AMRL-TR-78-71

ADA065563

THE EFFECT OF DESIGNATED POLLUTANTS ON PLANTS

Third Annual Report

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NOVEMBER 1978

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AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

NOTICES

TECHNICAL REVIEW AND APPROVAL

AMRL-TR-78-71

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

A. A. Thomas

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

AIR FORCE/56780/5 February 1979 — 100

SECURITY CLASSIFICATION OF THIS PAGE (*When Data Entered*)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AMRL-TR-78-71	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (*and Subtitle*) The Effects of Designated Pollutants on Plants Third Annual Report		5. TYPE OF REPORT & PERIOD COVERED Annual Report 1 July 1977 - 30 June 1978
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) A. L. Granett O. C. Taylor		8. CONTRACT OR GRANT NUMBER(s) F-33615-76-C-5005
9. PERFORMING ORGANIZATION NAME AND ADDRESS The Regents of the University of California University of California, Irvine Irvine, Orange County, California 92664		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62202F 6302/04/17
11. CONTROLLING OFFICE NAME AND ADDRESS Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, OH 45433		12. REPORT DATE November 1978
		13. NUMBER OF PAGES 74
14. MONITORING AGENCY NAME & ADDRESS(*if different from Controlling Office*)		15. SECURITY CLASS. (*of this report*) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (*of this Report*)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (*of the abstract entered in Block 20, if different from Report*)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (*Continue on reverse side if necessary and identify by block number*)

Air Pollution	Humidity	Diurnal
Missile Exhaust Products	Solid Rocket Fuel	Seasonal
Hydrogen Chloride (HCl)	Dew	Vegetable Crops
Aluminum Oxide Particles (Al_2O_3)	Exposure Chambers	Phytotoxic Responses
Developing Seeds	Ornamental Plants	Probit Analysis

20. ABSTRACT (*Continue on reverse side if necessary and identify by block number*)

The phytotoxicity of hydrogen chloride (HCl) gas and aluminum oxide (Al_2O_3) particulates was studied in special plant exposure chambers. These pollutants were generated separately by diluting bottled gas or commercial alumina dust. In addition, generation was affected by open-burning of small pieces of solid rocket fuel. The characteristics of these burn products were investigated. Rocket fuel gases produced phytotoxic responses similar to that seen on plants exposed to commercial HCl gas. It was concluded that under conditions tested,

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-2- Abstract Continued

Al_2O_3 dust does not cause visible plant injury nor, when dust is mixed with HCl gas, is there any significant increase in plant damage compared to HCl gas alone. The effects of relative humidity (RH) and dew were studied; injury increased at elevated RH levels. Leaves with dew present before and during exposure to HCl had more damage than leaves without dew or those receiving dew after HCl exposure. It was found that seeds placed on filter paper exposed to high levels of HCl did not develop normally. Seeds on soil were not so affected although both soil and filter paper were shown to adsorb HCl gas from the chamber atmosphere. A number of plants were tested for sensitivity to HCl gas and compared by probit analysis. Seasonal and diurnal changes in plant sensitivities were investigated; light was an important factor in plant response. A cooperative experiment with another research group provided confidence in facilities, methods, and grading techniques.

PREFACE

This is the third annual report of work performed under the Environmental Toxicology Research sponsored by Air Force Contract F-33615-76C-5005 to the University of California, Irvine. The work under this portion of the contract covers the period from July 1, 1977 to June 30, 1978, and was conducted by members of the Statewide Air Pollution Research Center, University of California, Riverside. The study is a continuation of work designed to aid Air Force personnel to recognize and predict the phytotoxic responses of terrestrial plants to air pollutants released by Air Force operations. The study is concerned with the principal exhaust components of one form of solid rocket fuel: gaseous hydrogen chloride and aluminum oxide particulates. The investigations reported here were conducted under greenhouse and laboratory conditions to control external variables as much as possible. The plants studied included species grown commercially and those native to the vicinity of Vandenberg Air Force Base, California.

The cooperation and aid of Air Force contract monitors, Lt Colonel R. C. Inman and Major C. B. Harrah, Toxic Hazards Division, AMRL, Wright-Patterson Air Force Base, Ohio, has been appreciated. The authors also wish to acknowledge the critical advice of R. J. Oshima and A. G. Endress of the Air Pollution Research Center and the able technical assistance of D. Duncan, L. A. Neher, C. L. Simpson and D. A. Small during various parts of the project. The assistance of University of California students A. M. Edwards, S. K. Hollingsworth, R. Kizer, D. H. Lick, and M. R. Schulte has also been appreciated. A portion of this work was carried out at North Carolina State University with the cooperation and help of Drs. W. W. Heck and W. M. Knott and their staffs. Dr. L. D. Strand, Jet Propulsion Laboratory, Pasadena, kindly supplied solid rocket fuel and information on its composition, characteristics, and general handling.

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INTRODUCTION

This project is part of the study on the effects of potential environmental pollutants released through Air Force operations on terrestrial and aquatic organisms. This particular phase of the study is to determine the effects of hydrogen chloride (HCl) gas and aluminum oxide (Al_2O_3) particulates on selected plant species. These potential pollutants are formed as by-products when solid rocket fuel burns (Goldford, 1976). Specifically, we are concerned with the phytotoxicity of gases and particulates released by the large Space Shuttle lift-off booster rocket engines on vegetation in the vicinity of the launch (Anonymous, 1976).

Our basic approach continued to be to expose plants to known concentrations of the pollutant in special chambers located in a greenhouse where most plants were grown (Granett and Taylor 1976, 1977). The chambers were supplied pure HCl gas diluted with filtered greenhouse air. Studies were also conducted using solid rocket fuel (SRF) as a source of pollutant. The Al_2O_3 was sized and diluted into the intake air stream with a special generator (Neher et al., 1977).

Pollutant concentrations were measured with wet chemistry techniques and a Geomet HCl monitor, using the chemiluminescent principle (Gaarder and Jensen, 1977, Gregory et al., 1974, Susko, 1977). The Geomet underwent extensive tests to determine parameters of reliability.

Environmental conditions such as light, temperature, and humidity can affect the reaction of a plant to air pollution stresses (Guderian, 1977). By minimizing changes in these factors as much as possible, the plant responses could be better determined. These conditions are interrelated and change diurnally and seasonally. Attempts were made to characterize the interaction of environment and phytotoxicity.

Plant response was measured by symptoms visible 24 to 48 hours after exposure. A common initial reaction was severe wilt which sometimes disappeared but normally developed into bifacial necrotic areas with abaxial glazing found after less severe episodes. Probit statistics were used to analyze twenty species. The probit technique allowed comparisons between species and among varieties. Methods of injury estimation were compared by workers in California and in North Carolina to determine the extent of personal biases in grading symptoms.

MATERIALS AND METHODS

Exposure equipment

The equipment used for exposing plants has been described elsewhere (Granett and Taylor, 1977). This included one rectangular and two cylindrical chambers. The 0.6 m^3 rectangular chamber was constructed of Lexan plastic. Filtered greenhouse air was forced through a small conditioning chamber into the large chamber with a high velocity fan. An exhaust fan drew the air through at nearly two air changes per minute at a slight (1/4-inch water) negative pressure. HCl from a large tank of dry gas was metered into the intake air stream. For one experiment, gas volatilized from an acid solution was introduced into the air stream.

Two 1.05 m^3 cylindrical chambers, constructed with a steel frame and Teflon film, were used for most exposure work (Jeffries et al., 1976). These chambers had a common exhaust fan providing two air changes per minute. Dry HCl gas flowing to the intake of either chamber was controlled with fine adjustment valves.

Mixing paddles were installed in both the rectangular and cylindrical chambers to stir the gases; these rotated at 120 rpm during each exposure. The Al_2O_3 generator (Neher et al., 1977; Granett and Taylor, 1977) fed dust to the intake of one of the cylindrical chambers. Solid rocket fuel was occasionally burned in these chambers to provide both HCl and Al_2O_3 simultaneously.

The large cylinder of dry, 40% HCl in nitrogen was contained in an insulated shield outside the greenhouse as a safety precaution. Heat tape and a thermostat kept the cylinder at or above 15C during the winter. After fumigations were completed for the day, nitrogen gas purged residual HCl from the supply tubes, the tank regulator, and the flowmeters.

HUMIDITY EXPERIMENTS

For increased humidity in the exposure chambers, a live steam line was fed into a 0.14 m^3, wooden-framed mylar-covered pre-chamber (0.4m x 0.6m x 0.6m) and the moistened air was forced through extensions of the intake tubes of the cylindrical chambers with a high velocity fan. Either one or both of the cylindrical chambers received the moist air. Chamber air flow was adjusted as necessary when the high humidity air duct was in place by mechanically reducing the exhaust flow rate or by increasing the velocity of the input fan. Relative humidity in the cylindrical chambers was measured by wet and dry bulb thermocouples in the exhaust lines.

Dew experiments were performed in the evening or early morning when natural dew might be expected. Plant leaves were thoroughly wetted by incubation in a Percival DC-20 Dew Chamber. This took 20 to 120 minutes depending on environmental conditions and dew chamber adjustments.

SOLID ROCKET FUEL

Solid rocket fuel (SRF) was made available by Jet Propulsion Laboratory, Pasadena, so that the phytotoxic effects of actual fuel exhaust products could be investigated. The fuel was supplied in various shapes; it was easily cut to pieces having a 8 mm diameter and as long as desired. Concentrations were determined by fuel weight. The first experiments were made with fuel coated with a burn retardant. The SRF composition (Table 1), the retardant material (Table 2), and the expected exhaust products (Table 3) were known (Nadler, 1976; Strand, 1977). The fuel burned completely after ignition at 250-300C. In unconfined, open burns such as these, the pieces sputtered and sparkled.

To start the fuel burning, model rocket ignitors constructed of thin nichrome wire were inserted into a slit in the SRF piece. The ignitor and fuel were attached to alligator clips on a screen- and asbestos-enclosed platform within the exposure chamber. Wires from the clips to an ignition box completed the power circuit (Figure 1). The circuit was tested with a continuity checker and, if satisfactory, ignition proceeded. A 6 volt lantern battery provided enough current to heat the nichrome and ignite the fuel.

TABLE 1.
COMPOSITION OF SOLID ROCKET FUEL

Oxidizer	
Aluminum chloride	69.80%
Aluminum	12.00%
Burn Rate Modifier	
Iron oxide	0.20%
Binder	
Polybutyldyn acrylonitrile (PBN)	12.04%
Curing Agent	
Dow Epoxy Resin - 331	1.96%

TABLE 2.
COMPOSITION OF BURN RETARDANT OF SOLID ROCKET FUEL

Methyl ethyl ketone (MEK)	87%
Ethyl cellulose (Ethocel)	10%
Tri-cresyl phosphate	3%

TABLE 3.
THEORETICAL EXHAUST PRODUCTS OF SOLID ROCKET FUEL

Product Species	Product Weight (Grams per 100g consumed propellant)
HCl	20.90
Cl_2	0.06
CO	24.37
N_2	8.50
H_2O	10.39
H_2	2.11
CO_2	4.32
OH & H	0.02
Solid Particulates	
Aluminum oxide	28.34
Aluminum chloride	0.02
Iron chloride	0.97

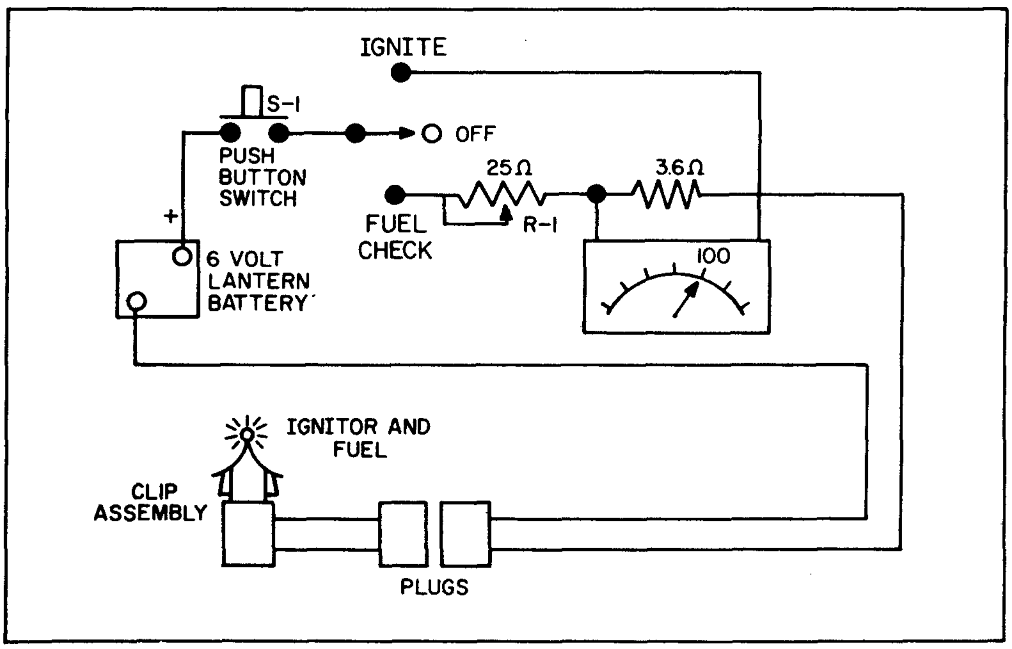

Figure 1. Circuit diagram of fuel circuit continuity testor and ignitor. R-1 is adjusted so meter reads 100 when clip assembly is shorted and both S-1 and FUEL CHECK switches are closed. After fuel is in place continuity is satisfactory if meter reads 90-100 with S-1 and FUEL CHECK switches closed. Fuel is ignited by closing S-1 and IGNITE switch. Circuit designed and built by L. M. Kienitz, Statewide Air Pollution Research Center staff.

DATA MEASUREMENTS

HCl gas concentration in the chamber atmosphere was measured most reliably by bubbling 15 liter air samples through 20 ml of 0.01 N nitric acid. Chamber air was drawn by means of a small vacuum pump through the air-scrubbing bubbler and a Precision Scientific Wet Test Meter. The bubbled solution was analyzed for chlorine with an Aminco Model 4-4433 automatic titrator. HCl concentrations were calculated as mg HCl per m^3 chamber air. At Riverside, California, 1 ppm HCl equals 1.52 mg HCl m^{-3}. At very high (at or over 85%) humidity levels, it was found useful to place the bubbler system within the chamber to avoid excessive moisture buildup. A Geomet model 401S HCl monitor was also available for measuring gas concentration. This instrument was used with some success after determining its limitations, conducting meticulous calibrations, and taking various precautions (Dawburn and Kinslow, 1976). The Geomet was not operated at very high humidity levels.

Temperatures were measured on a mercury thermometer hanging inside each chamber. Relative humidity (RH) was calculated by reading the output of wet and dry bulb thermocouples in the chamber exhaust line, by a sling psychrometer, or by a battery operated psychrometer. Light intensity was measured on a Yellow Springs Instrument Co. model 65 Radiometer, and light in the photosynthetically active region (PAR) was measured with a Li-Cor Radiometer model LI-185.

PLANT PRODUCTION

All plants in studies described in this report were grown in a greenhouse located at the University of California, Riverside, equipped with charcoal air filters, evaporative coolers, and steam heat. The environment was further modified by blowers and window white-washing as needed during the summer. Daytime temperature maxima were between 34 and 40C while night temperatures reached 18 to 23C. Where feasible, a drip system supplied deionized water. Plants were fertilized once to several times a week with a complete nutrient solution described by Hoagland and Arnon (1950). The plants grew in sterilized UC Mix II containing equal parts of sandy loam, peat, and redwood chips (Lerman, 1977).

Plants used in the study are listed in Table 4. Some were used in previously described investigations (Granett and Taylor, 1977). Citrus plants from earlier experiments were grafted with orange or lemon buds. When growth of these buds had proceeded, plants were exposed again. Grape plants were established from dormant shoot cuttings.

Plants were exposed to HCl gas in a set manner. They were watered prior to exposure, care being taken to avoid wetting the leaves. After exposure the plants were removed to greenhouse benches. Stress was often seen as a transitory wilted condition. Injury, occurring immediately to 24 hours post-exposure, was manifested as abaxial glazing or bifacial necrosis depending on exposure conditions and plant sensitivity. Injury was recorded up to 48 hours post-exposure since symptoms did not disappear.

TABLE 4.
LIST OF PLANT SPECIES AND VARIETIES USED IN PHYTOXICITY STUDIES

Plant	Scientific name	Variety
Aster	*Callistephus chinensis* (L.) Nees	Early bird white
Avocado	*Persea americana* Mill.	Haas and Bacon
Barley	*Hordeum vulgare* L.	CM 67
Bean	*Phaseolus vulgaris* L.	Pinto, U.I. III
Briza	*Briza maxima* L.	Ornamental quaking grass
Calendula	*Calendula officinalis* L.	Flame beauty
Citrus	*Citrus limo* (L.) Burm. f.	Rough lemon seedlings Lisbon lemon
Citrus	*Citrus sinensis* (L.) Osbeck	Valencia orange
Coreopsis	*Coreopsis grandiflora* Nutt.	Sunburst
Grape	*Vitis vinifera* L.	Johannesberg Reisling Cabernet Sauvignon
Marigold	*Tagetes patula* L.	French dwarf double goldie
Marigold	*Tagetes erecta* L.	American,Senator Dirksen
Petunia	*Petunia hybrida* Vilm.	White cascade
Radish	*Raphanus sativus* L.	Comet
Salvia	*Salvia splendens* Ker-Gawl	Patens
Tomato	*Lycopersicon esculentum* Mill	Ace
Wallflower	*Cheiranthus allioni* L.	Golden bedder
Zinnia	*Zinnia elegans* Jacq.	White gem, Cherry gem

INSTRUMENTATION AND CALIBRATION

GEOMET HCl MONITOR

The HCl chemiluminescent monitor, Geomet model 401S, has provided very useful real time data on fluctuation in HCl concentration in our exposure chambers. The concentration measured, however, did not always agree with bubbler samples taken during the same exposure. To determine the reliability of the Geomet, a calibration board was built (Figure 2) that provided a constant source of HCl gas. The gas was metered from a pressurized tank containing about 150 mg HCl m^{-3} in nitrogen through a valve and flowmeter into a tube where the HCl was diluted with compressed air before delivery to a 5.8 liter bell jar chamber. There were small stirring paddles to mix the gas within the chamber. Gas in the bell jar could be sampled by the Geomet or a bubbler.

After construction, the calibration board was tested for variability in delivery and for bubbler efficiency. Variability was tested at two HCl flow rates. Three bubbler samples were taken at each flow setting and all six measurements were replicated three times. The results showed considerably more variation (9.3/7.3 = 1.3) at the higher concentration (Table 5). Due to coarse flowmeter valves, the calibration board could not be reset to exactly the same value, but at any particular setting gas concentration remained reasonably constant.

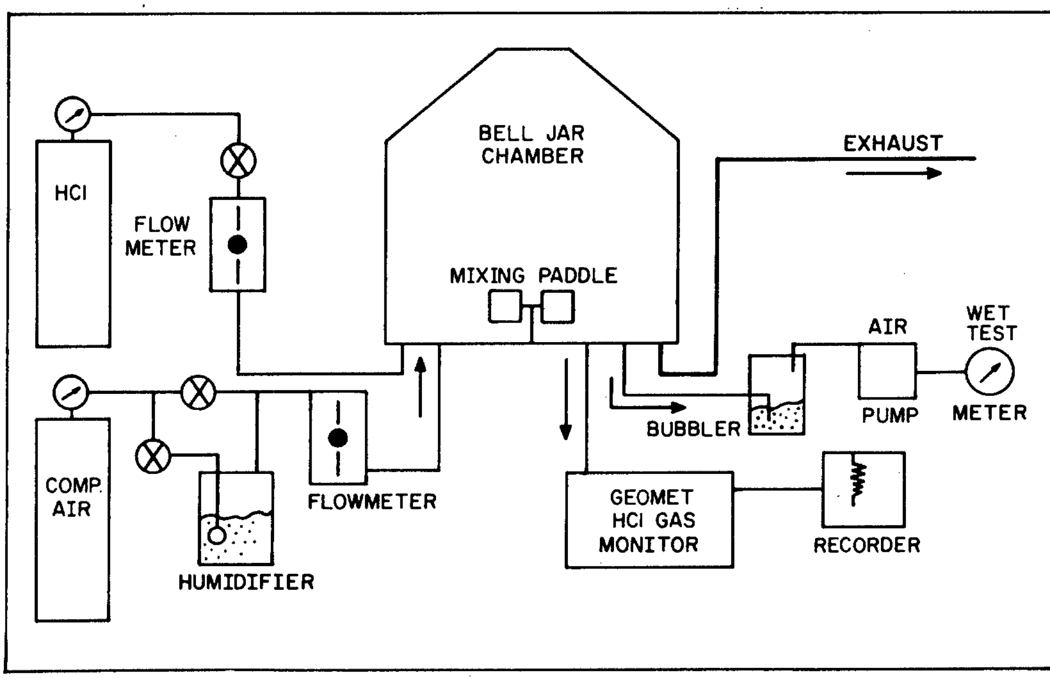

Figure 2. Schematic diagram of the Geomet calibration test panel.

TABLE 5.
VARIABILITY IN THE HCl CALIBRATION BOARD USING BUBBLER MEASUREMENTS

($\bar{x}$) HCl Concentration ($mg\ m^{-3}$)	Standard Deviation (s)	Coefficient of Variability [$cv = (\frac{s}{x})\ 100\%$]	Variance $\left(S\bar{x} = \sqrt{\frac{s^2}{n}}\right)$
16.91[1]	1.24	7.33%	0.41
53.28	4.96	9.31%	1.65

[1]Mean value of 9 samples

The bubbler efficiency was tested by installing a second scrubber in series with the board bubbler or by having 0.1N NaOH soaked and dried filter paper between the bubbler and the wet test meter. For increased sensitivity, 30 liter air volumes were drawn and 15 ml titration samples were run instead of the normal 15 liters of air and 5 ml samples. Tests were run at 17 and 53 mg HCl m^{-3} chamber concentration. In no case could any HCl be detected in the second bubbler or in elutions of the filter paper.

The Geomet operated by drawing air samples through a coated ceramic tube. Reactions in the tube produced detectable chemiluminescence when HCl was present. The ceramic tube is charged by allowing a coating solution (Table 6) to partially dry in it. Once charged, the tube reacted with HCl for a limited concentration-time period after which recharging was necessary. The 20 minutes drying time recommended in the Geomet instruction manual seemed excessive.

TABLE 6.
CERAMIC TUBE COATING SOLUTION FOR GEOMET HCl MONITOR

$NaBrO_3$	6 g
NaBr	29 g
$LiCl_2$	2 g
Double distilled water	to make 100 ml

Newly coated tubes were allowed to dry for 0.5, 1, or 20 minutes, to test most efficient drying time. The elapsed time needed for a 10% decay in reading was recorded (Table 7) and the results indicated that fast drying times did not shorten tube life. Four different tubes were tested. The ceramic tube usually attaches directly into a port of the luminescence cell; however, for convenience a short, flexible Teflon tube was placed between our instrument and its detector tube. This Teflon tube did not significantly affect the ceramic tube decay time (Table 7).

TABLE 7.
CERAMIC TUBE DRYING TIME EFFECTS GEOMET RESPONSE

Drying Time (minutes)	Time (min.) to reach 10% decay: Ceramic Tube Alone	Time (min.) to reach 10% decay: Ceramic Tube With Teflon Extension
0.5	8.25	6.75
1.0	8.13	8.00
20.0	6.63	7.75

The useful charge life was measured at four HCl concentrations with four tubes. The response of the tubes varied but all exhibited decreased service life as the gas concentration increased (Table 8).

TABLE 8.
USEFUL LIFE OF FOUR CERAMIC SAMPLE TUBES

HCl Concentration ($mg\ m^{-3}$)	Time (min.) to reach 10% decay				
	A	B	C	D	Average
11	22	25	19	39	26
42	6	6	10	15	9
61	4	4	8	10	6
78	2	2	5	7	4

Once the board and sample tubes were calibrated, attention focused on developing a procedure to calibrate the Geomet. The instrument was allowed to warm up for 15-30 minutes, the readout was zeroed with intake pumps off, and the calibrate light output was recorded. The calibration board generated a known concentration of HCl gas which was established with bubbler samples then measured with the Geomet. The Geomet pump was shut off after reading was made and the zero was re-established as needed. Using the ratio:

$$RR = \frac{\text{Known HCl Concentration}}{\text{Instrument Reading}} \times \text{Calibrate Light Reading}$$

the Geomet was adjusted to read RR for the new calibrate light readout. Zero, HCl source, and calibrate light were checked again until HCl readings were stable.

Each test day the Geomet was calibrated by the above procedure. The calibration board was set at about 95 and 10 mg m^{-3} and gas was measured twice at each setting by the Geomet and bubbler. This operation was repeated four times during the day and replicated five times over a three week period. The results (Figure 3) indicated Geomet and bubbler sample agreement at low concentrations. At high concentrations the Geomet and bubbler differed noticeably. Some problems in achieving reproducible high HCl levels could be traced to declining tank concentration over the three week period and unsteady high flowmeter settings. The Geomet, however, should have followed the large changes in HCl concentration and should not have differed from the bubbler samples as greatly. The grouping of the Geomet measurement may indicate an upper limit of Geomet response had been reached.

SOLID ROCKET FUEL STUDIES

Scope

Studies were initiated to investigate the generation of HCl and Al_2O_3 by burning SRF. Technical manipulation of the material was straightforward and was described above. Present studies included the particle morphology, static chamber gas concentration, particle adsorption or deposition, and phytotoxicity under regular and dew conditions.

SRF morphology

Al_2O_3 particulates generated by SRF were compared to manufactured dust by microscopically viewing material collected on Gelman AE-glass fiber and 0.2 μ Nucleopore filters. The dust generator and gas system supplied HCl gas plus Al_2O_3 particulate at about 20 mg m^{-3} each in the chamber atmosphere. A vacuum pump drew a sample of the atmosphere through the two filters. After the generators were turned off and the chamber was exhausted, a 473 mg piece of SRF was burned. Chamber particles were collected on a new set of filters.

Pieces of the filters were mounted on aluminum plugs and a thin layer of gold was evaporated onto the sample in preparation for viewing in a JEOLCO model U-3 scanning electron microscope. The fuel generated prodigious amounts of Al_2O_3 and the filters were heavily loaded with particulates (Figures 4A, 5A, and 6). Observations were made at aggregation edges so that individual pieces could be resolved. The particles were mostly very tiny, 2 μ or less, and often aggregated into linear arrays similar to that reported by Nimo (1974a). They appeared to stick to the glass fibers (Figure 5A). The manufactured Al_2O_3 had the form of larger, looser, uneven masses 5 μ or larger in diameter which did not cover the glass fibers (Figures 4B and 5B). On the filter exposed to fuel gases, large spheres about 150 μ diameter were occasionally found (Figure 6). The spheres sometimes had smaller particles on the surface and inside, the latter visible through surface cracks. Dawburn and Kinslow (1974) described similar particles which those authors thought to be formed in the high heat of burning. Our observations suggested the Al_2O_3 produced by actual burns appear similar but smaller than the commercial material used in our previous studies.

Chamber concentration and decay after SRF burn

HCl concentration was measured in the Lexan chamber after various amounts of fuel were burned using the Geomet chemiluminescent HCl monitor (Table 9). The Geomet showed that the chamber concentration rapidly declined after ignition (Figure 7). Several burns of about 400 mg each were made and different portions of the decay were monitored with both bubbler samples and the Geomet (Table 10). The bubbler sampled 10 liters of air in 2 minutes. Both bubbler and Geomet indicated almost no detectable HCl 10 minutes after ignition.

Burns in this series indicated that more complete mixing occurred if the stirring paddles were rotating. When the stirring paddles were off, maximum concentration peak was delayed nearly 40 seconds after 103 mg fuel was burned and peak was not as high nor was decay as rapid as expected (Figure 7B). Stirring paddle efficiency was further demonstrated by comparing SRF burns with dry gas (Table 11). For the dry gas, the chamber was brought to a specific concentration, allowed to equilibrate with the exhaust fan operating and then, at time zero, the gas and fan were

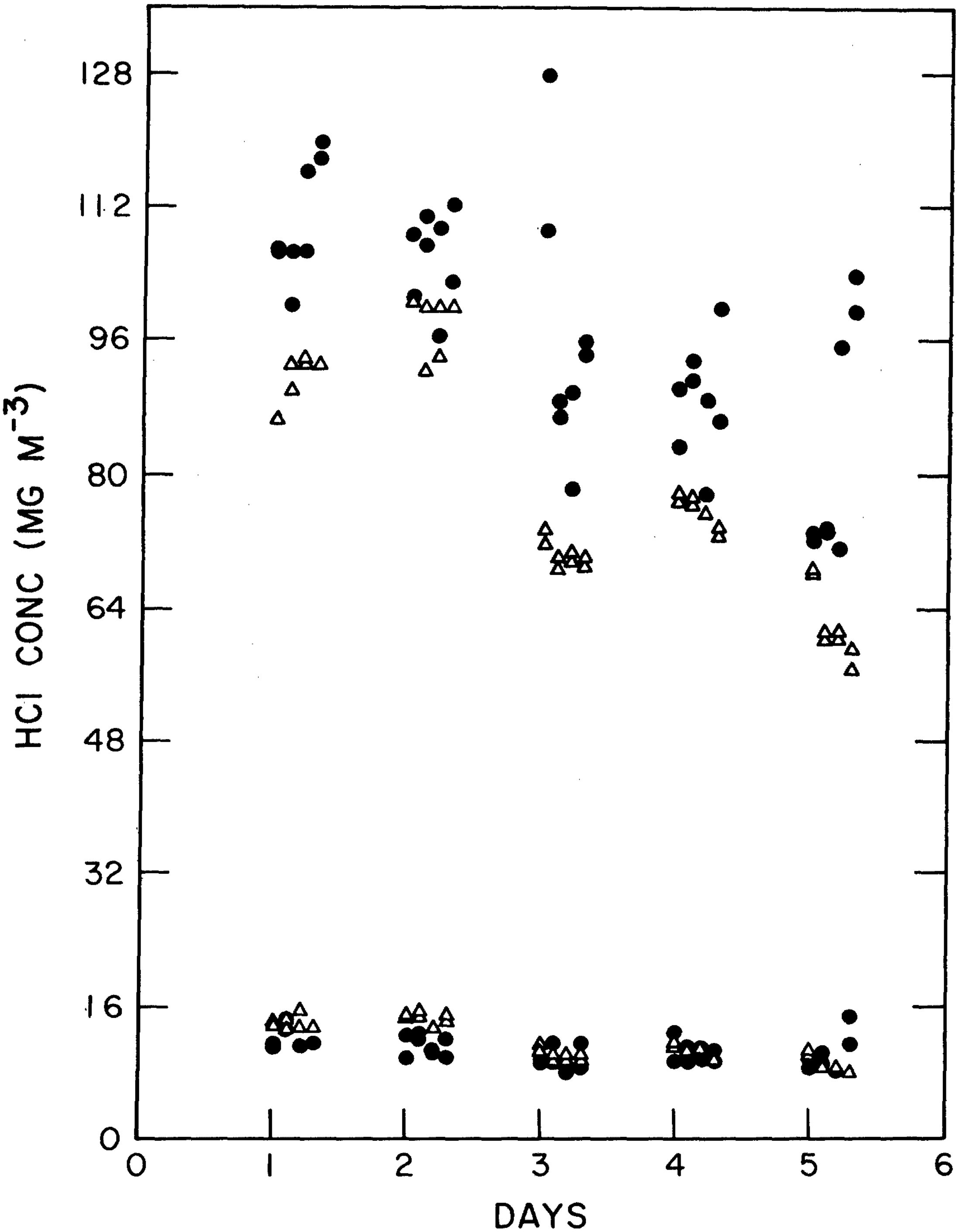

Figure 3. Geomet calibration analysis. Concentrations measured with Geomet HCl monitor (Δ) and bubbler (•) at a high and low HCl concentration on 5 different days at 4 times during each day and two measurements per concentration level for each time.

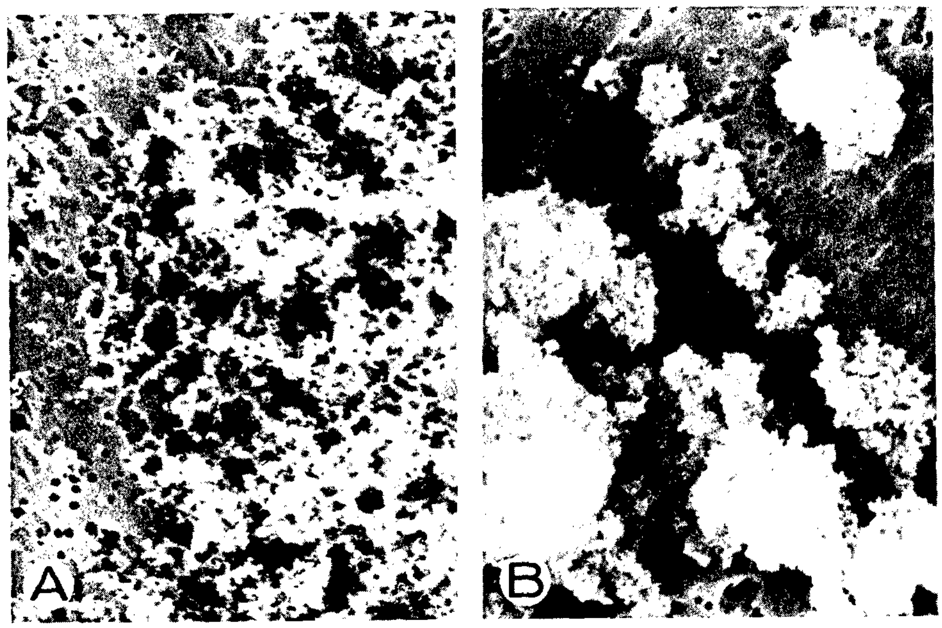

Figure 4. Scanning electron micrographs of Al_2O_3 particles on Nucleopore filters. A. SRF burn; B. HCl gas and commercial Al_2O_3 dust product. Magnification is about 5000x.

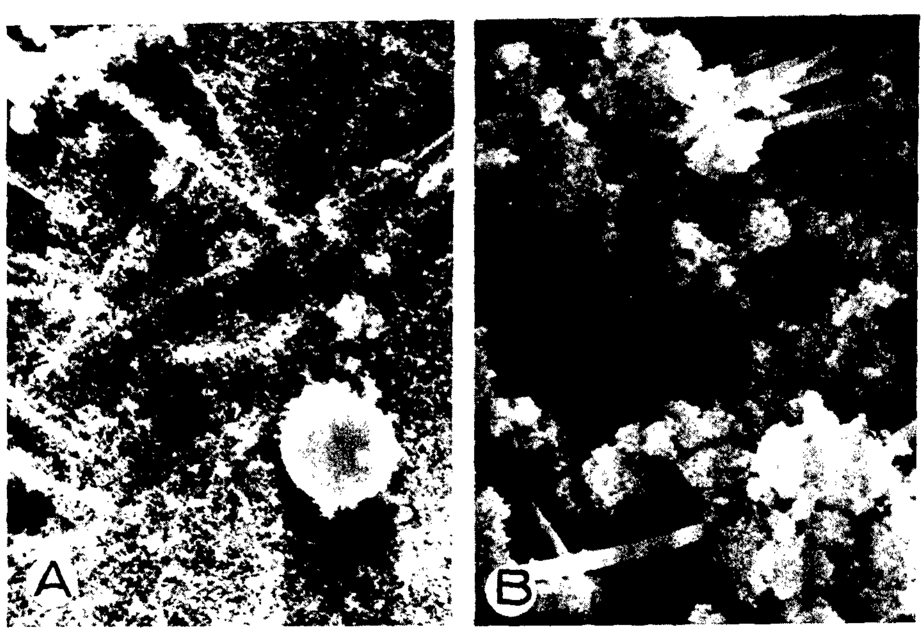

Figure 5. Scanning electron micrographs of Al_2O_3 particles on AE-glass fiber filters. A. SRF burn; B. HCl gas and commercial Al_2O_3 dust product. Magnification is about 5000x.

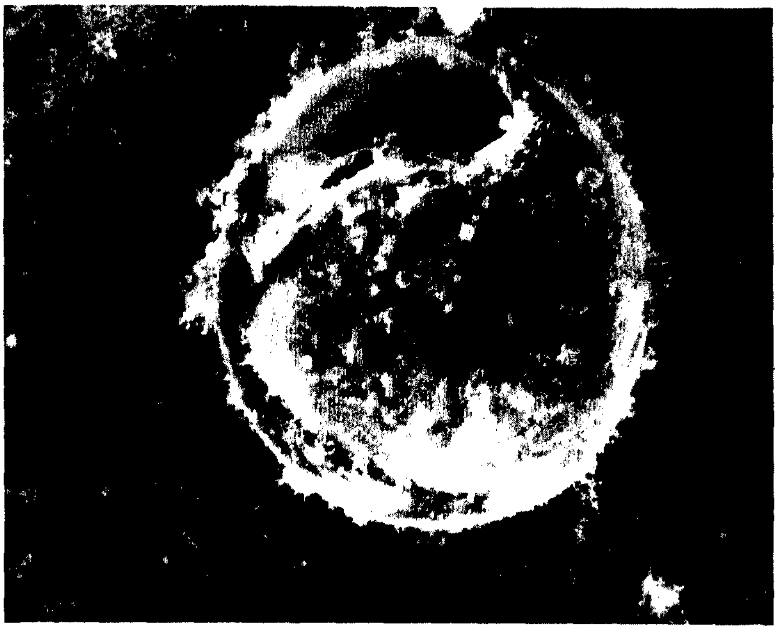

Figure 6. Scanning electron micrograph of a large particle found in the product of a SRF burn. Magnification is about 4000x.

TABLE 9.
MAXIMUM HCl GAS CONCENTRATION DETECTED BY GEOMET AFTER IGNITING SRF

Fuel Weight (mg)	Maximum HCl Concentration (mg m^{-3})
72	7
103	7
221	34
405[1]	40[1]
512	94
727	117

[1]Average of 5 different burns, all others are data of one burn

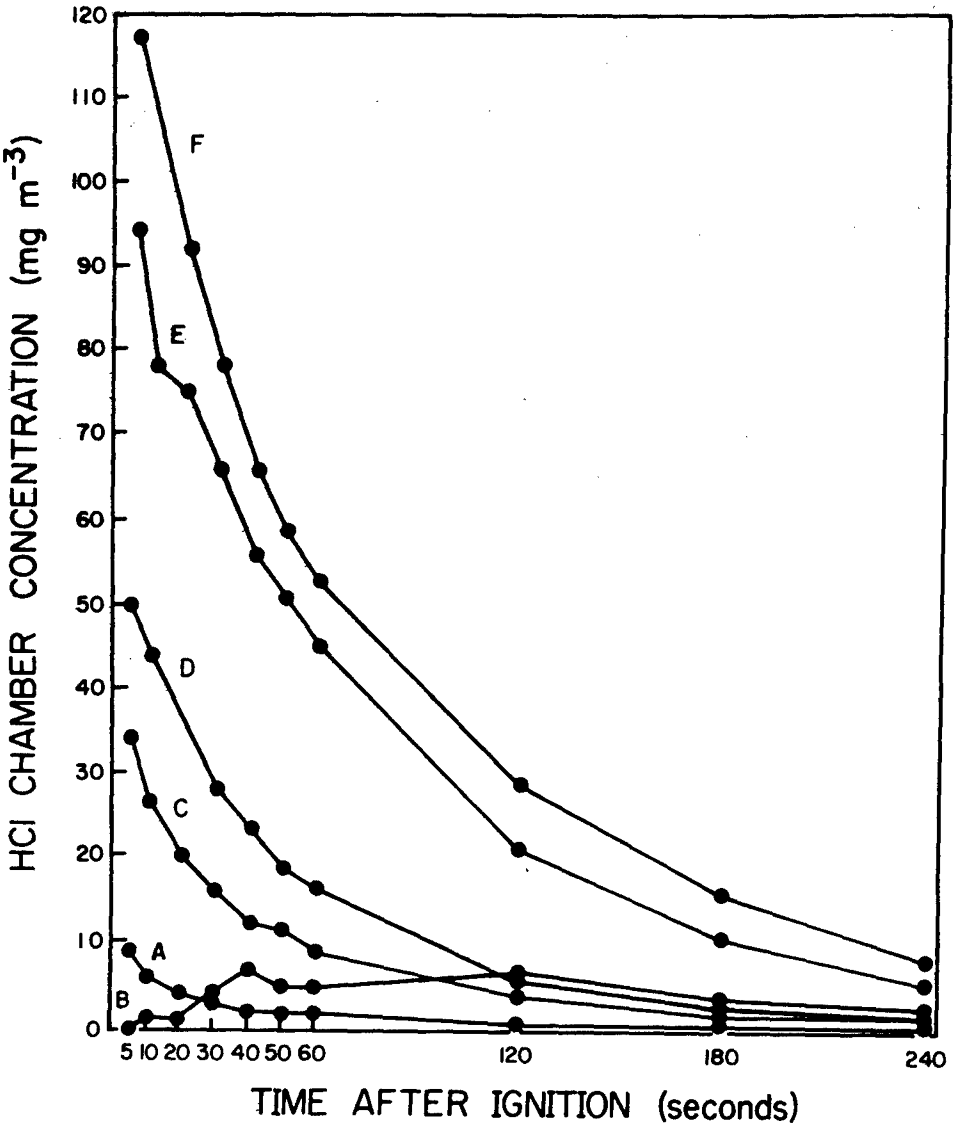

Figure 7. Decay in chamber HCl concentration during first four minutes after ignition of SRF of different sizes. A. 72 mg fuel; B. 103 mg fuel, see text note; C. 221 mg fuel; D. 418 mg fuel; E. 512 mg fuel; F. 727 mg fuel.

TABLE 10.
CHAMBER HCl CONCENTRATION DETERMINED WITH BUBBLER WET CHEMISTRY AND CHEMILUMINESCENT MONITOR AFTER SOLID FUEL BURN

Bubbler Sample Time (min. post-ignition)	Bubbler Measurement ($mg\ m^{-3}$)	Geomet[1] ($mg\ m^{-3}$)
0- 2	16.8	36.8
0.5-2.5	13.1	25.4
2- 4	3.3	9.1
3- 5	6.0	5.7
4- 6	3.3	2.2
6- 8	0.6	1.4
7- 9	0.1	1.1
9-11	0.2	0.9
10-12	0	0

[1]Averaged reading during bubbler sample time

TABLE 11.
DECAY IN CHAMBER HCl CONCENTRATION IN RELATION TO STIRRING PADDLES

		HCl Concentration, $mg\ m^{-3}$		
Generation	Stirring Paddle	Bubbler	Geomet Maximum	Decay Curve Slope
Solid fuel	Rotating	22.0	81.7	-0.329
Solid fuel	Stationary	10.6	43.0	-0.087
Tank	Rotating	26.6	80.0	-0.241
Tank	Stationary	48.2	82.0	-0.122

turned off. For SRF, burns were made in the normal way with the exhaust fan off. Bubbler samples were begun at 1 minute after gas was shut off or after ignition.

Chamber HCl concentration values were taken from the Geomet recording chart at t = 30 seconds, then every minute during the exposure. By comparing the maximum concentration, C_{max}, with each value, C_i at time i, decay lines were constructed for the treatments. The slope and intercepts for the three replicas were averaged for presentation (Figure 8). The natural log function describes normal decay phenomenon. Here the flattened response as well as increased concentration variability when the paddles were stationary indicate incomplete gas mixing. From the slope of the decay lines it seems that the solid fuel may be more reactive than pure HCl gas and that the paddles increase the possibility of the gas reacting with chamber surfaces.

Deposition or adsorption of HCl from SRF

The deposition or adsorption of HCl from the chamber atmosphere following the burning of fuel was examined by distributing filter paper discs at three different chamber heights and on the floor. Those on the floor were in petri plates which could be uncovered from outside the chamber. About 700 mg of fuel was ignited in the closed chamber. All three hanging discs and one of the discs on the chamber floor were exposed to the propellant gas for 10 minutes. By uncovering the other discs during the period, discs were exposed for 9.5, 9, 8, 5 or 3 minutes. The stirring paddles were stationary during four of the burns. Bubbler samples were started one minute after ignition. The filter discs were eluted in 0.1 N NaOH and chlorine content determined with the automatic titrator (Table 12).

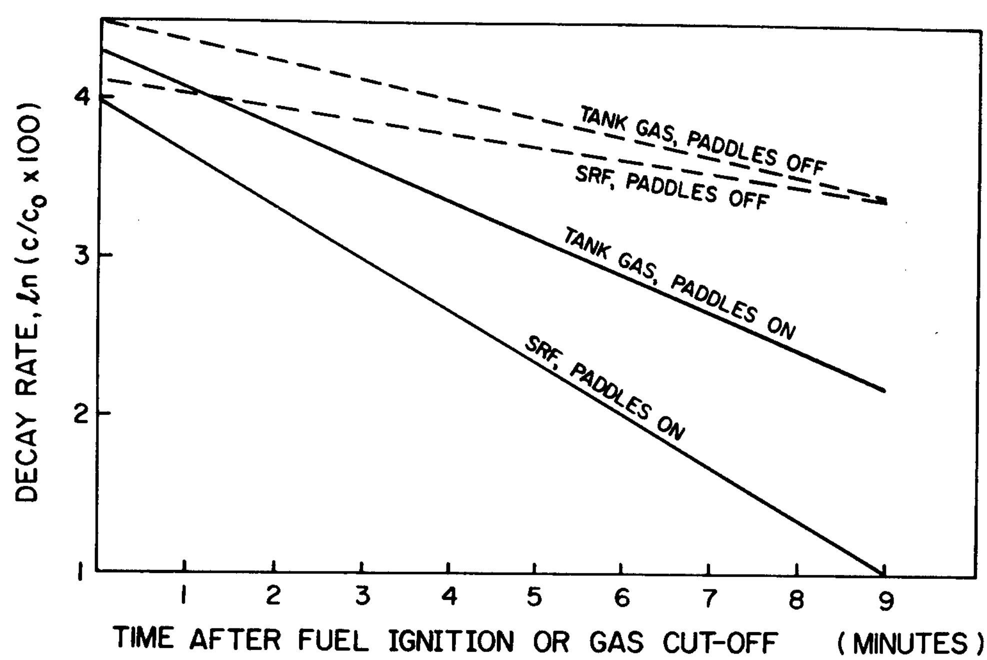

Figure 8. Effect of stirring paddles on decay in HCl gas concentration in Lexan chamber. Gas was from pressurized tank or SRF. A Geomet instrument monitored the HCl concentration of three replicas for each treatment and slope and intercept values were averaged. Lines are of the form

$$\ln\left(\frac{C_i}{C_{max}} \times 100\right) = A + Bt,$$ where C_i is average concentration,

C_{max} is maximum concentration, t is time in minutes, A is the intercept point, and B is the slope.

Incomplete mixing was indicated by the higher levels of chamber HCl seen when the paddles were stationary and by the chlorine adsorbed on the hanging filter paper discs. Discs higher in the chamber adsorbed more chlorine when there was no stirring to distribute the gas. With the paddles rotating, there were no significant differences among the chlorine found on the discs at the three heights.

There was a trend toward more adsorption with time, but only when the mixing paddles were moving. Any HCl droplets present were very small since much chlorine stayed high in the chamber and adsorbed more readily to the vertical rather than to the horizontal surfaces.

TABLE 12
DEPOSITION OF HCl IN LEXAN CHAMBER AFTER BURNING 700 MG SOLID FUEL

Deposition surface		Stirring Paddle		
		Rotating		Stationary
CHAMBER ATMOSPHERE (Peak HCl level, mg m^{-3})		15.5	*	47.4
FILTER DISCS (Chlorine, $\mu g\ cm^{-2}$)				
Position	Exposure Time (min.)			
High	10	10.2		9.7
Medium	10	10.5	*	5.8
Low	10	9.5	*	5.1
Floor	10	6.1		4.3
Floor	9.5	5.3		3.9
Floor	9	5.3		4.1
Floor	8	3.2	*	5.5
Floor	5	2.3		3.7
Floor	3	1.6	*	2.2

*Significant difference at 5% level between rotating and stationary values

Injury on plants exposed to gases generated by SRF

Radish and bean seedlings were exposed for 10 minutes to gas generated by burning fuel weighing 72 to 727 mg (Table 13). Six plants of each species were exposed and the number of leaves injured was counted and an estimate of the leaf area injured made. Injury on both species consisted of severe initial wilt visible 15-30 minutes after exposure. Gradually, most wilted leaves regained turgor and only limited necrotic areas remained, similar to injury from pure dry HCl gas. Radish seedlings were more sensitive than beans, which had a sharp injury threshold between 400 and 500 mg fuel (Figure 9).

Plant injury increased with exposure time if the fuel weight was kept constant at 400 mg (Table 14). The bean plants again reacted with steeper injury threshold than the more sensitive radish plants (Figure 10). Enough gas was generated by 400 mg fuel to injure either species as long as exposure time was sufficiently long. It was not clear why damage continued to increase 10 minutes after ignition, yet chamber HCl could no longer be detected.

TABLE 13.
INJURY ON PLANTS EXPOSED 10 MINUTES TO HCl GENERATED BY SRF

Fuel Weight (mg)	Geomet maximum (mg HCl m^{-3})	Stress		%-Leaves Injured		%-Leaf Area Injured	
		Bean	Radish	Bean	Radish	Bean	Radish
72	7	0	0	0	11	0	1
221	34	+	+	0	34	0	5
418	50	0	0	0	53	0	15
512	94	+	+	100	94	87	61
727	117	+	+	100	100	85	56

TABLE 14.
INJURY ON PLANTS EXPOSED FOR 2 TO 20 MINUTES TO HCl GAS GENERATED BY 400 MG OF SOLID FUEL

Exposure time	Geomet maximum (mg HCl m^{-3})	Stress		%-Leaves Injured		%-Leaf Area Injured	
		Bean	Radish	Bean	Radish	Bean	Radish
2	37	+	0	0	32	0	2
10	50	0	0	0	53	0	15
15	15	+	+	25	73	3	13
20	80	+	+	100	97	87	68

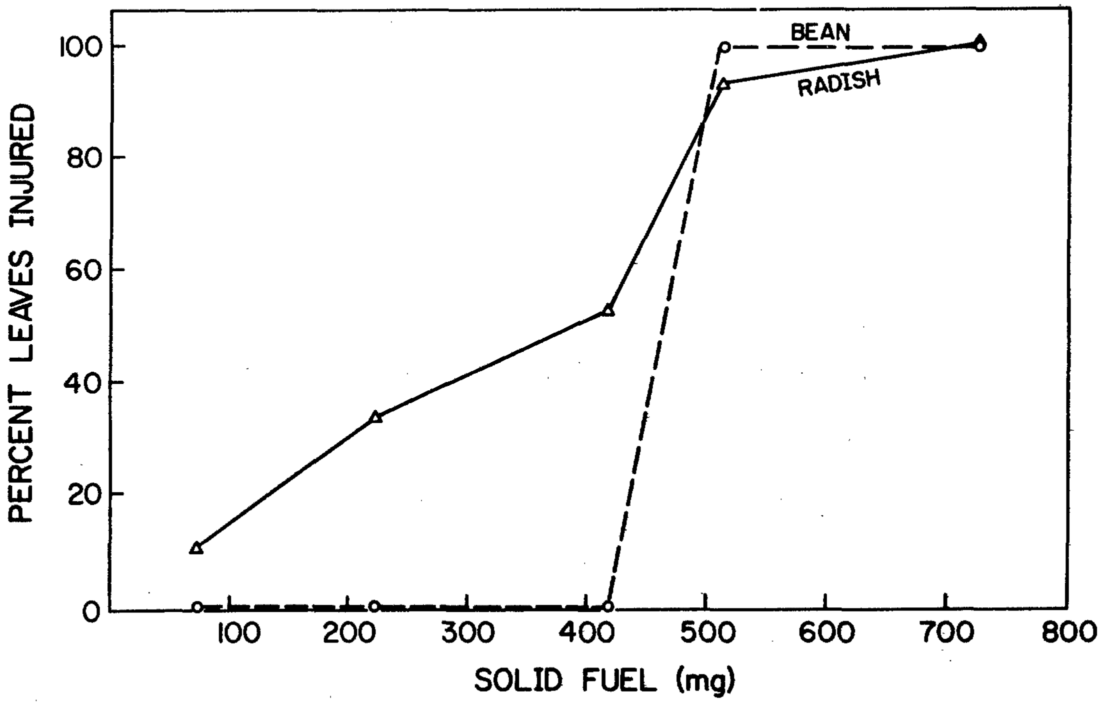

Figure 9. Leaf injury on plants after exposure to gas generated by SRF for 10 minutes after fuel ignition.

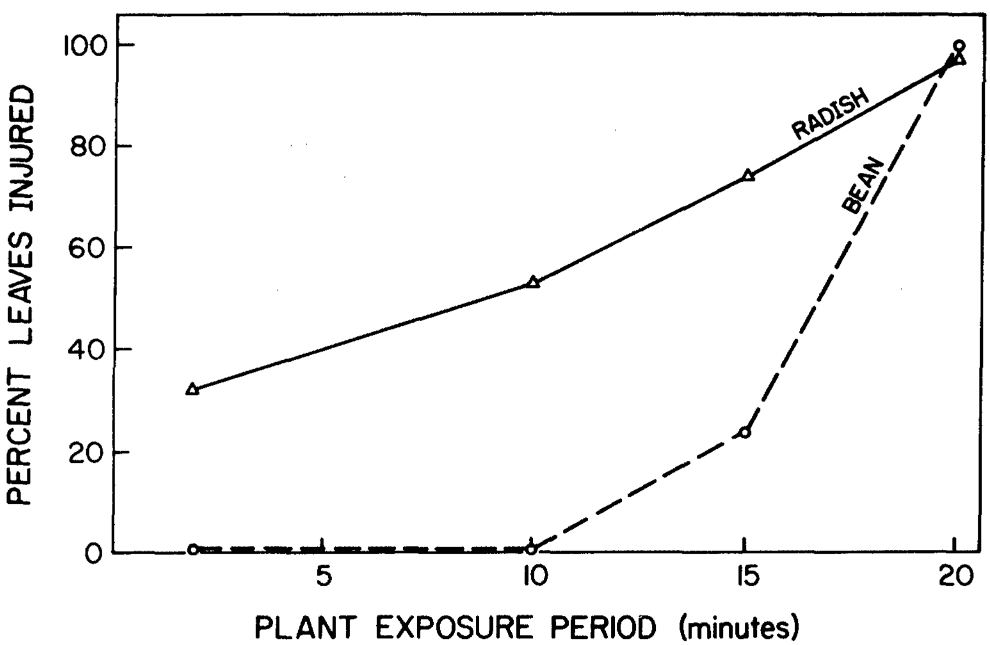

Figure 10. Leaf injury on plants exposed for certain periods to gases generated by burning 400 mg solid rocket fuel.

In another test, groups of ten 13-day-old pinto bean seedlings were exposed for 10 minutes to gases from 100, 200, 400 and 800 mg of burning SRF (Table 15). The series was replicated three times. With this larger population, the injury data could be submitted to probit analysis which indicated 10% injury threshold at 118 mg and 50% threshold at 269 mg fuel (Figure 11).

TABLE 15.
CHAMBER HCl CONCENTRATION AND PLANT DAMAGE ON PINTO BEAN SEEDLINGS EXPOSED FOR 10 MINUTES TO GAS FROM SRF

Solid Fuel (mg)	Chamber HCl Concentration (mg m^{-3})	Injury %-Leaves	Injury %-Leaf Area
100	12.6	0	0
202	21.9	22	4
399	52.3	73	27
800	96.1	98	90

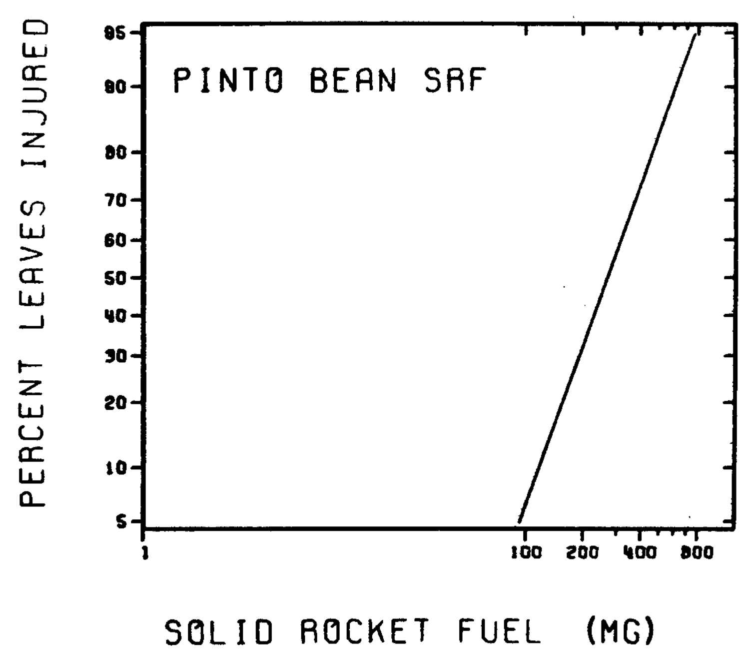

Figure 11. Probit analysis of pinto beans exposed for 10 minutes to gas generated by SRF.

Interaction of SRF gas and dew on plant injury

In this experiment, dew was formed on zinnia plants before or after exposure to one of four levels of HCl generated by SRF. There were two plants for each treatment and two replicas. Exposures were done at night under dark conditions.

Significantly more leaf injury occurred when dew had been formed on the plants before exposure than after or when there had been no dew (Figure 12). These results compare well with those found with dry gas. Plants receive minimal pollution injury in darkness perhaps because of stomatal closing or reduced photosynthetic activity. In the field, however, dew occurs in the dark during the late evening and early morning hours. With dew on the leaves, there may be as much plant injury as with daytime exposures.

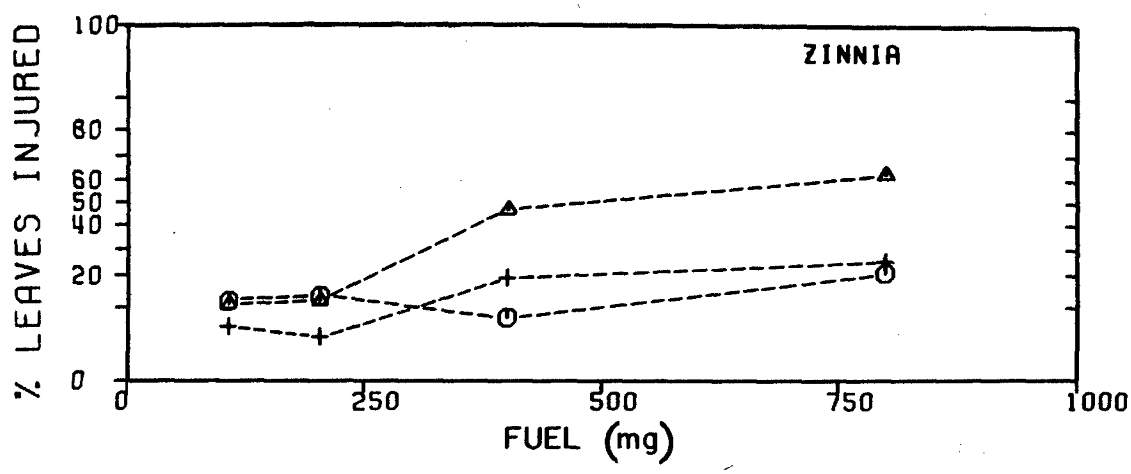

Figure 12. Interaction of dew and HCl gas generated by solid rocket fuel on percent leaves injured on zinnia seedlings. Code: Δ = dew before, 0 = dew after, + = no dew.

Conclusions about SRF

Solid rocket fuel produced large amounts of HCl and Al_2O_3 when small pieces were burned without confinement. The experiments described here were with single short burns which produced instantaneous high concentrations of pollutants. This was different from most experimental work with HCl and other air pollutants where there is a dynamic replacement of the chamber atmosphere and the pollutant level is kept constant. The SRF system, however, may be a more accurate portrait of a ground cloud from a rocket launch where gas concentrations rapidly reach a maximum then dissipate. Gases formed when SRF pieces 1 g and less were burned induced plant injury similar to that caused by pure HCl gas alone. Injury occurring on test plants increased as more fuel was burned or exposure time increased. Nimo et al. (1974b) showed that a considerable amount of the total HCl produced by burning rocket fuel was adsorbing on their chamber walls and could be recovered by thoroughly washing the chamber surfaces. Plant and chamber surfaces adsorbing varying amounts of HCl could account for our variable chamber concentrations.

PHYTOTOXICITY

EFFECT OF HCl GAS ON SEEDS

In an earlier report (Granett and Taylor, 1977), the effect of HCl gas on seeds was discussed. Further studies have been undertaken to better characterize this interaction. Tomato and barley seeds in petri plates on either filtered paper or soil were exposed to one of six HCl gas concentrations or to filtered air. Immediately after exposure some groups of seeds were transferred to another plate to provide four treatments: HCl-exposed seeds on (1) HCl-exposed- or (2) air-exposed-media and air-exposed seeds on (3) HCl-exposed- or (4) air-exposed-media. There were ten seeds in each of two plates for each treatment. Media was moistened and the seeds were allowed to germinate in a dark chamber at 22C. Germination rate and seedling length were reduced in those groups of seeds grown on paper exposed to high HCl concentrations (Table 16). The development of seeds germinated on paper was hampered whether the seeds themselves had been exposed to HCl or not. Development was not reduced in those treatments involving soil.

In treatments where there was any effect, tomato seeds were more sensitive than barley. In affected groups, seed germination was reduced only slightly even at very high gas concentrations. Seedlings were considerably stunted by moderate levels of HCl gas.

Since the support medium, particularly paper, seemed to influence seed development, the adsorption of HCl gas by paper and soil was investigated. Dry or moist filter paper discs or soil in open petri plates were exposed to concentrations of HCl gas for 20 minutes. The discs were eluted and analyzed for chlorine as outlined in a previous section and soil chlorine was measured using standard techniques described by Richards (1954) (Figure 13). There was significant uptake of detectable chlorine in the media after exposure. Soil moisture did not influence adsorption, but more chlorine was extracted from moist filter paper than from dry discs.

These studies led to the conclusion that tomato and barley seeds were not directly affected by single 20-minute exposures to gaseous HCl in concentrations as high as 170 mg m^{-3}. If seeds were on filter paper during exposure, however, their subsequent development was affected by gas adsorbed by the paper. Both filter paper and soil could adsorb HCl directly from the polluted atmosphere, but soil afforded considerable protection to seeds from HCl. Although the chlorine detected in exposed soil increased directly with HCl concentration, there was no significant decrease in seed germination or in stunting of seeds grown in this soil.

The pollution doses considered here were limited to a short 20-minute period although actual gas concentrations during the period far exceeded

TABLE 16.
DEVELOPMENT OF TOMATO AND BARLEY SEEDS ON FILTER PAPER OR SOIL EXPOSED TO HCl GAS FOR 20 MINUTES

Analysis	Percent Germination		Total Length (mm)	
	Paper	Soil	Paper	Soil
HCl Concentration ($mg\ m^{-3}$)				
3	94 a[1]	97	59	102
12	95 a	96	46	97
23	96 a	98	43	89
50	94 a	96	40	94
88	79 b	96	32	96
168	79 b	95	31	92
Treatment				
Seeds & Media, HCl	84	95	21 a[1]	95
Media, HCl; Seeds, Air[2]	83	97	22 a	92
Seeds, HCl; Media, Air[2]	95	98	63 b	87
Seeds & Media, Air	96	96	62 b	100
Species				
Barley	87	99	50 a[1]	120
Tomato	92	94	34 b	72

[1]Values in same column followed by the same letter are not significantly different at 5% level. Means with no letters were analyzed but were not significantly different, within columns, from each other using Duncan's test

[2]Seeds were transferred from petri plates in which they were exposed to obtain the treatment

those expected from normal sources of HCl pollution. Perhaps longer time periods, measured in hours or days, or multiple exposures would directly damage seeds. It is expected that extraordinarily high HCl doses would be necessary to create soil conditions detrimental to even sensitive seeds.

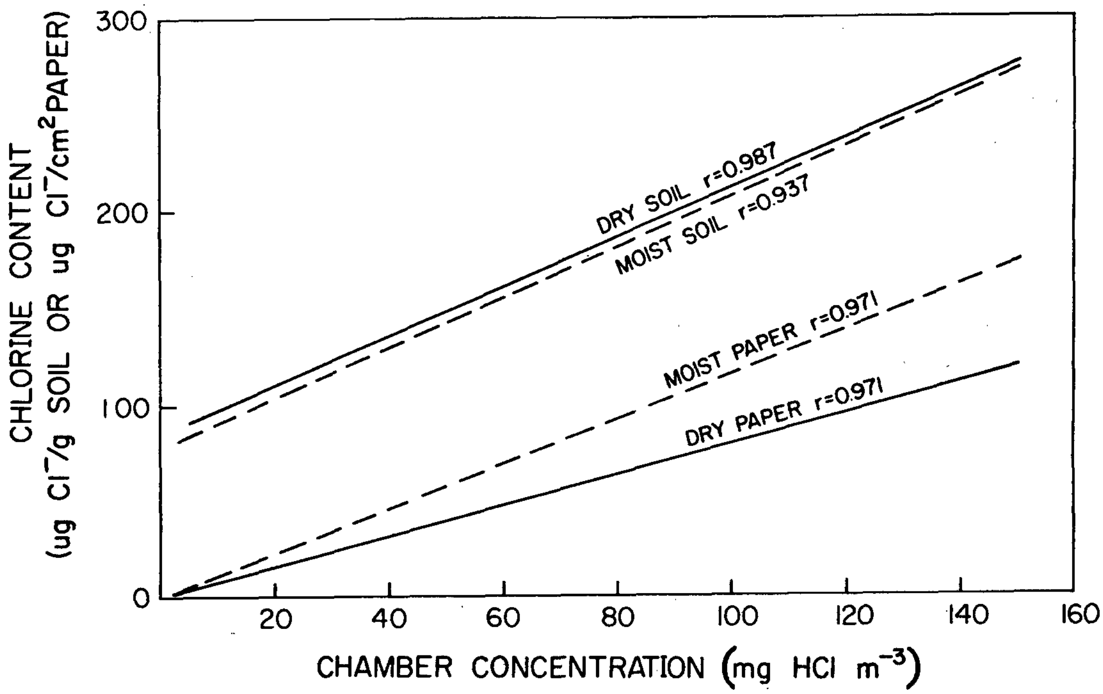

Figure 13. Extraction of chlorine from filter paper discs or from 50 g samples of soil exposed to HCl gas for 20 minutes.

RESPONSE OF PLANTS TO Al_2O_3 DUST

Al_2O_3 as a pollutant

Aluminum oxide, as a co-pollutant of solid rocket fuel exhaust, has been investigated during the past several years. A device was constructed and previously described that generates predictable concentrations of dust (Granett and Taylor, 1977; Neher et al., 1977). Numerous plants have been exposed to Al_2O_3 + HCl or Al_2O_3 alone with no strong evidence that the Al_2O_3 can injure plants alone or can significantly increase injury attributable to HCl. Current research, described below, resulted in similar findings.

Heavy applications of Al_2O_3 dust

One approach was to ascertain whether plants were affected by massive amounts of Al_2O_3. Zinnia seedlings were hand-dusted with a visible coat of Al_2O_3 prior to 20-minute exposures to HCl gas at different concentrations. There was slightly more injury on the dusted plants (Table 17) but this increase was not statistically significant at the 10% level (Table 18).

TABLE 17.
HEAVY DUSTING OF ZINNIA LEAVES WITH Al_2O_3 PRIOR TO EXPOSURE TO HCl GAS, LEAF INJURY.

HCl Concentration[1] ($mg\ m^{-3}$)	Percent Leaves Injured	
	No Dust	Dust
14	6	20
21	47	53
30	69	81

[1]Average of 4 exposures

TABLE 18.
HEAVY DUSTING OF ZINNIA LEAVES WITH Al_2O_3 PRIOR TO EXPOSURE TO HCl GAS, ANALYSIS OF VARIANCE OF PERCENT LEAVES INJURED

Source of variation	Degrees of freedom	Sum of squares	F-value
Concentration (C)	2	3435.3	36.99**
Dust treatment (D)	1	219.8	4.73
Replicas (R)	1	29.3	0.63
C x R interaction	2	198.9	2.14
D x R interacton	1	20.4	0.44
Error	4	185.8	---
Total	11	4089.5	---

** = 1% level of significance

In a companion experiment, groups of eight dusted pinto beans were exposed to low concentrations of HCl gas. The dusted leaves had less damage than undusted (Table 19) and the statistical analysis (Table 20) revealed significant differences in injury only with gas concentrations (C), not with the dust treatment (D).

TABLE 19.
HEAVY DUSTING OF PINTO BEANS WITH Al_2O_3 PRIOR TO EXPOSURE TO
HCl GAS, LEAF INJURY

HCL Concentration[1] (mg m^{-3})	Percent leaves injured	
	No dust	Dust
9	7	7
11	2	2
17	29	22

[1]Average of 4 exposures

TABLE 20.
HEAVY DUSTING OF PINTO BEANS WITH Al_2O_3 PRIOR TO EXPOSURE TO
HCl GAS, ANALYSIS OF VARIANCE OF PERCENT LEAVES INJURED

Source of variation	Degrees of freedom	Sum of squares	F-value
Concentration (C)	2	875.4	12.47*
Dust treatment (D)	1	37.1	1.06
Replicas (R)	1	993.7	28.32**
C x R interaction	2	548.5	7.81*
D x R interacton	1	126.4	3.60
Error	4	140.4	---
Total	11	2721.5	---

* = 5% and ** = 1% levels of significance

Effect of humidity on plant injury caused by Al_2O_3 + HCl

In the presence of high humidity, Al_2O_3 and HCl gas may coalesce forming large aerosols (Stephens and Steward, 1977). During rocket launches, water is liberated and large amounts of water vapor are present in the exhaust gases. Low greenhouse humidities and dry gas generation techniques may be inhibiting Al_2O_3 and HCl interactions in our experiments.

In one study, groups of six pinto bean and six marigold plants were exposed to HCl and HCl + Al_2O_3 at a 1:1 weight ratio for 20 minutes at either 50 or 85% relative humidity (RH). Humidity was produced and maintained by introducing live steam to the chamber air intake. At the higher gas concentration, 20 mg HCl m^{-3}, plants reacted with more injury at 85% than 50% RH (Table 21). There was no difference between HCl with or without Al_2O_3. At 10 mg HCl m^{-3}, however, there was a mixed reaction; at 50% RH, there was more injury when dust was present, while at 85% RH there was more injury with the HCl gas alone.

TABLE 21.
PERCENT LEAVES INJURED OF PLANTS EXPOSED TO HCl GAS AND HCl + Al_2O_3 (HCl+) FOR 20 MINUTES AT 50 OR 85% RELATIVE HUMIDITY

	Pinto Bean				Zinnia			
	10 mg m^{-3}		20 mg m^{-3}		10 mg m^{-3}		20 mg m^{-3}	
Relative Humidity	HCl	HCl+	HCl	HCl+	HCl	HCl+	HCl	HCl+
50%	8	33	75	75	19	43	72	80
85%	8	0	92	92	38	3	80	87

In repeating this experiment, pinto bean and zinnia plants were exposed to a combination of one of four HCl levels, one of four RH levels, and with or without Al_2O_3 dust present in the atmosphere. Al_2O_3 dust made no apparent difference in injury (Table 22) nor was there a clear relationship between injury and humidity level, but there was more injury as HCl concentration increased.

Conclusion of dust work

These experiments provided further evidence that Al_2O_3 did not significantly contribute to injury on plants caused by HCl gas under the conditions tested which included HCl:dust at a 1:1 ratio, Al_2O_3 as a concentrated dust, and various RH levels. Methods of mixing dust with HCl gas were checked previously (Granett and Taylor, 1977). SRF generates Al_2O_3 and HCl gas simultaneously but plants exposed to SRF gases appear very much the same as plants exposed to pure HCl gas. Baldwin (1974) found dry dust did not adsorb HCl or other gases. From our work it is not clear whether Al_2O_3 dust as well as water droplets adsorb HCl at higher humidities,

TABLE 22.
SUMMARIES OF PLANT INJURY AFTER EXPOSURE TO HCl GAS AND HCl + Al_2O_3 AT DIFFERENT LEVELS OF HUMIDITY

Treatment		Pinto Bean		Zinna	
Al_2O_3	present	87		44	
	absent	76		43	
HCl (mg m^{-3})	10.3	50	z[1]	40	yz
	10.6	73	yz	28	z
	13.4	89	xy	46	yz
	15.9	99	x	62	y
Humidity	53%	71	b	58	a
	66%	97	a	47	a
	74%	76	b	61	a
	81%	75	b	15	b

[1]Values followed by the same letters are not significantly different at the 5% level by Duncan's multiple range test

although decreases in injury at these RH levels when Al_2O_3 is present may be due to gas removed from the atmosphere by adsorption. Although aluminum dust may produce or mediate processes at a cellular or biochemical level, present tests could not detect any damage response from Al_2O_3 alone or any change in plant damage response to the combination of Al_2O_3 + HCl from that of HCl alone.

THE EFFECT OF RELATIVE HUMIDITY ON THE RESPONSE OF PLANTS TO HCl GAS

Increased RH during exposures

Relative humidity effects were discussed above in relation to Al_2O_3 interaction. Humidity levels appeared significant in the severity of plant injury, although the effects seemed inconsistent. A separate study, without Al_2O_3 dust, was designed to better clarify the interaction of HCl gas and RH.

Groups of pinto beans and French marigolds were exposed for 20 minutes to gas at 5, 10, 20, or 40 mg HCl m^{-3} at 50, 70, or 85% RH. HCl air samples were taken with the bubbler inside the chamber to avoid condensation problems. Plant injury increased with elevations in either gas concentration or RH (Figures 14).

Dew during HCl exposures

Since increased humidity and excess water vapor around a plant leaf can coalesce in nature as dew, dew formation and its interaction with HCl

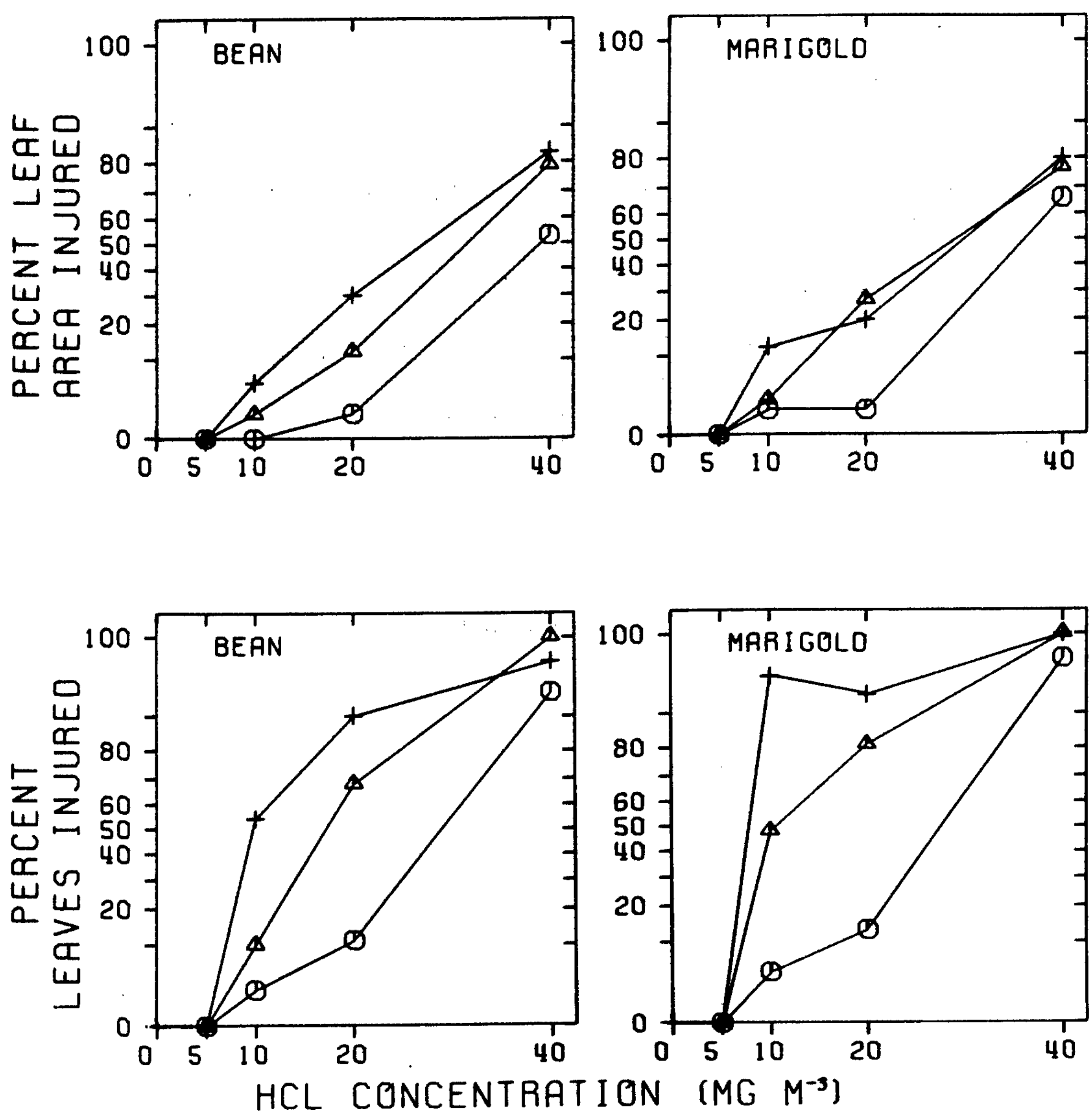

Figure 14. Leaf injury after exposure to 20 minutes of HCl gas at 50% (0), 70% (Δ), and 85% (+) RH.

gas was investigated. Bean, zinnia, marigold, and radish seedlings were grown under normal greenhouse conditions. Just prior to or just after exposure to HCl gas, the plants were placed in a dew chamber so that leaves became covered with a thin film of moisture. A set of HCl-exposed plants which did not receive dew served as a third treatment.

The first series of plants was treated with dew and exposed to HCl either in the early morning or at night. Since time of day did not influence plant reactions, all subsequent exposures were at night. All four species tested exhibited significant differences in injury response between plants receiving dew before exposure (DBE) and those plants dewed after exposure (DAE) or not at all, (ND) (Figure 15). DBE plants had more injury in all cases while injury on the DAE and ND plants was nearly the same.

This experiment showed that dew, like elevated RH, increased plant sensitivity to HCl gas. Since high humidity and dew are common phenomena under field conditions, this increased sensitivity should be kept in mind.

RH and the transformation of HCl gas to an aerosol

We have long wondered how much HCl in the fumigation chambers was present in the aerosol state particularly at higher RH. To check on the amounts of aerosols formed, air from the chamber was exhausted through a five-stage cascade impactor and a final 8 x 10 filter for 30 seconds at 40 cfm. The glass fiber filters had been coated with 0.1N NaOH and oven-dried to better retain impacted HCl. The humidity levels tested were 38%, 65%, and 85% RH. HCl gas, at about 20 mg m^{-3}, was generated by either diluting pressurized dry gas or by vaporizing aqueous acid solutions. After each sampling period, the filters were removed and were analyzed for total chlorine and pH.

In each case percent chlorine per cm^2 was calculated for each filter from all five stages of the cascade impactor and for the 8 x 10 inch final filter (Figure 16). Theoretically, a gas should contact all cascade filters to the same degree and there should be no difference in the chlorine recovered from each stage of the impactor (Figure 16-D). At low humidity levels, the chlorine was close to theoretical; but larger particles, possibly impacted room dust adsorbing gas, were present as seen by the increased chlorine in filters 2 and 3 (Figure 16-C,E). More large aerosols were found at higher humidity levels (Figure 16-A,B,F), possibly signifying that water droplets being formed removed some of the HCl gas from the air. The acid aerosols then impacted only the earlier stages of the cascade impactor. Data from the vaporizing generation system (Figure 16-E,F) compared well to the dry gas data (Figure 16-A,B,C).

All pH values were high because NaOH was used in filter preparation; however, the cascade filters exposed to HCl gas showed a decrease in pH over non-exposed controls and, as the humidity increased, the pH became lower (Table 23). Since more HCl gas was removed by the cascade filters, the pH of the final filter increased at the higher humidity levels.

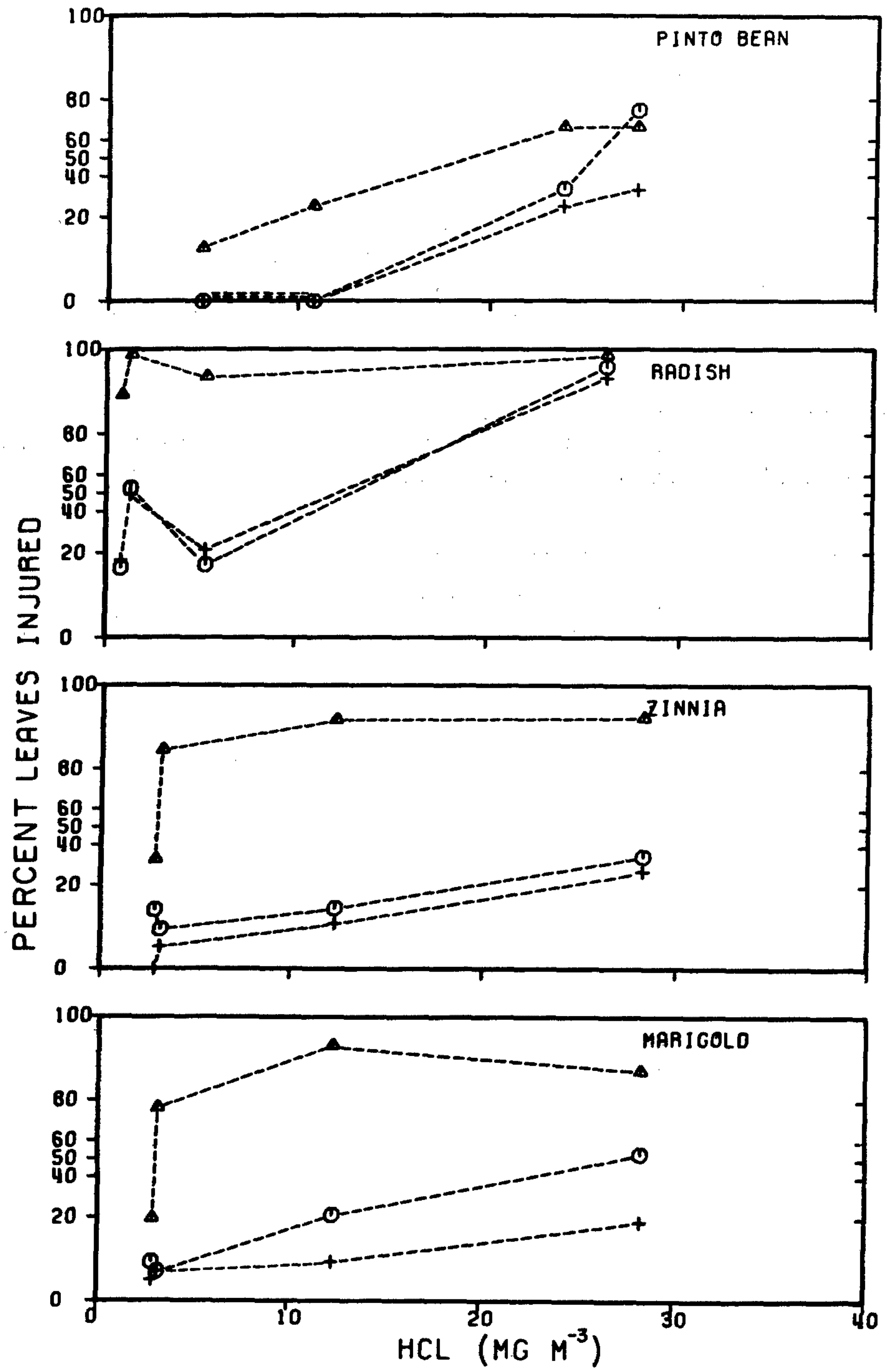

Figure 15. Effect of dew on plant injury caused by HCl gas where plants are treated with dew before (Δ) or after (0) exposure or receive no dew at all (+).

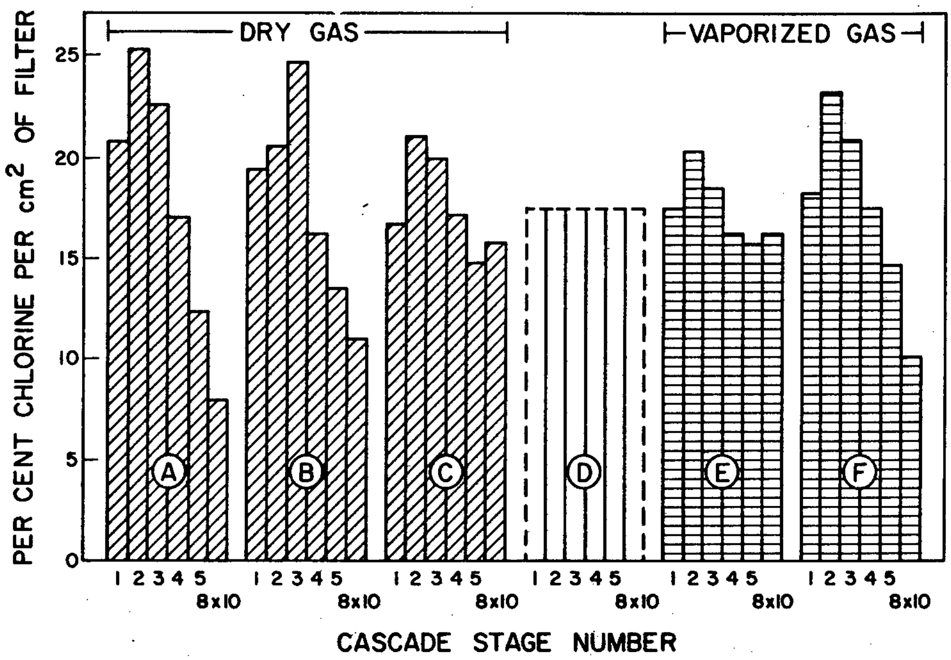

Figure 16. Histogram of chlorine recovered from cascade impactor drawing air for 30 seconds from an exposure chamber containing 20 mg HCl m^{-3} at different RH levels. A. Dry gas, 85% RH; B. Dry gas, 65% RH; C. Dry gas, 38% RH; D. Theoretical, no aerosols; E. Vaporized acid, 38% RH; F. Vaporized acid, 85% RH.

TABLE 23.
pH OF COATED GLASS FILTERS FROM CASCADE IMPACTOR

Treatment	Average of cascade filters	8 x 10 inch final filter
38% RH	8.84	9.40
70% RH	8.79	9.51
85% RH	8.76	10.32
Control (no HCl)	9.15	10.40

One further concern in the humidity experiments was whether the steam used to create the RH levels was contributing aerosols. This possibility was checked using a Climet model 208 particle analyzer. A particle distribution was derived by counting particles of 0.3, 0.5, 1, 3, 5, and 10 μ. The procedures involved sampling with 100-fold dilution for one minute intervals. Both the Lexan chamber and the cylindrical chambers were tested. Greenhouse air was analyzed as a check. HCl, in addition to steam, was introduced into the Lexan chamber for two measurements. Only slight differences were noted in aerosol production and in particle size distribution. Size distributions for the two chambers at several RH levels were prepared, and revealed no appreciable difference in aerosol size distribution. In all cases the mean particle diameter, representing 50% of the cumulative number could not be calculated but was less than 0.4 μ and 90% of all particles were less than 5 μ. There seems to be a slight increase in particle numbers as humidity is increased, but size distribution does not change (Figures 17 and 18). There is no shift when HCl is added. Higher particle numbers seen in the Lexan chamber compared to the cylindrical chamber were probably due to increased residence time for the steam in the cylindrical chamber intake. This time may have resulted in aerosol impaction or evaporation before particles reached the chamber. There does not appear to be any increase in aerosol size in distribution when steam is used to increase relative humidity.

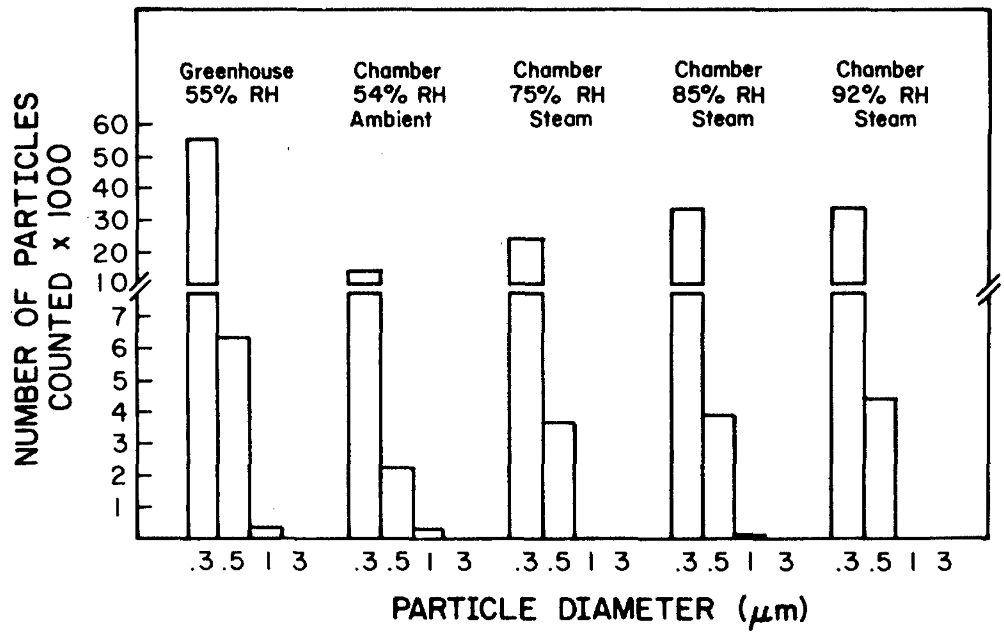

Figure 17. Histogram of particle size distribution of air in cylindrical chamber at different RH levels.

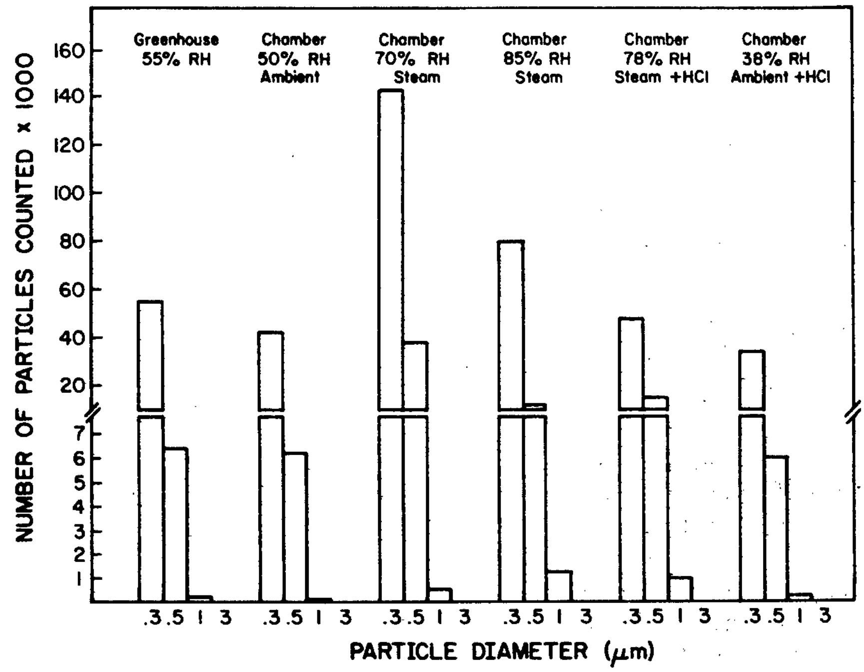

Figure 18. Histogram of particle size distribution of air in Lexan chamber at different RH levels and HCl gas concentrations.

PROBIT ANALYSIS OF PLANT SENSITIVITY TO HCl GAS

Probit analysis procedures

Findings were previously presented on plant sensitivity to 5, 10, or 20 minute exposures by linear regression or probit analysis (Granett and Taylor, 1977). Probit analysis was a more severe test and fewer significant lines were formed with the data. When probit regression lines are created from a dataset, no individual points remain (Finney, 1971) whereas linear regression demands the presentation of all points as well as significant lines. During the past year several more plant species were exposed to HCl gas in tests designed to yield threshold levels. In these tests, as in the past, a plant population was grown to be exposed at a specific age. Staggered plantings allowed several days of exposures. Concentration levels were chosen so that most damage was in the 20-80% injury range where probit analysis was most valid. Order of exposures was randomized. The exposures lasted 5 or 20 minutes and were in the cylindrical chambers using pressurized HCl gas. Bubbler samples were taken for each exposure and the titrated, calculated figure was the dose-concentration value. Both surfaces of each mature leaf were graded 24 to 48 hours after exposure with a 0 to 4 system, where 0 = 0%, 1 = 1-25%, 2 = 25-50%, 3 = 50-75%, and 4 = 75-99% of the leaf area was injured. Sometimes severe injury was graded as 5 = 100%. The kind of injury, usually glazing or necrosis, was also noted. Since probit analysis is valid only for numerical proportions, not estimations, only the proportion, number leaves injured over total number of leaves fumigated, was used in the probit program.

Exposures were summarized for use in the analysis as the program reads the number of leaves injured and number of leaves exposed. The weighted percentages are given angular transformation before regression statistics are calculated[1]. Dose was transformed to $\log_{10}$ mg HCl m^{-3} for analysis. Only significant probit regression lines were plotted, each with the same axis scales (Figures 19-23). To aid in interpreting the figures, the y axis was expressed as percent leaves injured instead of the arc sin equivalents.

<u>Sensitivities of varieties</u>--In the avocado and citrus experiments, plants were tested to determine whether seedlings and grafted varieties differed in their sensitivity to HCl. First, seedlings were exposed to HCl gas and response was measured. Several weeks later when new growth had replaced the injured leaves, a commercial variety was grafted on the seedlings. Several months were allowed for further growth before the grafted plants were exposed to HCl. With avocados (Table 24) ten exposures provided useful information on general sensitivity but a significant probit regression line was obtained by only one of the grafted varieties (Figure 19). Young avocado plants were found to be resistant to high concentrations of HCl gas and the seedlings were more sensitive than the grafted varieties.

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[1]The transformation, injury = arc sin (percent injury)$^{1/2}$ was used to correct for binomial distribution of percentages (Little and Hills, 1972).

TABLE 24.
LEAF INJURY ON AVOCADO PLANTS EXPOSED TO HCl GAS FOR 20 MINUTES

HCl Concentration ($mg\ m^{-3}$)	Percent Leaves Injured		
	Seedlings	Bacon	Hass
0	0	0	0
12	15	32	29
24	9	38	68
51	6	71	78
110	47	87	96

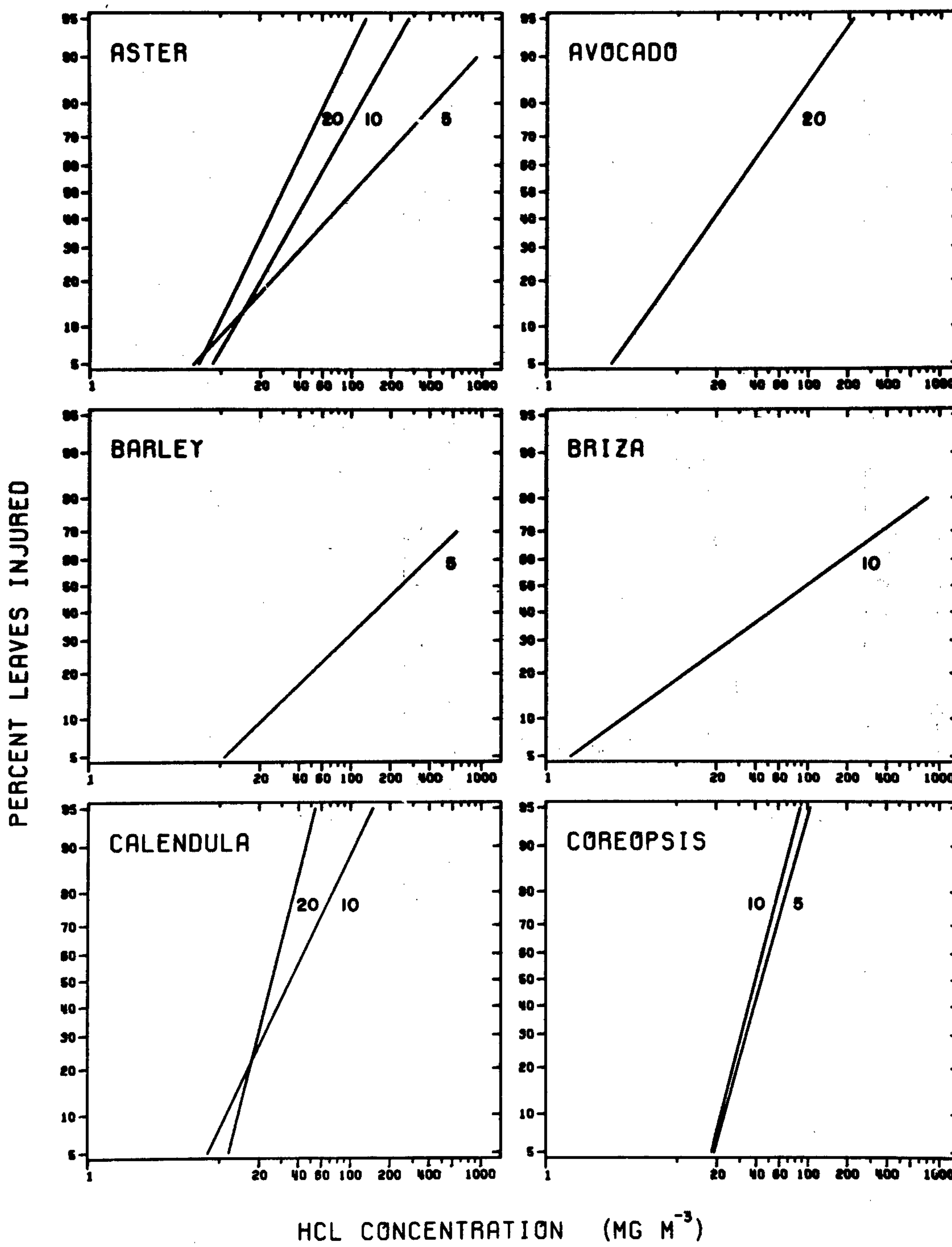

Figure 19. Probit analysis of six plant species: aster, avocado, barley, briza, calendula and coreopsis. Probit scale is the probability that a certain percent of the total leaves exposed will be injured at a given concentration ($\log_{10}$ scale) after 5, 10, or 20 minute exposures to HCl gas.

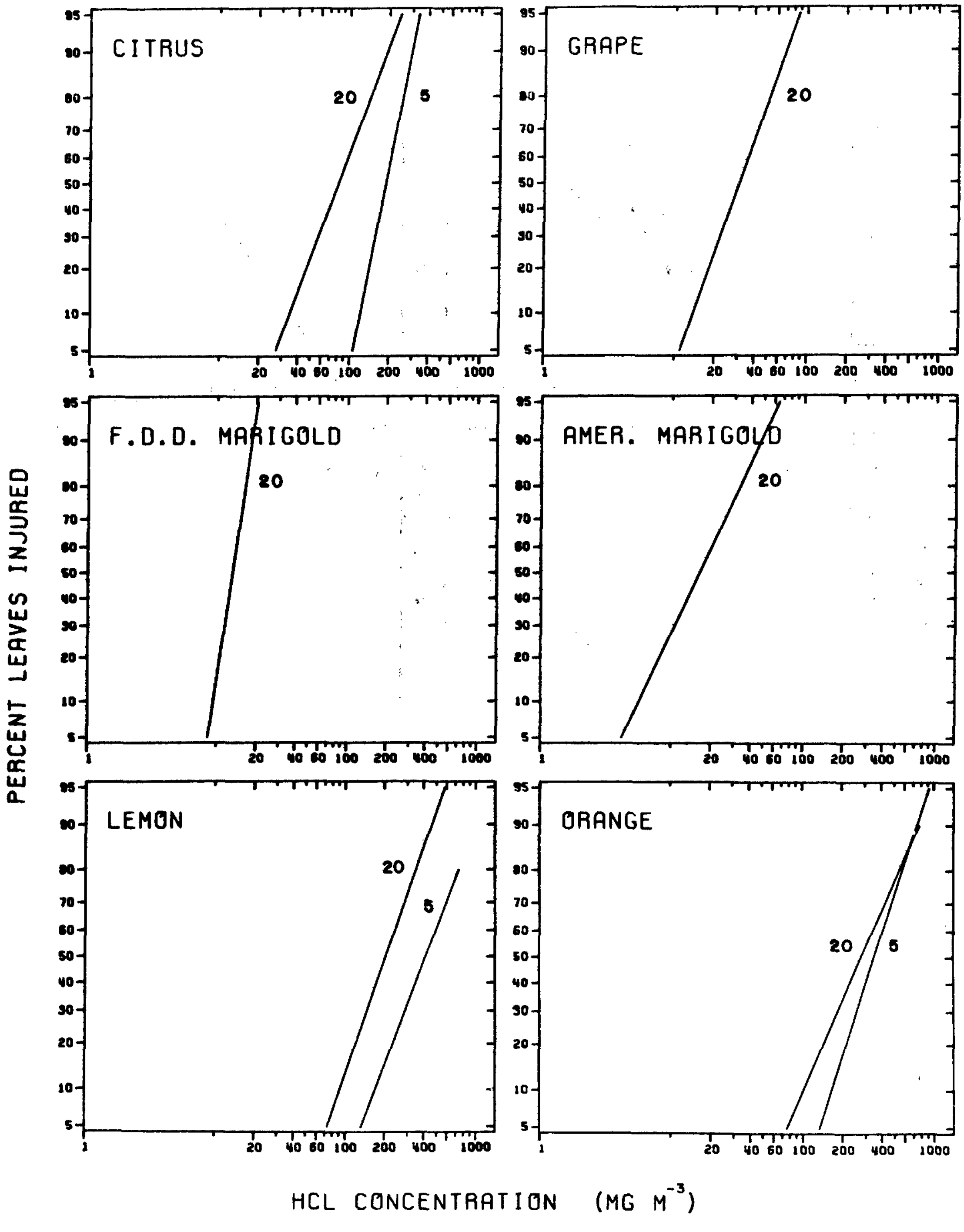

Figure 20. Probit analysis of five plant species: citrus seedlings, lemon, orange, grape, French marigold, American marigold. Probit scale is the probability that a certain percent of the total leaves exposed will be injured at a given concentration ($\log_{10}$ scale) after 5, 10, or 20 minute exposures to HCl gas.

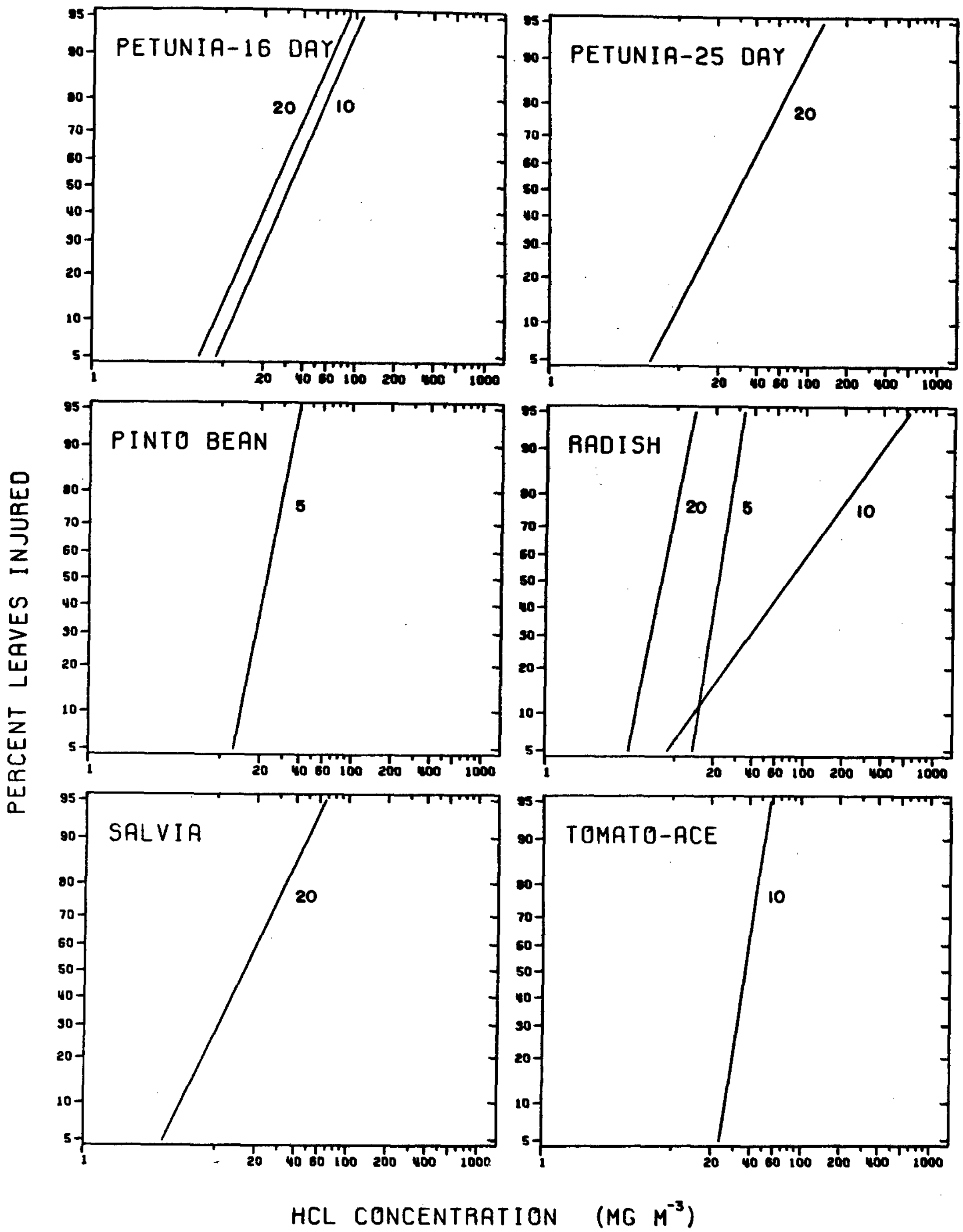

Figure 21. Probit analysis of five plant species: 16-day-petunia, 25-day-petunia, pinto bean, radish, salvia and tomato. Probit scale is the probability that a certain percent of the total leaves exposed will be injured at a given concentration (log_{10} scale) after 5, 10, or 20 minute exposures to HCl gas.

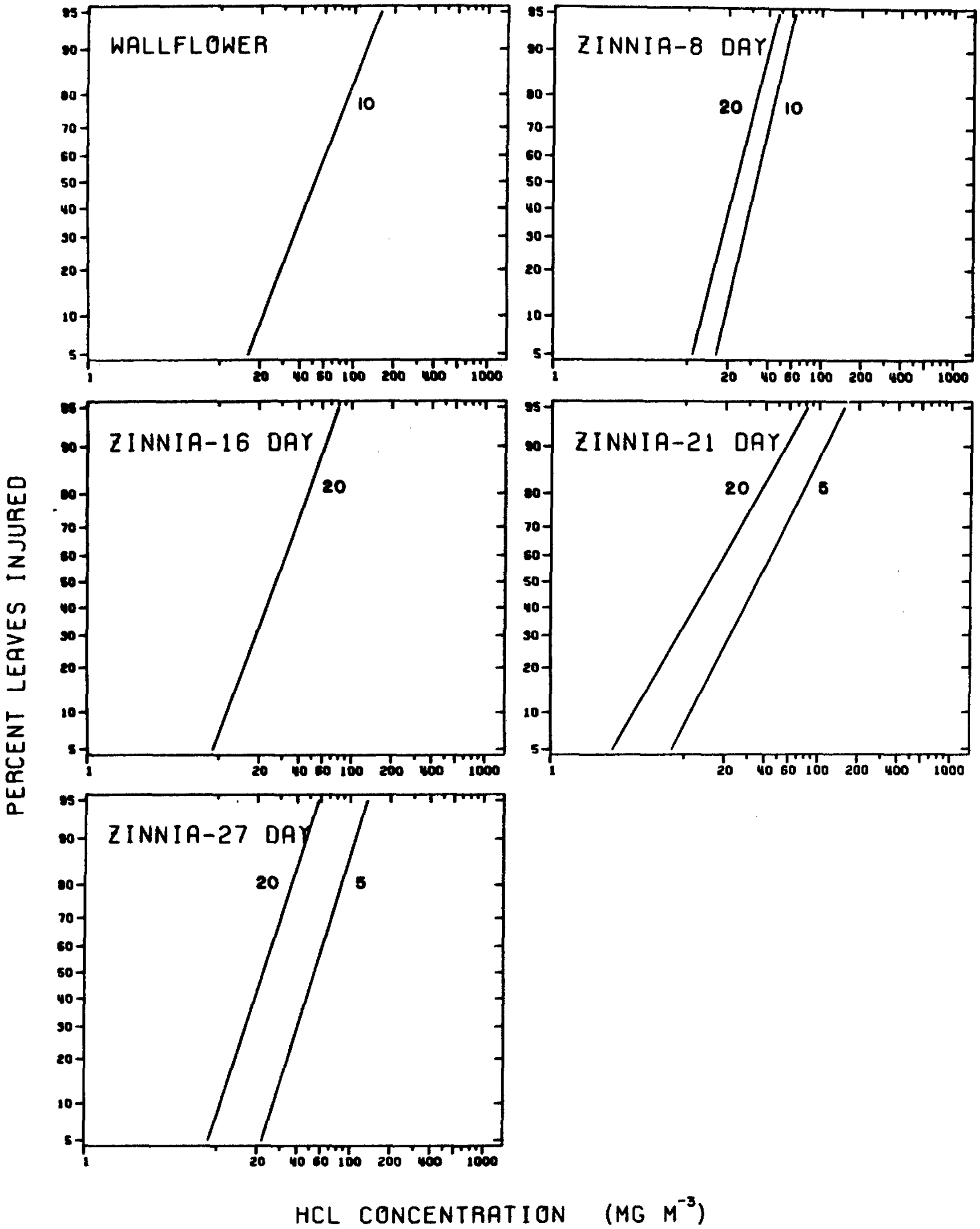

Figure 22. Probit analysis of two plant species: wallflower, 8-, 16-, 21-, and 27-day-zinnia. Probit scale is the probability that a certain percent of the total leaves exposed will be injured at a given concentration ($\log_{10}$ scale) after 5, 10, or 20 minute exposures to HCl gas.

Citrus plants also proved tolerant to high concentrations of HCl gas (Table 25 and Figure 20). In this case, rough lemon seedlings were exposed to HCl, then buds of varietal scions were grafted to the seedlings. In the subsequent exposures, the leaves of the grafted wood were more sensitive to pollutant injury than the rootstock leaves had been. Phytotoxic concentrations were considerably higher than might be expected under most orchard conditions.

TABLE 25.
LEAF INJURY ON CITRUS PLANTS EXPOSED TO HCl GAS

HCl gas Concentration[1] ($mg\ m^{-3}$)	Percent Leaves Injured					
	5-minute Exposure			20-minute Exposure		
	Seedlings	Orange	Lemon	Seedlings	Orange	Lemon
81				68	1	6
100	6	1	0			
126				66	14	52
166	9	9	54			
168				91	56	82
217	75	8	34			
228	85	23	54			

[1]Average of 5 exposures

Probit summary--The probit work to date is summarized in Table 26 and Figure 23. The estimates for dose concentrations necessary to injure 10% and 50% of the leaf population, EDD_{10} and EDD_{50} (Estimated Damaging Dose), respectively (Table 26) were calculated from the slope intercept data used for the graphs (Figures 19-23).

All the probit lines of the same time period were superimposed on one graph to create Figure 23. The 5-minute lines appear to cluster in two places whereas the 10-minute lines were generally steeper and higher along the concentration axis than the 20-minute lines.

TABLE 26.
ESTIMATED DAMAGING DOSES (EDD) FOR 10% AND 50% EXPECTED INJURY ON PLANTS EXPOSED TO HCl GAS FOR 5, 10, OR 20 MINUTES

	Exposure Time					
	5 Minutes		10 Minutes		20 Minutes	
Species	EDD_{10}	EDD_{50}	EDD_{10}	EDD_{50}	EDD_{10}	EDD_{50}
Aster	12	102	13	49	9	30
Avocado, Bacon					5	26
Barley	21	238				
Briza			4	98		
Calendula			11	35	14	25
Coreopsis	23	45	22	40		
Citrus -Rootstock	121	189			35	82
Citrus -Lemon	171	415			92	205
Citrus -Orange	166	351			101	279
Grape					14	31
FDD Marigold					9	13
Amer. Marigold					6	16
Petunia (16d)[1]			12	32	9	24
Petunia (25d)					8	28
Pinto Bean	14	22				
Radish	15	21	14	75	5	8
Salvia					5	16
Tomato			26	37		
Wallflower			21	51		
Zinnia (8d)	19	32			13	23
Zinnia (16d)					11	27
Zinnia (21d)	11	35			4	15
Zinnia (27d)	27	54			11	22

[1]Numbers in parenthesis refer to age, in days, of plants exposed.

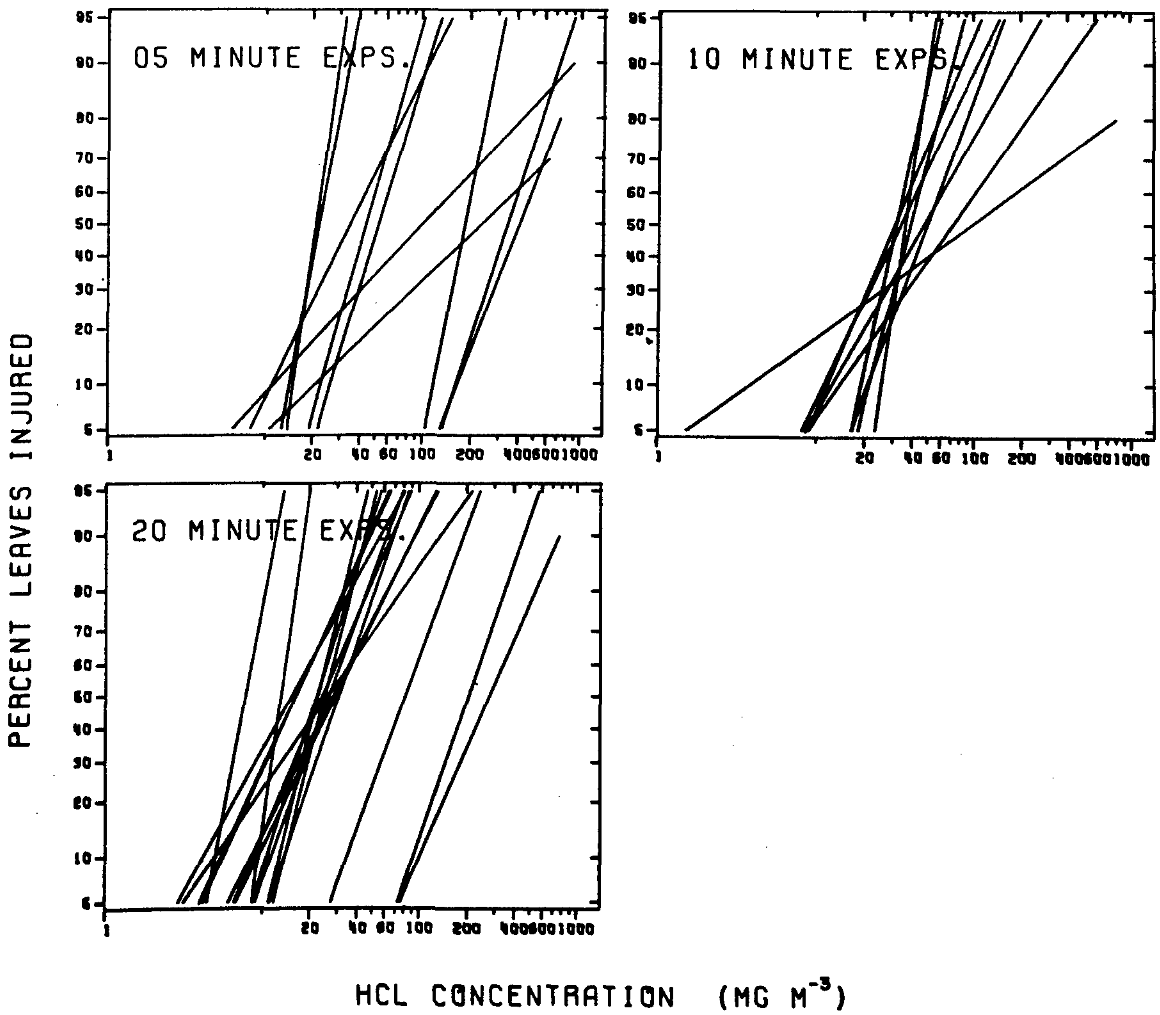

Figure 23. Summary of probit analysis for all plant species considered, separated by time of exposure. Probit scale is the probability that a certain percent of the total leaves exposed will be injured at a given concentration (log_{10} scale).

SEASONAL AND DIURNAL SENSITIVITY OF PLANTS

The response of plants to HCl has seemed more variable than to other gaseous pollutants. It has been more difficult, for example, to determine the threshold sensitivity of pinto beans. Guderian (1977) indicated that plant reactions to HCl differed with plant age, and season of the year. We have also noted that environmental factors can influence plant reactions. The present study was to further investigate how a population of plants may react to the gas at different times of the day or year. In addition to plant reaction, there was also interest in daily or seasonal fluctuations in the exposure system.

Populations of plants were grown at several times during the year. Throughout the day of exposures, theoretically constant chamber concentrations of 10 and 25 mg HCl m^{-3} were maintained by keeping the gas flowmeters at the same settings. Twenty-minute exposures were begun two hours before sunrise and continued hourly, ending two hours after sunset. During each exposure, bubbler samples were taken and light in the photosynthetically active region (PAR in μ-einsteins m^{-2} sec^{-1}) total light intensity (LI in ergs cm^{-2} sec^{-1}), temperature, and RH were recorded.

In each chamber, six pinto beans and four radish plants were usually exposed. In the last series, four zinnia seedlings were included with the beans and radishes. Plants were graded 48 hours post-exposure. HCl concentration, light, and injury were graphed to compare each experiment (Figures 24-30). The chamber gas concentration did not vary more than 5 mg m^{-3} over the entire day indicating that pressures in either the gas tank or the chambers were not greatly affected by midday heat buildup. "Greenhouse effect" heating in the chambers was minimized with Teflon film which allowed good heat exchange. Large air flow (almost 2 changes per minute) also helped keep chamber temperatures near ambient greenhouse levels. Flowmeter settings adequately maintained gas concentration. Since flowmeter settings were not changed from dawn to dust, reliability in resetting the flowmeters to desired concentrations was not determined.

Light intensity approximated a normal bell curve in most cases, but PAR varied more. It did not peak as early as LI and dropped more rapidly in the afternoon. LI was closely related to temperature; temperature influenced RH with RH decreasing as the temperature increased.

Both bean and radish were sensitive indicators of HCl gas. A small amount of injury was noted on plants exposed in the dark with the amount increasing rapidly as light increased. Plant injury reached maximum levels before LI values peaked, injury then declined through the rest of the day.

The trends seen in the individual experiments can be seen more clearly in the summary tables (Tables 27 and 28). Considering the seasonal changes (Table 27) the light values were much less during the winter and early spring compared to the bright summer. The rainy, overcast weather during the December and April exposures contributed to even lower LI. Chamber temperature did not vary as greatly as light levels. RH was usually low during the sunny winter days with increasing values during rainy weather. Summer RH was high because the greenhouse evaporative coolers operated during the heat of the day. On a diurnal basis (Table 28), light and temperature reached maximum values at about 11 am, the same time the lowest RH was reached.

Over the season the HCl at the low level averaged 13.2 ± 2.9 mg HCl m^{-3} while the high level exposures averaged 27.3 ± 2.2 mg m^{-3}. There was not a detectable relationship between the HCl chamber concentration and the season. On the diurnal level, a relationship may exist between the light and temperature measurements and the gas concentration; the early morning concentrations were lower than midday levels. Since the

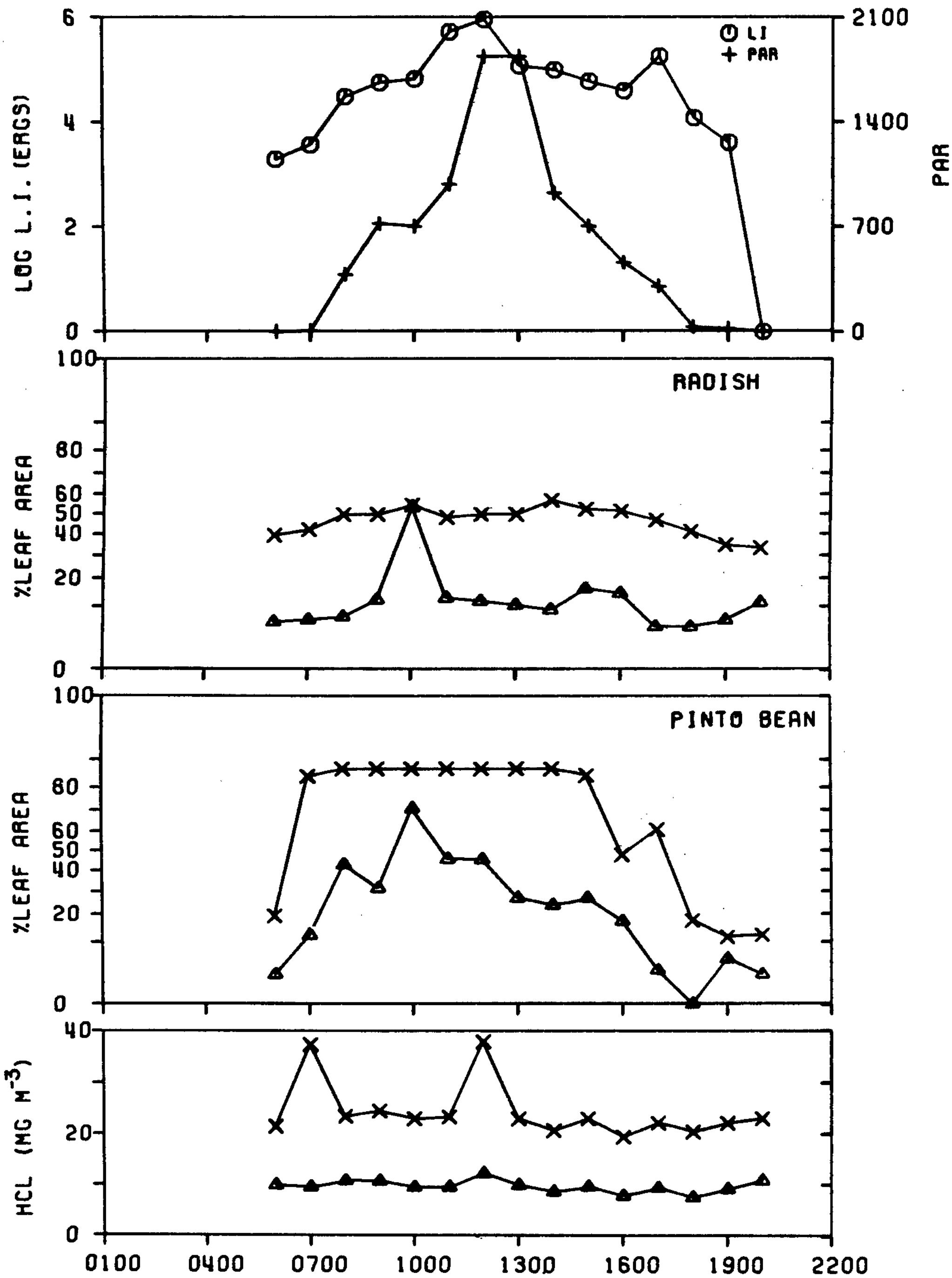

Figure 24. August diurnal experiment in which bean and radish plants were exposed to one of two HCl gas levels hourly during the day. Top to bottom: Light measurements, radish injury, bean injury, and gas concentration. X = high HCl, Δ = low HCl level.

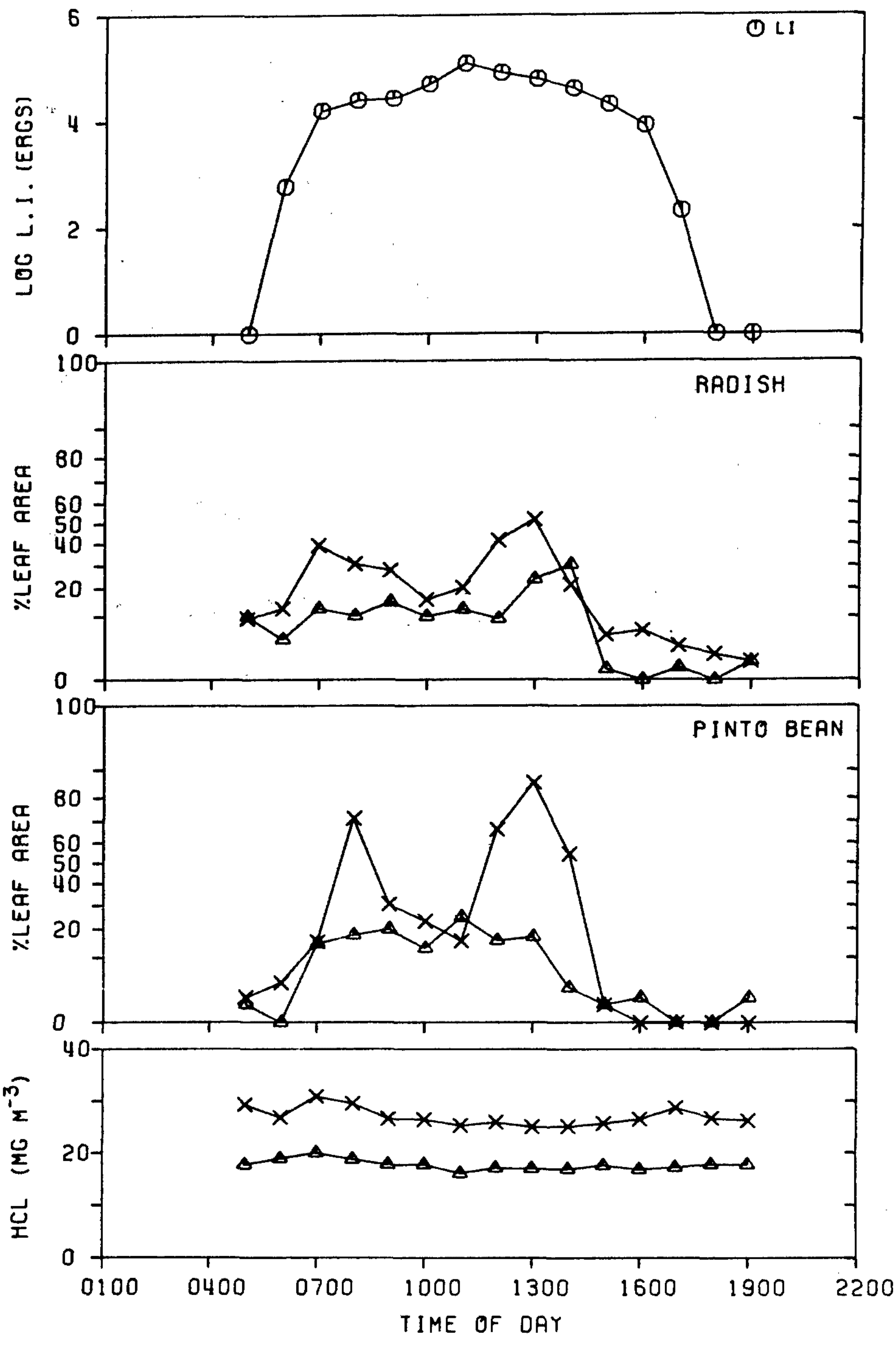

Figure 25. November diurnal experiment in which bean and radish plants were exposed to one of two HCl gas levels hourly during the day. Top to bottom: Light measurements, radish injury, bean injury, and gas concentration. X = high HCl, Δ = low HCl level.

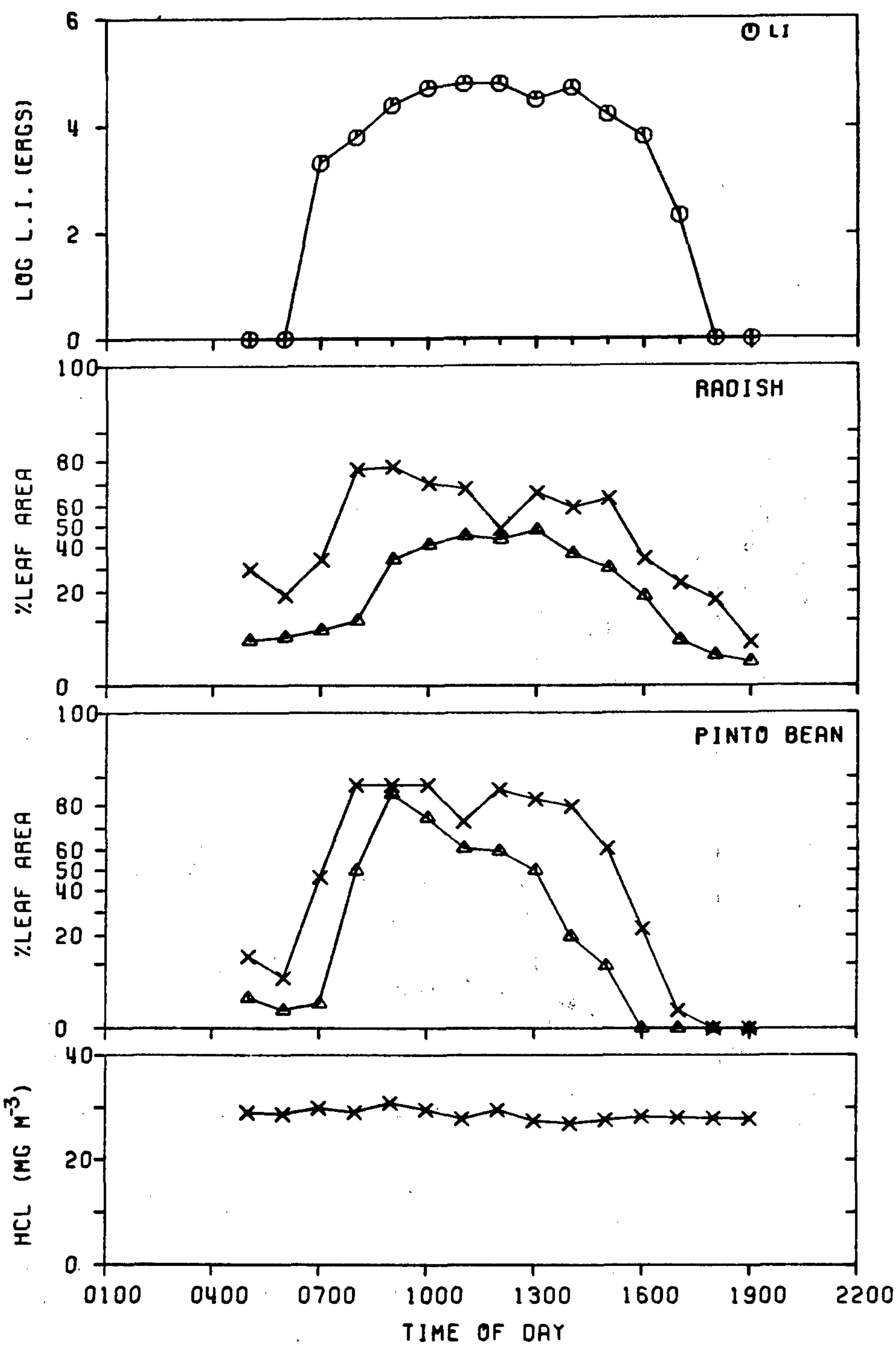

Figure 26. December diurnal experiment in which bean and radish plants were exposed to one of two HCl gas levels hourly during the day. Top to bottom: Light measurements, radish injury, bean injury, and gas concentration. X = high HCl, Δ = low HCl level.

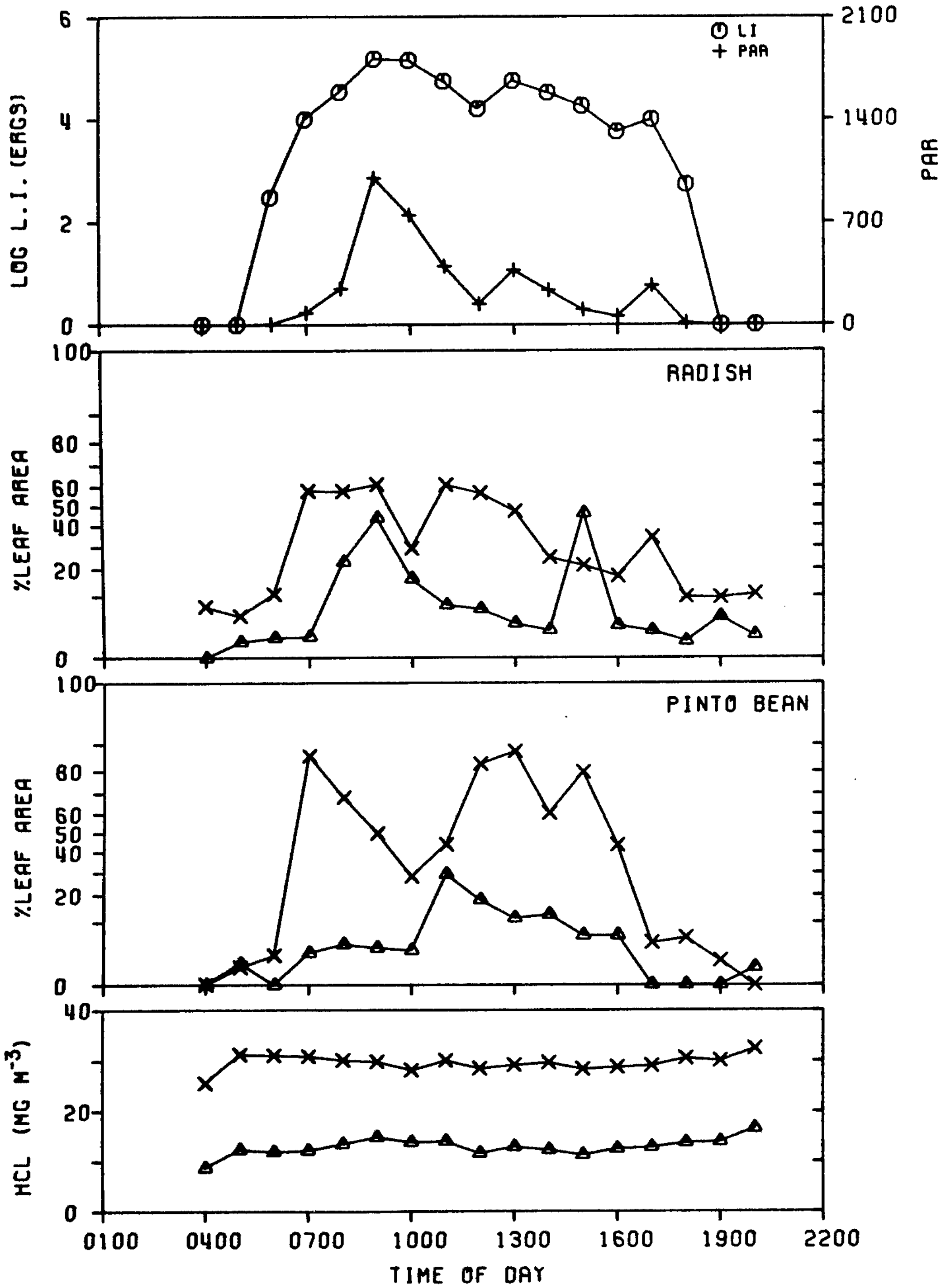

Figure 27. February diurnal experiment in which bean and radish plants were exposed to one of two HCl gas levels hourly during the day. Top to bottom: Light measurements, radish injury, bean injury, and gas concentration. X = high HCl, Δ = low HCl level.

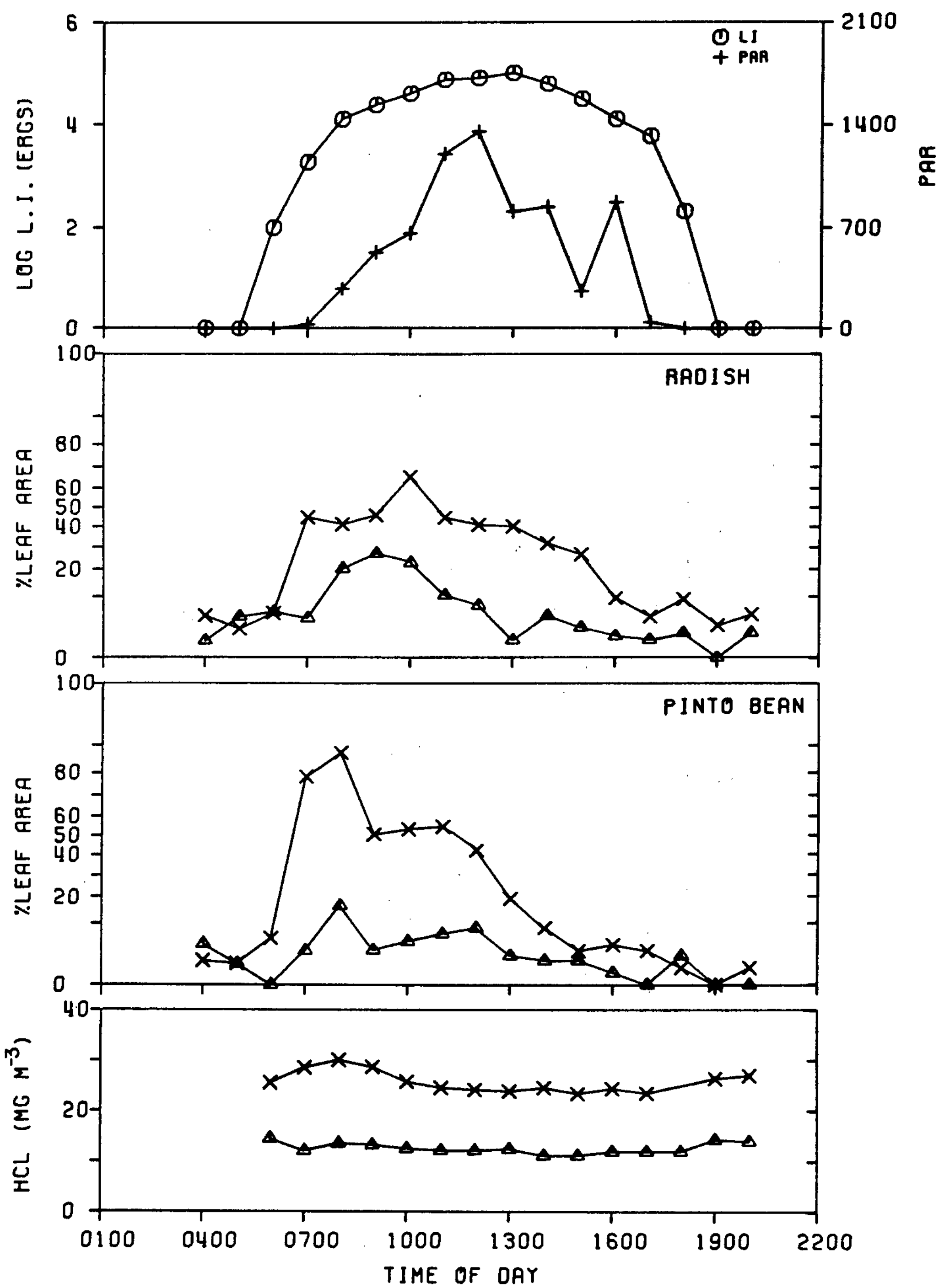

Figure 28. April diurnal experiment in which bean and radish plants were exposed to one of two HCl gas levels hourly during the day. Top to bottom: Light measurements, radish injury, bean injury, and gas concentration. X = high HCl, Δ = low HCl level.

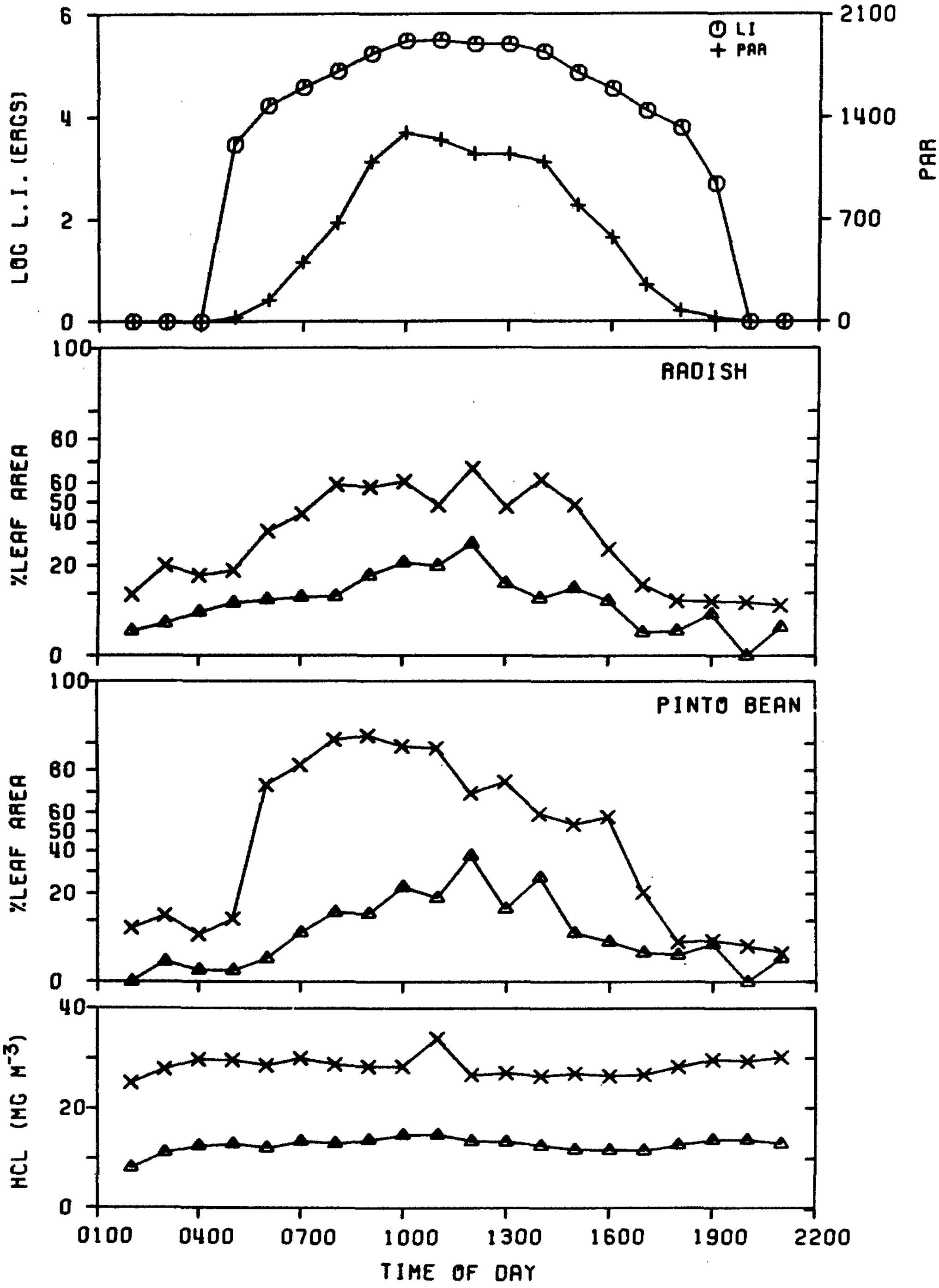

Figure 29. June diurnal experiment in which bean and radish plants were exposed to one of two HCl gas levels hourly during the day. Top to bottom: Light measurements, radish injury, bean injury, and gas concentration. X = high HCl, Δ= low HCl level.

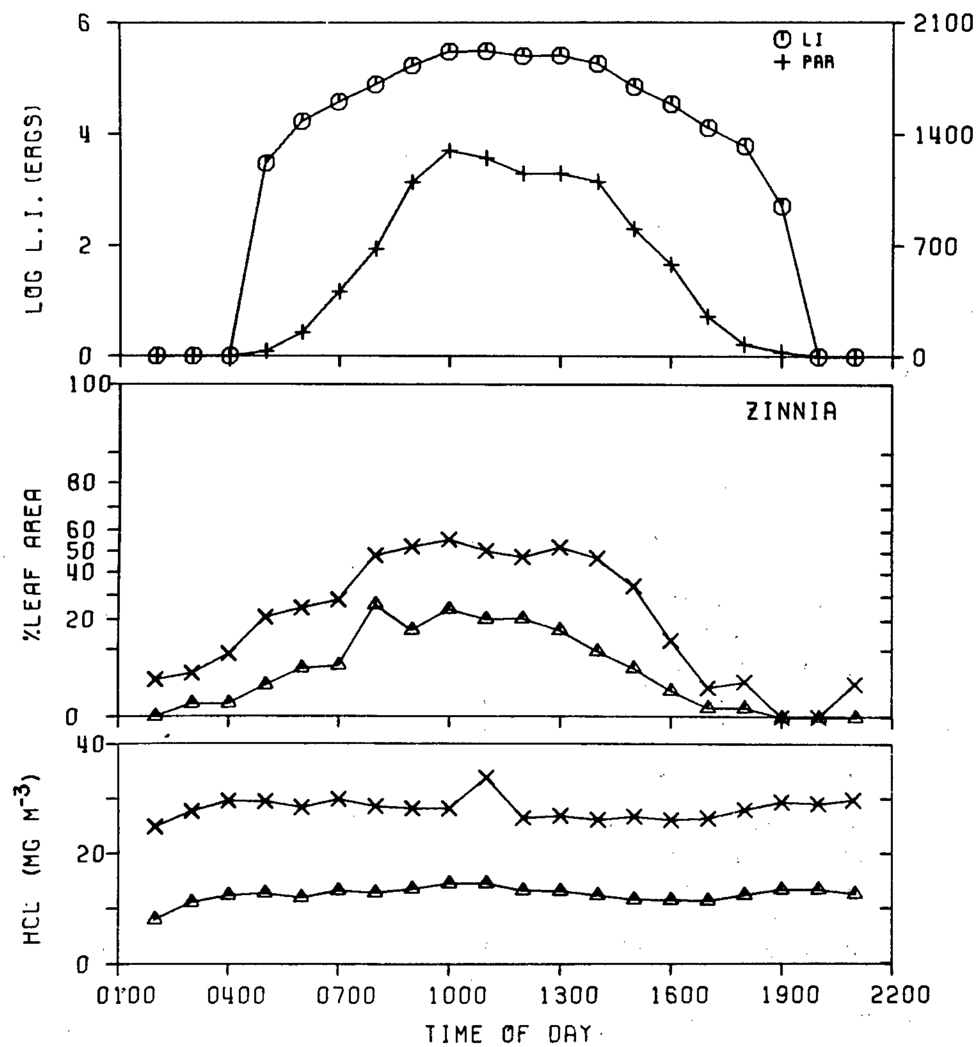

Figure 30. June diurnal experiment in which zinnia plants were exposed to one of two HCl gas levels hourly during the day. Top to bottom: Light measurements, zinnia injury, and gas concentration. X = high, Δ = low HCl level.

HCl concentrations do not decrease in the late evening, it may take several hours for the tank, regulators, supply tubes, and chamber surfaces to equilibrate.

There was some evidence that there was less injury during the winter months. The diurnal sensitivity of both species corresponded best with the change in light levels with greater injury occurring at midday rather than early morning, late afternoon, or night. At low HCl levels, beans were most sensitive from noon to 2 pm while radishes were sensitive earlier, from 8 am to 1 pm. Both species were highly sensitive from 8 am to 4 pm at the higher HCl levels.

TABLE 27.
SEASONAL SUMMARIES OF SIX DIURNAL EXPERIMENTS

Variable	Time of Year					
	AUG	NOV	DEC	FEB	APR	JUN
PAR light (μ einsteins m^{-2} sec^{-1})	593	NA[1]	NA	404	215	503
Light intensity (10^5 ergs cm^{-2} sec^{-1})	14.2	3.0	2.0	2.6	3.1	8.7
Temperature (C)	30	27	23	26	23	31
RH (%)	66	27	52	39	51	67
Low HCl: Concentration (mg m^{-3})	10	18	NA	13	13	12
Bean %-leaves injured	84	56	55	18	23	40
Radish %-leaves injured	60	49	66	33	30	55
Zinnia %-leaves injured	—	—	—	—	—	37
Bean %-leaf area injured	19	6	19	2	3	6
Radish %-leaf area injured	11	7	19	5	6	7
Zinnia %-leaf area injured	—	—	—	—	—	5
High HCl: Concentration (mg m^{-3})	24	27	29	26	30	28
Bean %-leaves injured	100	59	86	61	71	91
Radish %-leaves injured	99	73	93	73	84	94
Zinnia %-leaves injured	—	—	—	—	—	74
Bean %-leaf area injured	64	16	94	18	31	43
Radish %-leaf area injured	46	16	45	21	28	31
Zinnia %-leaf area injured	—	—	—	—	—	20

[1]NA = Data not available

TABLE 28.
DIURNAL SUMMARY OF SIX SEASONAL EXPERIMENT, EACH FROM BEFORE SUNRISE TO AFTER SUNSET

Pacific Standard Time	PAR	TEMP (C)	RH (%)	Low Level HCl ($mg\ m^{-3}$)	Low Level Percent Leaves Injured Bean	Low Level Percent Leaves Injured Radish	High Level HCl ($mg\ m^{-3}$)	High Level Percent Leaves Injured Bean	High Level Percent Leaves Injured Radish
0200	0	23	69	8	0	18	NA[1]	0	0
0300	0	23	72	9	0	34	25	62	88
0400	10	22	59	11	12	12	28	100	95
0500	39	21	54	13	9	46	28	14	66
0600	132	22	55	14	14	44	30	50	75
0700	395	22	53	13	4	50	27	68	79
0800	838	24	51	14	52	69	31	100	97
0900	853	30	44	14	87	71	28	100	98
1000	958	32	41	14	78	72	28	100	94
1100	1120	33	40	14	83	76	27	99	98
1200	1043	32	42	13	94	69	28	99	98
1300	774	32	42	13	91	80	28	100	99
1400	465	32	41	13	95	63	26	100	96
1500	491	30	42	12	75	56	26	99	93
1600	217	28	48	12	52	38	26	90	86
1700	30	25	50	12	24	20	26	63	79
1800	12	23	56	12	3	14	26	42	80
1900	0	24	52	13	1	17	27	27	70
2000	0	24	58	14	8	15	27	17	51
2100	0	27	64	14	5	22	28	35	80

[1]NA = Data not available

This series of investigations showed that the chamber concentrations of our generating equipment produced HCl at reasonably constant levels throughout the day and from season to season. Injury seemed most dependent on light with plants reaching maximum sensitivity as highest light levels were approached and plants in succeeding exposures being injured less. This seems plausible since stomates are light and water dependent and are known to influence pollutant uptake (Guderian, 1977). Dugger et al. (1962), among others, showed that sugars build up in the presence of light and influence plant sensitivity to ozone.

COOPERATIVE RESEARCH TO COMPARE FACILITIES AND TECHNIQUES

Need for cooperative research

A concern of research workers in all fields is to determine if their experimental results have external validity, that is, whether other workers can repeat the same or similar experiments and obtain the same conclusions. Since there is a National Aeronautics and Space Administration (NASA) project whose efforts parallel our own, it seems advantageous to compare techniques of handling HCl, grading plant damage, and, finally, analyzing results. The major investigators on the NASA contract at North Carolina State University agreed and a series of experiments were designed.

Experimental design

Zinnia and radish seedlings were exposed to four HCl gas concentrations (0, 7.5, 15, 30 mg m^{-3}) and at four time lengths (10, 20, 40, 80 minutes). Two plants of each species were exposed for each treatment and replicas were made on 3 successive days. Plant leaf area injury was evaluated 24 hours post-exposure in two ways 1) the Riverside method of estimating area damaged (UCR) and 2) the North Carolina technique (NCS). Percent leaves injured was calculated by the computer by evaluating the number of leaves with any area injury (estimated by UCR or NCS methods) divided by the number of leaves exposed. Two workers, one from each campus, independently made both estimates. Plant tops were oven dried; radish root fresh weights were measured at harvest.

After initial independent trials at the respective campuses, the first cooperative exposures were at North Carolina. Two weeks later the series of exposures were replicated in Riverside. Only the results of the cooperative work have been analyzed to date.

Campus facilities

The North Carolina facilities (NCS) were similar in many ways to the Riverside equipment (UCR). The chambers were of the same dimensions and design although four were available at NCS and only two at UCR. Dry HCl gas under pressure was the common pollutant source at both campuses. There were two notable differences, the NCS greenhouse was open and without filtered air and Geomet instruments were used as the standard HCl monitoring device at NCS. At UCR, greenhouse air was charcoal filtered and bubbler samples were taken to determine chamber HCl concentrations.

Data analysis

The data from the two weeks of exposures consisted of 192 plants per week (each campus), each plant being rated by two observers in two different ways. Only one set of weights were taken per plant. Analysis of variance tables were created for each variable measured. An accurate estimation of each variable's effect was obtained by using several different error mean square terms.

Plant weights

Plant weight data consisted of zinnia and radish dry weights and radish root fresh weights, all material harvested seven days after exposure. Since only one person measured weights, there was no chance for personal technique to come into play and the analysis could be viewed as a measure of plant variability at the two campuses (Tables 29 and 30). The analysis of variance for both dry and fresh weights shows very little campus effect: the plant weights did not vary signficantly between the two campuses. This was interpreted as meaning the rate of plant growth was reasonably similar despite the different climates and greenhouse conditions. HCl concentrations (T) and length of exposure (L) significantly decreased plant weights by harvest-time; reductions increased with treatment severity (higher T, longer L). Further analysis indicated that the HCl effect was linear while the exposure period had both linear and more complex elements. There was also a very significant interaction term (L x T) between the two treatments. Examination showed that this significance was created by the high concentration treatments causing maximum response well before the exposure (L). There was no significant interaction with the campus factor and either T or L which indicated that similar plant responses were achieved at both campuses as far as plant weights were concerned.

Plant injury

The data for plant injury was modified by deleting all control fumigations (0 mg m^{-3} at all exposure lengths) since injury response would be zero for all cases and subsequently the standard deviations would also be zero. This would violate one of the critical assumptions of analysis of variance: all standard deviations must be equal. The data was thus reduced to the response of 144 exposed plants, but since the separate graders were considered, there were 288 separate entries in the analysis. Each observer graded every leaf for visually necrotic and/or glazed area twice, by the UCR method (0-4) and the NCS method (0-100% in 5% increments). Percent area injured by the UCR technique was later computed by weighting and averaging the observed 0-4 estimates (0%, 12.5%, 37.5%, 62.5% and 87.5%, respectively). Analysis of variance for each method were compared (Tables 33 and 34). For analysis of percent leaves injured, the number of leaves with any injury was retrieved for each plant from the recorded data for both methods of area estimation. Differences occasionally occurred when very young or old leaves were measured by one but not the other grader. Angular transformation (arc sin) of the percent leaves injured data was accomplished prior to analysis. Analysis of variance for each method were compared (Tables 31 and

TABLE 29.
ANALYSIS OF VARIANCE FOR TOP DRY WEIGHTS AFTER EXPOSURE TO 0-20 mg HCl m^{-3} (T) FOR 0-80 MINUTES (L) AT TWO CAMPUSES (C).

		Radish			Zinnia		
SOURCE OF VARIATION	DF	SS	F	CV	SS	F	CV
C	1	0.3674996D 00	2.06		0.2530007D 01	2.33	
ERROR A	4	0.7129104D 00		71.9 %	0.4337305D 01		54.1 %
T	3	0.3962215D 01	28.27 ***		0.1005911D 02	9.53 **	
LINEAR	1	0.3781944E 01	80.96 ***		0.9623279E 01	27.35 ***	
QUADRATIC	1	0.1796365E 00	3.85		0.4356056E 00	1.24	
RESIDUAL	1	0.6343119D-03	0.01		0.2236384D-03	0.00	
C X T	3	0.2825286D 00	2.02		0.2119345D 00	0.20	
ERROR B	12	0.5605391D 00		36.8 %	0.4221617D 01		30.8 %
L	3	0.1879359D 01	31.48 ***		0.2387331D 01	7.82 ***	
LINEAR	1	0.1566508E 01	78.71 ***		0.2201203E 01	21.63 ***	
QUADRATIC	1	0.2135457E 00	10.73 **		0.1423227E 00	1.40	
RESIDUAL	1	0.9930475D-01	4.99 *		0.4380547D-01	0.43	
LXT	9	0.1821701D 01	10.17 ***		0.2495739D 01	2.72 *	
C X L	3	0.3117912D-01	0.52		0.6949711D 00	2.28	
C X T X L	9	0.4193747D 00	2.34 *		0.1593088D 01	1.74	
ERROR C	48	0.9552823D 00		24.0 %	0.4885046D 01		16.6 %
ERROR	96	-0.8215650D-14		0.0 %	0.2922949D 01		9.1 %
TOTAL	191	0.1099259D 02			0.3633910D 02		

* = 5% level, ** = 1% level, *** = 0.1% level of significance

TABLE 30.
ANALYSIS OF VARIANCE OF RADISH ROOT FRESH WEIGHT FOR PLANTS EXPOSED TO HCl GAS (T) FOR 0-80 MINUTES (L) AT TWO CAMPUSES (C)

SOURCE OF VARIATION	DF		SS	MS	F	CV
C	1		0.1083001D 02	0.1083001E 02	0.36	
ERROR A	4		0.1215236D 03	0.3038089E 02		130.7 %
T	3		0.6222926D 03	0.2074308E 03	21.88 ***	
LINEAR		1	0.6072017E 03	0.6072017E 03	64.05 ***	
QUADRATIC		1	0.8253652E-01	0.8253652E-01	0.01	
RESIDUAL		1	0.1500837D 02	0.1500837E 02	1.58	
C X T	3		0.3617913D 01	0.1205971E 01	0.13	
ERROR B	12		0.1137528D 03	0.9479401E 01		73.0 %
L	3		0.2599735D 03	0.8665782E 02	15.04 ***	
LINEAR		1	0.1535331E 03	0.1535331E 03	26.65 ***	
QUADRATIC		1	0.1033017E 03	0.1033017E 03	17.93 ***	
RESIDUAL		1	0.3138739D 01	0.3138739E 01	0.54	
LXT	9		0.1823791D 03	0.2026433E 02	3.52 **	
C X L	3		0.9967072D 01	0.3322357E 01	0.58	
C X T X L	9		0.6532825D 02	0.7258695E 01	1.26	
ERROR C	48		0.2765764D 03	0.5762009E 01		56.9 %
ERROR	96		-0.2046363D-11	-0.2131628E-13		0.0 %
TOTAL	191		0.1666241D 04			

** = 1%, *** 0.1% level of significance

TABLE 31.

ANALYSIS OF VARIANCE FOR PERCENT LEAVES INJURED ON ZINNIA AND RADISH PLANTS. PLANTS WERE EXPOSED TO HCl GAS (T=TREATMENTS) FOR DIFFERENT TIME PERIODS (L=LENGTH) AT TWO CAMPUSES (C) AND GRADED BY TWO METHODS, NCS AND UCR, BY TWO PERSONS (P).

			Zinnia					
			NCS Method			UCR Method		
SOURCE OF VARIATION	DF		SS	F	CV	SS	F	CV
C	1		0.7073715D 04	11.60 *		0.6208194D 04	8.75 *	
ERROR A	4		0.2439482D 04		43.4 %	0.2839530D 04		45.0 %
T	2		0.7262346D 05	74.56 ***		0.6028690D 05	51.24 ***	
LINEAR		1	0.6776169E 05	139.14 ***		0.5685270E 05	96.63 ***	
RESIDUAL		1	0.4861770D 04	9.98 *		0.3434204D 04	5.84 *	
C X T	2		0.1506005D 04	1.55		0.6097997D 03	0.52	
ERROR B	8		0.3896014D 04		38.8 %	0.4706688D 04		41.0 %
L	3		0.4870775D 05	54.69 ***		0.4176206D 05	43.14 ***	
LINEAR		1	0.3169611E 05	106.77 ***		0.2631424E 05	81.55 ***	
QUADRATIC		1	0.1576566E 05	53.11 ***		0.1426593E 05	44.21 ***	
RESIDUAL		1	0.1245979D 04	4.20 *		0.1181881D 04	3.66	
LXT	6		0.1487060D 05	8.35 ***		0.1810819D 05	9.35 ***	
C X L	3		0.1414057D 04	1.59		0.1419980D 04	1.47	
C X T X L	6		0.7346722D 04	4.12 **		0.5713654D 04	2.95 *	
ERROR C	36		0.1068726D 05		30.3 %	0.1161690D 05		30.3 %
P	1		0.4056388D 03	5.00		0.7941318D 02	2.52	
P X C	1		0.3859128D 02	0.48		0.5926632D 03	18.83 *	
ERROR D	4		0.3246413D 03		15.9 %	0.1259000D 03		9.5 %
P X T	2		0.3666638D 03	1.26		0.2610099D 03	0.85	
P X C X T	2		0.3848161D 03	1.32		0.9349035D 02	0.30	
ERROR E	8		0.1163455D 04		21.2 %	0.1226345D 04		20.9 %
P X L	3		0.2076985D 03	1.11		0.3104285D 03	1.67	
P X T X L	6		0.5495668D 02	0.15		0.1412330D 03	0.38	
P X L X C	3		0.1332708D 03	0.71		0.2906916D 03	1.57	
PXLXTXC	6		0.5943216D 03	1.59		0.2229352D 03	0.60	
ERROR	36		0.2247656D 04		13.9 %	0.2228307D 04		13.3 %
TOTAL	143		0.1764868D 06			0.1588443D 06		

			Radish					
SOURCE OF VARIATION	DF		SS	F	CV	SS	F	CV
C	1		0.8367052D 03	3.07		0.2814451D 04	6.37	
ERROR A	4		0.1091225D 04		36.9 %	0.1767883D 04		43.4 %
T	2		0.9022808D 05	366.12 ***		0.8130043D 05	427.09 ***	
LINEAR		1	0.8956113E 05	726.83 ***		0.8045131E 05	845.26 ***	
RESIDUAL		1	0.6669510D 03	5.41 *		0.8491198D 03	8.92 *	
C X T	2		0.4872495D 03	1.98		0.1270113D 04	6.67 *	
ERROR B	8		0.9857778D 03		24.8 %	0.7614312D 03		20.2 %
L	3		0.2807259D 05	42.48 ***		0.3087712D 05	42.50 ***	
LINEAR		1	0.2230539E 05	101.25 ***		0.2300120E 05	94.99 ***	
QUADRATIC		1	0.5717512E 04	25.95 ***		0.7514781E 04	31.03 ***	
RESIDUAL		1	0.4968616D 02	0.23		0.3611432D 03	1.49	
LXT	6		0.5168982D 04	3,91 **		0.4070774D 04	2.80 *	
C X L	3		0.7492015D 02	0.11		0.2986631D 03	0.41	
C X T X L	6		0.4195256D 04	3.17 *		0.3893867D 04	2.68 *	
ERROR C	36		0.7930519D 04		33.1 %	0.8717242D 04		32.1 %
P	1		0.5943304D 03	27.83 **		0.4384399D 03	55.05 **	
P X C	1		0.5551799D 03	26.00 **		0.1961666D 02	2.46	
ERROR D	4		0.8542726D 02		10.3 %	0.3185963D 02		5.8 %
P X T	2		0.1392828D 03	2.70		0.2124315D 03	5.74 *	
P X C X T	2		0.5442799D 02	1.05		0.3663843D 03	9.91 **	
ERROR E	8		0.2064607D 03		11.3 %	0.1479264D 03		8.9 %
P X L	3		0.1276153D 03	0.78		0.6701397D 02	0.40	
P X T X L	6		0.2561995D 03	0.78		0.4020030D 03	1.20	
P X L X C	3		0.1666565D 03	1.02		0.3855960D 02	0.23	
PXLXTXC	6		0.8331973D 03	2.55 *		0.7875554D 03	2.35	
ERROR	36		0.1958889D 04		16.5 %	0.2013072D 04		15.4 %
TOTAL	143		0.1440490D 06			0.1402968D 06		

* = 5%, ** = 1%, *** = 0.1% level of significance

TABLE 32.

ANALYSIS OF VARIANCE OF GRADER INFLUENCE ON PERCENT LEAVES INJURED. ZINNIA AND RADISH SEEDLINGS WERE EXPOSED TO HCl GAS (T=TREATMENTS) FOR DIFFERENT TIME PERIODS (L=LENGTH) AT TWO CAMPUSES (C) AND GRADED BY TWO TWO METHODS, NCS AND UCR, BY PERSONS FAMILIAR WITH EACH METHOD

		Zinnia					
		NCS Grader NCS Method			UCR Grader UCR Method		
SOURCE OF VARIATION	DF	SS	F	CV	SS	F	CV
C	1	0.1377501D 04	9.27 *		0.1182065D 04	4.93	
ERROR A	4	0.5945483D 03		26.0 %	0.9597102D 03		30.9 %
T	2	0.4208945D 05	399.98 ***		0.3660544D 05	245.68 ***	
LINEAR	1	0.4161889E 05	791.02 ***		0.3624218E 05	486.49 ***	
RESIDUAL	1	0.4705579D 03	8.94 *		0.3632612D 03	4.88	
C X T	2	0.1405689D 03	1.34		0.1470163D 04	9.87 **	
ERROR B	8	0.4209141D 03		15.5 %	0.5959810D 03		17.2 %
L	3	0.1300395D 05	26.26 ***		0.1682335D 05	37.94 ***	
LINEAR	1	0.1005399E 05	60.90 ***		0.1245361E 05	84.25 ***	
QUADRATIC	1	0.2830006E 04	17.14 ***		0.4217480E 04	28.53 ***	
RESIDUAL	1	0.1199512D 03	0.73		0.1522557D 03	1.03	
LXT	6	0.2846681D 04	2.87 *		0.1945837D 04	2.19	
C X L	3	0.1394838D 03	0.28		0.9618988D 02	0.22	
C X T X L	6	0.3660671D 04	3.70 **		0.1650053D 04	1.86	
ERROR	36	0.5943357D 04		27.4 %	0.5321158D 04		24.2 %
TOTAL	71	0.7021712D 05			0.6664994D 05		

		Radish					
SOURCE OF VARIATION	DF	SS	F	CV	SS	F	CV
C	1	0.4078631D 04	9.32 *		0.1482261D 04	3.10	
ERROR A	4	0.1749939D 04		35.7 %	0.1914606D 04		36.5 %
T	2	0.3160595D 05	39.30 ***		0.3405245D 05	88.83 ***	
LINEAR	1	0.2985861E 05	74.26 ***		0.3180449E 05	165.94 ***	
RESIDUAL	1	0.1747337D 04	4.35		0.2247963D 04	11.73 **	
C X T	2	0.3540760D 03	0.44		0.5519680D 03	1.44	
ERROR B	8	0.3216760D 04		34.3 %	0.1533334D 04		23.1 %
L	3	0.2222706D 05	58.95 ***		0.2104557D 05	39.01 ***	
LINEAR	1	0.1344189E 05	106.94 ***		0.1472099E 05	81.85 ***	
QUADRATIC	1	0.8059520E 04	64.12 ***		0.6066594E 04	33.73 ***	
RESIDUAL	1	0.7256548D 03	5.77 *		0.2579796D 03	1.43	
LXT	6	0.6989130D 04	9.27 ***		0.9692157D 04	8.98 ***	
C X L	3	0.7475721D 03	1.98		0.9381265D 03	1.74	
C X T X L	6	0.4730824D 04	6.27 ***		0.2818147D 04	2.61 *	
ERROR	36	0.4524919D 04		19.2 %	0.6474555D 04		22.4 %
TOTAL	71	0.8022486D 05			0.8050317D 05		

* = 5%, ** = 1%, *** = 0.1% level of significance

TABLE 33.

ANALYSIS OF VARIANCE FOR PERCENT LEAF AREA INJURED ON ZINNIA AND RADISH PLANTS. PLANTS WERE EXPOSED TO HCl GAS (T=TREATMENT) FOR DIFFERENT TIME PERIODS (L=LENGTH) AT TWO CAMPUSES (C) AND GRADED BY TWO METHODS, NCS AND UCR BY TWO PERSONS (P).

		Zinnia					
		NCS Method			UCR Method		
SOURCE OF VARIATION	DF	SS	F	CV	SS	F	CV
C	1	0.2272054D 04	99.97 ***		0.2852894D 04	52.22 **	
ERROR A	4	0.9090917D 02		22.5 %	0.2185180D 03		31.0 %
T	2	0.6399424D 05	411.78 ***		0.5594950D 05	327.06 ***	
LINEAR	1	0.6239291E 05	802.95 ***		0.5548831E 05	648.73 ***	
RESIDUAL	1	0.1601329D 04	20.61 **		0.4611918D 03	5.39 *	
C X T	2	0.6160709D 03	3.96		0.2787570D 03	1.63	
ERROR B	8	0.6216390D 03		41.6 %	0.6842746D 03		38.8 %
L	3	0.1435486D 05	43.26 ***		0.1497744D 05	57.18 ***	
LINEAR	1	0.1204845E 05	108.92 ***		0.1237236E 05	141.71 ***	
QUADRATIC	1	0.2258260E 04	20.41 ***		0.2554345E 04	29.26 ***	
RESIDUAL	1	0.4815351D 02	0.44		0.5073550D 02	0.58	
LXT	6	0.8269263D 04	12.46 ***		0.4447126D 04	8.49 ***	
C X L	3	0.6376812D 03	1.92		0.3889157D 03	1.48	
C X T X L	6	0.3551015D 04	5.35 ***		0.2772655D 04	5.29 ***	
ERROR C	36	0.3982268D 04		49.6 %	0.3143032D 04		39.2 %
P	1	0.1750387D 03	17.62 *		0.1310740D 04	212.11 ***	
P X C	1	0.1295082D 02	1.30		0.9925137D 02	16.06 *	
ERROR D	4	0.3973040D 02		14.9 %	0.2471824D 02		10.4 %
P X T	2	0.1849654D 03	12.63 **		0.2220012D 03	12.92 **	
P X C X T	2	0.3044883D 02	2.08		0.1846130D 02	1.07	
ERROR E	8	0.5856261D 02		12.8 %	0.6872864D 02		12.3 %
P X L	3	0.2248899D 02	0.88		0.8746447D 02	2.93 *	
P X T X L	6	0.3245148D 02	0.63		0.4137214D 03	6.94 ***	
P X L X C	3	0.1542470D 02	0.60		0.2566352D 02	0.86	
PXLXTXC	6	0.9629393D 02	1.88		0.1340214D 03	2.25	
ERROR	36	0.3070164D 03		13.8 %	0.3579071D 03		13.2 %
TOTAL	143	0.9936[illegible]38D 05			0.8847580D 05		

		Radish					
SOURCE OF VARIATION	DF	SS	F	CV	SS	F	CV
C	1	0.6940330D 04	19.40 *		0.5108081D 04	9.97 *	
ERROR A	4	0.1430692D 04		64.3 %	0.2049541D 04		76.2 %
T	2	0.7207536D 05	98.13 ***		0.5787441D 05	147.88 ***	
LINEAR	1	0.7205238E 05	196.20 ***		0.5780411E 05	295.39 ***	
RESIDUAL	1	0.2298339D 02	0.06		0.7030755D 02	0.36	
C X T	2	0.5365433D 03	0.73		0.1775993D 03	0.45	
ERROR B	8	0.2937847D 04		65.2 %	0.1565482D 04		47.1 %
L	3	0.4965843D 05	92.69 ***		0.4131751D 05	88.27 ***	
LINEAR	1	0.4221004E 05	236.36 ***		0.3554541E 05	227.81 ***	
QUADRATIC	1	0.6639109E 04	37.18 ***		0.5220285E 04	33.46 ***	
RESIDUAL	1	0.8092883D 03	4.53 *		0.5518140D 03	3.54	
LXT	6	0.2127477D 05	19.85 ***		0.1181835D 05	12.62 ***	
C X L	3	0.2106647D 04	3.93 *		0.1247487D 04	2.67	
C X T X L	6	0.9733470D 03	0.91		0.1026600D 04	1.10	
ERROR C	36	0.6429132D 04		45.4 %	0.5617159D 04		42.1 %
P	1	0.1630383D 02	0.27		0.1657170D 02	0.35	
P X C	1	0.1919183D 02	0.32		0.1879184D 01	0.04	
ERROR D	4	0.2382619D 03		26.2 %	0.1890518D 03		23.2 %
P X T	2	0.5547693D 02	3.49		0.7687501D 02	1.10	
P X C X T	2	0.2672506D 01	0.17		0.2958170D 02	0.42	
ERROR E	8	0.6349318D 02		9.6 %	0.2789747D 03		19.9 %
P X L	3	0.1933612D 01	0.03		0.5504096D 02	0.71	
P X T X L	6	0.5548231D 02	0.48		0.2001460D 03	1.29	
P X L X C	3	0.3459870D 02	0.60		0.5730128D 02	0.74	
PXLXTXC	6	0.1230278D 03	1.07		0.2191482D 03	1.41	
ERROR	36	0.6915153D 03		14.9 %	0.9336464D 03		17.2 %
TOTAL	143	0.1656651D 06			0.1298604D 06		

* = 5%, ** = 1%, *** = 0.1% level of significance

TABLE 34.

ANALYSIS OF VARIANCE OF GRADER INFLUENCE ON PERCENT LEAF AREA INJURED. ZINNIA AND RADISH SEEDLINGS WERE EXPOSED TO HCl GAS (T=TREATMENT) FOR DIFFERENT TIME PERIODS (L=LENGTH) AT TWO CAMPUSES (C) AND GRADED BY TWO METHODS, NCS AND UCR, BY PERSONS FAMILIAR WITH EACH METHOD.

			Zinnia					
			NCS Grader NCS Method			UCR Grader UCR Method		
SOURCE OF VARIATION	DF		SS	F	CV	SS	F	CV
C	1		0.9709654D 03	55.16 **		0.2008195D 04	43.59 **	
ERROR A	4		0.7040641D 02		20.9 %	0.1842859D 03		25.3 %
T	2		0.2868950D 05	281.28 ***		0.3085881D 05	313.63 ***	
LINEAR		1	0.2789186E 05	546.92 ***		0.3076927E 05	625.44 ***	
RESIDUAL		1	0.7976385D 03	15.64 **		0.8954525D 02	1.82	
C X T	2		0.1862977D 03	1.83		0.1329606D 03	1.35	
ERROR B	8		0.4079858D 03		35.5 %	0.3935710D 03		26.1 %
L	3		0.6938000D 04	44.74 ***		0.8500416D 04	59.66 ***	
LINEAR		1	0.5635277E 04	109.02 ***		0.7219238E 04	152.00 ***	
QUADRATIC		1	0.1279927E 04	24.76 ***		0.1271646E 04	26.77 ***	
RESIDUAL		1	0.2279528D 02	0.44		0.9531237D 01	0.20	
LXT	6		0.4266926D 04	13.76 ***		0.1528938D 04	5.37 ***	
C X L	3		0.2777127D 03	1.79		0.2716401D 03	1.91	
C X T X L	6		0.1262966D 04	4.07 **		0.2007912D 04	7.05 ***	
ERROR	36		0.1860776D 04		35.8 %	0.1709852D 04		25.7 %
TOTAL	71		0.4493153D 05			0.4759658D 05		

			Radish					
SOURCE OF VARIATION	DF		SS	F	CV	SS	F	CV
C	1		0.3114799D 04	9.71 *		0.2457006D 04	11.14 *	
ERROR A	4		0.1282572D 04		60.2 %	0.8818466D 03		49.4 %
T	2		0.3798617D 05	119.61 ***		0.2750721D 05	77.14 ***	
LINEAR		1	0.3798209E 05	239.19 ***		0.2740263E 05	153.69 ***	
RESIDUAL		1	0.4078264D 01	0.03		0.1045852D 03	0.59	
C X T	2		0.2622464D 03	0.83		0.6323794D 02	0.18	
ERROR B	8		0.1270341D 04		42.4 %	0.1426423D 04		44.5 %
L	3		0.2511399D 05	97.79 ***		0.2144474D 05	66.58 ***	
LINEAR		1	0.2130350E 05	248.87 ***		0.1828809E 05	170.33 ***	
QUADRATIC		1	0.3399449E 04	39.71 ***		0.2697450E 04	25.12 ***	
RESIDUAL		1	0.4110388D 03	4.80 *		0.4592044D 03	4.28 *	
LXT	6		0.1127088D 05	21.94 ***		0.5676221D 04	8.81 ***	
C X L	3		0.1061781D 04	4.13 *		0.7017634D 03	2.18	
C X T X L	6		0.4170563D 03	0.81		0.6411791D 03	1.00	
ERROR	36		0.3081696D 04		31.1 %	0.3865218D 04		34.5 %
TOTAL	71		0.8486153D 05			0.6466485D 05		

* = 5%, ** = 1%, *** = 0.1% level of significance

In general, these ANOVA tables all show that treatments (T) and time period (L) are very significant (at the 0.01% level) in the injury recorded regardless of grader or method. The significant L x T interactions come about because, at higher HCl concentrations, maximum injury levels were reached at less than the longest time periods. For instance, 90-100% injury was achieved at 20, 40 and 80 minutes at 30 mg m^{-3} while plants exposed to 15 mg m^{-3} experienced 30, 70 and 80% injury levels for the same time periods. The injury at high concentration could not increase in the same manner since maximum injury had been achieved sooner. The statistical significance thus does not imply biological significance.

The means of the injury data were linearly related to the HCl concentration (T). The relation with time period (L) was also linear with quadratic and cubic components. This means that a simple, straight line relationship could not be described. When analysis was made of a log distribution of the L variables, the relationship became more linear with one quadratic component.

In many cases the C (campus) variable was significant. This means that injury was different at the two campuses. This could arise in several ways. Actual injury was probably affected by such environmental factors such as temperature (higher at UCR), humidity (higher at NCS) and light levels (higher at UCR). Another point of difference was the method of measuring HCl which probably caused plants to receive somewhat different doses at the different campuses. Still another important point was that the reading of injury was changed somewhat during the second week (at UCR). Glazing had not been rated as strongly by the NCS grader initially and this was corrected in the subsequent series of exposures. This small grading change contributed to the significant differences between campuses (C) and particularly in the P x C interaction terms. Zinnia plants were more subject to glazing and more differences were noted in their analysis. The UCR method, when used to estimate leaf area injured, also indicated some variability between the two weeks (Table 34).

Cooperative experiment conclusions

This experiment showed that HCl-induced injury was similar on plants raised and exposed to HCl gas under different environmental conditions using different facilities 3000 miles apart. Regardless of the location for exposing plants or the method used for estimating plant injury, the basic plant responses to gas concentrations and length of exposures were essentially the same. It was important that one observer could determine and estimate damage by another person's techniques without serious problems to the overall analysis. This was gratifying both because the analysis provided further evidence of the nature of HCl phytotoxicity and because it lent external validity to and added confidence in our other experimental work.

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☆U.S.Government Printing Office: 1979 — 657-002/363