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THE EFFECT OF DESIGNATED POLLUTANTS ON PLANTS

Fourth Annual Report

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AMRL-TR-79-73

This report has been reviewed by the Office of Public Affairs (PA) 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

ANTHONY A. THOMAS, MD
Director
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Hydrogen fluoride (HF)	Phytotoxic responses	

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

The phototoxicity of hydrogen chloride (HCl) gas was studied with particular emphasis on various external plant stresses. Greenhouse grown plants and indoor exposure chambers were utilized to test the effect of viral infection, insecticide treatment, weed killer applications, or antioxidant protectants on injury caused by HCl gas. Plant sensitivity did not differ between virus-infected and non-viral plants. Increased injury, however, was seen on plants exposed to HCl gas after one of the insecticide treatments. An experimental antioxidant compound which protects plants from ozone injury did not increase tolerance to HCl

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gas. Since greenhouse conditions eliminate many natural stresses, HCl exposures were conducted in field plots both in Riverside and at Vandenberg Air Force Base, California. Some plants were fumigated only once while others received weekly doses. Field plants were generally more tolerant than the same species grown in the greenhouse. Further, plants exposed at Vandenberg were more sensitive than those at Riverside. Of the plants tested, a native species at Vandenberg was most resistant to HCl. HCl for the field work was generated either by diluting pressurized dry gas in a large volume of flowing carrier gas or by open-burning of small pieces of solid rocket fuel. Portable chambers remained over the field plants only for the duration of the 15-minute exposures. HF gas is considerably more phytotoxic than HCl. Work with this pollutant was limited to a review of the literature and an experiment on the uptake of fluoride salts through the roots.

PREFACE

This is the fourth annual report of work performed by members of the Statewide Air Pollution Research Center, University of California, Riverside during the period from July 1, 1978 to June 30, 1979. The project is sponsored by Air Force Contract F-33615-76C-5005 to the University of California, Irvine. Research is conducted to aid Air Force personnel in recognizing and predicting phytotoxic responses of terrestrial plants to air pollutants released by Air Force operations. Investigations are concerned with the exhaust products of solid rocket engines, particularly hydrogen chloride or hydrogen fluoride gases and aluminum oxide particles. These experiments were conducted in the laboratory, greenhouse and field during the past contract year. Plants selected for use include species native to or grown commercially in the vicinity of Lompoc and Vandenberg Air Force Base, California.

The cooperation and aid of Air Force contract monitor Lt. Col. C. B. Harrah, Toxic Hazards Division, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, has been appreciated. The authors acknowledge the technical aid of Dr. T. Bruhns, T. Carson, D. Duncan, L. A. Neher and C. L. Simpson during various portions of this project. Dr. Bruhns researched and wrote the fluoride literature review and Mr. Duncan was responsible for the photography. Assistance of University of California students, S. K. Hollingsworth, J. Meyers, M. R. Shulte and J. Phelen has also been appreciated. The advice and cooperation of Major A. L. Young, Ph.D., Occupational and Environmental Health Laboratory, Brooks Air Force Base, Texas, during the soil studies has been valuable. Dr. L. D. Strand, Jet Propulsion Laboratory, Pasadena kindly supplied rocket fuel and Dr. E. L. Jenner, E.I. duPont deNemours, Wilmington, Delaware, supplied antioxidant plant protectant.

TABLE OF CONTENTS

Table of Contents (continued)

Table of Contents (continued)

LIST OF ILLUSTRATIONS

LIST OF TABLES

INTRODUCTION

This project is part of a 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 limited to toxic exhaust products of rocket fuels, in use or planned. The investigations concern effects of some of these toxic materials on selected plant species. Previous annual reports in this series dealt with aluminum oxide particles (Al_2O_3) and gaseous hydrogen chloride (HCl) (Granett and Taylor, 1976, 1977, 1978). The present report details continued HCl work and includes work and literature on gaseous hydrogen fluoride.

In the last annual report, Granett and Taylor (1978) concluded that there was little detectable phytotoxic synergism between HCl and Al_2O_3 and no detectable injury from plant exposure to Al_2O_3 alone. Therefore, no further work with pure Al_2O_3 was undertaken. Last year, basic techniques

for generating and detecting HCl from solid rocket fuel exhaust were developed. The present report describes field experiments in which plants were exposed to gases, particularly HCl, derived from solid rocket fuel combustion. These studies were designed to compare the sensitivity of field grown plants with that of greenhouse grown plants.

During the spring of 1979, field exposures were conducted at Vandenberg Air Force Base using a portable chamber supplied with a steady-state level of dry HCl gas. The reactions of plants exposed at Vandenberg were compared to those of others grown and exposed in the greenhouse or field at Riverside, California.

Certain stresses have been shown to affect the sensitivity of plants to air pollutants; nutrient level, relative humidity, light, and temperature alter plant response to gaseous HCl (Granett and Taylor, 1978). Plant diseases and certain chemicals can also alter plant response (Wukasch and Hofstra, 1977; Papple and Ormrod, 1977; Bisessar and Temple, 1977; Koiwai et al., 1974; and Manning et al., 1974). In experiments reported here, plants were stressed by infection with tobacco mosaic virus (TMV) or by treatment with an antioxidant plant protectant before exposure to HCl.

An investigation into the phytotoxic effects of plants to short exposures of HF gas was begun by a thorough literature review and a study on the uptake of fluoride from the soil.

MATERIALS AND METHODS

EXPOSURE EQUIPMENT

The design of exposure chambers used in these studies has been described elsewhere (Granett and Taylor, 1978). The greenhouse experiments were conducted in steel-framed, 1.05 m^3 cylindrical chambers covered with a Tedlar film with an exhaust fan and circulating paddles (Jeffries et al., 1976; Heck et al., 1978). The pollutant, supplied as a 40% mixture of dry HCl in nitrogen, was controlled by a regulator, needle valve, and flowmeter. The exhaust fans were off during experiments when solid rocket fuel was burned.

Four chambers were used during the 1978 field experiments. These consisted of rectangular wooden frames suporting a Tedlar bag. The 4 by 3.5 by 3 foot chamber contained 1.19 m^3 and covered one experimental cell in the field plot (Figure 1). A small fan consisting of a motor and four cardboard circulating paddles was mounted on a steel stake weighted with a concrete base (Figure 2). The fan was placed in the center of an unexposed cell before the chamber was in place. Two alligator clips and an insulated platform, also mounted on the stake, held the igniting wire and solid rocket fuel and was controlled by a special switch box previously described (Granett and Taylor, 1978). The fan and ignition system were powered by batteries outside the chamber.

The spring field fumigations in Riverside and Vandenberg were conducted

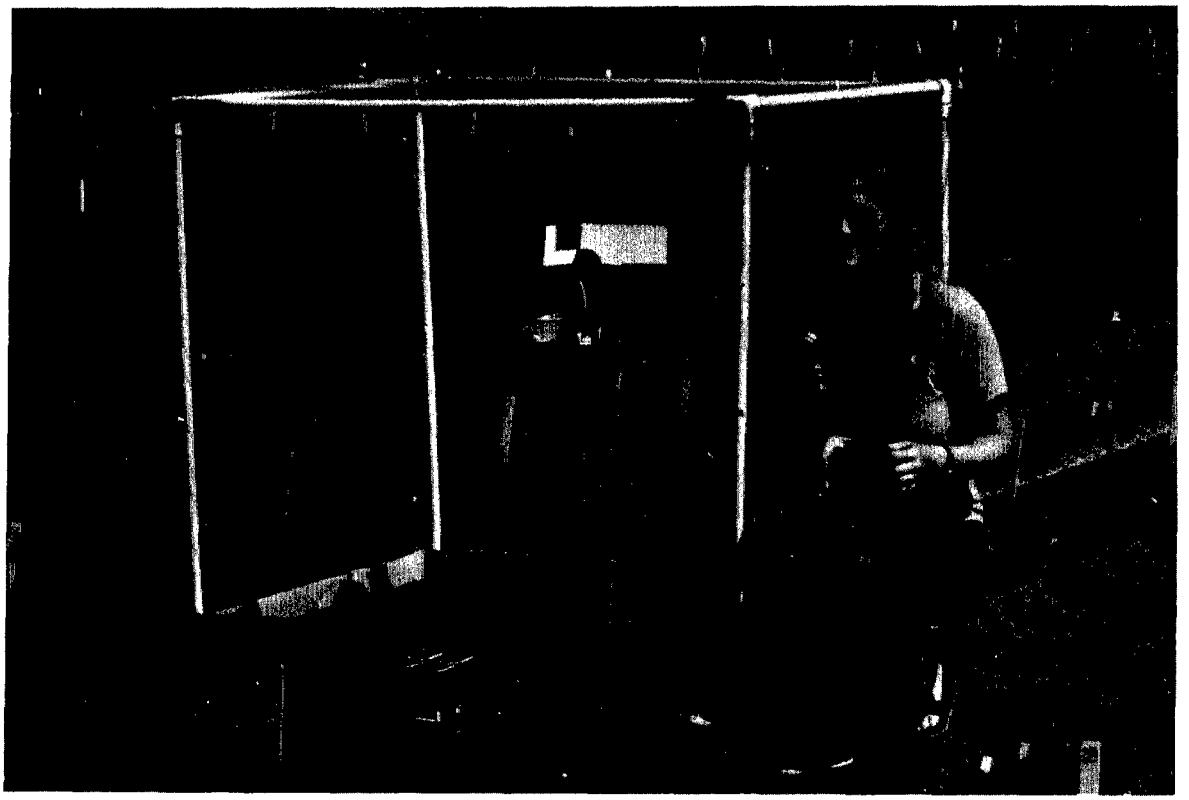

Figure 1. Above. Photograph of field exposure chamber for solid rocket fuel.

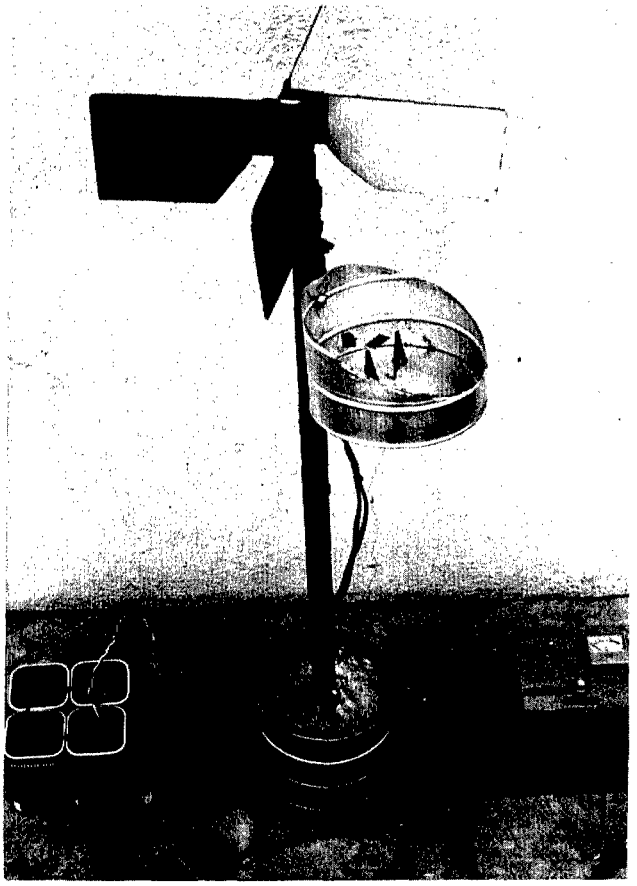

Figure 2. Left. Photograph detailing circulating fan, stake and fuel ignition cips for SRF exposure chamber.

with portable Tedlar-covered chambers of a similar size (1.2 m^3) but different construction. The skeletal structure was schedule 40, 1-inch OD PVC tubing with PVC slip-fit connectors. The lower frame was weighted with sand and sealed with paraffin. Eye bolts were installed to enable staking during windy periods. The Tedlar bag and 4-inch wide canvas skirts were joined to the base with split, schedule 125, 1-inch OD PVC (Figure 3). The entire structure could easily be dismantled for transport. A dynamic HCl generator was devised for use with these new chambers. Pieces of flexible plastic-covered drier hose carried HCl and carrier gas into and out of the chamber. The intake hose was attached to a squirrel cage blower which created positive pressure in the chamber. The blower was mounted on a generation control board which also supported the 6.9 liter tank of 30% HCl in nitrogen, a flow meter, and a needle value (Figure 4). Switches controlled the fan, an external sampler pump and other equipment. When an electrical outlet could not be reached by a 250-foot extension cord, a 400 watt gas generator was employed. A battery-operated fan circulated air and gas within the chamber.

SOLID ROCKET FUEL

Solid rocket fuel (SRF) stock was cut to pieces 0.5 by 0.5 by 1.0 cm, weighed, and fitted with model rocket ignition wires. Ignition occurred when a 1.5-volt dry cell heated the thin nichrome ignition wire.

FIELD AND PLANT PRODUCTION

Plants were grown at the Statewide Air Pollution Research Center greenhouse in 4-inch plastic pots or 16-ounce styrefoam cups, filled with a UC soil mix medium (Lerman, 1976), and were watered as needed and fertilized regularly with a modified nutrient solution (Hoagland and Arnon, 1950). Field grown pinto beans were sown in the field; flower crops were either sown in the field or transplanted to the field when 14-16 days old. Transplanting was simplified by seeding in peat pots.

Field preparation at the university plot consisted of plowing in fertilizer at the rate of 80 pounds nitrogen per acre and applying a pre-emergence weed killer, Dacthal, at 12 pounds per acre. Formed beds 30 inches wide and 6 inches high had central irrigation troughs. Zinnias were planted on one side of the bed and marigolds on the other. The plants were kept relatively pest-free by applying insecticides as needed.

At Vandenberg, one 25 by 50 foot plot was prepared near Space Launch Complex 5 by rototilling and raking the area. A fence was installed to exclude rabbits, gophers, and deer. Plants were grown in Riverside and transplanted at 2 weeks of age. A systemic fertilizer-insecticide was applied around each plant. Plants were watered by hose and by rain. Other exposure sites in the same area consisted of native vegetation.

VIRUS AND CHEMICAL INTERACTIONS

In a series of experiments, tobacco mosaic virus (TMV) was appplied to tobacco plants either before or after exposure to gaseous HCl. The virus

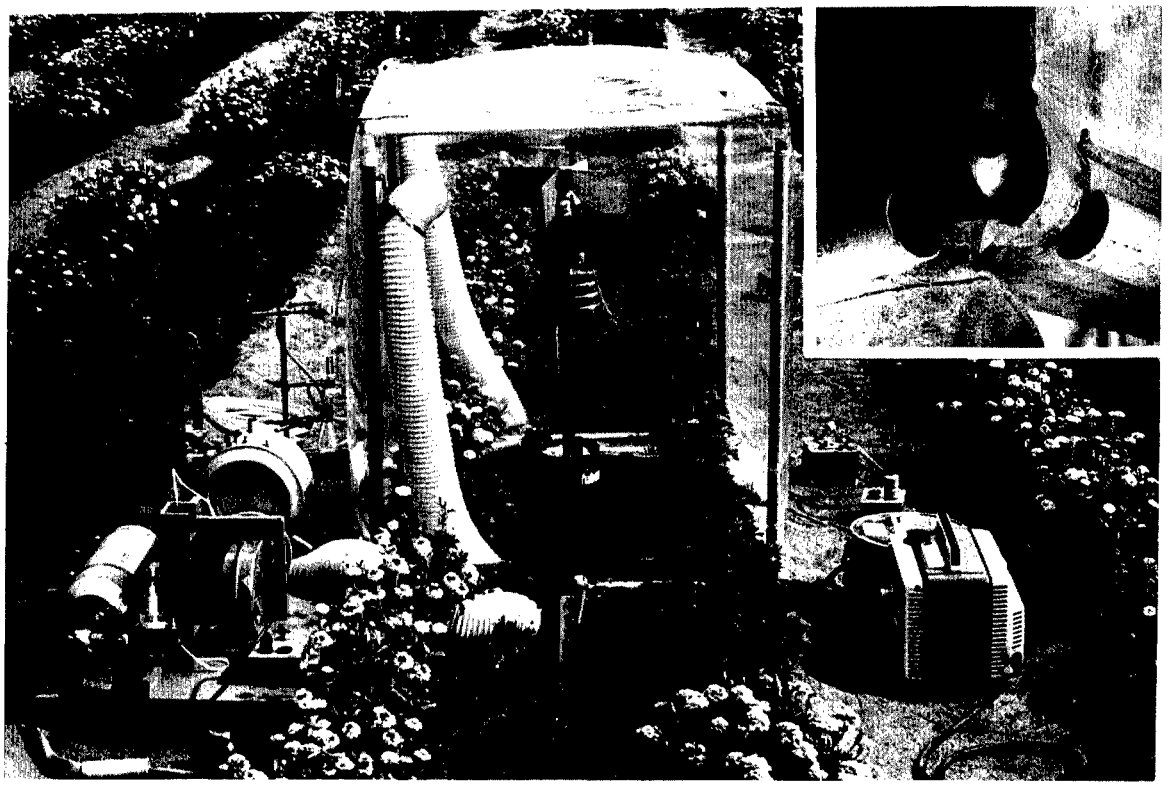

Figure 3. Photograph of field chamber designed for steady-level HCl exposures; insert details eye bolt for anchoring, canvas skirt, and PVC assembly clip.

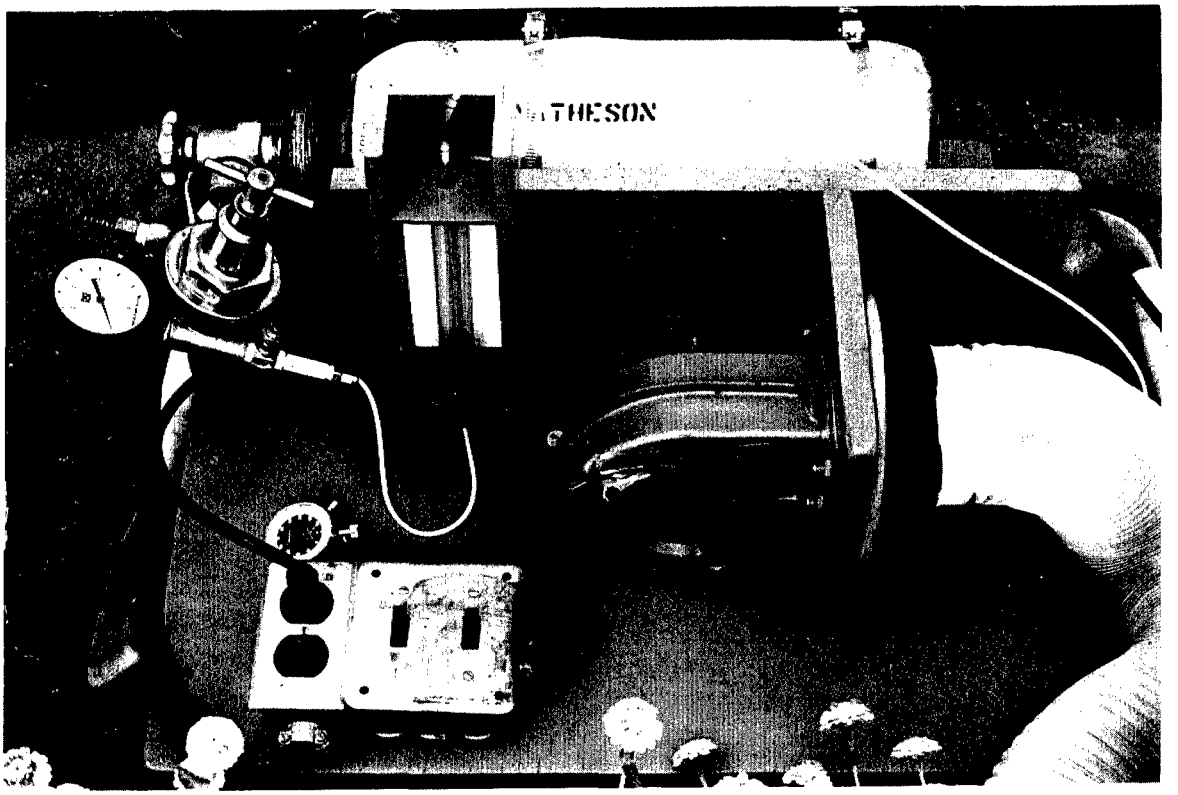

Figure 4. Photograph showing generation and blower assembly for supplying HCl gas to field chamber.

was a common U-1 strain (Granett and Shalla, 1970) which produced local lesions on some tobacco varieties, and systemic leaf mosaic and chlorosis on other, less sensitive, varieties.

DPX-4891 was tested for its effectiveness in protecting plants from HCl. This experimental duPont chemical, N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N-phenylurea, is supplied as 50% wettable powder (Gilbert et al., 1977; Carnahan et al., 1978). Solutions were made with distilled water and applied to plants as a spray and soil drench one day prior to HCl gas or ozone exposure. Plants were graded one to two days later.

MEASUREMENTS

Injury

Plants were examined for injury by gaseous HCl about 24 hours after the exposure. By this time plants had recovered from any initial wilting; leaf glazing, chlorosis, or necrosis had developed. Leaf necrosis was the most common injury although abaxial glazing was observed when stress was less severe. Visible injury was graded by estimating on a 1 to 4 scale the area of affected leaf surface. The type of injury was also noted. From the recorded data, percent plants injured per exposure, percent leaves injured and estimated percent leaf area injured were calculated.

Gas Concentrations

Concentration of HCl gas in the exposure chamber was determined by bubbling 15 liters of chamber atmosphere through 20 ml of 0.01 N nitric acid and analyzing the resulting solution with an Aminco Model 4-4433 automatic titrator. A Geomet Model 401B HCl monitor was also used for sampling. This chemiluminescent device is capable of real-time HCl determination and was useful for evaluating relative gas concentration.

Environmental Parameters

Temperature in the chambers was measured with mercury thermometers or a digital electronic device. Relative humidity (RH) was calculated from wet and dry bulb readings on a sling or battery operated psychrometer. Light intensity was read on a Yellow Springs Instrument Co. Model 65 Radiometer, and light in the photosynthetically active region (PAR) of the spectrum was measured with a Li-Con Model LI-185 Radiometer.

PHYTOTOXICITY INTERACTIONS

ANTIOXIDANT COMPOUND

Ozone, one of the major phytotoxicants in polluted urban air, is a strong oxidant. Many compounds have been screened for their protective activity and some have proven useful for experimental work, although not yet

commerically feasible (Koiwai et al., 1974; Rich et al., 1974; Carnahan et al., 1978; and Gilbert et al., 1977). Since HCl can enter the plant through stomata, the effect of an antioxidant was tested. Zinnia plants treated with antioxidant were subsequently exposed to 20 minutes of HCl or 2 hours of ozone. DPX 4891 was applied as an aqueous solution of 0, 500, 1000 or 2000 ppm by spraying leaves of zinnia seedlings until they dripped; the remainder was poured onto the soil around the plant in 40 ml aliquots. Each treatment was tested on three plants. Leaf injury was measured two to four days after HCl or ozone exposure. Some injury occurred at the higher ozone concentrations (Table 1) and was greatly reduced in those plants receiving the chemical treatments (Clark et al., 1978).

TABLE 1
LEAF INJURY ON ZINNIA PLANTS EXPOSED TO
2 HOURS OF OZONE 24 HOURS AFTER SPRAY AND DRENCH
APPLICATIONS OF DPX-4891 ANTIOXIDANT

Ozone Concentration (ppm)	Antioxidant (ppm) % Leaves Injured				% Leaf Area Injured			
	0	500	1000	2000	0	500	1000	2000
20	0	0	0	--	0	0	0	--
50	100	44	0	--	18	4	0	--
30	0	0	0	0	0	0	0	0
50	67	0	0	0	9	0	0	0
80	100	11	0	0	36	0.1	0	0

With HCl-treated plants, injury increased with gas concentration but no significant reduction in HCl damage accompanied antioxidant treatment (Table 2).

TABLE 2
LEAF INJURY ON ZINNIA PLANTS EXPOSED TO 20
MINUTES OF HCl GAS 24 HOURS AFTER SPRAY AND
DRENCH APPLICATIONS OF DPX-4891 ANTIOXIDANT

HCl Concentration ($mg\ m^{-3}$)	Antioxidant (ppm) % Leaves Injured				% Leaf Area Injured			
	0	500	1000	2000	0	500	1000	2000
9.2	62	33	33	--	7	6	6	--
35.7	100	100	100	--	81	82	81	--
8.0	6	6	0	11	0	0	0	1
18.5	100	88	81	89	42	44	38	55
35.0	100	100	100	100	78	76	66	86

SYSTEMIC VIRUS INFECTION

Plants in nature are rarely perfectly healthy and diseases add stress to plant systems. Viruses usually invade the whole plant, although some plants are hypersensitive to specific viruses and invasion by that virus is limited to a few cells. These cells die and effectively contain the pathogen from further spread. Some virus treatments reduce injury caused by ozone (Davis and Smith, 1974, 1976; Brennan and Leone, 1969). Both systemic and local infections were studied to determine if gaseous HCl had an effect on either the reaction of the plant to the virus or on virus replication in the host.

Tobacco plants are sensitive indicators of air pollution (Menser, 1969) and may respond differentially to HCl depending on the TMV infection (Brennan and Leone, 1970). Nicotiana tobaccum var. Bell W-3, 4 and 5 weeks old, were inoculated with water or with TMV prepared by grinding infected tobacco leaves in a mortar and pestle, diluting with water, and filtering the mascerate through cheese cloth. The inoculum was applied to carborundum-dusted leaves using cotton swabs. Four weeks later, when the virus was well established and newly emerging leaves showed mosaic symptoms, the plants were divided into groups for 20 minute exposures to HCl gas. Two diseased and two virus-free plants were simultaneously exposed to each of five HCl levels; two replicas were performed. Plants were graded 48 hours after exposure and the data were summarized and analyzed (Tables 3 and 4).

HCl concentration had a significant influence on plant injury, but did not affect the virus. Virus stress under these conditions neither increased nor reduced injury caused by HCl gas.

TABLE 3
INJURY ON BELL W-3 TOBACCO PLANTS
SYSTEMICALLY INFECTED WITH TMV AT TIME OF EXPOSURE TO HCl GAS.
AVERAGE OF TWO REPLICAS

HCl Concentration ($mg\ m^{-3}$)	% Leaves Injured		% Leaf Area Injured	
	-TMV	+TMV	-TMV	+TMV
12	57	43	13	8
16	60	51	17	10
28	45	69	24	26
54	100	100	68	69
87	100	100	71	73

TABLE 4
ANALYSIS OF VARIANCE FOR LEAF AREA INJURY ON BELL-W3 TOBACCO PLANTS SYSTEMICALLY INFECTED WITH TMV PRIOR TO EXPOSURE TO HCl GAS

	Degrees of Freedom	Sum of Squares	F-Values
Concentration	4	7153.31	15.76 ***
V (Virus present, absent)	1	13.33	0.12
C x V	4	382.33	0.84
Error	10	1134.38	
Total	19	8683.35	

*** = significance at 0.1% level

LOCAL LESION VIRUS INFECTION

The Pennbell variety of tobacco is hypersensitive to TMV infection; inoculation is quickly followed by death of cells. Injury becomes visible as distinct local lesions, the number of which indicates the virus concentration of the inoculating solution (Corbett and Sisler, 1964). The present study determined whether the virus-host relationship was affected by air pollution stress. Systemically infected tobacco leaves were mascerated in two volumes of 0.05 M phosphate buffer, pH 7.0, filtered through cheesecloth, and diluted with one volume buffer plus carborundum. Aliquots of the diluted filtrate were frozen to provide virus inoculum. At 36, 24, 12, 2 and 0 hours before plants were exposed to a pollutant, a frozen aliquot was thawed, diluted 10-fold with water, and applied to fully expanded half leaves. Each treatment was applied to two half-leaves on each plant prior to rinsing leaves with water. The fumigations consisted of about 18 mg HCl m^{-3} for 20 minutes, about 30 pphm ozone for 90 minutes, and charcoal filtered air for 90 minutes. Plants were returned to the greenhouse benches following exposures and graded 24 to 48 hours later. Local lesions were counted 72 hours after virus inoculation, i.e. 36 to 72 hours after exposure.

Minimal injury resulted from HCl (Table 5) and none from ozone. An analysis of variance of the virus-induced local lesions indicated differences in lesion averages for different inoculation times, but not among averages for the three fumigations (Table 6).

TABLE 5
INJURY ON TOBACCO AFTER EXPOSURE TO 18 mg m^{-3} HCl GAS FOR 20 MINUTES; TOBACCO PLANTS WERE INOCULATED WITH TMV 0-36 HOURS PRIOR TO EXPOSURES

	TMV Inoculations (hours before exposure)				
	36	24	12	2	0
% Leaf area injured	2.2	1.2	1.9	8.8	4.8
Total number local lesions	265	201	423	156	59
Average number local lesions per half leaf	6.63	5.03	10.58	3.90	1.48

TABLE 6
AVERAGE NUMBER OF LOCAL LESIONS ON TOBACCO HALF-LEAVES INOCULATED 0-36 HOURS BEFORE EXPOSURE TO AN AIR POLLUTANT

	Inoculation Time (hours pre-exposure and P.S.T.)					
	36 10 PM	24 10 AM	12 10 PM	2 8 AM	0 10 AM	
Pollutant						Average
HCl	6.6	5.0	10.5	3.9	1.4	5.5
Ozone	14.0	3.6	4.8	6.3	5.4	6.8
None	8.6	2.6	7.6	5.5	6.8	6.2
Average	9.7 x[1]	3.7 z	7.6 y	5.2 yz	4.5 z	6.7

[1]Averages followed by the same letters are not significantly different at 5% level by Duncan's Multiple Range Test.

The five inoculation treatments for the unexposed plants produced data (local lesion numbers) not statistically different (33% chance of being the same) from each other (Table 7) since all five treatments consisted of inoculations with the same virus suspension and counts were made 72 hours after each application. HCl and ozone data, however, were significantly different with less than 1% chance of the means being equal in either case. The data were adjusted so the pollutant-free control for each treatment equaled 100 local lesions then corresponding values for the other pollutants could be calculated (Figure 5). Ozone inhibited virus development with TMV inoculated 0 to 12 hours before exposure and stimulated the development with inoculations 24 or 36 hours post-exposure. With HCl, TMV development and

TABLE 7

RESULTS OF STATISTICAL ANALYSIS OF VARIANCE OF THE AVERAGE NUMBER OF VIRUS LOCAL LESIONS DEVELOPING ON PLANTS EXPOSED TO HCl, OZONE OR NO POLLUTANT. VIRUS WAS INOCULATED 0-36 HOURS BEFORE EXPOSURE

	None	Ozone	HCl
F-statistic	1.4878	14.2045	60.5237
Significance (Q)	33.44%	0.60%	0.02%
(P)	66.56%	99.40%	99.98%

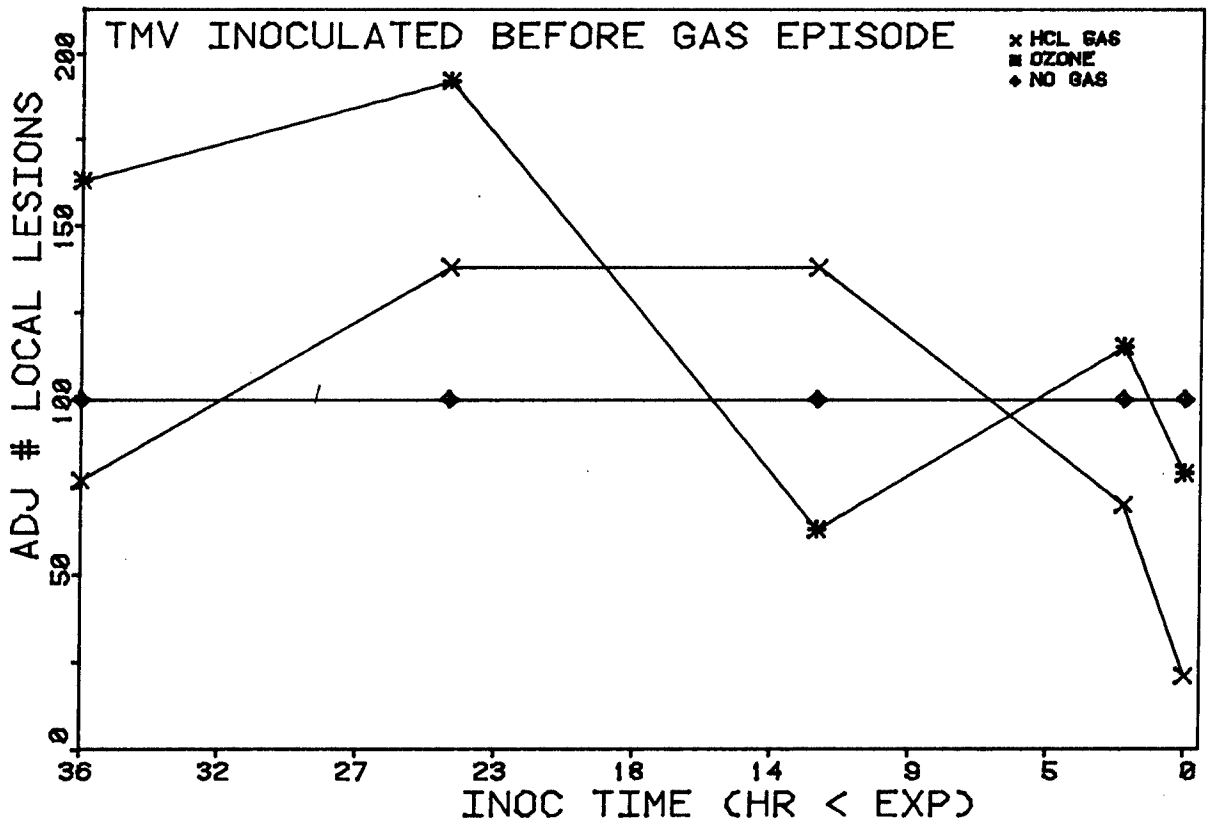

Figure 5. Effect of air pollutants on the development of TMV local lesions; mathematical adjustment made so pollutant-free control always yielded 100 lesions.

expression was enhanced when inoculated at 12 or 24 hours before exposure, but inhibited at 0, 2 or 36 hours prior to exposure. These results indicate that virus development may be altered by exposing plants to HCl.

SOLID ROCKET FUEL

A pungent odor persists for at least 15 minutes after igniting solid rocket fuel (SRF) in a closed chamber, whereas HCl cannot be detected 10 minutes after ignition of a large amount of fuel. Several investigations concerned the phytotoxic nature of the odor.

Persistence of Phytotoxic Compounds

Zinnia plants were exposed to gas generated from 400 gm pieces of SRF. Seedlings were removed from the exposure chambers at 2 to 40 minutes after ignition and later graded for injury (Table 8).

TABLE 8
INJURY ON ZINNIA PLANTS EXPOSED TO GAS FROM 400 mg SRF FOR 2 TO 40 MINUTES IN A CLOSED CHAMBER

Exposure Period (minutes)	% Leaves Injured	% Leaf Area Injured
2	27.2	2.0
5	55.5	5.7
10	82.3	19.4
15	74.3	17.8
20	61.4	12.8
30	46.8	5.0
40	96.7	29.6

Amount of injury increased as the plant exposures increased from 2 to 10 minutes. As exposure times further increased to 15, 20 and 30 minutes, less injury developed. After a 40-minute exposure, however, nearly 100% of

the leaves were injured (Table 6). After a certain period, a constant injury level, determined by fuel weight, would be expected. Differences in fuel density, chamber wall outgassing, depletion of carbon dioxide, or build-up of toxic gases may have been responsible for the actual responses seen.

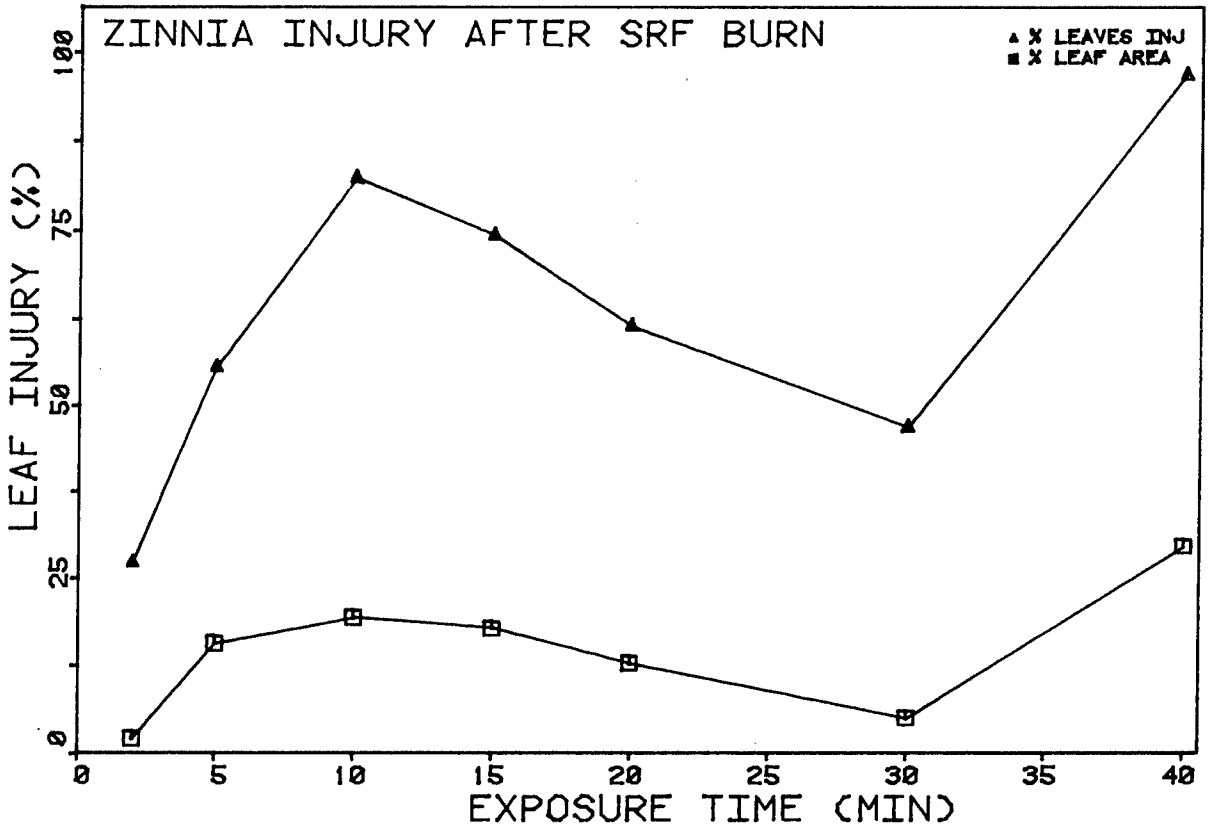

Figure 6. Leaf injury on zinnia plants removed from a closed chamber 2 to 40 minutes after ignition of 400 mg of SRF.

Decay of HCl and Oxidants in Exposure Chamber

Further tests were carried out to characterize the odor persisting after burning SRF. SRF was ignited and the resultant gases were monitored with a Geomet device for HCl and a Mast instrument for total oxidants. Chamber air was also sampled with a bubbler. Three burns were so monitored (Figure 7A and B). Geomet readings indicated a rapid drop in HCl gas concentration. Average readings were compared to determinations from bubbler

samples taken at the same time; the Geomet read high at the higher concentrations (Table 9).

Rate of total oxidant decline was apparent when the scale was expanded tenfold (Figure 7B) and appeared more gradual than the HCl rate measured with the Geomet (Figure 7A). HCl concentration halved 5 minutes after the fuel started burning while 75% of the peak total oxidants still remained present.

The Mast instrument was calibrated by comparing ozone reading to a Dasibi detector. A separate test verified that the Mast would not detect pure HCl gas.

TABLE 9
CHAMBER HCl CONCENTRATIONS ACCORDING TO GEOMET AND BUBBLER, AVERAGE OF THREE SRF BURNS (mg m^{-3})

Time during fumigation (minutes)	GEOMET READINGS Time after bubbler started (seconds) 0	60	120	180	Avg.	BUBBLER
0-3	57	51	41	33	46	32
7-10	19	17	15	14	16	10
17-20	6	5	5	5	5	5

These experiments suggest that oxidants are responsible for the odor following ignition of SRF when little HCl remains. In a final test, pieces of SRF were burned in a chamber and the resultant gas was sampled with NO_2 detection tubes in a Matheson Model 8014 gas detector pump. Less than 0.5 ppm NO_2 was detected after burning 400 mg fuel. With 49,300 mg, only 2.75 ppm NO_2 was measured. This NO_2 may have been from vaporized paint from the burning platform. NO_2 does not appear to cause the SRF odor.

FIELD EXPERIMENTS

GENERAL RATIONALE

Most experimental work on this contract has been conducted in the greenhouse and laboratory where conditions are controlled to avoid unmeasured interacting stresses such as diseases, water stress, severe temperature fluctuations, ambient air pollution, and so forth. The current series of experiments were undertaken to determine whether greenhouse work could accurately predict field responses. During the summer of 1978,

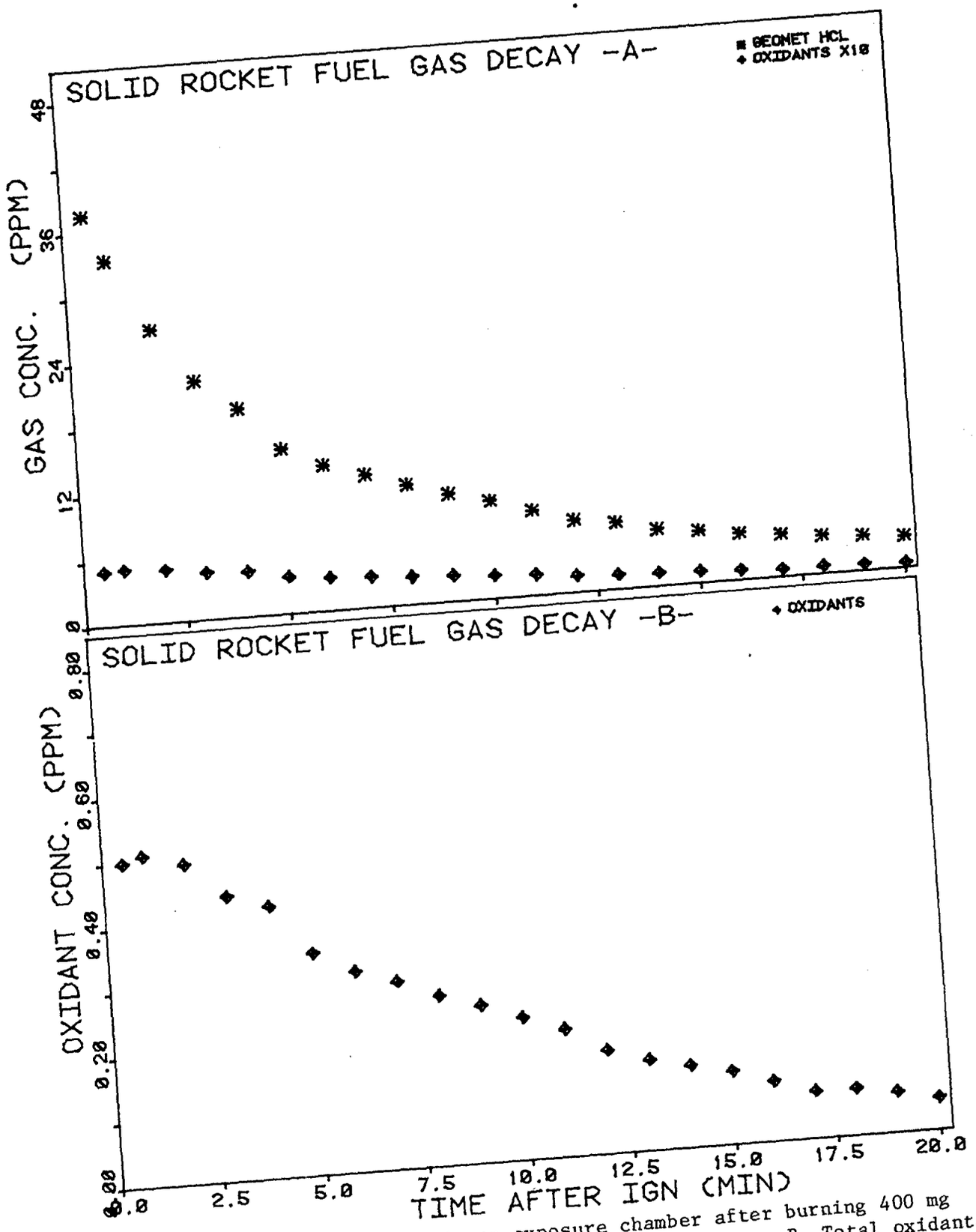

Figure 7. Detection of gases in an exposure chamber after burning 400 mg SRF. A. Average HCl and total oxidants from three burns. B. Total oxidants on an expanded scale.

plots were prepared in Riverside, California, and zinnia, marigold and bean plants were exposed under temporary chambers to gas generated by rocket fuel. Chambers with a dynamic air flow and a continuous dry gas generation system were introduced in the spring of 1979 when fumigations were carried out at Vandenberg Air Force Base as well as in Riverside.

RIVERSIDE FIELD EXPOSURES WITH SOLID ROCKET FUEL

Preliminary Greenhouse Experiments

Several greenhouse tests were performed prior to exposing plants in the field. Originally, special tapes were planned for sowing seeds in the prepared field plots. The tapes conveniently held the seed then dissolved in moist soil. Burpee Seed Company, Riverside, sold zinnia seed tapes but pinto beans had to be hand-encapsulated in tape generously supplied by Union Carbide Corporation, Watsonville, California. Greenhouse tests indicated a lower germination rate with zinnia seed tapes compared to direct seeding.

The field was prepared by University Agricultural Operations Department personnel who disced and shaped the plots and applied fertilizer and pre-emergence weed killer.

Since treatments with insecticides during the course of the field work were probable, interactions between the plants, weed killer, several insecticides and HCl were investigated. In greenhouse tests, the weed killer Dacthal was incorporated into the soil. The insecticides Diazinon, Orthene, and Cygon were applied as sprays at 0.24 mg per gallon two days before exposure to HCl gas. Bean plants received 19 mg m^{-3} HCl gas for 20 minutes and zinnia seedlings were exposed to 25 mg m^{-3}. Plants were graded 24 hours after exposure. A significant increase in injury was noted in the plants treated with Cygon, a systemic insecticide (Table 10). Vein necrosis appeared on the lower leaves of beans treated with Diazinon, although this chemical did not affect zinnias as severely. Tip necrosis was noted with several treatments but was most pronounced after Cygon and was probably a reaction to the chemical rather than to HCl gas.

This preparatory work indicated that plant sensitivity to HCl gas would not be affected through the judicious use of Diazinon, Orthene, or Dacthal. The combination of Cygon and Dacthal, however, increased pollutant-caused injury.

Preliminary Field Trial

The greenhouse experiments correctly predicted low seed germination in the field. The seed tapes were found too time consuming and a system of germinating all seeds in peat pots in the greenhouse was adopted. Plants were transplanted to the field two weeks after sowing. At time of exposure, the bean and zinnia plants were smaller and had thicker leaves than greenhouse-grown plants. The first trial consisted of 12 exposures, each burning one piece of solid rocket fuel. The chamber, with the stirring paddles rotating, remained over the test cells for 15 minutes after ignition.

TABLE 10
INJURY CAUSED BY INTERACTION OF HCl GAS WITH CHEMICALS USED IN FIELD OPERATIONS

Treatment	Pinto Bean[1] % Leaves Injured	Pinto Bean[1] % Leaf Area Injured	Zinnia[1] % Leaves Injured	Zinnia[1] % Leaf Area Injured
Control	56 z[2]	8 a	40 b	7 c
Dacthal Only	68 z	10 a	47 b	7 c
Diazinon + Dacthal	96 y	16 a	40 b	6 c
Orthene + Dacthal	68 z	13 a	45 b	9 c
Cygon + Dacthal	100 y	15 a	82 b	12 c

[1]Average of 10 plants.

[2]Averages followed by same letter are not significantly different at 5% level by Duncan's Multiple Range Test.

Gas concentrations were determined by fuel weight; theoretically, 20.9 mg HCl is released for every 100 mg propellant burned (Nadler, 1976). The peak HCl concentrations (Table 11) were achieved at the completion of ignition and levels decreased rapidly thereafter (Granett and Taylor, 1978). Each cell consisted of ten 21-day-old zinnia and ten 13-day-old bean plants. Senescence, ozone injury, and insect damage destroyed some plants and tended to mask injury due to HCl gas. HCl injury, graded 4 days after exposure (Table 11), consisted of foliar glazing. Even at highest concentrations, beans exhibited very little injury.

TABLE 11
INJURY ON ZINNIAS AND PINTO BEANS AFTER EXPOSURE TO GASES GENERATED BY ROCKET FUEL IN A PRELIMINARY FIELD TRIAL

Fuel Weight (mg)	Theoretical Peak HCl Concentration (mg m^{-3})	Beans No. Plants[1]	Beans % Leaves Injured	Zinnias No. plants[1]	Zinnias % Leaves Injured
250	44	26	0.0	32	2.0
500	88	33	2.1	28	20.2
1000	176	30	12.7	26	43.4
1500	263	34	12.0	30	52.6

[1]Total number of plants exposed in 3 replicas.

Sensitivity Trials

Bean, zinnia and marigold plants were exposed to HCl gas generated from SRF fuel weighing 300, 600, 900, 1500 and 1800 mg and yielding total chamber HCl concentrations of 53 to 316 mg m^{-3}. Summarized data (Table 12) were submitted for probit analysis (Figure 8). Using the probit lines, the fuel necessary to injure 10, 25, 50 and 75% of the leaves exposed could be estimated (Table 13). Field grown marigold plants were more sensitive to SRF gas than were zinnia or bean plants. Beans grown and exposed to SRF-generated gas in the greenhouse were considerably more sensitive than field grown plants (Table 13 and Figure 8). In addition, greenhouse and field beans had steeper probit lines than the marigolds and zinnias.

TABLE 12
INJURY ON BEAN, ZINNIA AND MARIGOLD SEEDLINGS AFTER FIELD EXPOSURE TO GAS GENERATED BY SRF; AVERAGE OF 30-40 PLANTS

Fuel Weight	% Plant Leaves Injured			% Leaf Area Injured		
(mg)	Bean	Zinnia	Marigold	Bean	Zinnia	Marigold
300	0	9.3	3.6	0	0.8	0.2
600	0	26.4	57.1	0	3.7	7.0
900	8.8	31.9	59.7	1.1	3.2	9.5
1200	52.4	50.0	89.6	11.2	9.2	32.5
1500	90.3	65.5	88.6	27.2	4.4	36.2
1800	73.8	66.7	100.0	23.2	30.8	49.0

TABLE 13
INJURY PROBABILITY OF PLANTS EXPOSED TO GAS FROM SRF

Species	Injury Level 10%	25%	50%	75%
Beans	758[1]	960	12471	1622
Zinnia	313	581	1155	2300
Marigold	337	460	690	1038
Beans in greenhouse	118	174	269	416

[1]Fuel weight, in mg, needed to cause injury on 10 to 75% of the leaves exposed.

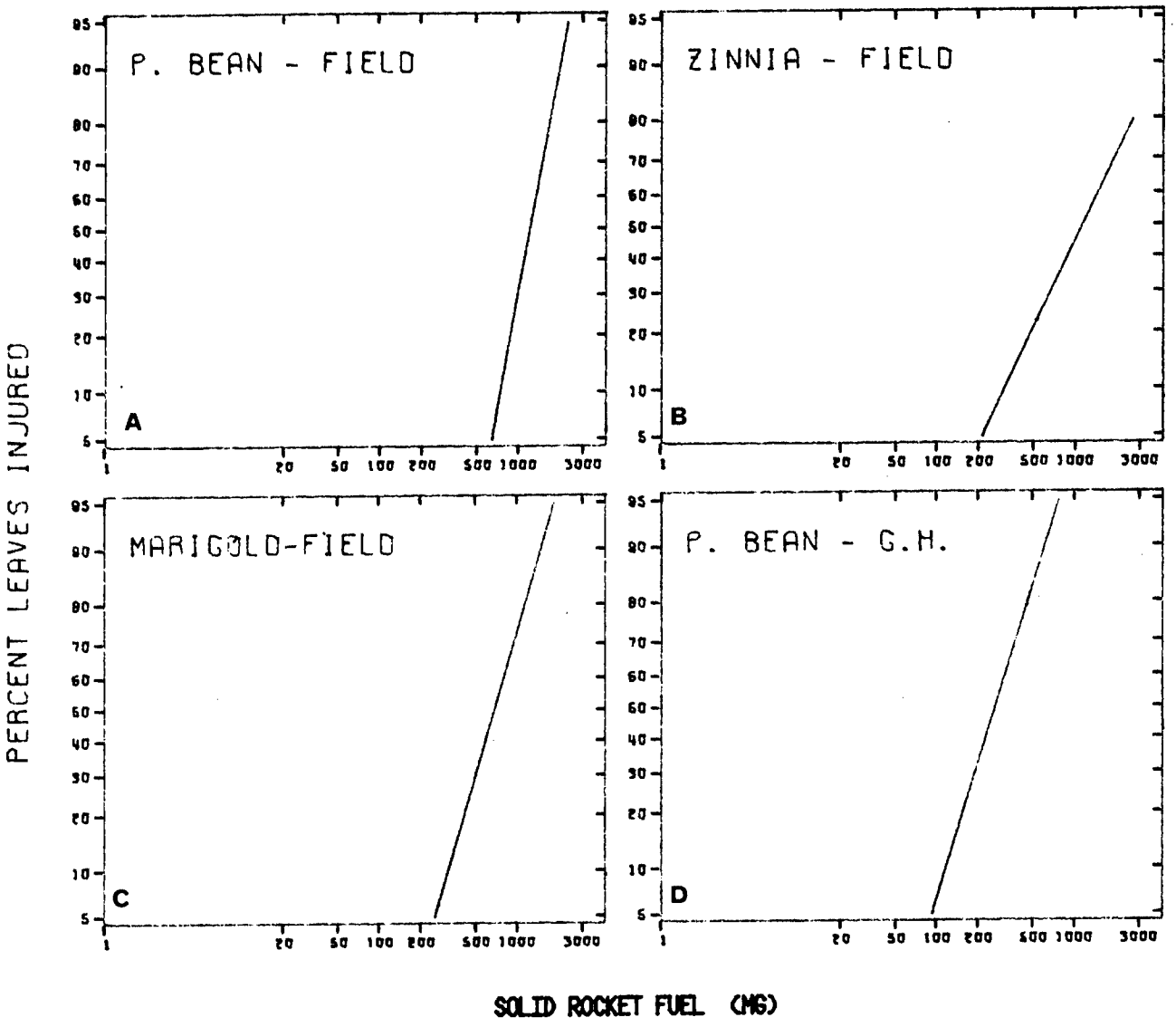

Figure 8. Probit analysis of plants exposed to gas generated by SRF: A. Pinto bean, B. Zinnia, and C. Marigold under field conditions; D. Pinto bean under greenhouse conditions. Concentration axis is $\log_{10}$ of fuel weight.

Yield Experiments

Multiple Exposures in the Field

The third field experiment consisted of weekly 15 minute exposures of zinnia, marigold, and bean plants. Four gas concentrations determined by SRF weights were replicated four times in each block. The block of treatments was repeated the next day. Fuel weights of 0, 300, 800, and 1800 mg

for the first five weeks were subsequently increased 35% to 0, 405, 1080, and 2430 mg in order to increase plant response. Plants were germinated in peat pots in the greenhouse; beans were transplanted three days after sowing while the zinnia and marigolds were transplanted at 11 days. Plants were sprayed with 5 gm per gallon Orthene as needed to control insects. Beans were first exposed to HCl at 15 days after seeding; the ornamentals were 19 days old at first exposure (Table 14).

TABLE 14
DATES IN 1978 FIELD YIELD EXPERIMENT

Plants	Greenhouse Sown	Transplant to Field	Week of First Exposures	Week of Last Exposures	Number Exposures	Week of Harvest
Zinnia	Jul 7	Jul 18	Jul 24	Nov 6	14	Nov 20
Marigolds	Jul 7	Jul 18	Jul 24	Nov 6	14	Nov 20
Pinto Beans	Jul 17	Jul 20	Jul 31	Oct 9	9	Oct 23

Plants incurred mild injury at higher fuel weights for the first two weeks. In successive weeks, however, visible injury was no longer seen. To test equipment and the hypothesis that older field-grown plants acquire tolerance, young greenhouse-grown plants were brought to the field and exposed under the same conditions. Greenhouse plants sustained injury in proportion to fuel burned (Table 15). Less leaf area injury occurred when field zinnia plants shared chamber space with the greenhouse plants, presumably because a fixed concentration of generated HCl (e.g. from 2430 mg fuel) was now available to a greater total leaf area. Greater injury developed on plants under sunny compared to cloudy conditions.

Samples of the marigold and zinnia plants were taken one week after final exposures and several weeks before harvest, were oven dried, finely ground in a Worley mill, and analyzed for chlorine content (Table 16). Only the marigold plants receiving the highest weekly exposure had statistically greater chlorine content than the controls.

TABLE 15
INJURY ON GREENHOUSE AND FIELD GROWN PLANTS RESULTING FROM EXPOSURE TO SRF GASES UNDER FIELD CONDITIONS

Fuel (mg)	Weather	No. Replicates	% Leaves Injured	% Leaf Area Injured
		PINTO BEAN PLANTS FROM GREENHOUSE		
405	cloudy	3	11.1	0.7
1080	cloudy	3	31.0	2.1
2430	cloudy	3	68.1	8.6
2430	clear	1	88.9	66.8
		ZINNIA PLANTS		
Greenhouse plants on bare soil				
2430	cloudy	1	100.0	63.2
2430	clear	1	100.0	85.4
Field and greenhouse plants			Greenhouse / Field	Greenhouse / Field
405	clear	3	1.8 / 0.0	0.3 / 0.0
1080	clear	3	50.7 / 0.0	9.9 / 0.0
2430	clear	3	100.0 / 0.0	44.2 / 0.0

TABLE 16
CHLORINE CONTENT OF MARIGOLD AND ZINNIA PLANTS EXPOSED WEEKLY TO GAS GENERATED BY SOLID ROCKET FUEL

Fuel Weight (mg)	Chlorine Content ($mg\ Cl^-/100g$) Marigold	Zinnia
0	2.017	1.727
405	2.015	1.720
1080	2.276	1.542
2430	2.309*	1.799

*Significantly different from control at 5% level.

The first two axillary flowers from exposed zinnia and marigold plants were tagged and collected after drying on the plant. Pinto bean pods were collected during the season as they matured. Mature, unshriveled seeds were counted, weighed, and germination rates for subsamples from each cell were determined. The final results of the yield experiment consisted of the seed numbers, weights, and germination rates for all three species. The yields indicated no significant change in parameters associated with the dry seeds (Table 17 and 18). Marigold seeds failed to germinate on soil, on moist filter paper, or in petri plates. Results indicate no effect on yield of field-grown plants stressed weekly with SRF-generated gases at concentrations which did not consistently cause visible injury.

TABLE 17
PINTO BEAN SEEDS HARVESTED FROM PLANTS EXPOSED WEEKLY TO SRF GAS

Fuel Weight (mg)	Average Plants per Cell (#)	Pods per Cell			Total Seeds per Cell (#)	Mature Seeds per Cell		
		Mature (#)	Immature (#)	Total (#)		Average (#)	Weight (mg)	Germination rate (%)
0	7.5	88	42	130	272	231	232	82
405	7.8	94	31	125	288	240	240	87
1080	7.8	87	41	128	283	226	231	88
2430	7.5	78	38	116	239	198	228	89
ANOVA Significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

TABLE 18
SEED HARVESTED FROM ZINNIA AND MARIGOLD PLANTS EXPOSED WEEKLY TO SOLID ROCKET FUEL GAS

Fuel Weight (mg)	Number Fertile-Appearing Seeds per Plant		Average Weight per Seed (µg)		% Germination	
	Zinnia	Marigold	Zinnia	Marigold	Zinnia	Marigold
0	28.0	154.0	6.77	1.03	83.5	0
405	26.7	155.4	6.25	1.04	75.5	0
1080	29.2	150.5	6.92	1.11	81.5	0
2430	24.8	147.2	6.65	0.97	83.5	0
ANOVA Significance	N.S.	N.S.	N.S.	N.S.	N.S.	-

The seed data was summarized (Tables 17 and 18) following analysis of variance calculations. The statistical tests failed to show any significant differences between the treatments (fuel weight) for any seed parameter. In fact, of all factors measured, only the chloride content of marigold plants exposed to gas from 2430 mg SRF was significantly different from the control plants (Table 16). One probable reason for this is that plants become more resistant with age and weekly gas treatments had little effect. Photographs taken 9 weeks after exposures began indicated differences; plants exposed to the higher concentrations were smaller, less bushy, and had fewer flowers than control plants (marigolds had 11, 36, 12, and 4 flowers and zinnias had 34, 34, 35, and 25 flowers on plants exposed to 0, 405, 1080, and 2430 mg fuel, respectively). These differences were no longer present at harvest 8 weeks later. Another reason for lack of significant differences may be that plants were not greatly stressed by the HCl generated. The highest dose, 2430 mg fuel, released about 20.9% or 508 mg HCl. In comparison, only 14 mg HCl m^{-3} for 15 minutes would deliver the same 508 mg HCl to plants exposed in a dynamic system with two air changes per minute, a treatment which previous work had shown to cause only minor injury to most greenhouse plants. Results indicate that field plants are more resistant to HCl gas than greenhouse plants and, like greenhouse plants, they are more tolerant at certain ages.

Yields After Single Exposures to HCl

Above results prompted a greenhouse test in which 13-day-old bean plants were exposed once to 20 minutes of dry HCl gas, at one of four concentrations. Pods were harvested as they matured and dried. Seeds from the first 10 pods were counted, weighed, and 50-seed subsamples from each treatment were tested for germination rate (Table 19). When plants were 7 weeks old, all remaining pods were harvested. No significant differences were found between the four treatments with respect to numbers of pods and seeds, seed weight, or germination rate. Plants more severely stressed with

TABLE 19
YIELD OF PINTO BEAN PLANTS EXPOSED TO ONE EPISODE OF HCl GAS THEN KEPT TO MATURITY IN THE GREENHOUSE[1]

HCl Concentration ($mg\ m^{-3}$)	Avg. Total Mature Pods per Plant (no.)	Age When 1st Pod Matured (days)	Average of First 10 Pods: Seeds per Pod (no.)	Weight per Seed (mg)	Germination Rate (%)
0	19.8 a[2]	82 z	3.3 b	304 c	85 d
25	20.5 a	88 y	3.4 b	319 c	81 d
49	23.3 a	90 y	3.2 b	309 c	80 d
87	24.3 a	97 x	3.3 b	323 c	77 d

[1]Averages of 5 plants in each of 3 replicates

[2]Averages followed by the same letter not different at the 5% level of significance by Duncan's Multiple Range Test.

HCl did show a delay in pod maturity. The harvested seeds had an average weight of 297 mg; more than seeds from the field trials, 193 mg, but less than commercial seeds, 317 mg. Single doses of HCl do not affect final seed yield or germination, but may delay maturity.

Soil Analysis

There has been a question as to whether soil would adsorb enough chlorine from the air to affect plant growth. After the field yield experiment, soil samples were taken at three depths from plots representing each of the four treatments. Subsamples were sent to the Air Force Occupational and Environmental Health Laboratory at Brooks Air Force Base, Texas, for analysis of certain elements (Tables 20, 21). Other samples were analyzed for pH and chlorine by standard methods and were submitted to the Agricultural Cooperative Extension Service, University of California, Riverside (Table 22).

TABLE 20
ANALYSIS OF SOIL FROM UCR FIELD AFTER 14 WEEKS EXPOSURE TO GAS FROM SRF[1]

SRF Fuel (mg)	Soil Depth (cm)	Hydrocarbons (mg/kg)	Nitrates (μg N/g)	Cr (ppm)	Cu (ppm)	Fe (ppm)	Pb (ppm)	Mn (ppm)	Ni (ppm)	Zn (ppm)
0	0-1	16	1.5	2.9	3.4	1550	6.0	160	2.0	22.0
	1-2	<6	2.0	3.6	4.6	1650	9.0	190	0.5	20.0
	2-3	<6	3.2	<2.5	4.6	900	2.0	110	2.5	16.0
405	0-1	12	2.5	5.0	4.6	1350	4.5	180	4.0	22.0
	1-2	<6	3.8	2.9	6.3	1300	7.5	180	0.5	19.5
	2-3	<6	4.2	2.9	5.4	1200	16.5	195	1.5	22.0
1080	0-1	<6	1.8	2.2	4.2	1150	10.0	140	<0.5	18.0
	1-2	<6	3.0	5.0	4.6	1400	25.0	185	4.0	37.5
	2-3	8	4.5	3.6	4.6	1500	11.0	160	1.0	22.0
2430	0-1	<6	2.2	4.3	5.0	1650	6.5	195	9.5	25.5
	1-2	<6	1.5	3.6	5.0	1500	1.5	170	3.5	24.5
	2-3	<6	2.0	2.9	4.2	1350	15.0	170	2.0	20.5

[1]The following values were the same for all samples: Cadmium, <0.5 ppm; Hexavalent chromium, <1.0 ppm; silver, <0.5 ppm; and beryllium, <0.5 ppm.

TABLE 21
SUMMARIES OF ANALYSES OF SOIL FROM FIELD PLOTS EXPOSED WEEKLY TO GAS GENERATED BY SRF

SRF Wt. (mg)	Hydro-carbons (mg/kg)	Nitrates (μg N/g)	Cr (ppm)	Cu (ppm)	Fe (ppm)	Pb (ppm)	Mn (ppm)	Ni (ppm)	Zn (ppm)
0-1 cm depth only									
0	16	1.5	2.9	3.4	1550	6.0	160	2.0	22.0
405	12	2.5	5.0	4.6	1350	4.5	180	4.0	22.0
1080	<6	1.8	2.2	4.2	1150	10.0	140	20.5	18.0
2480	<6	2.2	4.3	5.0	1650	6.5	195	9.5	25.5
Averages for all three soil levels									
0	9.3	2.2	3.0	4.2	1367	5.7	153	1.7	19.3
405	8.0	3.5	4.0	5.4	1283	9.5	185	2.0	21.2
1080	6.7	3.1	3.6	4.5	1350	15.3	162	1.8	24.2
2430	6.0	1.9	3.6	4.7	1500	7.7	178	5.0	23.5
Average for all four fuels at same soil level									
Level									
0-1	10.0	2.0	3.6	4.3	1425	6.8	169	4.0	21.9
1-2	6.0	2.6	3.5	5.1	1462	10.8	176	2.1	24.1
2-3	6.5	3.5	3.0	4.7	1238	11.1	159	1.8	20.1

TABLE 22
LABORATORY ANALYSIS OF FIELD SOIL EXPOSED WEEKLY TO GASES GENERATED BY SRF

Fuel Weight[1] (mg)	Soil Depth (cm)	pH[2]	Electric Conductivity (mmhos/cm)	Cation Exchange Capacity (CEC) (me/100g)	pH[3]	Cl^- (mg/g)
0	0-1	8.25	1.85	8.15	8.50	1.007
	1-2	--	--	--	8.45	1.185
	2-3	--	--	--	8.43	1.074
405	0-1	8.25	1.70	8.40	8.50	1.025
	1-2	--	--	--	8.45	1.060
	2-3	--	--	--	8.43	1.105
1080	0-1	8.25	2.05	9.40	8.60	1.071
	1-2	--	--	--	8.48	1.138
	2-3	--	--	--	8.40	1.000
2430	0-1	8.20	2.05	8.65	8.43	1.080
	1-2	--	--	--	8.43	1.026
	2-3	--	--	--	8.38	0.936

[1]Fuel was ignited within 1.2 m^3 chambers placed over plot. Chamber was removed after 15 minutes.

[2]pH of soil brought to field capacity with water, average of 2 plots.

[3]pH measured after mixing 50 g soil with 150 ml distilled water for 10 minutes.

No relationship between gas exposure and elemental analyses was apparent (Table 20). No differences were detected in the emission spectrum of the soil although a possible decrease of hydrocarbon and nitrate levels existed in soil exposed to gases from 2430 mg fuel compared to the other treatments (Table 21). The general lack of relationships between fuel size and soil parameters was consistent in other data as well (Table 22). Periodic movement of irrigation water through the field possibly contributed to lack of differences from site to site.

In addition to the above work, soil from an area not previously exposed to rocket gases was sampled before and several hours after 2430 mg SRF was burned. A chamber remained over the dry ground 15 minutes after ignition. Soil analysis revealed a decrease in pH, electric conductivity, and chlorine content after exposure and an increase in cation exchange capacity (Table 23). Element analyses of these soil samples yielded differences whose meaning is unclear (Table 24). Any soil differences resulting from the fuel gases appear insignificant compared to site and sample differences.

TABLE 23
ANALYSIS OF FIELD SOIL BEFORE AND AFTER ONE EXPOSURE TO GASES GENERATED BY BURNING 2430 mg SRF

Sample	Depth (cm)	pH[1]	Electric Conductivity (mmhos/cm)	Cation Exchange Capacity (CEC) (me/100 g)	pH[2]	Cl^- (mg/g)
Before	0-1	8.15	2.55	8.15	8.25	0.940
	1-2	--	--	--	8.20	0.992
	2-3	--	--	--	8.15	
After	0-1	8.10	1.95	8.50	8.15	0.730
	1-2	--	--	--	8.15	0.790
	2-3	--	--	--	8.15	0.934

[1]pH of soil brought to field capacity with water, average of 2 plots.

[2]pH measured after mixing 50 g soil with 150 ml distilled water for 10 min.

TABLE 24
ANALYSIS OF TOP cm SOIL SAMPLED FROM AN AREA BEFORE AND AFTER BURNING 2430 mg SRF

Sample	Hydrocarbons (mg/kg)	Nitrates (μg/g)	Cr (ppm)	Cu (ppm)	Fe (ppm)	Pb (ppm)	Mn (ppm)	Ni (ppm)	Zn (ppm)
Before	<6	9.0	2.2	3.8	1200	9.5	160	4.0	18.5
After	<6	2.2	1.5	4.2	1600	<2.5	150	<0.5	16.0

Conclusions of Field Studies with SRF

Several generalizations may be made from this field work. Field plants were injured by gas generated by rocket fuel, but relatively large quantities were necessary. Weekly exposures showed that plants were more sensitive when younger. Part of this tolerance with age may be that the increased surface area of the growing plant receives a reduced amount of HCl per unit area. With minimal injury, as in most of these tests, plant growth

and yield was not affected by weekly doses of generated gas: numbers, weight, or germination rate of seed harvested from exposed plants did not differ significantly from unexposed plants. Soil sampled from plots exposed once or weekly to SRF gas was analyzed and no soil parameters could be correlated with fuel weight.

Field Exposures with Dry HCl Gas

Riverside Preliminary Experiments

New portable field chambers, (Figure 3) were constructed. In conjunction with the chambers, an HCl generation system and power control unit (Figure 4) was developed by which a constant level of gas could be supplied under field conditions from a tank of 30% HCl. Initial tests of the equipment involved bean and zinnia plants predisposed to outdoor conditions in an open lath house. The highest flow rate was 271 cc per minute and little injury occurred. Subsequently, the flowmeter was replaced, HCl concentrations were elevated, and greater injury resulted (Table 25). To achieve the high gas concentrations required for field work, the blower intake was partially blocked to reduce air movement through the chamber from 80 seconds to 120 seconds per change. A calibration curve was prepared by making incremental changes in the flowmeter setting and measuring resultant chamber concentration. No plants were used in these tests but measurements were repeated with the chamber over moist ground (Figure 9). During all exposures the circulating paddles were rotating in the middle of the chamber.

TABLE 25
TEST EXPOSURES WITH PORTABLE FUMIGATION CHAMBER

Flow ($cc\ min^{-1}$)	Chamber HCl Concentration ($mg\ m^{-3}$)	Visible Injury			
		% No. Leaves		% Leaf Area	
		Bean	Zinnia	Bean	Zinnia
148	6	0	4	0	1
524	56	25	12	2	3
1418	103	50	88	23	36

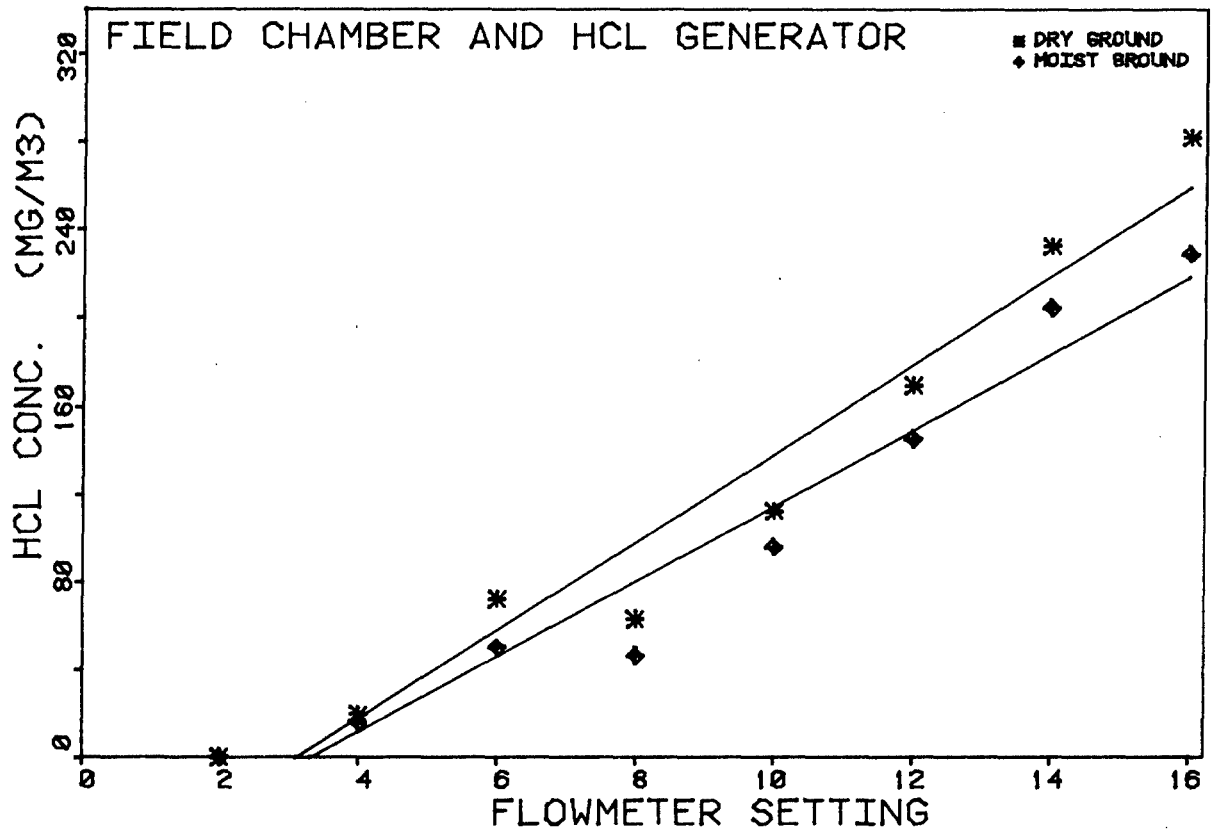

Figure 9. Calibration curves for Tedlar-covered field chamber with positive pressure air flow of one change every two minutes. Dry HCl gas was diluted into air intake from 30% tank. HCl concentrations, in mg m^{-3}, were determined by bubbler samples.

Vandenberg Plot

A 25 by 50 foot plot was developed adjacent to Space Launch Complex 5 in South Vandenberg Air Force Base. The area was cleared of weeds, rototilled, and raked. Peat pots containing two-week-old plants were transferred from the Riverside greenhouse and transplanted so that each experimental cell consisted of one row of 10 zinnia plants and a parallel row of 10 marigolds.

During the three weeks between transplanting and exposure, considerable plant injury occurred, particularly leaf tip-burn caused by strong sea winds. Too few zinnia plants remained for testing since they were more sensitive to this stress than marigolds.

Twelve exposures were made by singly enclosing the surviving marigold cells with the portable chamber and providing HCl gas for 15 minutes. Bubbler samples taken during the exposure were later titrated to determine chamber HCl concentrations. Plants were graded for injury; relatively high gas levels were necessary to effect significant injury (Table 26 and Figure 10).

Leaves of the exposed plants were removed, oven-dried, eluted in weak nitric acid, and their chloride content was determined by titration (Table 26). Tissue chloride content seemed unaffected by the HCl exposures, even at the highest concentration in these tests.

TABLE 26
INJURY AND CHLORINE IN MARIGOLD LEAVES EXPOSED TO HCl GAS FOR 15 MINUTES AT VANDENBERG AIR FORCE BASE

HCl Concentration (mg m^{-3})	Percent Leaves Injured	Percent Leaf Area Injured	Tissue Chloride (Cl^- ug/mg)
55	51	8	32.3
115	94	38	25.0
159	95	32	29.6
171	99	59	27.6

Native Species at Vandenberg

In addition to the marigolds, wild lupine plants, Lupinus longifolius, were exposed to HCl gas. Lupine was selected since it was abundant near the prepared plot and it was in flower at this time of year. Four cells were chosen, marked, and exposed for 15 minutes to HCl gas. Plant injury, seen after 48 hours, consisted chiefly of leaf necrosis and was graded on the basis of leaf injury or non-injury (Table 27 and Figure 11). The HCl concentrations necessary for significant injury to this native species were considerably higher than for the marigolds planted nearby.

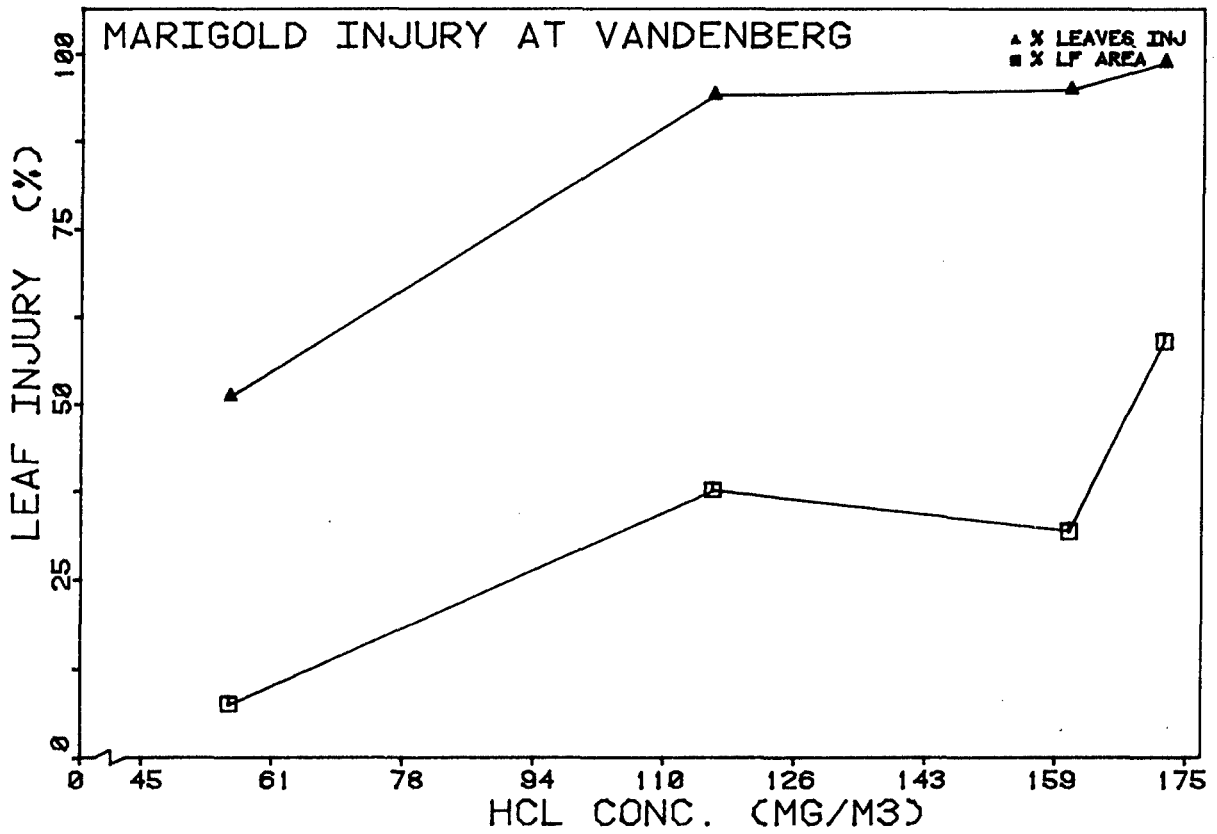

Figure 10. Leaf injury on marigold plants grown and exposed 15 minutes to HCl gas at Vandenberg Air Force Base.

TABLE 27
INJURY ON NATIVE LUPINE PLANTS EXPOSED AT VANDENBERG AFB TO HCl GAS FOR 15 MINUTES

HCl Concentration (mg/m^{-3})	Percent Leaf Injury
77.4	23
120.7	43
196.6	61
247.7	86

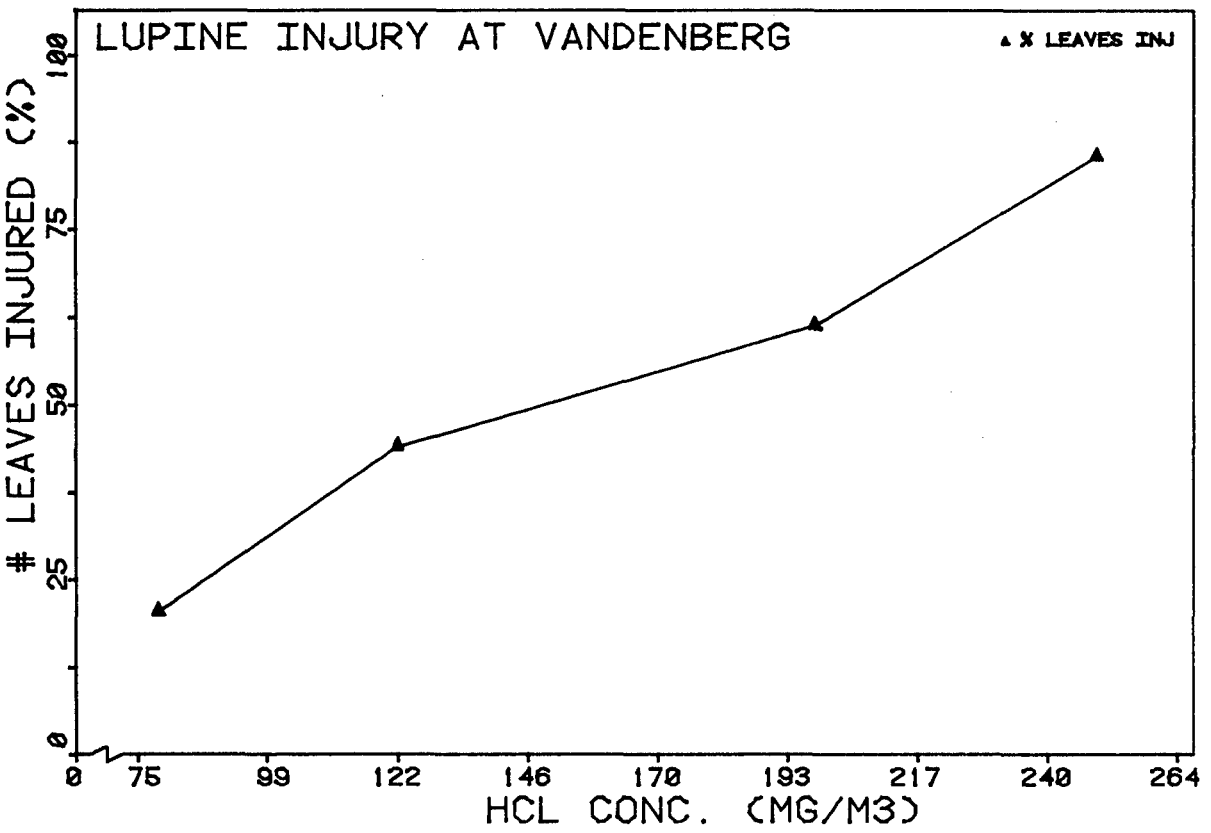

Figure 11. Leaf injury on lupine plants exposed to HCl gas at Vandenberg AFB.

Riverside Field Work

The original design of the Vandenberg plot was planted at Riverside. Since the plot was protected from the wind and could be better maintained, both marigolds and zinnias survived. The experiment consisted of two blocks of 8 cells; each cell had 10 plants per species and was exposed to one of eight different gas concentrations. The two blocks were similarly exposed on different days, thus producing two replicates. A total of 40 plants were exposed to each concentration. Plant injury was assessed 48 hours after exposure (Table 28). Highly significant differences among concentrations but not between blocks were realized.

TABLE 28
LEAF INJURY ON ZINNIA AND MARIGOLD PLANTS GROWN AND EXPOSED IN A FIELD IN RVERSIDE, CALIFORNIA; AVERAGE OF 4 CELLS OF 10 PLANTS EACH

HCl Concentration (mg m^{-3})	Marigold		Zinnia	
	No. Leaves Injured (%)	Leaf Area Injured (%)	No. Leaves Injured (%)	Leaf Area Injured (%)
2	44.7 z[1]	2.7 z	39.2 yz	2.9 z
7	36.7 z	2.4 z	24.3 z	2.9 z
8	42.9 z	3.1 z	33.4 yz	2.8 z
14	51.1 yz	6.1 z	46.5 y	3.4 z
19	54.2 yz	9.8 y	40.3 yz	3.1 z
28	69.7 xy	23.8 x	66.4 x	7.8 z
36	87.9 x	42.6 x	90.6 z	15.2 y
45	89.1 x	51.8 x	72.0 y	14.9 y

[1]Averages followed by the same letter are not significantly different at 5% level by Duncan's Multiple Range Test.

Probit Analysis and Comparisons

Probit analysis (Finney, 1971) allows the comparison of injury rates and therefore the sensitivity of different plants to HCl gas (Granett and Taylor, 1978). Probit lines (Figure 12) were derived for the field work with a computer program, and from these lines the expected HCl concentration necessary to produce a given amount of injury could be calculated (Table 29). In general, greenhouse plants are more sensitive than field plants to HCl gas. The greenhouse plants, in addition, have a narrower range between minor (25%) and severe (95%) injury. Field plants need very high gas concentrations for severe injury. The plants at Vandenberg, particularly the native lupine, need more gas to reach injury levels comparable to the Riverside plants.

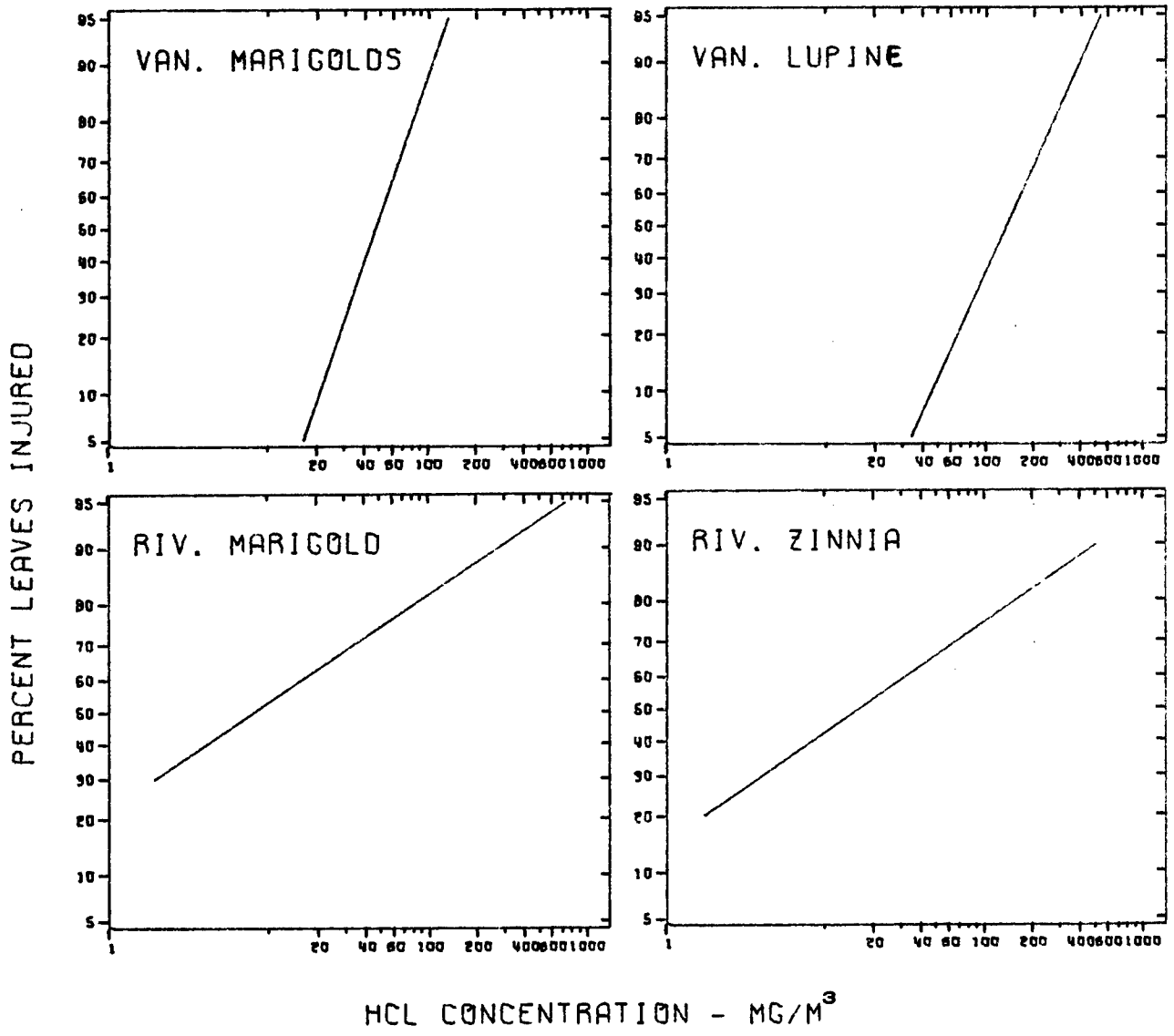

Figure 12. Probit analysis of plants exposed to HCl gas under field conditions at Vandenberg AFB and Riverside, California.

TABLE 29
TOLERANCE OF PLANTS TO GASEOUS HCl COMPARED BY PROBIT ANALYSIS. DOSE, IN mg m^{-3}, NECESSARY FOR EXPECTED LEAF INJURY

Expected Injury Level	Riverside Greenhouse		Riverside Field		Vandenberg Field	
	Marigold	Zinnia	Marigold	Zinnia	Marigold	Lupine
25%	11	15	1	3	31	79
50%	13	22	8	17	48	139
75%	16	33	52	101	75	246
95%	20	58	741	1342	136	558

HYDROGEN FLUORIDE

REVIEW OF EFFECTS

The effects of hydrogen fluoride (HF) gas on plants are more detrimental than those of HCl (Treshow and Pack, 1970). HF is one product of experimental rocket engine fuels. To date, however, most information on fluorinated plasticizers in these fuels is classified.

The appendix to this report is a detailed review of the pertinent HF literature. Little information was found dealing with short term, high concentration doses that might result from a post-launch ground cloud.

DRENCH EXPERIMENT

An initial experiment was undertaken to determine the effects of sodium fluoride (NaF) on the growth of pinto bean plants and to establish fluoride analytical procedures. The soil of nine- and 20-day-old potted plants was drenched with 0, 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} M NaF solutions. Additions of sodium chloride (NaCl) adjusted total ionic strength of all treatments to 10^{-1} M, except for one control which contained no added salt. Plant injury ranged from no effect to wilt, senescence, and death. Injury, height, and petiole length were recorded every other day and averages were calculated (Table 30).

TABLE 30
EFFECT OF NaF SOIL DRENCH ON BEAN PLANTS

Code	NaF (M)	T.I.S.[1] (M)	Injury (0-5) Young	Injury (0-5) Old	Petiole length (cm) Young	Petiole length (cm) Old	Final height (cm) Young	Final height (cm) Old
T0	0	0	0.6 z[2]	0.0 z	6.8 x	6.9 x	12.9 x	18.6 x
T1	0	10^{-1}	3.0 x	1.9 xy	3.2 y	6.8 x	12.2 xy	14.6 y
T2	10^{-4}	10^{-1}	2.4 xy	1.6 y	3.6 y	6.9 x	11.5 y	14.9 y
T3	10^{-3}	10^{-1}	1.4 yz	1.9 xy	3.8 y	6.3 xy	12.3 xy	13.1 y
T4	10^{-2}	10^{-1}	3.0 x	2.8 x	3.8 y	5.7 y	12.8 x	15.5 y
T5	10^{-1}	10^{-1}	5.0 w	5.0 w	--	--	--	--

[1]Total ionic strength was adjusted with NaCl.

[2]Data in each column followed by the same letter were not significantly different by Duncan's Multiple Range Test.

Injury was estimated for each leaf on a 0-5 basis, 0 indicating lack of injury and 5 indicating death. Notably, there was injury with treatment T1, in which only NaCl contributed to the ionic strength. This injury suggests that osmotic effects, apart from fluoride effects, are significant in salt uptake from soil. Age was apparently a factor in plant reaction. Reduced petiole lengths were found on young plants treated with high ionic strength solutions (T1-4), but on older plants, the petiole lengths were reduced only with the highest nonlethal NaF level (T4). There was no apparent correlation between height of young plants and salt or fluoride treatment; all older plants treated with high ionic strength solutions (T1-4) were shorter than the T0 plants. Principal results of this work were to confirm that a relatively high fluoride concentration, between 10^{-2} and 10^{-4} M, consistently injured plants regardless of total ionic strength and that some concentration between 10^{-1} and 10^{-2} M consistently killed the plants.

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APPENDIX

ACCUMULATION AND TOXICITY OF FLUORIDE IN PLANTS:

A LITERATURE REVIEW

Of all common atmospheric pollutants, fluoride (here referred to as F, even if in the ionic state) produces phytotoxic effects at the lowest concentrations. Most atmospheric F has its source as an escape from certain industrial processes, especially fertilizer, metal, and brick manufacture; rocket engine exhaust may also contain considerable concentrations of F (N.A.S., 1971). Atmospheric F is generally found as HF, although SiF_4 and various other gases may occur. Particulate fluorides, also released as a pollutant, are generally unavailable or nontoxic to plants and pose their greatest hazard to grazing livestock. This review, therefore, is concerned only with gaseous and dissolved F.

CLASSICAL INJURY

Visible phytotoxic effects attributable to F, termed classical injury because they have been reported since the 19th century (N.A.S., 1971), are confined mostly to leaf tissue and consist of chlorosis (yellowing due to chlorophyll degradation), necrosis (tissue collapse and death), and, in some cases, lesion formation and abscission. Monocotyledonous plants, because of their parallel veination, generally incur tip injury. Large apical portions of gladiolus leaves become necrotic due to a single F exposure; additional injury from subsequent exposures remains separated from the initial by distinct brown bands (Hitchcock et al., 1962; Treshow and Pack, 1970). In Sorghum, apical necrosis is usually subtended by a chlorotic zone which is absent or very restricted in gladiolus, but both species may have additional intercostal necrotic and chlorotic markings (Hitchcock et al., 1963). Injury in sweet corn, unlike that of most monocots, consists of bifacial lesions which are most abundant in the distal half of older leaves (Mandl et al., 1975). In pine, injury consists of tip necrosis of the current year's needles and is separated from healthy tissue by a reddish-brown band (Carlson et al., 1979). Injury in dicotyledonous plants is similar to that of monocots, except that marginal as well as apical necrosis and chlorosis occur. Apricot leaves develop semicircular, marginal lesions which are separated from healthy tissue by a narrow, reddish-brown abscission layer; the necrotic tissue separates at this layer, leaving the leaf undamaged except for the scalloped edges (Treshow and Pack, 1970). Injury to citrus consists of extensive chlorosis, especially at the leaf tip, and limited amounts of necrosis following high exposure (Leonard and Graves, 1972); in addition, leaf abscission is common in both young and older leaves after exposure to high F concentrations (MacLean et al., 1968).

Fluoride-induced chlorosis and necrosis are caused by morphological and physiological changes in the leaf. Chloroplasts and cell walls are the major sites of F accumulation (Chang and Thompson, 1965; 1966b), and

derangement and dispersion of chloroplasts from their peripheral location are among the first structural changes leading to chlorosis (Wei, 1973). The intrathylacoidal spaces of the grana may sustain initial chloroplast injury, as indicated by electron microscopy, and effects on enzyme systems located in this area may precede visible external injury (Lhoste, 1979). Fluoride may also affect the early stages of chlorophyll synthesis (McNulty and Newman, 1961).

Development of necrosis may be preceded by a dark green, "water-soaked" appearance of the injured leaf tissue. In apricot and other fruit trees, this appearance results from collapse of the spongy mesophyll and lower epidermis, followed by distortion of the palisade layer and finally collapse of the upper epidermis (Solberg and Adams, 1956). In pine, however, mesophyll collapse does not occur until after occlusion of the resin ducts and phloem (Stewart et al., 1973). Autocatalysis of pigments in Coleus following mesophyll breakdown indicates the loss of structure and function which accompanies mixing of the cellular constituents (Lamprecht and Powell, (1977). Poovaiah and Wiebe (1969) considered tylosis formation in the petiole and leaf xylem of geranium following fumigations that produced marginal necrosis to constitute evidence that F may influence tissue function distant from the site of visible injury.

NON-CLASSICAL EFFECTS AND "HIDDEN" INJURY

In addition to the above classical effects, Thomas (1956) suggested that certain "hidden" effects may be present in visibly undamaged tissue. These hidden effects could presumably cause reduction in growth and yield. Hill et al. (1958) found no evidence for hidden injury to the photosynthesis or growth rates of various tomato cultivars exposed to F, even at concentrations producing visible injury. However, a large body of more recent evidence indicates effects upon the metabolism, growth, flowering and fruiting, yield, and genetics of various other plants at F concentrations below that producing visible injury as well as effects upon uninjured areas of damaged leaves exposed to higher F concentrations. Biochemical bases for some of these effects have been suggested.

Metabolism

Fluoride at concentrations below that producing visible damage stimulates respiration (Applegate and Adams, 1960a) as do higher concentrations prior to development of necrosis (Applegate and Adams, 1960b; Yu and Miller, 1967; McNulty and Newman, 1957). Onset of visible injury results in a rapid respiratory decline. The initial stimulation has been attributed to ATPase inhibition and to increased energy requirements arising from the need for cellular repair (Yu and Miller, 1967). Subsequent respiratory decline apparently results from both general metabolic inhibition stemming from formation of metal-fluoride complexes (McCune and Weinstein, 1971; Ordin and Altman, 1965) and from inhibition of specific F-sensitive enzymes, including competitive inhibition of the succinic dehydrogenase system in the tricarboxylic acid cycle (Lovelace and Miller, 1967a,b) and interference with the enolase system (Miller, 1958).

Specific and nonspecific nonrespiratory metabolic effects are indicated by the high F-sensitivity of enzymes involved with sucrose biosynthesis (Yang and Miller, 1963b), by F-associated changes in enzyme activity (McCune et al., 1964), and by changes in the pool size of various cellular metabolites, including carbohydrates, organic acids, and amino acids (Weinstein, 1961; Yang and Miller, 1963a), and starch and polysaccharides (Adams and Emerson, 1961).

Growth and Yield

The greatest F-induced effect on growth is a change in the mass of various plant parts, but minor modification of shape may occasionally occur. Decrease in size and weight of vegetative parts is most common. Sorghum and gladiolus plants subjected to F exposures which produced noticeable injury were significantly smaller than plants receiving milder exposures (Hitchcock et al., 1963). Yield of turnip and alfalfa were reduced when foliar F concentration exceeded 60 parts per million (ppm) (Hansen et al., 1958), and dry weights of leaves and stems of tomato plants were reduced by relatively high F exposures (MacLean et al., 1976). Both ambient and experimental F exposure caused reduced top growth in citrus (Leonard and Graves, 1972; Brewer et al., 1969). In addition, fruit yield was significantly reduced by relatively high levels of ambient atmospheric F (Leonard and Graves, 1966; 1972). The reduced growth and yield were accompanied by a decrease in leaf size and total leaf area (Leonard and Graves, 1972; Brewer et al., 1969). Fumigation of ten crop plants resulted in decreased leaf and stem weights of some species at relatively high F concentrations, but also resulted in increased weights at low F concentrations (Pack and Sulzbach, 1976). The concentrations at which weight increase or decrease occurred varied from species to species.

The reduced growth has several possible causes. It could result from the decreased photosynthesis associated with leaf injury and reduced leaf area, as is apparently the case with citrus (Leonard and Graves, 1972; Brewer et al., 1969) and perhaps with Sorghum and gladiolus (Hitchcock et al., 1963). It could also result from mitotic inhibition, as indicated by reduced cell division in root tips exposed to F (Galal and Abd-alla, 1976; Nitsan and Lang, 1965). Reduction of cell division and elongation is apparently a response to F-induced reduction of endogenous RNA levels and activity (Chang and Thompson, 1966a; Pilet, 1969), to altered RNA structure (Chang, 1968), to increased RNase activity (Pilet, 1969), and perhaps to inhibition of DNA synthesis (Chang and Thompson, 1966a).

Aside from the morphological changes of reduced top growth, F has caused production of long, spindly stems in citrus (Matsushima and Brewer, 1972); the spindly stem growth was concomitant with reduction of mass in tomato (MacLean et al., 1976).

Fruiting

Classical F injury has been reported for a number of fleshy fruits. Symptoms in peach, apricot, cherry, and pear are basically similar, but suture red spot (soft suture disease) of peach is the most serious and

best known (Treshow and Pack, 1970). The effect is characterized by premature ripening of a localized spot along the fruit suture lines and sometimes by splitting of the fruit at the suture. Reddening of the spot may occur 2-4 weeks early, resulting in overripe, soft areas in the mature fruit. Development of suture red spot is determined early in fruit development when atmospheric F concentrations too low to produce foliar necrosis may injure up to 90% of the peaches. Subsequent F levels are not critical, and F accumulation in the fruit is negligible.

Reduction of fruit number and size has been observed in several plant varieties. A relatively high F concentration of 6 $\mu g\ m^{-3}$, which produced considerable foliar injury, reduced the size of tomato fruits and caused complete or partial seedlessness (Pack, 1966). A mean ambient F concentration of 0.6 $\mu g\ m^{-3}$, which produced no foliar injury, did not affect tomato fruiting but reduced the fresh mass of marketable bean pods by almost 25 per cent (MacLean et al., 1977). In addition, experimental F exposures lowered the starch content and affected the physical appearance of bean seeds (Pack, 1971). Ten crop plants grown primarily for their seed or fruit responded variously to different F concentrations, but the most common response was development of fewer seeds (Pack and Sulzbach, 1976). The decreased seed production sometimes occurred at relatively low F concentrations which produced no foliar injury. This independence of direct effect on fruiting from foliar injury may result from reduced fertilization, since fumigation of either the pollen or maternal parent may result in reduction of pollen germination, pollen tube growth, and pollen retention on the stigma (Sulzbach and Pack, 1972). In strawberry, fruit deformation occurs at 0.5 $\mu g\ F\ m^{-3}$ in the absence of foliar injury, and fruit weight is reduced at 2.0 $\mu g\ F\ m^{-3}$, with only very mild concommitant foliar injury (Pack, 1972). Here also, F apparently affects fertilization and seed development independently of the foliage.

Mutagenicity

A number of mutagenic effects have been attributed to F. Aqueous NaF solutions induce chromosomal aberrations resulting in bridges, fragments, or both in onion root tips (Mohamed et al., 1966a; Temple and Weinstein, 1978). The same aberrations were observed in mitotic and meiotic smears of tomato plants fumigated with HF (Mohamed et al., 1966b), and progeny from these fumigated parents included some plants that developed abnormally and resembled known mutants and other plants that had chromosomal aberrations (Mohamed, 1968). No visible damage was apparent in the fumigated plants, and the chromosomal effects diminished after recovery in a F-free atmosphere. In maize, 3 $\mu g\ F\ m^{-3}$ induced asynapsis, translocation, and inversions in addition to bridges and fragments (Mohamed, 1970). In all cases, the frequency of chromosomal aberrations tended to increase with longer fumigation periods (Mohamed, 1969). In a separate study, fumigation at a comparable exposure level produced no visible mutation or chromosomal aberration in tomato, and the difference in results is attributed to the different genetic backgrounds of the two tomato varieties (Temple and Weinstein, 1978). The mutagenic mechanism of F is unknown, although it may

be due to direct or indirect inhibition of DNA synthesis (Mohamed, 1969). This mechanism was also suggested as the cause for the reduced number of mitotic figures in corn seedling roots (Chang and Thompson, 1966a).

In addition to the above mutagenic effects upon the chromosomes, F can potentially affect the genetic system by altering recombination and inducing polyploidy. The recombinational index increased from 5.5 to 15 between one set of linked markers in maize in response to 3 μg F m^{-3} for 4 days; an adjacent chromosomal region showed no recombinational change (Mohamed, 1977). Such dichotomous responses for different chromosomal regions are not uncommon (Lifschytz, 1971). Failure of cytoplasmic division following chromosomal division was reported in maize (Mohamed, 1970); occurrence of such an event in the apical meristem could produce autopolyploidy, while occurrence during premeiotic division could result in unreduced gametes and allopolyploidy.

The above evidence indicates that F has mutagenic effects upon chromosomes, at least under certain conditions. Since no agents are known which cause chromosome breakage without causing gene or point mutations (Serres, 1978), these results imply a more widespread mutagenic effect. No direct evidence is available, however, for mutagenic effects of F with respect to specific or multiple locus genic systems, single strand DNA breaks, lethal genes, cytoplasmic mutations, sister-chromatid exchanges, or somatic recombination. Thus, F effects upon the preponderance of genetic factors are completely unknown, although a variety of monitoring and screening systems for detection and evaluation of possible mutagenic effects is now available (Constantin, 1978; Nilan, 1978; Serres, 1978).

UPTAKE AND ACCUMULATION

Fluoride can be absorbed into the roots from the soil and into the leaves from the atmosphere or from leaf surface deposits. Although the relatively low amounts of F normally found in plants growing in uncontaminated areas (2-20 ppm dry weight) are absorbed largely from the soil, no definite relationship exists between plant and soil concentration (N.A.S., 1971). Rather, uptake of F, both naturally occurring and from experimental addition in moderate amounts, depends as much on the nature of the soil as on the fluoride concentration (Hansen et al., 1958; Hitchcock et al., 1971). Fluorides in the amounts added to the soil by way of air or fertilizer pollution are largely inactivated by the soil and rendered unavailable to plants (Hansen et al., 1958; Daines et al., 1952), although large scale gaseous and particulate F pollution may significantly affect vegetation and soil (Thompson et al., 1979).

Most F absorbed in response to pollution is taken in through the leaves (MacIntire, 1952) and transported by the transpirational stream to the leaf tips and margins where it accumulates (Hitchcock et al., 1962; MacLean et al., 1973, Ledbetter et al., 1960; Jacobson et al., 1966). Fluoride absorbed by the roots also accumulates in the leaves, although large amounts remain in the roots (Daines et al., 1952; Ledbetter et al., 1960). Leaf concentrations often increase to thousands of times that found in the surrounding ambient air (Leonard and Graves, 1972) but, after

cessation of exposure, little F from older leaves having a high concentration is translocated into newly developed leaves having a low concentration (Hitchcock et al., 1963). Irreversible binding of F does not generally occur (Ledbetter et al., 1960) except in a few unusual plants that synthesize highly toxic carbon-fluorine compounds (N.A.S., 1971). The ease with which the soluble F is removed from the leaves of some plants by repeated, mild washing suggests a two-way interchange between the leaf interior and exterior (Ledbetter et al., 1960). This exchange may largely account for the post-fumigational loss of up to 50% of accumulated F (Weinstein, 1961; Hitchcock et al., 1971). Some plants, however, have relatively little translocation to the leaf exterior (Jacobson et al., 1966), and F loss may result mostly from abscission of older leaves or necrosis of tissue having high F concentrations.

At the subcellular level, the sites of F accumulation in citrus leaves were determined to be, in decreasing order, chloroplasts, cell walls, soluble proteins, and mitochondria (Chang and Thompson, 1965; 1966b). In tomato, the order of chloroplasts and cell walls were interchanged (Ledbetter et al., 1960); however, this order may result from lack of correction for chloroplast and chloroplast-fragment cross contamination (Chang and Thompson, 1965; 1966b).

The large number of variables affecting F accumulation results in a great amount of variation for foliar F content under both natural and experimental conditions. Atmospheric F concentration and duration of exposure, the product of which has been termed the "exposure factor" (Adams et al., 1957), are the basic variables. Fluoride accumulation increases with both increased atmospheric F concentration and increased length of exposure (Hitchcock et al., 1962; Pack and Sulzbach, 1976). In alfalfa, for example, a 7-day exposure to filtered air with addition of 0.0, 1.04, 1.89 and 6.09 $\mu g\ F\ m^{-3}$ resulted in plant F concentrations of 4.8, 9.8, 13.9 and 75.1 ppm, respectively (Hitchcock et al., 1971). In timothy and red clover, exposure to 7.0 $\mu g\ F\ m^{-3}$ for 0, 3, 5, 7 and 10 days resulted in tissue contents of 3, 103, 152, 188 and 240 ppm F, respectively (MacLean et al., 1969b). However, corn and tomato plants fumigated for equal exposure factors accumulated more F with higher concentrations than with longer exposure (Leone et al., 1956), and F accumulations in response to the two variables were neither reciprocal nor consistent within equal exposure factors for a variety of citrus and ornamental species (MacLean et al., (1968). Furthermore, timothy and red clover accumulated more F for similar exposure factors when exposed to 1.9 $\mu g\ F\ m^{-3}$ during alternate 48 hour periods than when exposed to 1.6 $\mu g\ F\ m^{-3}$ continuously (MacLean et al., 1969b). Thus, at least under certain conditions, F concentration is more influential on accumulation than is duration of exposure, and use of exposure factor as a measure of the rate at which exposed plants accumulate F is imprecise.

Other environmental factors also affect F accumulation. Soybean plants fumigated during 6 daytime hours per day accumulated more than twice as much F as plants fumigated for 6 nighttime hours per day (Poovaiah and

Wiebe, 1973), and alfalfa exposed to 5.0 μg F m^{-3} adsorbed more F onto leaf surfaces with night fumigation than with day, absorbed more F into the leaves during day fumigation, but, after 40 total hours of exposure as compared to 24 total hours, took up the same amount with night exposures and twice as much with day (Benedict et al., 1965). High temperature (26C) reduced F accumulation in gladiolus, but increased it in sunflower (MacLean and Schneider, 1971), and high root temperatures in four herbaceous crop plants apparently favored translocation of F from the tops to the roots (Benedict et al., 1965). Relative humidity affected the distribution of accumulated F in gladiolus (MacLean et al., 1973). In addition to these environmental factors, F accumulation is influenced by plant and tissue age (Hitchcock et al., 1962; Leonard and Graves, 1972; Carlson et al., 1979, MacLean and Schneider, 1971) and by the nutritional status of the plant (Pack, 1966; MacLean et al., 1969a; 1976).

Fluoride injury clearly results from accumulation since foliar F contents equivalent to atmospheric concentrations are far too low to produce injury. Furthermore, when transpiration, and thus F accumulation, was restricted by placing plastic bags over the apical portions of gladiolus leaves, the injured zone shifted to just below the bagged area and corresponded to the zone of highest F accumulation (Jacobson et al, 1966).

SUSCEPTIBILITY AND INJURY

Plants vary greatly in their inherent susceptiblity to injury by F. Relatively susceptible varieties (gladiolus, apricot) may be injured by continuous exposure to 0.1 parts per billion (ppb) F or by foliar accumulation of 20 ppm (20 μg F per g dry weight) (Hitchcock et al., 1962). Resistant varieties (cotton, camellia) may remain uninjured from a hundred times that exposure (Treshow and Pack, 1970) or foliar accumulation of 4000 ppm F (Jacobson et al., 1966). Varieties highly sensitive to injury generally accumulate considerably less F than more tolerant plants (Hitchcock et al., 1962, 1963; Jacobson et al., 1966; MacLean and Schneider, 1971). The higher accumulation rate in more resistant varieties commences at the beginning of exposure, before injury develops, and is thus a result of intrinsic physiological factors rather than injury in the susceptible varieties. Fluoride sensitivity is generally correlated with taxonomic similarity (Adams et al., 1957) but not strictly so. Thus, Larix occidentalis and L. leptolepsis are highly susceptible while L. decidua is resistant (N.A.S., 1971), gladiolus is generally susceptible but cv. Snow Princess is much more sensitive than cv. Elizabeth the Queen (Hitchcock et al., 1962), and several varieties of Sorghum and sweet corn vary in relative susceptibility (Hitchcock et al., 1963; Mandl et al., 1975). Sensitivity of plants from different seed lots of the same variety may differ greatly (Treshow and Pack, 1970), but the extent of intravarietal genetic variation and the amenability of F susceptibility to selective modification are largely unknown.

The physiological basis for the difference in inherent susceptiblities is somewhat obscure. Different sensitivities among 42 varieties of gladiolus are generally correlated with stomatal frequency and stomatal well

diameter (Hendrix and Hall, 1957); thus, potential for gas exchange may be one factor. In addition, differences in translocation and ultimate disposition of F in cotton, tomato and gladiolus suggest that transpiration rate and internal-external transport of F in the leaf may affect susceptibility (Jacobson et al., 1966).

Within plants, younger tissues are often more susceptible to F injury than older tissues (Hitchcock et al., 1971; MacLean et al., 1976). In pine, the current year's needles are highly sensitive and may be injured when foliar F concentrations reach 8-10 ppm; older needles are much more resistant (Carlson et al., 1979). The two youngest leaves of gladiolus are very sensitive, older leaves less so (MacLean et al., 1973). In citrus, young leaves are more susceptible to both chlorosis and abscission (Leonard and Graves, 1966; 1972).

Plant injury and susceptibility to injury may vary, sometimes inconsistently, in response to different exposure conditions and environmental factors. In citrus, for example, high concentration-short term exposure to HF produced severe foliar injury to plants containing quantities of F that, if accumulated over a longer period, would induce little or no visible injury (MacLean et al., 1968). Conversely, different foliar F levels in forage crops resulting from continuous-low and intermittent-moderate HF fumigations were not reflected in the amount of foliar injury; both treatments produced similar, moderate necrosis and chlorosis (MacLean et al., 1969b). Daily exposures of 39 plant varieties to 1.5 ppb F tended to produce greater visible leaf injury than did twice-weekly exposures to 5 and 10 ppb F (Adams et al., 1957). Plants in the field generally developed more injury when growing under unfavorable, inconsistently irrigated conditions than when grown without water stress, but greenhouse grown plants were most sensitive under optimal conditions which produced turgid, succulent growth (Treshow and Pack, 1970).

Differences in injury resulting from intermittent exposure may be due to physiological and metabolic recovery. Intermittent exposures of citrus to HF gas or NaF sprays were apparently less toxic than continuous HF exposure because they allowed the absorbed F to be chemically inactivated or translocated between exposures (Brewer et al., 1969). The starch-nonstarch polysaccharide ratio of pine normalized during intermittent F exposures of relatively high concentration, but remained altered during more continuous fumigations at lower concentration (Adams and Emerson, 1961). Tomato and bean plants having reduced growth and altered levels of organic acids and chlorophylls recovered after cessation of exposures chosen to maximize biochemical effects and minimize injury (Weinstein, 1961).

A large number of plant species, especially those of agricultural or horticultural value, have been tabulated as to relative F susceptibility on the basis of: a) the amount of leaf injury following a given F exposure level, b) the exposure level required to produce a minimal or given amount of injury, or c) the amount of F present in injured tissue (N.A.S., 1971; Weinstein, 1977; Treshow and Pack, 1970; Pack and Sulzbach, 1976; Woltz and Waters, 1978a,b; Zimmerman and Hitchcock, 1956). Although the first

of these criteria is most widely used, the different exposure times and F concentrations employed during different investigations generally produce less than directly comparable results. The inherent variation in plant susceptibilities and the sensitivity of exposure systems to environmental vagaries preclude more than a coarse determination of relative susceptibilities. However, a number of generalizations have been suggested (N.A.S., 1971):

a) Several economically important conifers and other woody ornamentals are highly susceptible, several other conifers are intermediately so.

b) Most broad-leaved deciduous trees and shrubs are intermediate to tolerant.

c) Common vegetable and field crops are mostly moderately susceptible to tolerant. Sweet corn and some other grasses are exceptions, being highly susceptible, and many species have not been tested.

d) Fruit and berries are generally moderately to highly susceptible,

e) Herbaceous ornamentals are heterogeneous; some groups (especially the monocots) include highly susceptible varieties while other groups (Caryophyllaceae and Brassicaceae) include relatively tolerant varieties.

Due to the great variation, these generalizations are of little value in predicting susceptibilities of untested varieties. Furthermore, being based on visible foliar injury, they do not necessarily reflect relative susceptibilities to "hidden" injury, fruit damage, or loss in yield. Tomato, for example, is tabulated as being more susceptible than bean (N.A.S., 1971); but yield of both seed and fruit is more sensitive in bean than in tomato. Similarly, strawberries are tabulated as intermediate to resistant, but fruit quality and weight are reduced by F concentations well below that producing foliar injury (Pack, 1972). Clearly, more information on relative susceptibilities based on criteria other than visible leaf injury is needed, and proposed tests based on short-term foliar effects may have limited value (Davison et al., 1974).

METHODOLOGY

Most experiments dealing with F involve exposure of plants to the gas, evaluation of resulting injury and, usually, analysis of F accumulated in the tissue. Two exposure methods have been widely used; one consists of fumigating plants in an exposure chamber that maintains a predetermined F concentration (Hitchcock et al., 1962; 1963; Woltz and Waters, 1978a) and the other utilizes ambient atmospheric F for treatment (Carlson et al., 1979). A combined method utilizes fumigation with ambient air in chambers; control treatments consist of filtered air (Leonard and Graves, 1966, 1972; Thompson, 1969). Both methods require sampling and analysis of the experimental atmosphere to determine F content. Fumigation chambers usually consist of greenhouses or frames covered with clear plastic. Since these chambers generally eliminate the micrometeorological characteristics

found outside, fumigation experiments cannot duplicate plant response to F in ambient field conditions (Mukammal, 1976).

When ambient air is not used, F must be introduced into the chamber by a generating and delivery system. Usually HF solution is atomized and/or volatilized and the resulting gas is introduced into the chamber (Hill et al., 1958; Mavrodineanu et al., 1962; Mandl et al., 1971). Compressed HF gas is avoided because of the extreme safety hazard.

A variety of air sampling and analysis techniques are available. Manual sampling involves passing a known amount of contaminated air through an impinger or alkali-coated filter to capture the F (Jacobson and Weinstein, 1977). If contamination with particulate F is a possibility, samples are prefiltered through either an acid-treated or membrane filter. Fluoride in the aqueous sample is measured by titration, spectrophotometry, or selective electrode potentiometry.

Analysis of tissue samples was classically performed by the Willard-Winter method (Willard and Winter, 1933) which involves ashing and alkali fusion at high temperature (600C), distillation of the dissolved melt to remove interfering ions, and finally thorium nitrate titration. These time consuming processes are largely eliminated by selective electrode potentiometry. Release of bound F is accomplished by either alkali fusion (McQuaker and Gurney, 1977; Baker, 1972), digestion with organic acids (Johnson, 1976), or oxygen bomb combustion (Levaggi et al., 1971), followed by pH adjustment and direct potentiometric F determination. One method involves potentiometric measurement of the slurry produced from digestion of dried samples in H_2SO_4 and NaOH (Jacobson and Heller, 1970). Known addition or direct measurement methods allow rapid detection of F levels as low as 0.02 ppm (Orion Research, 1977).

Continuous and semi-automated methods for F analysis of air and plant materials are available (Weinstein et al., 1965; MacLean et al., 1967). Although these methods are very useful for monitoring long-term experiments or ambient air concentrations, their high cost renders them inappropriate for short-term, single-concentration experiments, especially since tissue samples must be ashed and alkali-fused.

DISCUSSION AND CONCLUSIONS

A priori, one might expect that exposed plants become damaged if they accumulate F beyond some threshold level and that this accumulation is proportional to exposure level. In fact, previously presented evidence indicates that these assumptions are at best only broadly applicable. Figure A1 indicates the variables associated with each of the events leading to F induced injury in plants. Each event is affected not only by its own set of variables, but also by the variables of all previous events. Injury, being the last event of the series, is influenced by the greatest number of variables and is therefore most inconsistent.

As exposure increases from zero to the lowest phytotoxic levels, the initial factor limiting injury is inherent susceptibility. Variables

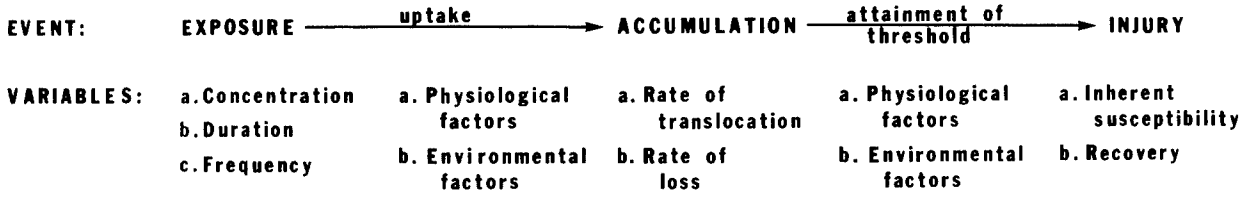

Figure A1. The sequence of events leading to fluoride injury and the variables affecting each event.

associated with exposure and accumulation are less important, and sensitive plants will be injured while resistant varieties remain uninjured. As exposure increases to moderately phytotoxic levels, accumulation gains in importance and may become the limiting factor. Other variables remain important, exposure affecting the rate of accumulation and susceptibility affecting the threshold of injury, but most injury at this level nevertheless results from accumulation of F over a period of time. As exposure increases to highly phytotoxic levels, the exposure conditions themselves largely determine injury. Accumulation is very rapid, with translocation and loss being unimportant, and most varieties will receive injury.

Two factors, the above shift of importance from susceptibility to accumulation to exposure as exposure level increases and the previously mentioned large amount of variation for injury, make accurate prediction of injury exeedingly difficult. Prediction is feasible only in specific cases where at least plant susceptibility and exposure conditions are known; more general predictions would be vague to the point of being meaningless. The converse of this point is illustrated by the quest for an air quality standard. If protection of all vegetation from injury is desired, essentially no amount of atmospheric F can be allowed because of the few highly sensitive varieties (Carlson et al., 1979); but even if slight injury to sensitive varieties can be tolerated, extremely low exposure standards must be set because of variation and differences in the response curves of different varieties (McCune, 1969).

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