson, J. A. *Science (Washington, D.C.)* **1981**, *211*, 1444.
Schloman, W. W. Jr.; Hively, R. A.; Krishen, A.; Andrews, A. M. *J. Agric. Food Chem.* **1983**, *31*, 873.
Traub, H. P.; Slattery, M. C. *Bot. Gaz. (Chicago)* **1946**, *108*, 295.
Taylor, K. W. Emergency Rubber Project, Department of Agriculture Forest Service Report 2189, 1946; pp 43–44.
Tsaregorodtseva, S. O. *Biol. Nauki (Moscow)* **1975**, *18*, 89.
Vostrowsky, O.; Michaelis, K.; Ihm, H.; Zintl, R.; Knobloch, K. *Z. Naturforsch., C: Biosci.* **1981**, *36C*, 724.
Weihe, D. L.; Nivert, J. J. In Proceedings of the Third International Guayule Conference, Pasadena, CA, 1980; Gregg, E. C., Tipton, J. L., Huang, H. T., Eds.; Guayule Rubber Society: Riverside, CA, 1983; pp 115–125.

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Effect of the Pollutant Ozone in Ambient Air on Lima Beans

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Eight lima bean genotypes with different phenotype seed characteristics were grown in two ozone atmospheres, i.e., glass houses with nonfiltered (NF) air and charcoal-filtered (CF) air. Tolerances to ozone (O_3) and seed yield were determined for each genotype. Chemical analyses were conducted on the seeds for Kjeldahl nitrogen, 16 amino acids, ammonia, four sugars, and starch. The four genotypes that were most resistant to O_3 had green cotyledons. The cotyledon color may be important for the identification of lima bean genotype resistance to O_3. Seed yield did not correlate with visible leaf tissue damage. Kjeldahl nitrogen and amino acid levels were higher in seeds grown in the low-O_3 atmosphere. Seeds of plants grown in NF air contained higher carbohydrate and starch content than those from plants grown in CF air. Cotyledon color or seed coat color were not related to changes in Kjeldahl nitrogen, amino acids, carbohydrate, or starch associated with the O_3 treatments.

INTRODUCTION

Ozone (O_3) gas is produced by photochemical reactions involving sunlight, the nitrogen oxides, and hydrocarbons from fuel combustion such as auto exhausts. In ambient air, O_3 concentrations are varied with elevated concentrations produced over long distances downwind of industrial complexes or major population centers. Light winds may carry O_3 and the precursor chemicals over extensive rural areas of the U.S. resulting in damage to sensitive plants. Five-year average (1978–1982) O_3 values for the 48 contiguous states are available (Adams et al., 1984). Ozone is considered in the U.S. to cause more plant damage than any other air pollutant (Heggestad and Heck, 1971). Mixtures of O_3 with other gaseous pollutants are also a concern since they may have additive or more than additive effects (Lefohn and Tingey, 1984).

Ozone, as a phytotoxic gas, can produce leaf damage, premature senescence, leaf drop, reduced growth, and lower seed yield in sensitive plant species (Lee et al., 1981). Injury to foliar plant structures occur when sensitive cultivars of some species are exposed for 2–4 h with concentrations of 0.05–0.12 ppm O_3 (Reinert et al., 1982).

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Studies to determine the effect of O_3 on 1209 lines of tomatoes from a worldwide collection (Clayberg, 1971) and on over 2000 plant introductions of *Phaseolus vulgaris* (Reinert et al., 1984) showed that most lines were susceptible to varying amounts of O_3 damage. In the tomatoes tested only four lines were found to be O_3 resistant, while in beans only 54 plant introductions were O_3 resistant. Flower and seed color in *P. vulgaris* did not appear to be related to O_3 sensitivity (Reinert et al., 1984). These two investigations emphasize the genetic variation of plants in their resistance and susceptibility to O_3. Geographical areas with relatively high O_3 levels such as Beltsville, MD, have allowed the development by plant breeders of cultivars that are more tolerant to O_3 than possible in areas of low O_3. Crops known to have high O_3 tolerances as a result of such plant breeding activity are alfalfa (Howell et al., 1971), potatoes (Heggestad, 1973), dry bean (Heggestad et al., 1976), cotton (Heggestad et al., 1977a), sugar beets (Menser, 1974), and barley and smartweed (Bennett et al., 1974). One snap bean cultivar, Gallatin 50, produced 15% more beans growing in nonfiltered (NF) air (high O_3) than when grown in charcoal-filtered (CF) air (Heggestad et al., 1977b). It is desirable that plant breeders know the tolerance or susceptibility of the genetic material used in producing new breeding lines and new cultivars.

An investigation by Dugger et al. (1962) showed that both a high level of carbohydrate (above 4 mg/g of fresh weight) and a low level (1–0.1 mg/g of fresh weight) prevented O_3 damage in pinto bean leaves while carbohydrate concentration in the midrange (2–4 mg/g of fresh weight) increased O_3 damage to the leaves. An additional study on leaves of rough lemon seedlings determined that carbohydrate decreased, reducing sugars increased, starch decreased, organic acids fraction increased, and amino acids fraction increased with O_3 treatments (Dugger and Palmer, 1969). These studies indicate that O_3 also affects other metabolic processes besides carbohydrate metabolism.

The above studies indicate the need for additional investigations on the effect of O_3 on horticultural crops. Therefore, a study was designed to determine the relative susceptibility of specific lima bean genotypes (*Phaseolus lunatus*) to O_3; relationship of cotyledon and seed coat color to O_3; and the effect of O_3 on the concentration of total nitrogen, amino acids, ammonia, carbohydrates and starch.

MATERIALS AND METHODS

Chemicals and Reagents. All chemicals and reagents were reagent grade. Solvents used for HPLC were of HPLC grade. Enzyme preparations were diluted from the commercial preparation 1 to 5 v/v.

Plant Material. Eight lima bean genotypes, Henderson Bush, Dixie Butter Pea, Fordhook 242, Jackson Wonder, Green Cotyledon Dixie Butter Pea (F_7 generation selection Dixie Butter Pea × Kingston), Green Cotyledon Jackson Wonder (F_7 generation selection Jackson Wonder × Bridgeton), Bridgeton, and 79 Mildew Resistant Fordhook, were grown in glass houses in individual 25.4-cm pots containing friable, moist potting soil during the summer of 1982 at Beltsville, MD. Individual seed were planted in each pot on April 20. Five plants made up a replication, and the experiment was replicated three times. Lima bean seeds were harvested for the first time on June 28. Seeds were harvested daily as they reached market maturity until July 15. After this date, the plant material was allowed to flower for a second time and a second seed harvest was obtained. Seed yields from the second harvest were small; therefore, seeds from each of the three replications were pooled so that adequate samples were available for chemical analysis. The harvested seeds were frozen, freeze-dried, and shipped to Athens, GA. On arriving at the laboratory, the seeds were placed in a −34 °C room for 24 h and redried on a freeze dryer (Vacudyne Pilot Freeze Dryer, Model 4PFD-CX, Chicago, IL). The redried seeds were ground (Wiley Mill, Model ED-5) to pass a 40-mesh screen and placed under vacuum over P_2O_5 until chemically analyzed.

Plant Damage. Individual leaf damage of the lima bean plants was visually assessed by comparing among the eight genotypes the percent leaf burn, leaf curl, and leaf chlorosis. Distinct differences in the plant damage were easily classified into three categories.

Glass House. Lima beans were grown in a standard glass house in NF air, utilizing a system of moist fiber pads for cooling. Lima bean plants in the low-ozone atmosphere were grown in an adjacent section of the glass house that had air forced through a bank of 12 activated carbon filters for O_3 removal and then through mist pads for cooling. Details of the filtration system for the glass house are previously reported (Heggestad et al., 1967). Environmental conditions such as temperature, light, and humidity between two glass house sections were similar. Ozone was determined by a Bendix 8002 ozone monitor (Bendix Corp., Lewisberg, WV). A Dafibi Model 1003PCH (Dafibi Environmental Corp., Glendal, CA) was used for calibration.

Total Nitrogen. Kjeldahl nitrogen was determined in duplicate by the micro Kjeldahl method 47.021–47.023 (AOAC, 1980).

Acid Hydrolysis. Duplicate hydrolyzates were prepared by weighing 20 g of finely ground lima bean seed (corresponds to 1 mg or less N) and carrying out the procedures as described by Moore and Stein (1963). Modification of the procedure occurred frm this point on. The sample was removed from the oven after 22 h and cooled and the hydrolyzate quantitatively transferred to a 50-mL graduated cylinder by washing with citrate buffer, pH 2.2 and 0.2 N Na^+ concentration (pH meter, Radiometer, Model PHM 64, London Co., Cleveland, OH). The graduated cylinder was placed in an ice bath, and the contents were stirred. Five milliliters of 12 N NaOH was added to the cold stirred sample. The sample was placed into a 24 °C water bath and brought to temperature and the pH adjusted to pH 2.2 with concentrated NaOH or pH 2.2 citrate buffer (0.2 N). Sample filtration was through a 0.45-μm microporous filter (HVLP 02500, Millipore Corp., Beford, MA) using a Millipore stainless-steel filter holder fitted to a 20-mL hypodermic syringe. The sample was stored at −30.5 °C prior to amino acid analysis.

Amino Acid Analysis. Amino acids were determined on a Durrum D501 amino acid analyzer (Palo Alto, CA) equipped with the Mark II data processor using the single-column method as described in the operation manual (Durrum Instrument Corp., 1972). Samples analyzed on the amino acid analyzer were in the order of a standard, 12 samples, and a standard.

Sugar Extraction. Duplicate samples of finely ground lima bean seeds (2 g) were placed in a 50-mL beaker, and approximately 25 mL of boiling methanol–water (75:25, v/v) was added. Each sample was stirred for 5 min and filtered under vacuum through Whatman No. 1 filter paper. The extraction was repeated three times, and the extracts were combined. The combined methanol–water extract was evaporated to dryness on a rotary evaporator, brought to a known volume with water, and filtered through 0.45-μm microporous filter (HVLP 02500, Milli-

Table I. Genotype Code, Genotype Name, Cotyledon Color, Seed Coat Color, Plant Damage, and Seed Yield for Lima Beans Grown in Polluted and Nonpolluted Air

genotype code	genotype	seed characteristics: cotyledon color	seed coat color	vis plant damage	total harvest[a]: CF[b]	NF[b]	harvest loss, %
1	Henderson Bush	white	white	S[c]	239	161	32.6
2	Dixie Butter Pea	white	white	S	325	175	46.1
3	Fordhook 242	white	white	VS[d]	530	512	3.4
4	Jackson Wonder	white	speckled	VS	406	176	56.6
5	Green Cotyledon Dixie Butter Pea	green	white	R[e]	356	112	68.5
6	Green Cotyledon Jackson Wonder	green	white	R	302	150	50.3
7	Bridgeton	green	green	R	316	205	32.1
8	79 Mildew Resistant Fordhook	green	green	R	666	463	30.5

[a] Harvest one + harvest two; grams of lima bean seeds fresh weight. [b] CF, charcoal-filtered air; NF, nonfiltered air. [c] Susceptible to ozone damage. [d] Very susceptible to ozone damage. [e] Resistant to ozone damage.

pore Corp.) as described in the acid hydrolysis procedure. The samples were stored at −30.5 °C for HPLC analysis.

Starch Hydrolysis. Five grams of the finely ground lima bean seeds was placed into a cellulose Soxhlet extraction thimble (Whatman 2.5 cm × 8 cm), a glass wool plug was placed in the thimble over the ground sample, and 125 mL of 95% ethanol was added to the Soxhlet flask. The Soxhlet extraction was carried out for 4 h at a distillation rate of 1 drop/s. The sample was cooled and the thimble air dried for 24 h to remove most of the ethanol. The thimble was placed into a vacuum oven at 60 °C for 2 h to remove the last trace of ethanol.

Duplicate samples of 150 mg of the alcohol-insoluble material were placed into a test tube (1.6 cm × 1.5 cm), and 2 mL of deionized water was added. To gelatinize the starch, the test tube with the sample was placed into a boiling water bath for 30 min. During the gelatinization step the sample was stirred several times. Immediately on removal from the water bath the sample was placed into an ice bath for cooling. To the cooled sample was added 0.5 mL of Tenase (α-amylase preparation, Miles Laboratories, Elkhart, ID), and the sample placed into a 68 °C water bath. After 60 min the sample was removed and cooled under running tap water, and 0.5 mL of Diazyme (glucoamylase, Miles Laboratories) and 0.5 mL of Clarase (fungal α-amylase, Miles Laboratories) were added. The sample was placed into a 55 °C water bath and stirred periodically. After 1 h the sample was removed from the water bath and cooled to room temperature, and the contents were quantitatively transferred to a 50-mL polycarbonate centrifuge tube and centrifuged at 10000g (Sorvall RC2B, Du Pont Co., Newtown, CN). The liquid in the centrifuge tube was carefully decanted from the pellet and filtered with a glass microanalysis frit support filter holder (XX10-025-02, Millipore Corp., Bedford, MA), with prefilter (AP-0025, Millipore Corp.), support filter (AP10-0025, Millipore Corp.), and a 0.45-μm microporous filter (HVLP-0025, Millipore Corp.). The pellet was resuspended in 15 mL of deionized water and centrifuged for a second time. The solution was removed and filtered, and the two filtrates were combined. The filtrate was quantitativity washed into a 50-mL Erlenmeyer flask, frozen in a dry ice–acetone mixture, and freeze-dried. The dried filtrate material was taken up in the smallest volume of deionized water possible, and the solution was passed through a 0.7 cm × 10 cm mixed-bed ion-exchange column (AG3X-4A, OH^- form; AG50W-8X, H^+ form; bed height 4 cm; BioRad Chemical Division, Richmond, CA) to remove metals, salts, amino acids, proteins, and the hydrolysis enzymes. Three void volumes of the eluent (neutral charged material) were collected from the column. The eluent was evaporated to dryness on the rotary evaporator and brought to a known volume. The sample was frozen at −30.5 °C until analyzed by HPLC. Glucose calibration standards were used to determine the percentage recovery from the ion-exchange column. Three void volumes were determined to give 99–100% recovery. Each day, duplicate blank samples of the enzyme used were prepared for the starch hydrolysis. The blanks were carried through all the analytical steps and analyzed by HPLC.

HPLC Analysis. Sugars were separated on a 0.41 cm × 30 cm, 600CH carbohydrate column (Alltech Associates, Inc., Deerfield, IL) operated at ambient room temperature (24 °C). Mobile phase consisted of 70:30 (v/v) acetonitrile–water with a flow rate of 2.5 mL/min. Column pressure was 70.3 kg/cm^2. The guard column (Waters Associates Inc., Milford, MA) was packed with CO:Pell Pac, cyano amino groups bonded to 25–37-μm glass beads (Whatman, Clifton, NJ). High-pressure liquid chromatography analysis was conducted on a Waters 510 pump, and peak detection was with a Waters 401 differential refractometer. Sample introduction was 20 μL by manual injection (Model 7125, Rheadyne, Inc., Cotati, CA). Peak heights and areas were determined by a recording integrator (C-R3A, Shimadzu Scientific Instruments, Inc., Columbia, MD). A standard curve prepared for each sugar at three concentrations was used to determine the sugar concentration.

Glucose was determined by HPLC as a measure of starch in the lima bean seeds. The column used in the analysis was an Aminex HPX87C (BioRad, 0.78 cm × 30 cm) using water at a flow rate of 0.6 mL/min as the mobile phase. Column temperature was maintained by a column heater (BioRad) at 85 °C. Column pressure was 38.7 kg/cm^2. The guard column was packed with Aminex A-5 (BioRad) in the Ca^{2+} form. Equipment used in the HPLC analyses was the same as described above. A standard curve of glucose at three different concentrations was used to obtain the concentration of the glucose. Analysis by HPLC of the blank showed the presence of glucose (1 mg/mL) and required sample correction. The corrected glucose concentration was multiplied by 0.90 to obtain the milligrams of starch.

Statistical Analysis. A completely random experimental design was employed, and the data were analyzed by the ANOVA program of the Statistical Analysis System (SAS) (Ray, 1982; Little and Hill, 1978).

RESULTS AND DISCUSSION

Lima bean genotypes used in the investigation are described by name, seed characteristic such as cotyledon and seed coat color, plant damage from O_3, and seed yield in Table I. Lima bean plants with the greatest visible tissue damage were Fordhook 242 (3) and Jackson Wonder (4). The genotypes Henderson Bush (1) and Dixie Butter Pea

Table II. Statistics for Treatment, Genotype, and Treatment Interactions for the Chemical Constituents Determined in Lima Bean Seeds Grown in Polluted and Nonpolluted Air[a]

	treatment	genotype	treatment × genotype
Kjeldahl nitrogen	**	**	**
essential amino acids			
threonine	**	**	**
valine	**	**	**
methionine	NS	**	NS
isoleucine	**	**	**
leucine	*	**	NS
tyrosine	**	**	**
phenylalanine	**	**	**
histidine	**	**	NS
lysine	NS	**	NS
nonessential amino acids			
aspartic acid	**	**	**
serine	**	**	**
glutamic acid	**	**	**
proline	**	**	**
glycine	**	**	**
alanine	**	**	**
arginine	**	**	**
ammonia	**	**	**
carbohydrates			
fructose	NS	**	**
sucrose	**	**	**
raffinose	**	**	**
stachyose	**	**	**
starch	**	**	**

[a] Level of significance: 5% (*); 1% (**); nonsignificant (NS).

(2) had tissue damage that was not as severe as the two genotypes above while Green Cotyledon Dixie Butter Pea (5), Green Cotyledon Jackson Wonder (6), Bridgeton (7), and 79 Mildew Resistant Fordhook (8) had only minimal tissue damage. The lima bean genotypes with the least O_3 damage to leaves all produced seeds that had green cotyledons. Cotyledon color may correlate with resistance in lima beans to foliar damage by O_3. Seed color does not appear to correlate with tissue damage of the lima bean genotypes. Seed yields produced from the first harvest were more uniform among the genotypes than the seed yields from the second harvest. Fordhook 242 (3) grown in NF air (high O_3) had extreme tissue damage but had only 3% loss of seed yield while Jackson Wonder (4), with the same amount of tissue damage, had a seed yield loss of 57%. The greatest loss of seed yield (69%) occurred in Green Cotyledon Dixie Butter Pea (5). This lima bean genotype had only slight tissue damage from the air pollution.

The highest hourly concentration of O_3 for May was 0.132 ppm and for June 0.110 ppm. The average hourly concentration for O_3 in May was 0.083 ppm and in June was 0.062 ppm. The seasonal 7-h mean (11:00 a.m. to 6:00 p.m.) for O_3 concentration with diurnal fluctuations for sections of a glass house with CF air, NF air, and ambient air for July 8 to Oct 1 are given in Figure 1. The peak 1-h mean diurnal O_3 concentration occurred at 2:00 p.m.

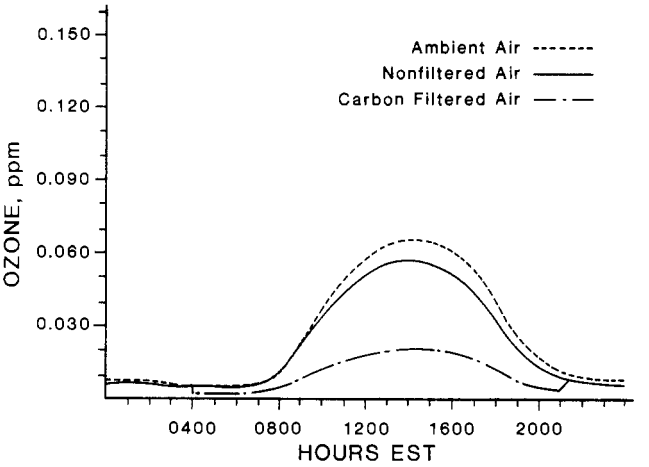

Figure 1. Seasonal 7-h mean O_3 concentrations with diurnal fluctuations for sections of glasshouse with charcoal-filtered air, with nonfiltered air, and in ambient air at 3 m above ground, 30 m from the glasshouse, 86 days from 8 July to 1 Oct.

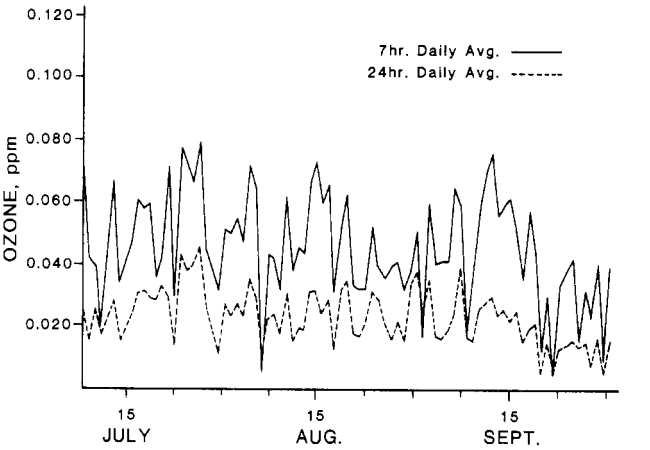

Figure 2. Ambient 7-h (0900–1600 EST) and 24-h O_3 concentrations from 8 July to 1 Oct at a field site 30 m west of the glasshouse location.

EST with the concentration of the ambient air (air outside of the glass house) 0.065 ppm, NF air 0.055 ppm, and the activated CF air 0.02 ppm.

The average daily O_3 concentration of ambient air for 7-h time span from 9:00 a.m. to 4:00 p.m. EST and the 24-h daily average of O_3 concentration in ambient air for July 8 to Oct 1 are given in Figure 2. Large fluctuations occurred in the O_3 concentrations for both the daily 7- and 24-h averages. Average daily O_3 concentrations in the 7-h time period ranged from less than 0.01 ppm to as high as 0.08 ppm.

Analysis of variance of the Kjeldhal nitrogen, amino acids, ammonia, sugars, and starch for the first harvest revealed highly significant treatment X genotype interactions except for methionine, leucine, histidine, and lysine (Table II). Significant treatment differences were found for leucine and histidine. In the CF air (low O_3), the

Table III. Mean Value for Significant Genotype Differences in the Amino Acids Methionine, Leucine, Histidine, and Lysine in Lima Bean Seeds[a]

	genotype[b]								
amino acids[c]	1	2	3	4	5	6	7	8	$S^{\bar{X}}$[d]
methionine	248	308	269	290	310	281	190	310	13
leucine	1631	1890	1015	1621	1755	1594	1312	1683	124
histidine	651	779	547	630	703	607	593	645	18
lysine	1111	1585	1142	1310	1387	1278	1223	1379	75

[a] Milligram/100 g of dry weight. [b] Genotype code, see Table I. [c] Means of duplicate analysis. [d] Standard error of mean, calculated with six error degrees of freedom.

Table IV. Means of Duplicate Analyses of Genotype and Treatments of Lima Bean Seeds from the First Harvest Grown in Polluted and Nonpolluted Air

	genotype[a]																
	1		2		3		4		5		6		7		8		
	CF[b]	NF[b]	CF[b]	NF[b]	CF[b]	NF[b]	CF[b]	NF[b]	CF[b]	NF[b]	CF[b]	NF[b]	CF[b]	NF[b]	CF[b]	NF[b]	S^{x}[c]
Kjeldahl nitrogen[d]	3.22	3.24	3.77	3.41	3.53	3.30	3.56	2.64	3.18	2.87	3.60	3.10	2.74	2.86	3.13	2.90	0.05
essential amino acids																	
threonine	906	882	1056	961	961	890	942	754	859	801	960	855	747	752	882	808	20
valine	1043	999	1234	1054	1131	1030	1124	877	1007	951	1117	964	874	897	1025	947	24
isoleucine	942	910	1157	1028	1042	972	1020	806	909	845	1053	881	799	829	964	910	22
tyrosine	677	667	791	796	711	700	708	531	661	623	766	652	572	608	657	610	28
phenylalanine	1179	1129	1433	1382	1321	1249	1330	974	1150	1046	1294	1049	977	1005	1215	1113	40
methionine	270	226	278	338	263	276	299	281	297	322	313	249	196	185	323	297	19[f,g]
leucine	1650	1612	2017	1948	1369	1420	1687	1555	1830	1679	1802	1385	1593	1472	1817	1548	175[g]
histidine	669	633	784	772	538	556	656	605	694	712	670	544	604	581	690	600	27[g]
lysine	1345	1295	1582	1588	1132	1152	1353	1266	1417	1355	1403	1152	1253	1193	1464	1284	107[f,g]
total	8681	8353	10332	9867	8468	8245	9119	7649	8824	8334	9378	7731	7615	7522	9037	8117	
nonessential amino acids[e]																	
aspartic acid	2244	2268	2773	2445	2485	2278	2344	1845	2162	2022	2568	2217	2021	2120	2229	2057	47
serine	1262	1218	1537	1345	1367	1126	1335	1019	1221	1135	1372	1189	1034	1068	1236	1127	22
glutamic acid	2649	2758	3316	2879	2897	2683	2812	2228	2513	2336	2976	2567	2324	2364	2641	2461	60
proline	936	907	1097	1032	1013	909	970	780	876	823	994	863	783	824	897	839	17
glycine	810	782	976	888	881	823	869	709	771	720	868	763	676	687	815	759	15
alanine	895	866	1052	946	966	898	955	774	861	807	948	838	750	768	880	838	17
arginine	1101	1037	1345	1274	1179	1106	1238	888	1032	959	1356	1100	943	992	1091	993	39
ammonia	425	406	566	551	502	480	503	382	366	336	550	470	410	439	455	425	18
total	10322	10242	12662	11350	11290	10303	11026	8625	9802	9138	10632	10007	8941	9262	10244	9499	
carbohydrate[h]																	
fructose	2.1	2.3	2.9	2.9	2.6	2.5	2.6	1.9	2.2	2.7	3.3	2.9	2.6	3.0	1.9	2.4	0.1[f]
sucrose	12.3	12.9	9.9	9.9	10.8	13.2	15.9	17.6	12.9	15.6	23.4	22.3	29.5	29.6	13.7	15.6	0.2
raffinose	3.4	3.8	3.6	2.8	4.4	4.3	2.4	2.7	4.4	3.2	4.4	4.3	4.6	1.9	2.1	3.4	0.1
stachyose	34.6	40.2	35.0	39.8	36.1	44.2	37.4	37.9	35.7	40.3	24.6	22.9	14.9	17.4	39.0	42.1	0.5
total	52.4	59.2	51.4	55.4	53.9	64.2	58.3	60.1	55.2	61.8	55.7	52.4	51.6	51.9	56.7	63.5	
starch	363.9	392.1	341.3	373.7	378.0	365.6	353.5	407.7	376.8	404.7	369.1	409.1	405.8	424.8	369.1	353.8	9.0

[a] Genotype code, see Table I. [b] CF, activated carbon-filtered air; NF, nonfiltered air. [c] Standard error of mean, calculated with three error degree of freedom. [d] Grams/100 g of dry weight. [e] Milligrams/100 g of dry weight. [f] Nonsignificant with respect to treatment. [g] Nonsignificant with respect to treatment X genotype. [h] Milligram/gram of dry weight.

Table V. Means of Duplicate Analyses of Genotype and Treatment of Lima Bean Seeds from the Second Harvest Grown in Polluted and Nonpolluted Air

	genotypes[a]											
	1		2		3		4		7		8	
	CF[b]	NF[b]	CF[b]	NC[b]	CF[b]	NF[b]	CF[b]	NF[b]	CF[b]	NF[b]	CF[b]	NF[b]
Kjeldahl nitrogen[c]	3.32	3.50	3.66	3.57	3.36	3.06	2.99	3.36	3.45	3.20	3.92	3.32
essential amino acids[d]												
threonine	951	941	958	994	893	818	803	853	921	841	990	842
valine	1098	1124	1147	1160	1116	997	918	999	1129	985	1243	1048
isoleucine	1029	993	1096	1058	958	867	867	952	981	891	1082	904
tyrosine	766	648	769	651	576	542	557	565	643	602	741	636
phenylalanine	1036	1218	1313	1292	1136	1030	1103	1187	1215	1021	1326	1111
methionine	228	223	228	215	208	191	192	186	211	198	233	198
leucine	1800	1759	1924	1848	1696	1530	1528	1683	1738	1654	1898	1582
histidine	707	726	732	734	651	587	591	675	681	623	728	600
lysine	1436	1384	1498	1472	1357	1247	1219	1327	1378	1248	1498	1258
total	9051	9016	9665	9424	8591	7809	7778	8427	8897	8063	9739	8179
nonessential amino acids[d]												
aspartic acid	2512	2495	2604	2504	2434	2192	2058	2314	2411	2163	2701	2247
serine	1347	1347	1426	1407	1302	1168	1107	1120	1364	1249	1474	1243
glutamic acid	2920	2888	3091	2930	2837	2568	2449	2710	2744	2578	3113	2604
proline	1031	994	1088	1018	929	848	878	946	959	882	1038	858
glycine	879	867	949	922	839	770	756	825	844	764	921	780
alanine	997	942	1031	1006	917	880	843	921	959	866	1010	864
arginine	1225	1202	1306	1204	1247	1096	1009	1094	1156	1005	1516	1153
ammonia	394	795	449	783	757	672	345	382	735	678	899	721
total	11305	12030	11944	11774	11262	10194	9445	10312	11172	10185	12672	10470
carbohydrate[e]												
fructose	1.7	3.3	2.8	3.4	1.9	1.9	1.4	2.0	1.4	2.1	2.6	2.2
sucrose	14.1	12.8	10.4	8.7	25.8	23.4	12.6	13.1	14.5	17.5	20.8	22.3
raffinose	3.6	4.6	2.8	4.6	4.8	4.2	3.2	2.9	2.9	4.4	4.9	6.8
stachyose	40.1	32.1	40.1	38.1	30.5	22.6	40.3	43.2	45.2	39.9	25.7	25.6
total	59.5	52.8	56.1	54.8	63.0	52.1	57.5	61.2	64.0	63.9	54.0	56.9
starch	309.7	266.8	342.4	301.4	352.4	395.7	308.7	279.5	341.4	278.9	375.6	355.8

[a] Genotype code, see Table I. [b] CF, charcoal-filtered air, NF, nonfiltered air. [c] Grams/100 g of dry weight. [d] Milligram/100 g of dry weight. [e] Milligram/gram of dry weight.

concentration of leucine was 1679 mg/100 g of dry weight for histidine 663 mg/100 g of dry weight while in the NF air (high O_3) the concentration of leucine was 1446 mg/100 g of dry weight and for histidine 625 mg/100 g of dry weight. Therefore, in the lima bean seed grown in CF air the essential amino acid concentrations for leucine and histidine were 14% and 6% higher than in the lima bean seed grown in the NF air with its elevated O_3 atmosphere. Lysine and methionine were the only amino acids and fructose the only sugar that were nonsignificant with treatments.

Significant differences were found among the genotypes for the essential amino acids methionine, leucine, histidine ($p \leq 0.01$) and lysine ($p \leq 0.05$) (Table III). The concentration (mg/100 g of dry weight) range among the genotypes was variable with methionine 190 to 310; leucine 1015 to 1890; histidine 593 to 779; and lysine 1111 to 1585. The lowest concentration for the essential amino acids (methionine and histidine) occurred in Bridgeton (7) while the highest concentration for the essential amino acids (leucine, histidine, lysine) occurred in Dixie Butter Pea (2).

The Kjeldahl nitrogen in the lima bean seeds of Henderson Bush (1) and Bridgeton (7) was not significantly affected by the pollution treatments (Table IV). In the remaining genotypes, Kjeldahl nitrogen content increased in the lima bean seeds grown in CF air. The sulfur amino acid cystine was found in extremely small amounts in only some of the genotypes and was therefore not reported. The essential amino acid tryptophan, which is destroyed during acid hydrolysis, was also not reported. The amino acid concentrations were generally higher in CF air. The total essential amino acids and the total nonessential amino acids, except for Bridgeton (7), reflected this trend. In all genotypes the essential amino acid with the highest concentration was leucine while the nonessential amino acid with the highest concentration was glutamic acid. The percent recovery for the amino acid determinations ranged from 79% to 88%. Cotyledon color and seed coat color do not appear to be factors related to changes in the amino acids associated with O_3 treatments.

The carbohydrate contents, however, appear to respond differently than the amino acids to the pollution treatments (Table IV). The overall trend for the sugars was to increase in the lima bean seeds grown in the NF air. These increases in sugar concentration occurred in all the genotypes except Green Cotyledon Jackson Wonder (6) which decreased. The oligosaccharide stachyose had the greatest increase in concentration of the galactose-containing sugars (raffinose, stachyose). Increases of these oligosaccharides in beans are important as these compounds appear to be responsible for flatulence (Fleming, 1981; Fleming, 1982).

Seed yield in the second harvest of lima bean seeds was variable, with most genotypes producing small amounts of seeds and two genotypes, Green Cotyledon Dixie Butter Pea (5) and Green Cotyledon Jackson Wonder (6), producing no seeds. Differences in seed yields between the first harvest and second harvest show the effect of O_3 stress on the lima bean plants. The three replications of each genotype-producing seed were combined to make one sample of each genotype that was sufficiently large to allow for the chemical analyses. Therefore, statistical analysis could not be conducted on data from the second harvest. In comparing seed quality from plants in CF and NF air for the second harvest, 79 Mildew Resistant Fordhook (8) grown in the NF air had the greatest Kjeldahl nitrogen and amino acid content (Table V). The data for amino acids are very similar to the first harvest. The lima bean seeds of the second harvest responded differently than in the first harvest with respect to carbohydrate and starch. The

higher sugar and starch content were obtained in seeds of plants grown in the activated CF air except for Jackson Wonder (4) and 79 Mildew Resistant Fordhook (8). Seed coat and cotyledon color do not appear to correlate with the compositional changes in lima bean seeds related to the O_3 treatments.

In summary, susceptibility to O_3 damage to leaves was determined for eight genotypes of lima beans. Greatest resistance to visible leaf injury from pollutants in ambient air was exhibited by genotypes with green cotyledons. Seed coat color was not related to leaf tissue injury. Seed yield was not related to the amount of visible plant tissue damage. The maximum Kjeldahl nitrogen and amino acid synthesis occurred in seeds from harvest one and two grown in CF air. Seeds from the first harvest grown in NF air and seeds from the second harvest grown in CF air had the highest carbohydrate and starch. Cotyledon or seed coat color was not related to O_3 effects on Kjeldhal nitrogen or starch. Treatment of ambient air O_3 on methionine, lysine, and fructose was not statistically significant. Additional studies are required to determine the effect of O_3 on the synthesis of biochemical components in bean seeds.

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Registry No. NH_3, 7664-41-7; starch, 9005-25-8; L-methionine, 63-68-3; L-leucine, 61-90-5; L-histidine, 71-00-1; L-lysine, 56-87-1; nitrogen, 7727-37-9; L-threonine, 72-19-5; L-valine, 72-18-4; L-isoleucine, 73-32-5; L-tyrosine, 60-18-4; L-phenylalanine, 63-91-2; L-aspartic acid, 56-84-8; L-serine, 56-45-1; L-glutamic acid, 56-86-0; L-proline, 147-85-3; glycine, 56-40-6; L-alanine, 56-41-7; L-arginine, 74-79-3; D-fructose, 57-48-7; sucrose, 57-50-1; D-raffinose, 512-69-6; stachyose, 470-55-3; ozone, 10028-15-6.

LITERATURE CITED

Adams, R. M.; Hamilton, S. A.; McCarl, B. A. "The Economic Effects of Ozone on Agriculture"; Environmental Protection Agency, Environmental Research Laboratory: Corvallis, OR, 1984; EPA-600/3-84-090, p 175.

Association Official Analytical Chemists "Official Methods of Analysis", 13th ed.; AOAC: Washington, DC, 1980.

Bennett, J. P.; Resh, H. M.; Runeckles, V. C. *Can. J. Bot.* **1974**, *52*, 35–42.

Clayberg, C. D. *HortScience* **1971**, *6*, 396–397.

Dugger, W. M., Jr.; Palmer, R. L. Proceedings, First International Citrus Symposium; University of California: Riverside, 1969; Vol 2, pp 711–715.

Dugger, W. M., Jr.; Taylor, O. C.; Cardiff, E.; Thompson, C. R. *Proc. Am. Soc. Hort. Sci.* **1962**, *81*, 304–315.

Durrum Instrument Corp. "Operation and Maintenance Manual, Durrum Amino Acid Analyzer Model D500"; Durrum Instrument Corp.: Palo Alto, CA, 1972.

Fleming, S. E. *J. Food Sci.* **1982**, *47*, 12–15.

Fleming, S. E. *J. Food Sci.* **1981**, *46*, 794–798, 803.

Heggestad, H. E.; Christiansen, M. N.; Craig, W. L.; Heartley, W.H. In "Air Pollution and Its Impact on Agriculture", Cottrell Centennial Symposium; California State College: Turlock, CA, 1977a; pp 101–127.

Heggestad, H. E.; Howell, R. K.; Bennett, J. H. *U.S. Environ. Prot. Agency, Res. Dev.* **1977b**, EPA-600/3-77-128, 37.

Heggestad, H. E.; Craig, W. L.; Meiner, J. P.; Saettler, A. W. *Proc. Am. Phytopath. Soc.* **1976**, *3*, 326.

Heggestad, H. E. *Am. Potato J.* **1973**, *50*, 315–328.

Heggestad, H. E.; Heck, W. W. *Adv. Agron.* **1971**, *23*, 111–145.

Heggestad, H. E.; Menser, H. A.; Bailey, W. A. Preprint 67-38h, Air Pollution Control Association Annual Meeting, Pittsburgh, PA, 1967; pp 1–6.

Howell, R. K.; Devine, T. E.; Hanson, C. H. *Crop. Sci.* **1971**, *11*, 114–115.

Lee, E. H.; Bennett, J. H.; Heggestad, H. E. *Plant Physiol.* **1981**, *67*, 347–350.

Lefohn, A. S.; Tingey, D. T. *Atmos. Environ.* **1984**, *18*, 2521–2526.

Little, T. M.; Hill, F. J. "Agricultural Experimentation Design and Analysis"; Wiley: New York, 1978.

Menser, H. A. *J. Am. Soc. Sugar Beet Technol.* **1974**, 19, 81–86.

Moore, S.; Stein, W. H. *Methods Enzymol.* **1963**, 820–923.

Ray, A. A. "SAS Users Guide: Statistics"; SAS Institute, Inc.: Cary, NC, 1982.

Reinert, R. A.; Dunning, J. A.; Heck, W. W.; Benepal, P. S.; Rangappa, M. *HortScience* **1984**, *19*, 86–88.

Reinert, R. A.; Heggestad, H. E.; Heck, W. W. In "Breeding Plants for Less Favorable Environments"; Christiansen, M. N., Lewis, C. F., Eds.; Wiley: New York, 1982; pp 260–267.

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Analogues of Phytoalexins. Synthesis of Some 3-Phenylcoumarins and Their Fungicidal Activity

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Little is known about the relationship between fungicidal activity of isoflavonoid phytoalexins and their physicochemical properties, in particular partition properties. As a first part of an investigation on the influence of partition coefficients on the fungicidal activity of analogues of isoflavonoid phytoalexins, a series of 3-phenylcoumarins, including ethers and side-chain derivatives of high lipophilicity, were synthesized and tested in vitro and in vivo for antifungal activity. The results seem to indicate that an increase of lipophilicity has a negative effect and a free OH is indispensable for activity.

INTRODUCTION

Phytoalexins are chemical compounds involved in the resistance of plants to fungal infection (Bailey and Mansfield, 1982). They exhibit fungistatic, fungicidal, and, in some cases, also antibacterial activity (Grisebach and Ebel, 1978). They have been studied especially by plant pathologists interested in understanding the mechanism of resistance of plants to diseases. Some suggestions on the possibility of using them as models for the synthesis of new fungicides have also been made (Polter, 1974; Ward et al., 1975). The work of Rathmell and Smith (1980)

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