

tion and analysis of all the data obtained under clear or cloudy conditions. The upper half of Table 1 shows the success of the system in alerting the pilot to subsequent encounters; the lower half shows the veracity of the alarms given. The false alarms include early data obtained when instrumentation and system problems were being experienced. The most recent data are from the 1979 CAT-dedicated missions carried out from the NASA Convair 990 aircraft. During these missions 36 turbulence events were encountered and alerts were given for 34 (94.4 percent). A total of 39 alarms were given, of which only five were false (12.8 percent). A comparison of the data from this last period with the total of all data shows a significant improvement in the system.

A typical time distribution of CAT alerts at a fixed altitude is illustrated in Fig. 2. Multiple alerts were received for each event. In 25 CAT encounters at an altitude of 12.5 km, the greatest number of alarms (31 percent) occurred between 2.5 and 4.5 minutes. More than half (57 percent) of the alarms were given between 2.5 and 6.5 minutes. This lead time is adequate for the pilot to warn the passengers and crew or to reduce the speed of the aircraft in order to minimize the impact of the turbulent area.

The CAT missions were flown without a cloud-detecting channel. The importance of eliminating cloud effects in future sensors is illustrated by the data of Table 2, which represent four nonturbulent flights with and without cloud effects. Fourteen false alarms were caused

by clouds. On the basis of these results we conclude that the expected number of false alarms in cloud-free areas is about one per 3½ hours of flight time.

The results of these tests of the infrared radiometer system indicated that CAT may be detected from 2 to 9 minutes before an encounter at least 89 percent of the time at altitudes of 5.8 to 12.5 km. In the absence of general models for accurate ground prediction of CAT, on-board warning of nonconvective turbulence events is the most practical means of obtaining operational air safety.

L. P. STEARNS

*Environmental Research Laboratories,
National Oceanic and Atmospheric
Administration,
Boulder, Colorado 80303*

P. M. KUHN

*Northrop Services, Inc.,
Moffett Field, California 94035*

R. L. KURKOWSKI

*Flight Systems Research Division,
NASA Ames Research Center,
Moffett Field, California 94035*

F. CARACENA

*Environmental Research Laboratories,
National Oceanic and Atmospheric
Administration, Boulder*

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first reported to cause synergistic effects with respect to leaf injury on tobacco (*Nicotiana tabacum* L.) (4). Subsequent reports indicate that plant responses to SO₂ + O₃ may be additive, more than additive, or less than additive, depending upon the species tested and the conditions of the experiments (5). Tingey *et al.* (6) found that only 3 of 11 species tested showed synergistic responses. Pollutant concentrations and SO₂/O₃ ratios also influenced the plant response.

In a field study, Oshima (7) observed a significant exposure dose-response interaction for mixtures of SO₂ and O₃ when the O₃ concentrations were just below the threshold value required to reduce the yield of red kidney bean (*Phaseolus vulgaris* L.). He added SO₂ at the rate of 0.10 part per million (ppm) or 262 µg m⁻³, about 6 hours per day on 45 days over an 11-week period to Los Angeles Basin air. The enhanced toxicity resulted when SO₂ was added to a 50:50 mix of carbon-filtered (CF) and nonfiltered (NF) air providing a total O₃ dose of 95 hours ≥ 0.10 ppm (196 µg m⁻³) (filtration through carbon removes O₃). In another field study, soybean [*Glycine max* (L.) Merr.] plants were exposed to 0.10 ppm of SO₂ in CF air for 6 hours per day for 133 days beginning 14 days after emergence; their yields were not reduced when compared to plants grown in exclusively CF air (8).

We report here results obtained in 1979 at Beltsville, Maryland, using open-top chambers in the field to assess the impact of low SO₂ doses added to Washington, D.C., metropolitan air on the yields of three snap bean (*P. vulgaris*) cultivars. The cultivars were selected because of their known differences in resistance to ambient oxidants. An 8-year field study investigating the susceptibilities of 387 snap bean cultivars and breeding lines to oxidant-induced leaf injury had shown that 70 percent were resistant to the exposures, 22 percent were intermediate, and 8 percent were highly susceptible (9). Open-top chamber experiments, initiated in 1972, indicated that endemic oxidant concentrations in suburban Washington, D.C., lowered the yields of five plant species tested (including snap beans) by an average of 10 percent (10). Mean yields of four snap bean cultivars were reduced 4 percent by ambient oxidants during the 5-year period from 1972 to 1976 (11). We used three of these cultivars, Astro, Bush Blue Lake 274 (BBL 274), and BBL 290, which, based on the yield response, were oxidant-resistant, oxidant-intermediate, and oxidant-susceptible, respectively. Cultivar BBL 290

Photochemical Oxidants Potentiate Yield Losses in Snap Beans Attributable to Sulfur Dioxide

Abstract. *Field-grown snap beans (Phaseolus vulgaris) were given recurring midday exposures to sulfur dioxide in open-top field chambers containing ambient photochemical oxidants. There was a linear correlation (correlation coefficient = -0.99) between increasing concentrations of sulfur dioxide and the yields of snap beans. Synergism was indicated for the mixtures of ambient ozone plus sulfur dioxide, leading to threefold greater yield losses in nonfiltered air than in charcoal-filtered air (to remove the ozone). Even the lowest sulfur dioxide dose in nonfiltered air reduced the yields of Astro, a cultivar that exhibited no visible pollutant-induced foliar injury.*

It is projected that sulfur oxide pollution will become increasingly important as more coal is burned to satisfy the demand for electricity and industrial energy (1). Sulfur dioxide (SO₂), presently the second most important plant-damaging pollutant in the United States (2), is primarily a point-source pollutant. Ozone (O₃), the most ubiquitous and

serious phytotoxic air pollutant affecting crop growth, originates from the action of sunlight on nitrogen oxides and hydrocarbons, products of automobile exhaust (2, 3). Only limited data are available on the impact of mixtures of SO₂ and O₃ on the yields of field-grown crops. Most data relate to foliar injury.

In 1966, O₃ and SO₂ mixtures were

showed foliar oxidant injury every season (9, 11) with an average 5-year yield loss of 14 percent. By contrast, Astro rarely showed oxidant injury to leaves and a significant yield loss occurred in only 1 year.

The following SO₂ concentrations were added to NF air in the open-top chambers: 0, 0.06 ± 0.01, 0.12 ± 0.02, and 0.30 ± 0.03 ppm; SO₂ concentrations in chambers with CF air were 0 and 0.30 ± 0.03 ppm. Treatments were replicated three times. Pressurized SO₂ was diluted with ambient air in a mixing manifold and discharged into the blower ducts of the chambers through Nupro (12) valves and flowmeters. The SO₂ concentrations were monitored with a pulsed fluorescence SO₂ analyzer (model 43 Thermo-Electron). A permeation tube calibrator (model 143 Thermo-Electron) was used to calibrate the analyzer. Eighteen chambers were monitored for successive 6-minute periods. Solenoids for each continuously drawn sample line were controlled by a stepping switch system that regulated the sequential chamber monitoring. The SO₂ dispersing and monitoring system was similar to that described by Heagle *et al.* (13) for O₃ delivery and control. Each cultivar was grown in a row plot 1.8 m by 0.7 m (5 cm between plants), with the cultivars in different quadrants in each replication to avoid location effects (11). The plots were seeded on 26 June. The SO₂ fumigations (6 hours per day, 5 days per week) were initiated 28 days after seeding (24 July) and continued until harvest, 31 days later. Fumigations were not conducted on days with rain or strong winds. Exposures occurred on 24 of 31 days prior to harvest. Pod production and the plant weights without roots were determined; leaf injury was assessed a few days before the harvest (Table 1).

Data on O₃ were obtained from the Maryland Health Department (14), from their site at the National Institutes of Health, Bethesda, Maryland (14 km west of Beltsville), and at our laboratory monitoring site. During June, July, and August, hourly peak O₃ concentrations were 0.14, 0.10, and 0.13 ppm, respectively. Daily maximum values for these months averaged 0.09, 0.08, and 0.09 ppm. Daytime (0900 to 2000 hours) means for the 3 months were 0.045, 0.038, and 0.041 ppm, respectively. Our records indicate that the O₃ values at Bethesda were about the same as those at Beltsville.

The SO₂ reduced snap bean yields more in NF chambers with ambient O₃ than in CF chambers (Table 1); that is, the yield reduction by the highest SO₂

Table 1. Effects of filtering air through activated carbon (to remove O₃) on snap bean yield losses attributable to SO₂ fumigations. The SO₂ was added 6 hours per day, 5 days per week (total, 24 days) between 24 July and 24 August 1979 (harvest date). The average July–August daily maximum O₃ value during the SO₂ fumigation period (0900 to 1500 hours) was 0.065 ± 0.025 ppm. The ambient SO₂ background concentrations averaged less than 0.002 ppm.

Treatment			Plants without roots* (kg/plot, 1.3 m ²)	Pods (kg/plot, 1.3 m ²)	Leaf† injury index (0 to 10)
SO ₂ (ppm)	Chamber air	Oxidants (O ₃)			
0	CF	Low‡	3.91 a	1.41 a	0 a
0	NF	Ambient	3.68 a	1.39 a	1.0 b
0.30 ± 0.03	CF	Low‡	3.51 a	1.19 a	1.7 b
0.30 ± 0.03	NF	Ambient	2.63 b	0.79 b	2.9 c

*Values in each column followed by the same letter are not significantly different at the 5 percent level of probability. †The leaf injury index value multiplied by 10 gives the mean visible injury expressed as a percentage of the leaf area affected. ‡Any chamber O₃ values above 0.015 ppm resulted from some ingress of air through the open tops of the chambers during periods of moderate wind. The charcoal filters removed 80 to 90 percent of the O₃ in air passed through the filters and blowers.

dose in NF air was 43 percent as compared to 16 percent in CF air. The plant biomass (minus roots) was reduced 29 and 10 percent, respectively. There was a negative regression between mean pod yield and SO₂ concentration (Fig. 1). Astro showed a measurable yield reduction in NF air even at 0.06 ppm SO₂.

The combined data for the three cultivars showed a highly significant negative correlation (correlation coefficient $r = -.99$) between mean bean yield and SO₂ concentration. The r value computed for all 36 data points (SO₂ times replications times cultivars), rather than the mean values, was lower but still significant ($r = -.69$). Intercept (a) and slope (m) values calculated for the aggregate data were the same as for the mean data. Testing the regression data for linearity as compared to a quadratic or cubic form showed that the linear regression exhibited the best fit to the experimental results. This was true for the individual cultivars as well as for the combined data

which reflected species response. No interaction was found for cultivar times SO₂, an indication of the homogeneity of the slopes for the three cultivars. Values of a , m , and r for the individual cultivars and for the combined data are shown in Fig. 1.

Astro, which exhibited no recognizable pollutant-induced foliar injury, was of special interest because the yield reduction under the conditions of the study was at least as great as that shown by two visibly injured cultivars. The symptoms of foliar injury, especially on BBL 290, resembled those of oxidant injury. A recent study rating injury on leaves of young seedlings caused by single exposures to O₃, SO₂, and mixtures of these two gases showed Astro to be the only one of 33 bean cultivars tested that showed no leaf injury (15). Our results with Astro indicate that such foliar injury ratings on young plants have little value in predicting the resistance of bean cultivars to yield losses after recurrent exposures to low doses of SO₂ and O₃. Even for older field-grown plants, the yield reductions were not closely related to foliar injury.

Our data reveal potentiated dose-response effects for SO₂ and O₃ mixtures. Mean yield reductions occurred even with the lowest SO₂ dose, although oxidants alone had no measurable effect on yield. At 0.30 ppm, SO₂ reduced yields threefold more in NF air than in CF air. It is possible that Heagle *et al.* (8) would have observed reduced soybean yields if the 0.10 ppm SO₂ had been added to NF air rather than to CF air. Since O₃ is so ubiquitous, experiments to determine the effects of SO₂ on crop yields will not reflect field conditions if SO₂ is added only to CF air.

The SO₂ concentrations used in this study can be found downwind of some large smelters and coal-fired electric

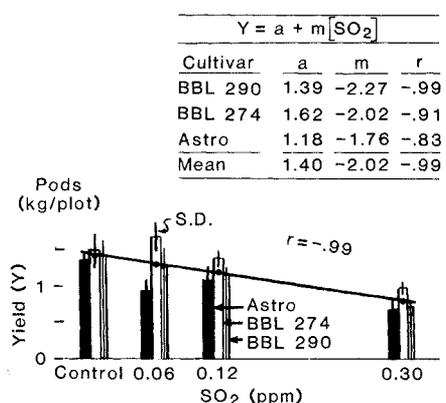


Fig. 1. Yields of three snap bean cultivars and combined mean yield for the species plotted as a function of the SO₂ concentration. Peak SO₂ concentrations in the control plots were ≤ 0.01 ppm; S.D., standard deviation. Inset: Regression parameters for the individual cultivar means and the combined cultivar means.

power-generating plants; however, the daily exposure duration and frequency used for at least the two highest concentrations, 0.12 and 0.30 ppm, are more extreme than is likely to occur at present in most industrial areas. The linear relationship between SO₂ concentration and mean yield loss for the three cultivars (Fig. 1) suggests that in seasons with favorable growing conditions, as in 1979 at Beltsville, SO₂ doses even lower than our minimum could reduce the productivity of susceptible crops when present in combination with ambient oxidants now present in the mid-Atlantic seacoast region. The yield data indicate that, of the three cultivars tested, BBL 274 has the most tolerance to mixtures of O₃ and SO₂.

HOWARD E. HEGGESTAD
JESSE H. BENNETT

Plant Physiology Institute, Science and Education Administration, Department of Agriculture, Beltsville, Maryland 20705

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6 March 1981; revised 15 May 1981

Morphine in Cow and Human Milk: Could Dietary Morphine Constitute a Ligand for Specific Morphine (μ) Receptors?

Abstract. Morphine has been found in cow and human milk at concentrations of 200 to 500 nanograms per liter. Multistep purification yields a material that has immunological, biological, pharmacological, and chemical properties identical to those of morphine. Similar morphine-like material, which has been tentatively identified in some common plant sources, may be a ubiquitous dietary constituent and a possible source for the material in milk. Since morphine (μ) receptors have a low affinity for enkephalins, and since morphine-like materials have been described in brain and intestine, it is possible that morphine in food may be the source of this material and a normal ligand specific for μ receptors.

Several hormones are present in the milk of human and other mammals (1-3). Recently, compounds displaying opioid activity in the receptor binding assay and guinea pig ileum were reported in chloroform-methanol extracts of lyophilized bovine milk (4). Further purification resulted in several active compounds, and the major one displayed opioid activity in the guinea pig ileum, a tissue that contains predominantly μ or morphine receptors (5, 6), but not in the mouse vas deferens, which contains predominantly δ or enkephalin receptors (5, 6). Because the amounts of these opioid activities were highly variable in milk (4, 7), a substance with opioid activity was isolated from an enzymatic digest of casein (7) in the belief that it represented a similar, peptic material (8). This compound, a heptapeptide named β -casomorphin (7), exhibited very low opioid activity (250 times less potent than normorphine in the ileum assay), whereas the NH₂-terminal pentapeptide exhibited higher activity (22 times less potent than normorphine). We recently reported (9) that the synthetic peptide NH₂-Tyr-Pro-Phe-Pro-NH₂ (morphiceptin), the tetrapeptide amide of β -casomorphin, also found in enzymic digests of β -casein, has morphine-like activities and is specific for μ receptors but not for δ receptors. We now report the presence of the alkaloid morphine in cow and human milk.

Morphine was first detected in lyophilized cow's milk that was homogenized (Polytron) with 2N glacial acetic acid in

methanol and then filtered. Amounts measured by a radioimmunoassay (RIA), which is specific for morphine (10), ranged between 0.3 and 5 ng per gram. In a typical experiment to further identify the morphine, 100 g of powdered milk or lyophilized skim milk (1 liter) was homogenized in 1 liter of 2N glacial acetic acid in methanol for 10 minutes at room temperature, filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure. Recovery at this step, with [³H]dihydromorphine as a marker, is around 90 percent. Although morphine is very soluble in chloroform and methanol, these solvents were much less effective for extraction, which suggests strong interaction with the proteins in milk. After extraction, additional protein was precipitated by the addition of acetonitrile (10 percent by volume). After centrifugation, evaporation of acetonitrile, and filtration the sample was applied on a preparative high-performance liquid chromatography (HPLC) column (11); the active fractions (H₂O and acetonitrile, 1:1 by volume) were lyophilized and further purified as described in Fig. 1. Although purification resulted in very low (about 4 percent) yields, the material was a single compound that had the same retention time as morphine in four different HPLC systems. (In addition to the procedures described in the legend to Fig. 1, the following were used: Whatman Partisil ODS and a mobile phase with 8 percent acetonitrile and 92 percent 10 mM am-

Table 1. Characteristics of morphine present in milk.

Property	Morphine in milk*	Morphine
Cross-reactivity with antiserum to morphine	Positive	Positive
Cross-reactivity with antiserum to codeine	Negative	Negative
IC ₅₀ † for δ receptors‡	33 nM	35 nM
IC ₅₀ for μ receptors§	0.7 nM	0.5 nM
ED ₅₀ in guinea pig ileum	0.9 μ M	0.9 μ M
Retention time in four HPLC systems	Identical to morphine	
Molecular ion	285.1365	285.1365

*Concentrations were calculated as equivalent to morphine by RIA. †Concentration of ligand causing 50 percent inhibition of specific binding. ‡Binding assays with [¹²⁵I]-labeled [D-Ala², D-Leu⁵]enkephalin (14). §Binding assays (14) with [³H]naloxone (0.38 nM). ||The concentration causing 50 percent depression of smooth muscle contractions.