Quality of Corn Grain from Plants Exposed to Chronic Levels of Ozone[1]

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ABSTRACT

Whole kernel corn, derived from field-grown corn plants that had been exposed to ambient ozone at chronic concentration levels, was examined to determine the effects of the stress imposed on the edible grain. Initially, the study examined the composition of the grain from a commercial field corn hybrid exposed to five levels of ozone ranging from 0.02 to 0.15 ppm. A second phase also included two other open-pedigreed hybrids that were more sensitive to ozone but in this case were exposed at three levels of ozone—0.02, 0.06, and 0.15 ppm. Stress effects from ozone were more readily manifested in the vegetative plant at lower levels of ozone than in the grain. Compositional changes in the grain recovered from plants exposed to all levels of ozone studied were minor for protein, amino acids, fat, fiber, ash, starch, and amylose content of starch. Essentially no difference in macromineral element levels was found at all levels of ozone exposure; however, levels of trace elements (zinc, iron, and copper) and methionine increased significantly with an increase in ambient ozone concentration. Responses to the same levels of ozone were different for the three corn genotypes.

Extensive studies have clearly established that ambient ozone at chronic levels adversely affects both growth and yield characteristics of a wide variety of crops. Ozone in the troposphere is a product of complicated chemical interactions (Shen et al 1977). Initially, nitric oxide (NO), a high-temperature combustion product, is oxidized in the atmosphere to nitrogen dioxide (NO_2), an intermediate product. Then photodissociation of NO_2 in the air by the absorption of ultraviolet radiation from sunlight produces atomic oxygen that in turn reacts with molecular oxygen to form ozone (O_3) (Graedel and Farrow 1975). The ambient O_3 thus produced then affects plant and animal life by direct contact. Ozone causes foliar injury on a wide variety of edible crop species, each of which may exhibit slightly different phytotoxic symptoms (Brennan 1975).

Visual examination of foliar injury caused by exposure to ambient oxidants such as O_3 and oxides of nitrogen has been used extensively to estimate suppression of vegetative growth in plants (Seidman et al 1965). This represents an important method of observation because it identifies when critical growth periods occur during phenology, and these effects can be monitored with the emergence of new leaves. In addition, at plant maturity both plant size and crop yield are important physical characteristics that can reflect the adverse effects of atmospheric pollutants or other stresses. In a specific study with soybean leaves, Tingey et al (1973) measured changes in soluble metabolites in the leaves as a result of ozone exposure; levels of protein, amino acids, total soluble sugars, sucrose, and starch decreased, whereas reducing sugar levels increased during the same growth period. Also, because little was known about possible deteriorative effects of food commodities exposed to ambient oxidants during storage, Brooks and Csallany (1978) examined both soybean and corn seeds (whole, halves, and ground) that had been grown under normal agronomic conditions but later exposed during storage to air, 15 ppm NO_2 or 1.5 ppm O_3. Their results showed that exposure of whole seeds induced no polyunsaturated fatty acid or tocopherol destruction because of their protective intact seed coats. When the seed coat of soybeans was broken, however, significant oxidation injury was caused, depending on both oxidizing ability and concentration of the gases used.

Even though many detrimental physical effects have been observed in growing plants exposed to ozone in both greenhouse and field studies, the effects on the composition and quality of the edible parts of the exposed plants have received only scant attention. The purpose of this study was to establish whether edible grain produced from field corn plants, grown and exposed in special field chambers at different adjusted levels of ambient ozone, would be adversely affected as a result of the total plant exposure. In this study the field-grown dent corn plants were stressed at levels of ozone characteristic of those concentrations observed in ambient air in the United States.

Cameron et al (1970) reported injury to field-grown sweet corn in California, where both growth and yield had been affected by ambient oxidants. In subsequent work they expanded their studies to examine injury to sweet corn, both in the field and in the greenhouse (Cameron and Taylor 1973). It is difficult to compare results of controlled ozone concentrations in greenhouse studies with those observed with constantly varying exposures to air pollutants in the field. One of the first reports of yield loss in a field-grown agronomic crop due to long-term, low-level ozone exposures was made in an early field-exposure chamber used by Heagle et al (1972) to expose two sweet corn cultivars at three controlled levels of ozone (0, 5, and 10 pphm for 6 hr per day). In this system, oxidant pollutants already present in ambient air were filtered out by an activated charcoal filter, and the resultant filtered air stream was passed through an ozone generation system where desired amounts of ozone were added to the air. The ozonated air was then introduced into the field chamber where the corn plants were growing.

Heagle et al (1973) successfully designed and evaluated an open-top field chamber for studying the effects of air pollutants on plants grown therein, where important factors such as air temperature, relative humidity, and especially direct sunlight irradiation were similar to ambient air conditions.

The present study utilized open-top field chambers to grow the corn plants to be stressed at different chronic ozone levels (Heagle et al 1979a,b). The corn grain harvested from the exposed plants was evaluated to determine whether composition or the nutritional quality had changed as a result of the stress imposed by the ozone. One phase of the study involved grain from a commercial field corn hybrid exposed to five levels of ozone ranging from 0.02 to 0.15 ppm. In a subsequent phase, the same commercial corn hybrid plus two open-pedigreed hybrids were grown at three ozone exposure levels of 0.02, 0.06, and 0.15 ppm. Compositional analysis of the grain included protein, fat, fiber, starch, amylose content of the starch, amino acid composition of the protein, and mineral constituents including both macro- and microelements.

MATERIALS AND METHODS

Plant Growth at Different Ozone Levels

In a preliminary greenhouse study, corn plants grown from seeds representing five open-pedigreed and six commercial field corn

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hybrids were exposed 7 hr daily for 21 consecutive days to levels of ozone ranging from 0.08 to 0.17 ppm O_3 (Heagle et al 1979a). On the basis of visible foliar injury and measured weights of stems and leaf tissues, three hybrids that exhibited different degrees of sensitivity to ozone were selected for the subsequent field studies.

The second phase of this study involved the commercial corn hybrid Coker 16, which was shown to be intermediate in sensitivity in the preliminary test. It was grown near Raleigh, NC, under field conditions to determine threshold ozone doses for plant injury and growth and yield effects (Heagle et al 1979a). The five different ozone treatment levels included ambient air concentrations (0.06 ppm O_3) plus four other treatments where open-top field chambers were used to adjust desired ozone air concentrations in the range of 0.02 ppm O_3 (charcoal-filtered air) to 0.15 ppm O_3 during an exposure period of 7 hr per day (Heagle et al 1979b). At all five ozone levels, plants were grown simultaneously both in a field of sandy-clay-loam soil (Cecil Appling Association) fertilized with (N-P-K, 14-0-14) at 500 kg/ha and NH_4NO_3 at 336 kg/ha and in pots adjacently placed. The potted plants in 15-L plastic pots were grown in a mixture containing (sandy-loam-soil:sand:pro-mix BX, in a 1:1:1 ratio). Corn plants (one per pot) were fertilized with 6 g (N-P-K, 14-4-6) and 5 g NH_4NO_3/pot. The pots were placed in rows adjacent to plants grown in the ground in the open-top field chambers at the adjusted O_3 concentration desired. Resultant physical characteristics, including plant height and weight of stover plus grain yield and size, were then measured to assess the effects of ozone exposure.

The third phase involved Coker 16 plus two other open-pedigreed hybrids—FR632 × FR619 (resistant in the greenhouse test) and H95 × FR64A (sensitive in the greenhouse test)—and hereafter designated respectively as (OP-1) and (OP-2). All three hybrids were grown under a field environment. However, only three ozone levels—0.02 ppm (charcoal-filtered air), 0.06 ppm (ambient air), and 0.15 ppm—were used. Harvested grain samples from the second and third phases (Heagle et al 1979b) were examined in this study. Each grain sample collected represented a composite yield from 40 plants (eight in each of five blocks) grown in open-top field chambers or ambient air. Analyses were run on two to six samples from the composite, depending on measurement.

Treatments and Analysis of Grain Samples

Fifty grams of each composited corn grain sample was ground to pass through a 40-mesh screen for protein (N × 6.25) and fiber analyses as determined by standard methods (AACC 1961). Fat was determined by extraction of the ground corn grain with hexane for 16 hr in a Soxhlet apparatus.

Ash was determined by ignition in a platinum dish at 575° C for at least 16 hr. Starch was measured polarimetrically with 90% dimethyl sulfoxide as the starch solvent with a 1-g sample of grain, using the method of Garcia and Wolf (1972). A Bendix electronic polarimeter equipped with a 0.5-dm cell was used for optical rotation measurements at 546 nm (mercury green). Amylose content of the starch was determined spectrophotometrically by measuring the absorbance of the amylose-iodine complex from an aliquot of the DMSO-starch solution (Wolf et al 1970).

Macro- and trace mineral elements were determined with approximately 10 g (in triplicate) of whole grain, which was wet-ashed with HNO_3 and $HClO_4$ for subsequent analysis by flame atomic absorption (Garcia et al 1974). Background correction for cadmium analysis was accomplished with a hydrogen continuum lamp with a Varian AA6DAB spectrophotometer. A colorimetric molybdenum-blue procedure was followed for phosphorus analysis of the ashed grain (AACC 1957).

For amino acid analysis, the defatted meal was hydrolyzed by refluxing in 6*N* hydrochloric acid for 24 hr. The ratio of meal to acid was 1 mg/2 ml, and the acid was prepared by diluting concentrated HCl 1 to 1 and bubbling N_2 through it for 1 hr. Hydrochloric acid was removed after hydrolysis on a rotary evaporator at 40° C with two water additions. Samples were finally dissolved in starting buffer and analyzed in a Glenco MM-100 amino acid analyzer. Methionine values were obtained by combining methionine, methionine-sulfone, and methionine sulfoxide values.

Analyses of variance were determined for each measurement. The effects of ozone level were assessed against interaction effects for determining significance. Where appropriate, the least significant difference (LSD) at the 0.05 level is shown.

RESULTS AND DISCUSSION

In the preliminary greenhouse study, Coker 16 exhibited intermediate sensitivity to ozone when compared to 10 other hybrids, and on this basis it was selected for field studies. Plants were field-grown and subjected to five different levels of O_3: that occurring in ambient air (0.06 ppm); plus 0.07, 0.11, and 0.15 ppm O_3 levels adjusted in field chambers; and 0.02 ppm (charcoal-filtered air). These latter four levels of ozone represented daily 7 hr per day applications that were maintained between 0930 and 1630 hr, as previously described (Heagle et al 1979b). Grain yield of Coker 16 at 0.11 and 0.15 ppm was 2 and 14% less, respectively, than at 0.02 ppm. Yield was not decreased at 0.06 or 0.07 ppm.

Effects on a Single Corn Hybrid at Five Ozone Exposure Levels

Table I shows significant and nonsignificant effects on the composition of Coker 16 whole-kernel corn harvested from plants grown in the field of sandy-clay-loam soil (Cecil Appling Association) and also of plants grown simultaneously in adjacently placed pots. From original data derived from the different measurements, analyses of variance were computed; a separate analysis was used for each measurement. Because there were 10 treatments involving Coker 16 only, it was possible to test nine effects or comparisons among means. A very large number of these effects were highly significant for practically all measurements; this is probably the result of the analysis procedure. The compositing of grain from 40 plants (from five blocks) tends to reduce the expected plant-to-plant variation. Thus, when samples are taken from the composite, variability estimates tend to reflect analytical variation rather than ear to ear variation. Therefore, when effects are assessed, the variability estimate is too low and, thus, many significant indications are obtained. In this case it was decided it was better to follow a more conservative approach and to use interaction effects for assessing treatments. This procedure was followed, and seven measurements were associated with significant variation.

When observations for grain derived from both pots and field soil were combined, results showed that significantly higher levels of amylose in starch, ash, and chromium were associated with charcoal-filtered air (0.02 ppm O_3) than with ambient air (0.06 ppm O_3). Grain from pots was associated with significantly lower levels of amylose and copper, but higher levels of chromium. Significant positive linear trends with ozone levels were observed with zinc and iron, but a negative linear trend with magnesium. Ozone added to ambient air (0.07, 0.11, and 0.15 ppm O_3) yielded significant increases over charcoal-filtered air (0.02 ppm O_3) and ambient air (0.06 ppm O_3) for both amylose and zinc. Individual means associated with the 10 treatments for the seven measurements that had significant effects are shown in Table I. However, for those measurements where variation was not significant, only the overall means are listed.

Even though exposure of Coker 16 plants at chronic ozone levels had no significant effect on the protein content of the harvested grain, the ground whole kernel corn was further examined to determine whether the amino acid levels of the protein itself had been affected. The effects on the levels of 17 individual amino acids were examined for the 10 ozone treatments, including both potted and field soil growth conditions, and no significant variation was found as a result of analysis of variance. It was concluded that no significant alteration of the amino acid pattern was evident for Coker 16 as a result of chronic ozone exposures.

Even though potted and field soil media were different, analytical measurements of the grain were similar. Of the macrominerals analyzed in the grain, only magnesium content declined at the highest ozone concentration (0.15 ppm), whereas

TABLE I
Effects of Air-Ozone Concentrations on Contents of Whole-Kernel Corn Coker 16 Hybrid, Field Grown[a]

		Potted, ppm O_3					Soil, ppm O_3					
		0.02	0.06				0.02	0.06				
		Charcoal	Ambient	0.07	0.11	0.15	Charcoal	Ambient	0.07	0.11	0.15	
Designation	Overall Mean	Filtered	Air	(O_3 Added to Ambient)			Filtered	Air	(O_3 Added to Ambient)			LSD
					Significant							
Ash, %		1.44	1.30	1.34	1.36	1.43	1.47	1.27	1.33	1.29	1.36	0.12
Percent amylose in starch		29.8	29.2	30.2	30.3	29.7	30.4	29.8	30.2	30.7	30.3	0.8
Magnesium, %		0.124	0.123	0.127	0.126	0.116	0.125	0.128	0.125	0.124	0.120	0.009
Zinc, $\mu g/g$		24.3	23.7	25.2	25.4	26.8	23.5	23.9	23.9	24.3	25.9	1.6
Iron, $\mu g/g$		16.9	16.6	16.1	17.8	18.9	16.2	15.9	16.3	16.2	17.4	2.0
Copper, $\mu g/g$		1.31	1.41	1.24	1.37	1.45	1.43	1.54	1.68	1.55	1.71	0.37
Chromium, $\mu g/g$		0.097	0.054	0.076	0.093	0.077	0.070	0.047	0.052	0.047	0.072	0.047
					Nonsignificant							
Fat, %	4.72 ± 0.04											
Fiber, %	2.61 ± 0.04											
Starch, %	67.2 ± 1.2											
Protein, %	9.98 ± 0.21											
Potassium, %	0.266 ± 0.014											
Phosphorus, %	0.326 ± 0.013											
Manganese, $\mu g/g$	5.79 ± 0.30											
Cadmium, $\mu g/g$	0.047 ± 0.009											

[a] On a dry weight basis, individual values for specific ozone treatments for protein represented the mean of six samples; fat, ash, and fiber mean of two samples, starch and amylose content of starch mean of two samples each analyzed twice (four analyses). Macro and trace elements represented the mean of three samples.

TABLE II
Significant Effects of Air-Ozone Concentration on Content of Whole-Kernel Corn From Three Hybrid Selections— Coker 16, (OP-1), and (OP-2), Field Grown

	PPM O_3			
	0.02	0.06	0.15	
Designation	Charcoal Filtered	Ambient Air	O_3 Added to Ambient	LSD
Methionine, g/16 g N	1.25[a]	1.33	1.67	0.19
Zinc, $\mu g/g$	25.3	23.7	31.3	1.5
Iron, $\mu g/g$	17.6	16.8	20.3	2.7
Copper, $\mu g/g$	1.45	1.53	1.91	0.19

[a] Each mean is for the three hybrids, two samples per hybrid for methionine or three samples for metals, on a dry weight basis.

three trace elements—zinc, iron, and copper—were the highest at 0.15 ppm O_3. Individual results for chromium showed greater variation due to less analytical sensitivity for chromium at these low levels. From the foregoing data it was concluded that Coker 16, a hybrid previously established by greenhouse studies to be intermediate in sensitivity to ozone had exhibited increases in zinc, iron, and copper in the kernel at 0.15 ppm O_3.

Effects on Three Corn Hybrids at Three Ozone Exposure Levels

The next phase of the study examined the composition of harvested grain from three field-grown corn hybrids, representing the two open-pedigreed hybrids OP-1 and OP-2 and Coker 16. The plants had been exposed to three levels of ozone: charcoal-filtered, 0.02 ppm; and ambient, 0.06 and 0.15 ppm. The weights of kernels per plant at 0.15 ppm were 12, 37, and 40% less than at 0.02 ppm for Coker 16, OP-1, and OP-2, respectively. The size of kernels per hybrid followed similar trends. Thus, it was presumed that compositional changes in the grain might also be different for each hybrid.

Analyses of variance were also determined for analytical measurements on the grain derived from the three hybrids. Three effects were estimated, the variation due to hybrid selection, to ozone treatment, and to the interaction of hybrid selection with ozone level. In 21 out of 32 analyses, the interaction was significant relative to sampling variation, and all effects were nonsignificant for only three measurements. Again, it seemed that sampling variation underestimated ear-to-ear variation. A conservative approach is to view the hybrid selection as providing replication and testing the variation due to ozone level against the selection-ozone interaction. This method was followed, and ozone effects were significant for methionine, zinc, iron, and copper, as shown in Table II.

Methionine levels increased with increasing ozone levels for all three hybrids. The greatest changes occurred in OP-1 and OP-2, where methionine levels increased by about 30 and 27%, respectively, between 0.02 and 0.15 ppm O_3. Methionine levels also increased under the same conditions for Coker 16, but only by 19%. For Coker 16 levels of zinc, iron, and copper in the grain increased at the 0.15 ppm O_3 level as had previously been established in the combined field soil and potted soil experiment. The effect of ozone stress was demonstrated even more with the open-pedigreed varieties OP-1 and OP-2, resulting in increased grain zinc content of 7.7 and 6.4 $\mu g/g$, respectively at the 0.15 ppm O_3 level; iron also increased by 3.2 and 4.3 $\mu g/g$, respectively. By the same comparison, copper content had even greater proportionate increases of 0.57 and 0.54 $\mu g/g$ at 0.15 ppm O_3.

Although Coker 16 showed no increase in cadmium content at the 0.15 ppm O_3 level both in the earlier combined field and potted soil experiment and this later experiment, both OP-1 and OP-2 hybrids did show their highest proportionate metal increase when cadmium levels increased by respective factors of approximately 1.7 and 2.1 times greater at the 0.15 ppm O_3 level than at the 0.02 ppm O_3 level. This further demonstrated hybrid differences in sensitivity to ozone stress.

Interestingly, levels of methionine increased for all hybrids with increasing ozone exposure levels. Relative increases in methionine were similar in magnitude to increases for Cu, Fe, and Zn, with Cu providing the closest correlation. It thus appears that biochemical changes that occur in the grain are manifested best in trace element and methionine concentration changes; they appear to be directly connected because of similar effects. Additional studies are required to formulate plausible conclusions regarding the relationship between methionine and trace metals.

For the three hybrids no significant variation in concentrations of protein, fat, ash, fiber, and three macroelements could be ascribed to different genotypes or ozone exposure conditions. However, starch content of different corn hybrids usually can be expected to vary. Analyses of variance did show that variation among hybrid selections were significant for starch, amylose content of starch, histidine, lysine, zinc, manganese, and chromium.

SUMMARY

This study assessed the effects of chronic levels of ambient ozone in field-grown corn plants, with research emphasis directed toward changes in composition of the edible grain. Overall, general conclusions involving plants grown at ozone levels ranging from 0.02 to 0.15 ppm were that stress effects due to ozone exposure were more readily manifested in the vegetative plant at lower levels of ozone than in the grain. Physical characteristics such as foliar injury, suppression of plant size, and crop yield were easily detectable at lower threshold concentration levels, whereas composition changes in the grain initially occurred at higher ozone levels (0.15 ppm). Compositional changes in grain recovered from plants exposed to all levels of ozone studied were minor for protein, most amino acids, fat, fiber, ash, starch, and three macrominerals (K, P, and Mg). However, levels of certain trace elements (Zn, Fe, Cu, and Cd for two open-pedigreed hybrids) and methionine generally increased with an increase in ambient ozone concentration. Genotype response to the same levels of ozone was different for three corn hybrids.

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