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Response of Selected Plant and Insect Species to Simulated Solid Rocket Exhaust Mixtures and to Exhaust Components from Solid Rocket Fuels

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ABSTRACT

RESPONSE OF SELECTED PLANT AND INSECT SPECIES TO SIMULATED SOLID ROCKET MOTOR EXHAUST MIXTURES AND TO EXHAUST COMPONENTS FROM SOLID ROCKET FUELS

Greenhouse exposure chambers were built and tested for use with hydrogen chloride (HCl) gas. A dispensing system for aluminum oxide (Al_2O_3) or alumina particles was designed and tested for use in one chamber. A controlled burn facility was designed and tested to expose plants to solid rocket fuel (SRF) exhaust in 10 ft diam by 8 ft tall field chambers. Both the greenhouse and field chambers were constructed for use as continuous stirred tank reactor (CSTR) type exposure chambers. The chambers were tested and found to model the ideal CSTR well at flow rates approaching one chamber volume per min.

The HCl concentrations predicted for the greenhouse chambers, based on calculations that considered chamber design and calibration data, compared well with concentrations actually measured in the greenhouse exposure chambers. Uniformity of plant response to HCl across and within the greenhouse chambers was shown using soybean and radish as test plants. The dispensing and monitoring of the Al_2O_3 system performed according to design criteria. Both the commercial particulate mixture of α- and γ- phases of Al_2O_3 and the burn particulate exhibited a bimodal size distribution. The controlled burn facility for field chamber exposures performed as designed permitting short term exposures of vegetation to combustion products of the SRF exhaust.

Horticultural practices were developed to grow 24 species native to Florida in the greenhouse. These 24 species, three agronomic species (9 cultivars) and nine horticultural species (16 cultivars) were screened for sensitivity to HCl and selected species were screened for sensitivity to Al_2O_3, to mixtures of Al_2O_3 + HCl, and to SRF exhaust. The more sensitive species were exposed to HCl and to SRF exhaust using a dose-response design.

For the most sensitive cultivated species (radish and soybean) threshold injury concentrations of HCl were 3 and 4 ppm (1 ppm = 1.5 mg/m^3) for an 80 min exposure and 9 and 16 ppm for a 10 min exposure. Comparative concentrations for the most sensitive native species (pennywort and arrowhead) were 5 and 12 ppm for 80 min and 16 and 30 ppm for 10 min. Plants were more sensitive to HCl during the fall and spring than during the winter. Increased humidity or water on the leaf surfaces during exposure made plants more sensitive to HCl but not to Al_2O_3. Chloride in soybean leaf tissue correlated well with the HCl dose (concentration x duration of exposure); chloride moved very little in the plant over time; previous chloride accumulation did not affect subsequent accumulation. The response of zinnia and radish to HCl were similar when exposed in Raleigh, N. C. or in Riverside, California.

Exposures of selected plants to large doses of Al_2O_3 (50 mg/m^3 during a 60 min period) did not cause injury or affect growth. Plants responded to mixtures of Al_2O_3 and HCl in the same way they did to HCl alone.

The relative exhaust mixtures were monitored by determining the HCl concentration. Results from exhaust mixture studies were similar to those found with greenhouse exposures to concentrations of HCl that were similar to those in the exhaust mixtures. However, there was some indication that chlorine (Cl_2) or other oxidants were changing the pattern of injury response.

Selected plants were exposed to Cl_2 or nitrogen dioxide (NO_2) to determine the relative sensitivity of the plants to these gases in relation to their sensitivity to HCl. The plants were 4 to 20 times more sensitive to Cl_2 than to HCl but were 2 to 4 times less sensitive to NO_2.

The ED_{50} for forager honey bees was about 100 ppm HCl for 120 min and, if our extrapolation is approximately right, about 150 ppm HCl for 30 min. The ED_{50} for the most sensitive life stage (pre-ovipositional adult) for the corn earworm was 102 ppm HCl for 60 min. The ED_{50} for lacewing larvae was about 150 ppm HCl for 60 min. Brood production of active bee colonies was temporarily affected by multiple exposures to SRF exhaust at HCl concentrations of about 10 ppm HCl. However, multiple exposures of bee colonies exposed to about 20 or 30 ppm of HCl caused a loss of brood production; brood production started to increase after the last exposure but two of the four colonies were not able to complete recovery and were lost.

1. GENERAL SUMMARY

1.1. Introduction

Space shuttle launches from Kennedy Space Center (KSC), Merritt Island, Florida are scheduled to start in late 1980 or early 1981. Preliminary investigations showed that the components of solid rocket motor (SRM) exhaust (Table 1) most likely to cause adverse effects to biological systems are aluminum oxide (Al_2O_3) or alumina and hydrogen chloride (HCl) gas (40). Alumina alone may or may not be toxic but in either case could be a carrier of HCl and could thus affect biological systems.

The area around the shuttle launch site is a part of the Merritt Island National Wildlife Refuge. It is a mosaic of different coastal ecological communities, each with its own mixture of flora and fauna. The ecology of the area has been intensively studied and characterized in a recent study funded by NASA (66). Within the refuge there are 1,016 hectares of land planted with citrus; the major commercial crop on Merritt Island with an appraised annual production value of $7.2 million. Honey production is also commercially important on the island.

The National Aeronautics and Space Administration (NASA) asked the USDA-SEA at North Carolina State University to investigate the effects of HCl, Al_2O_3, and SRF exhaust on selected plant and insect species. Our general mission was to determine if the exhaust clouds generated by shuttle launches could affect the native plants of the refuge, citrus production or the beekeeping industry.

Hydrogen chloride gas is known to injure sensitive plant species at concentrations above 5 ppm (1 ppm = 1.5 mg/m^3) in 60 min or longer exposures (42). The response of plants to shorter exposure times or to multiple exposures is not known. The response of insects to HCl has not been studied - but insects are sensitive to SO_2 and fluoride. No biological effects are known for Al_2O_3, but chemically active particulates can injure plants and cause growth reductions. The alumina may act as a carrier for HCl and thus indirectly cause injury to plants or insects.

The general objectives of this research were: (1) to determine the effects of SRF exhaust and component chemicals of the exhaust on selected native and cultivated plants; (2) to determine the ED_{50}s for honey bees, corn earworms, and common lacewings exposed to HCl; and (3) to study the behavior of honey bees exposed to SRF exhaust. Specific objectives were to:

(1) Develop a plant exposure system(s) for dispensing and monitoring the two major chemicals in SRF exhaust (HCl and Al_2O_3).

(2) Develop a plant exposure system for dispensing and monitoring SRF exhaust (controlled fuel burns).

Table 1. Composition of solid rocket motor (SRM) exhaust[1/]

Component	Composition by wt (%)[2/]
CO_2	30.7
Al_2O_3	22.4
H_2O	21.3
HCl	14.1
N_2	8.2
Cl_2	1.6
Cl_x, CO, NO_x, etc.	1.7

1/ The solid rocket fuel (SRF) consists of aluminum (Al, 16%) ammonium perchlorate (NH_4ClO_3, 70%) and a binder, polybutadiene-polyacrylonitrile (PBAN, 14%).

2/ The exhaust composition reflects the concentration of each component of SRM exhaust 3000 ft from the jet nozzle (ref. 52).

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(3) Develop horticultural practices for growing plants native to Merritt Island, Florida for use as test species in objective 4. These species may be used later at Merritt Island in a vegetation monitoring system.

(4) Determine dose-response relationships for short-term exposures of selected plant species to HCl, Al_2O_3, mixtures of the two and to SRF exhaust.

(5) Determine the effects of HCl, Al_2O_3, and mixtures of the two on honey bee; determine the effects of HCl on corn earworm and common lacewing; and determine the effects of SRF exhaust on honey bee colonies.

Additional details in several facets of this study may be obtained from earlier reports (31, 34, 35, 40) and from Master's theses developed as part of this project (14, 59, 70).

1.2. Facilities

Exposure chambers for greenhouse and field use, a HCl monitoring and dispensing system, an Al_2O_3 monitoring and dispensing system, and a controlled burn facility (for generating, dispensing and monitoring SRF exhaust) were designed, built and tested. The design, construction, and initial evaluation of all systems were done as thesis research by Alan G. Sawyer (59) and James D. Tyson (70). These theses give details of

calculations, methods, and their validation for the systems included in this study. They are attached as addenda to this report.

1.2.1. Facilities development

Four continuous stirred tank reactor (CSTR) chambers were constructed in a double-walled plastic greenhouse to expose plants and insects to HCl and Al_2O_3 (Figure 1). The CSTR design assures uniform chamber conditions. A 1000 w high-intensity multivapor lamp was positioned over each chamber to maintain adequate light for plant growth and response to pollutants. Air was charcoal filtered and drawn through each chamber at about one change/min (35 cfm).

Hydrogen chloride was dispensed from a bottle of about 25% HCl in dry nitrogen through rotometers into the inlet duct of each chamber. Concentrations from 0 to 150 ppm HCl could be produced in any of the four chambers by adjusting gas flow. The chamber concentration of HCl was monitored within each chamber with a Geomet[1/] HCl monitor.

One exposure chamber was modified to dispense and monitor Al_2O_3 (Figure 2). The dispensing equipment consisted of a motor-driven variable speed pump that drove a metal rod through a Teflon cylinder. The cylinder contained the Al_2O_3 in a sectioned carrier tube that opened into the inlet duct of the chamber. The Al_2O_3 was carried by the air stream into the chamber and was monitored by pulling air from the chamber at a specified flow rate through preweighed millipore filters. Two forms of Al_2O_3, α- and γ- forms, were mixed and used in these studies.

The field chamber system, for exposing plants to SRF exhaust, consisted of five 10 ft diam by 8 ft high chambers with tops, a "burn" box and associated air handling blower with ducts (Figure 3). The system used a constant flow blower (3000 cfm) that pushed ambient air through a "burn" box containing the burning fuel. The exhaust was carried through a tripartate plenum (flow-divider) which apportioned the exhaust into three exposure chambers. The exhaust exited through a second tripartate plenum into a scrubber chamber before release into the air. Variable-flow blowers for adding dilution air and baffles on the inlet ducts permitted the dispensing of different exhaust concentrations in each of the chambers. A fifth chamber was used as a control. The chambers were monitored for HCl and Al_2O_3 in the same manner as the greenhouse chambers.

The SRF was obtained from Thiokol Corporation as 1/4 in. thick, 4 in. x 6 in. slabs. The slabs were hand cut into 1/2 in. x 1/4 in. x 6 in. strips, dipped into a burn restrictor solution and air dried. The fuel was layed end to end in grooves on copper plates in the burn box

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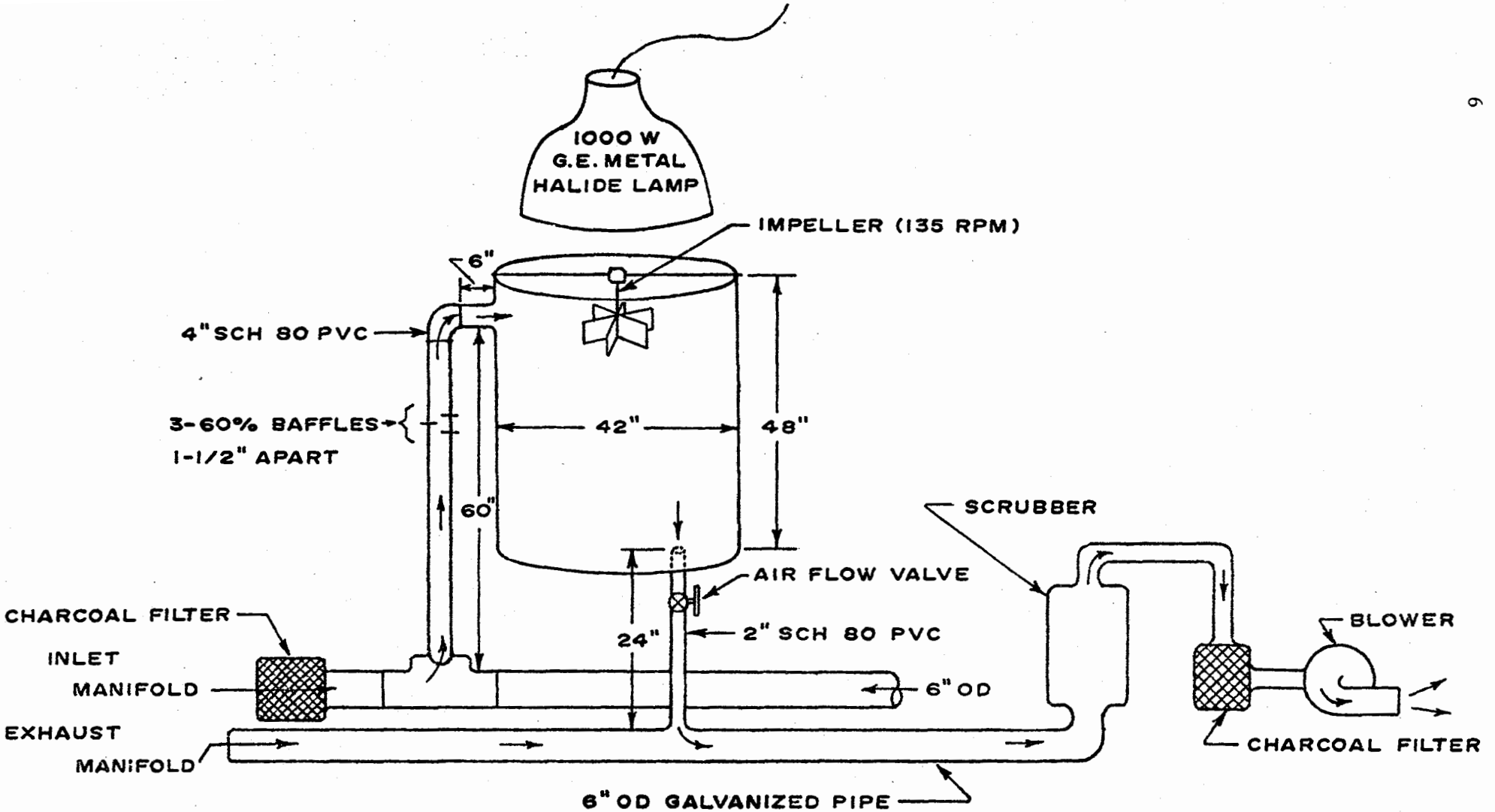

Figure 1. Schematic diagram of one exposure chamber, ∿ 1000 liters.

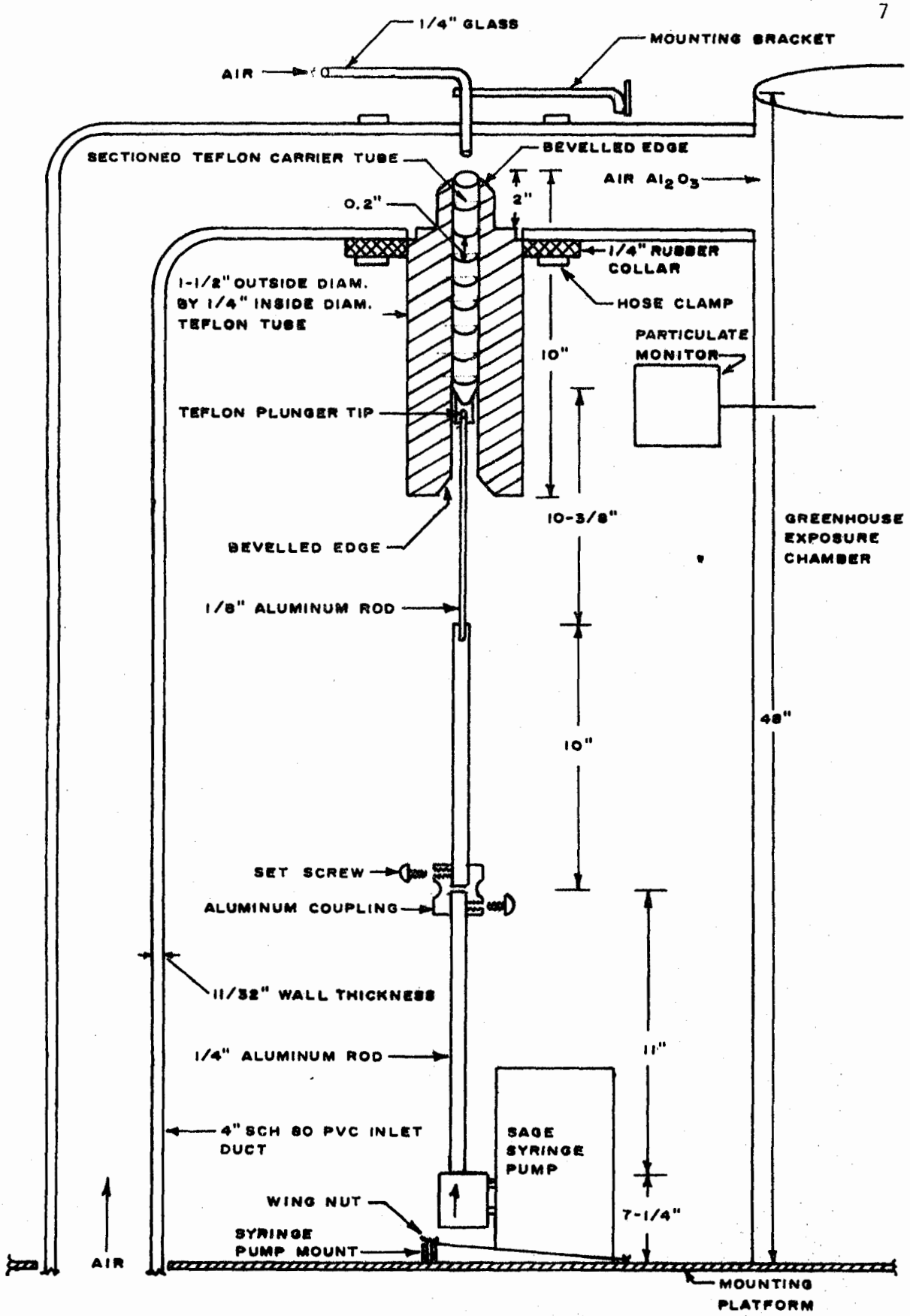

Figure 2. Schematic diagram of Al_2O_3 particulate dispensing system.

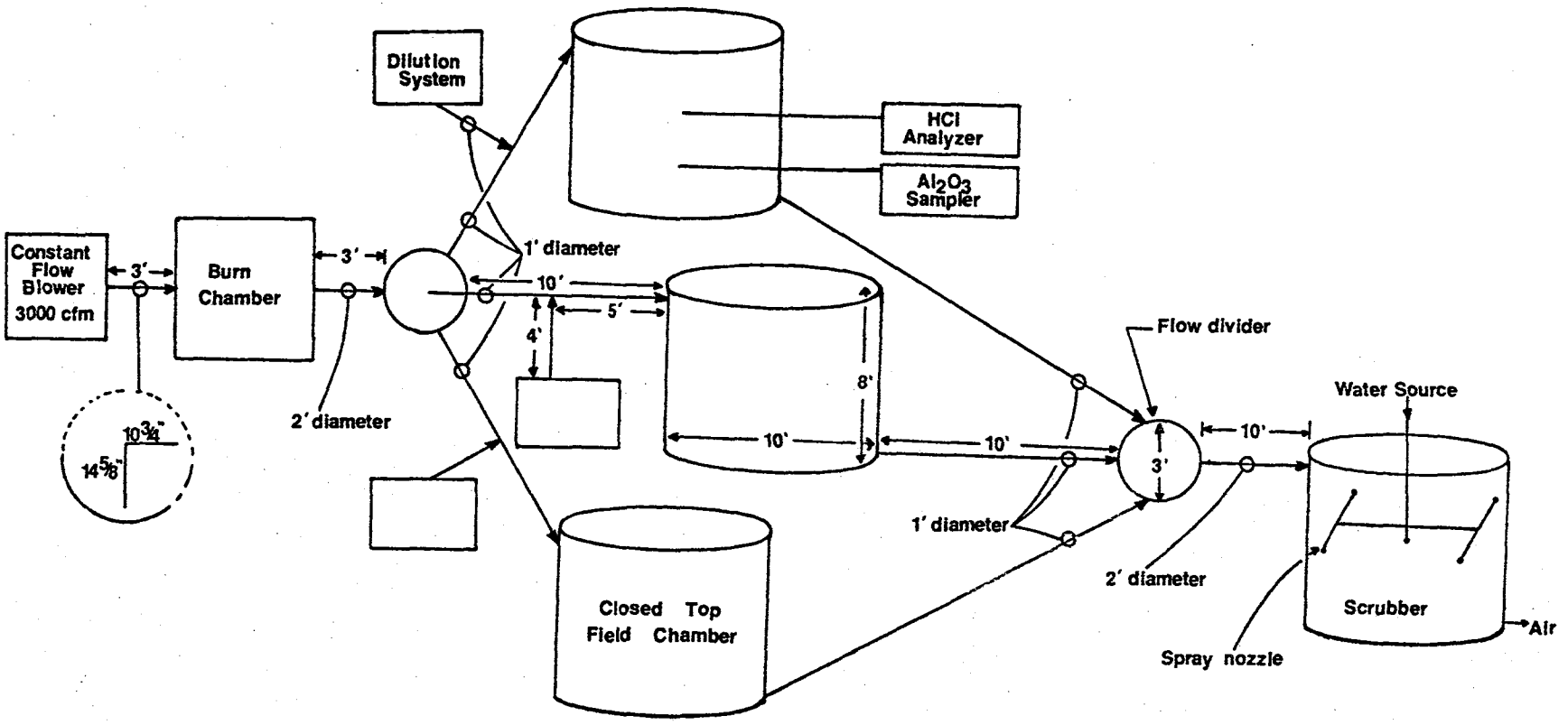

Figure 3. Schematic diagram of the field exposure system

and was ignited by a nicrome wire attached to a switch on the outside of the box. When two or three plates of fuel were necessary the second and third plates were ignited automatically as the fuel on the prior plate burned out.

1.2.2. Results and discussion

Stimulus-response tests were used to test the mixing and flow behavior of the greenhouse CSTR exposure chambers. The response (HCl concentration) of the chambers to a negative step-change in HCl was monitored at the chamber exit with the Geomet. Data were taken at low and high airflow rates and at varying absolute humidities. These tests indicated a deviation from ideal CSTR behavior, especially at lower airflow rates and at high humidities. Two flow behavior regions in the chamber are suggested that can be modeled as two ideal CSTRs connected in parallel. The model that was developed described the actual chamber response very well (Figure 4). The flow of the chambers more nearly exhibited an ideal CSTR behavior at the high flowrates and lower humidities; these conditions were used in most of the plant and insect exposures.

Gaseous HCl is hygroscopic and readily retained by moist surfaces. Preliminary tests indicated that it was difficult to transport the sample to the analytical reaction point without serious loss of HCl. Therefore, we monitored the chambers directly by inserting the ceramic collection tube of the Geomet through the chamber wall. Before calibration checks and biological exposures the chambers were normally equilibrated at a given HCl concentration for 15 minutes. When this was done, the HCl dispensing system performed as designed with good comparison between predicted HCl concentrations and HCl concentrations actually monitored. Tests, using plants as biological indicators, showed uniformity of foliar injury both across and within the greenhouse exposure chambers.

The Al_2O_3 particulate mixture consisted of a 90% non-reactive α-phase (>2 μm diam) and a 10% reactive γ-phase (<0.5 μm diam) by weight. The Al_2O_3 particulate dispensing system worked as expected, except at relative humidities above 95% when particulate packing occurred in the Teflon cylinder. The particulate monitoring system also performed well. The Al_2O_3 was collected on a 10 μm Nucleopore filter and analyzed for particle size using a scanning electron micrograph. The basic testing was done using a chamber loading of 50 mg/m^3 of Al_2O_3. Most of the γ-phase was collected using a 10 μm filter because it adhered to the larger α-phase particles. A biomodal distribution was found that simulated the distribution found in the SRM exhaust. The size range in the first mode was 2.3 to 6.2 μm and in the second mode was 9.2 to 18.3 μm.

The components of the field chamber system operated smoothly. Typical data from the stimulus-response tests in the field chambers indicated that all three exposure chambers simulated ideal CSTR behavior (Figure 5). The mean residence times in chambers 1, 2, and 3 were 0.72 min, 0.98 min and 0.91 min, respectively. These times were different because of the flow split from the burn chamber and dilution blower settings. Although the flow divider functioned well in splitting the airstream, baffles were

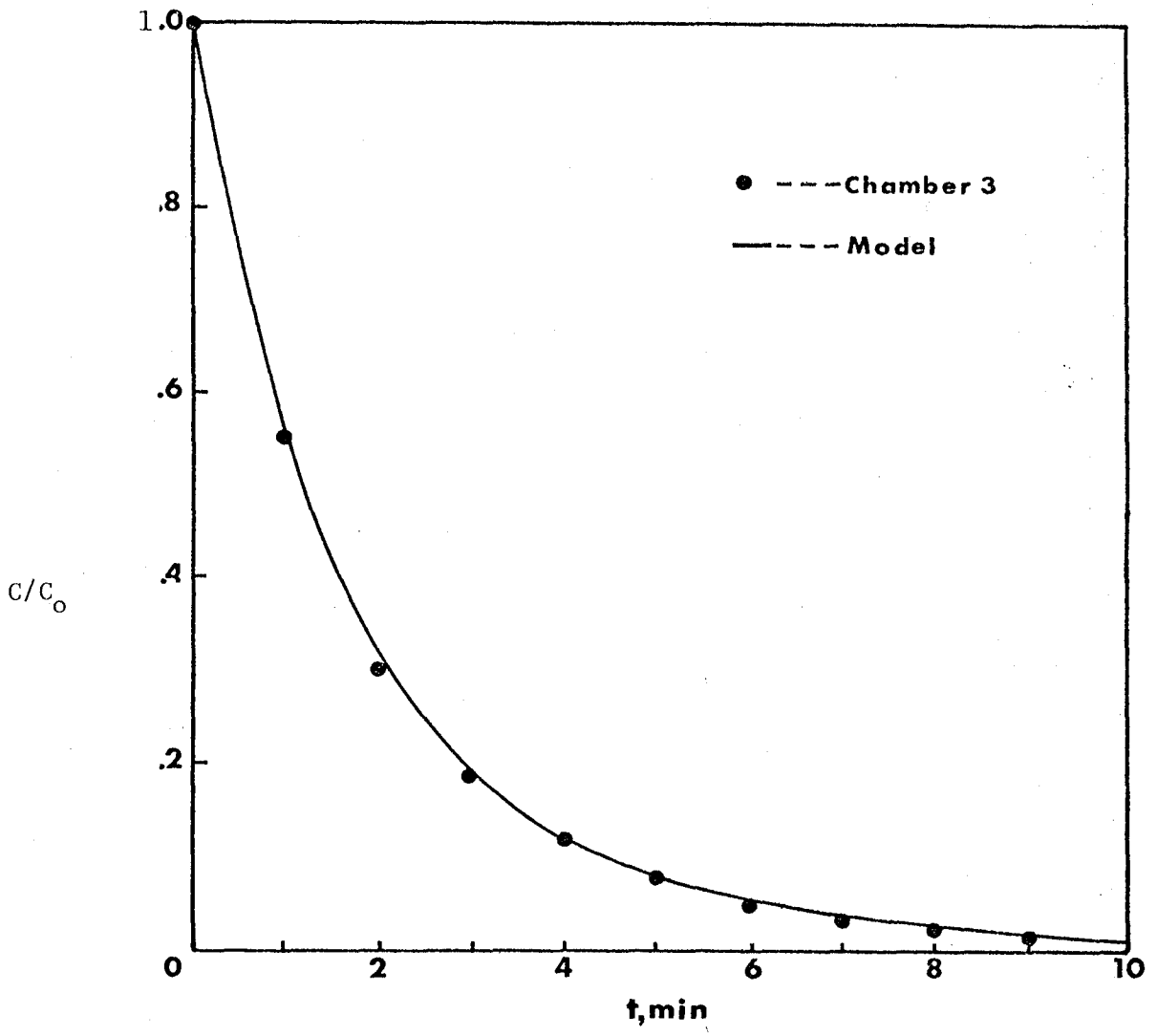

Figure 4. Model, using two parallel CSTRs, fit the data for the greenhouse chambers; C = chamber concentration, C_o = steady state concentration.

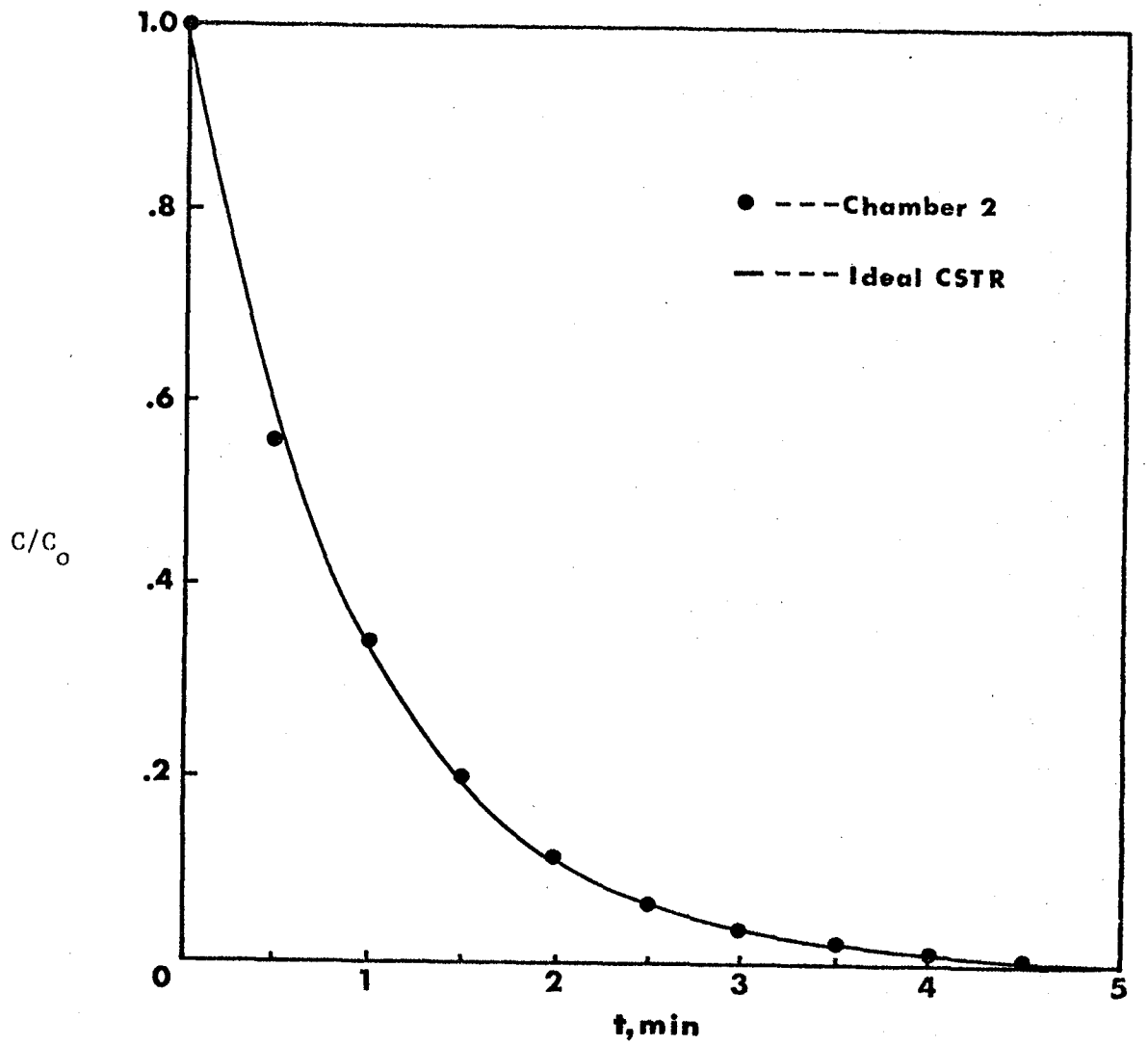

Figure 5. Ideal CSTR model fit to data for the field chambers; C = chamber concentration, C_o = steady state concentration.

inserted in the flow dividers and used with the dilution blowers to assure three distinct chamber exhaust concentrations. The scrubbing chamber cleaned a high percent of both the HCl and Al_2O_3 from the airstream. The actual HCl concentration in the field chambers was lower than predicted because of HCl adsorption on walls, higher chamber air flows than were predicted and the use of fuel sizes that were slightly less than the calculated sizes. The major difference in the exhaust Al_2O_3 was that less of the fine particulate was agglomerated on the large particulate and there was an absence of the very large Al_2O_3 particulates in the exposure chambers (we did not test for α- and γ-phases of Al_2O_3 from the SRF exhaust). Overall operation was satisfactory and permitted continuous exposure of vegetation to exhaust products of the SRF for periods up to an hour.

1.3. Effects: Vegetation

Twenty-four native plant species, 9 horticultural species (16 cultivars) and 3 agronomic species (9 cultivars) were studied in at least one of the phases of this project. A complete plant list can be found in the Appendix (7.1.).

1.3.1. Methods and procedures

Plants were grown in a greenhouse in plastic pots filled with a standard soil mixture. Horticultural and agronomic plants were thinned or transplanted 7 to 10 days after the seeds were planted so that each pot had one plant. Native plants were collected from Merritt Island, shipped bare rooted or as cuttings, and potted, one plant per pot. Slash pine (1 year seedlings), citrus (3-4 ft trees) and live oak (2-3 ft trees) were purchased from nurseries in the Merritt Island area and shipped as potted plants. Plants were watered as needed and most were fertilized once a week with 100 ml per pot of a VHPF nutrient solution (6-25-15 of NPK with micronutrients). The trees were fertilized every 6 months with the recommended amount of Agriform (20-10-5) time release pellets and once every two months with the VHPF nutrient solution.

Plants used in all exposures were grown in the greenhouse to a certain size, physiological age, or chronological age depending on the plant being tested. Plants grown from seed were exposed 14, 21, or 28 days after seedling depending on the species. During the cool winter months plants grew from seed at a slower rate, so these plants were exposed at a physiological age similar to that obtained by the plants in warmer conditions after 14, 21, or 28 days of growth. Trees and native plants were exposed after they became well established and had added substantial new stem and leaf tissue. Plants were selected for growth uniformity before each exposure and randomly placed in all treatments.

All plants were initially exposed to 0, 10, 20 or 40 ppm HCl for one hour in the greenhouse exposure chambers. Foliar injury was estimated for each plant 48 to 72 hr after the exposure. Injury was usually determined for individual leaves on a 0-100% basis (5% increments, including 1%). When plants had too many leaves for such an evaluation, the total plant was assigned a percentage injury value from 0-100% (10% increments, including 5%). Duplicate plants with 3 replications (on different days) of each exposure concentration were run for a total of 6 plants per treatment.

Selected plants were exposed to HCl and SRF exhaust using a dose (concentration x duration of exposure)-response design. The concentrations used in the dose-response exposures were determined by the results of the HCl screens. The HCl concentrations in the SRF exhaust exposures were maintained at about 0, 10, 20, and 30 ppm. Selected plants were exposed to different concentrations of Al_2O_3 and HCl plus Al_2O_3. For the Al_2O_3 tests, plants were exposed to totals of 20, 40 and 80 mg/m^3 of Al_2O_3 over a period of 60 minutes. In the HCl plus Al_2O_3 exposures, plants were exposed to 10 or 15 ppm HCl for 60 minutes with or without the simultaneous addition of 20 or 35 mg/m^3 of Al_2O_3, respectively. These ratios of HCl to Al_2O_3 were meant to simulate the ratios expected in the SRM exhaust. Plants in all exposures were graded for injury in a manner described for the HCl exposures and were harvested 7 days after exposure for dry wt determinations. Three replications over three consecutive days and 3 duplicate plants per replication were run in all of these experimental designs for a total of 9 plants per treatment.

In addition to the above basic designs that were part of the original proposal, we selected other experimental designs that permitted a better understanding of systems and the HCl effects. These designs, presented briefly here, are detailed in the body of the report.

(1) Seven plant species were subjected to multiple exposures of HCl.

(2) Radish 'Comet' and zinnia 'White Gem' were used in an inter-laboratory comparison of plant response.

(3) Soybean 'Dare' was used to study the uptake, distribution and translocation of Cl^- in plants. This is included primarily in the thesis research of Madeleine Engel which is attached as an addendum to this report.

(4) Radish 'Comet' was used to determine the effect of leaf misting, humidity, soil moisture and method of HCl generation on plant response to HCl.

(5) Radish 'Comet' was used to study the differential effects of light vs dark exposures, time of day and time of year on plant response to HCl.

(6) Four plant species (two cultivated and two native) were used in simple dose-response designs with nitrogen dioxide (NO_2) and chlorine (Cl_2). These were done to see if either gas could be of potential harm during shuttle flights.

(7) Radish 'Comet' and soybean 'Dare' were used to compare the uniformity of plant response within and across chambers (sections 1.2.2. and 3.3.1.2.).

Most of these designs used foliar injury and plant dry or fresh wt changes as the basic response measures.

1.3.2. Results and discussion

Large necrotic areas that were bifacial and interveinal were the typical symptoms of foliar injury from HCl. These areas were usually white to off-white in color and corresponded to areas that were water soaked near the end of and/or immediately after an exposure. At low doses, the necrosis usually occurred first on the margins and tips of the leaves but with higher doses injury also occurred toward the base and center of the leaves. After short exposures to low concentrations, many plants developed scattered chlorotic spots on the upper leaf surface which resembled oxidant injury. Snap bean and zinnia developed undersurface glazing reminescent of PAN injury. The tips of the needles of young candles in pine turned tan after an exposure to HCl. Low doses of HCl caused yellowish necrotic spots on the lower leaf surface of citrus and high doses caused bifacial chlorotic areas.

The response of the 49 different plants to the HCl screen was variable depending on species and cultivars within species (Appendix 7.2.). The relative sensitivity of these 49 species and selections (Table 2) was developed from Appendix 7.2. Table 2 shows six separations that are based on the average injury for each plant from the three HCl concentrations used (Appendix 7.2.) even though the average value does not give an indication of the dose-response curves. These curves are quite different for many of the plants tested and can be conceptually constructed from the data in Appendix 7.2. Agronomic and horticultural plants were generally more sensitive to HCl than the native plants or the citrus species.

Concentration x time (dose) response exposures showed that concentration was more important in causing foliar injury than exposure duration (Table 3). This is shown for radish in Table 3 by the dotted (----) lines connecting equal doses. These data indicate that for a given HCl dose a higher concentration for a shorter time period is more harmful than a lower concentration for a longer time period. Injury thresholds for the nine test species subjected to the complete dose-response design are shown in Table 4. These results confirm that the cultivated species studied, except for citrus, are more sensitive than the native species. Growth and/or yield reductions were restricted to plants which developed approximately 40% or more total foliar injury after an acute HCl exposure (growth and yield data are included in several tables in sections 4. and 7.4.).

The response of a plant to HCl was altered by its physiological age and the environmental conditions at the time of the exposure. Leaves which had just completed their expansion were the most sensitive to the pollutant. The older leaves were next in sensitivity and the new expanding leaves were the most resistant. Plants were usually most sensitive during the first 2 to 3 weeks of growth; exposures during this period had the most effect on biomass production. Yield was not usually altered unless the plant was injured at least a week or more before flowering or filling of the food storage structure.

Exposure of plants to the SRF exhaust showed that HCl was the principal phytotoxicant. The HCl concentration in the SRF exhaust that

Table 2. The relative sensitivity of 36 plant species (49 different plant selections) to foliar injury from exposure to HCl.[1/]

Sensitive	Moderately sensitive	Intermediate	Moderately resistant	Resistant	Not injured
celery	arrowhead	marsh elder	cattail	Boston fern	glasswort
	groundsel	sea lavender		camphor weed	
radish (Comet)	pennywort	switchgrass	croton*	fedder bush**	sea oats
	lettuce (Grand Rapids)	corn (Silver Queen, Coker 16)	muscadine	live oak	smooth cord-grass
soybean (Dare, Lee, Scott)	lima bean		railroad vine	*Paspalum***	
	radish (Cherry Belle)	snap bean (Burbee Dark)	sea ox-eye	primrose	tobacco (Bel B, Florida)
tomato (Yellow Pear)				sea grape	
	snap bean (BBL-290, Burbee Dark)	tomato (Tiny Tim)	sunflower	slash pine	
			wax myrtle	grapefruit	
	soybean (Hood)	zinnia (White Gem)		orange	
	tomato (Better Boy, Fantastic, Heinz, Roma, Tiny Tim)			tobacco (Bel W_3)	
	zinnia (White Gem)				

[1/] This classification was developed from results of a single 60-minute controlled greenhouse exposure (screen) to 0, 10, 20, 40 ppm HCl (Appendix 7.2.). This classification criteria are shown below.

Category	Threshold conc.	Average injury
Sensitive	<10 ppm	>50%
Moderately sensitive	≤10 ppm	36 to 49%
Intermediate	≤10 ppm*	20 to 35%
Moderately resistant	10 to 20 ppm	10 to 19%
Resistant	20 to 40 ppm	>0 to 9%
Not injured	>40 ppm	0

* The threshold for corn was between 10 and 20 ppm.

Table 3. Effect of HCl dose (concentration by duration of exposure) on foliar injury to selected plants.[1/]

Exposure duration (min)	Foliar injury (%) at four HCl concentrations (ppm)				Foliar injury (%) at four HCl concentrations (ppm)			
	0	5	10	20	0	10	20	40
	Radish (Comet)[2/]				Pennywort			
10	0	+	6	49	0	+	9	23
20	0	3	36	66	0	1	11	72
40	0	5	49	91	0	4	60	94
80	0	16	89	98	0	49	88	98
	(LSD - 0.05, 7.7%)				(LSD - 0.05, 8.2%)			
	Soybean (Dare)				Wax Myrtle			
10	0	0	+	9	0	+	1	15
20	0	0	1	70	0	+	3	21
40	0	+	14	76	0	1	5	53
80	0	6	69	94	0	1	12	45
	(LSD - 0.05, 7.6%)				(LSD - 0.05, 10.7%)			

1/ All values are average foliar injury (averaged over all leaves read) to test plants. The injury covers a 0-100% injury range estimated in 5% increments and averaged over 9 test plants (3 duplicates and 3 replicates). Data were analyzed by an analysis of variance and treatment means were separated by LSD (0.05). The + signifies less than 0.5% average injury.

2/ The dotted (----) lines represent equal doses of HCl and show that increasing concentration is more harmful than increasing duration of exposure. This is true for all plant species studies.

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produced a given foliar injury and subsequent reduction in biomass was similar to the HCl concentrations in the single gas exposures. The foliar injury symptoms, the differences in leaf susceptibility, and the relative species sensitivity suggest that the effects of the SRF exhaust on plants were due primarily to HCl. Plants developed some upper leaf surface stippling during the SRF exposures that was reminiscent of oxidant injury. The exhaust does contain Cl_2 which could cause plant injury and/or damage. However, in most instances the HCl should be the primary phytotoxic agent.

Table 4. The injury threshold HCl concentrations for selected plant species.[1/]

Plant species	Threshold HCl concentrations (ppm) at four exposure durations (min)			
	10	20	40	80
Radish, 'Comet'	9	6	5	3
Soybean, 'Dare'	16	12	7	4
Tomato, 'Betterboy'	16	12	11	5
Zinnia, 'White Gem'	16	12	8	6
Corn, 'Silver Queen'	15	12	11	10
Pennywort	16	14	11	5
Arrowhead	30	25	19	12
Wax myrtle	30	22	20	15
Marsh elder	35	26	21	16
Citrus, 'Valencia'	>80	>80	80	53

[1/] The values were obtained from evaluation of dose-response data contained in Tables 3, 13, 14, 15, and 33. Threshold injury was defined as 5% of the leaf area with visible symptoms.

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Alumina (Al_2O_3) did not cause foliar injury nor alter growth in any of the plants tested. A mixture of alumina and HCl caused injury similar in appearance and severity to that observed from exposure to HCl alone. If the Al_2O_3 particulate acts as an HCl carrier, it was not apparent in these exposures. We believe it is an essentially innocuous component of the exhaust. However, it could cause plant damage if the Al concentration increased in acid soils, since free Al^{+++} is toxic to plants.

The results of additional experiments on plant effects can be rather briefly summarized.

(1) The injury effects of two or more short exposures to HCl were additive for the plants tested.

(2) The Statewide Air Pollution Laboratory at the University of California, Riverside and the USDA Air Pollution Research Team at North Carolina State University undertook a comparative study of the effects of HCl exposures on radish and zinnia. The data was similar even though the two programs did not evaluate foliar injury in the same way and there were differences in climate, environment, and culturing techniques.

(3) The amount of chloride ion accumulated in soybean correlated positively with the amount of foliar injury and HCl dose. Tissue analysis for chloride may be useful in identifying HCl

as the cause of foliar injury except where the background chloride is high, as occurs on Merritt Island. However, chloride content within the tissue should not be used to determine ambient HCl concentrations.

(4) Radish were more sensitive to HCl when the relative humidity was high (85+%) or when the leaves were wet than at low humidities or when leaves were dry. Atmospheric and/or leaf surface moisture could alter the diurnal response pattern to HCl that is seen in most plants during air pollution exposures. These factors could affect the response of vegetation to SRM exhaust in the field.

(5) Radish showed a uniform response to HCl across time within days but not across seasons. Plants were as sensitive to dark as to light exposures. Plants were more sensitive to HCl during fall and spring exposures than during winter exposures.

(6) A single dose-response design was done for Cl_2 and NO_2. Four test species (radish, soybean, pennywort and marsh elder) were chosen because of their known sensitivity to HCl. The four species were from 4 to 20 times more sensitive to Cl_2 than to HCl but were 2 to 4 times less sensitive to NO_2 than to HCl. The expected Cl_2 component of SRM exhaust (1.6%) could be as injurious to sensitive vegetation as the HCl component (14.1%).

The HCl concentrations that caused injury to the most sensitive species were generally higher than the maximum (4 to 6 ppm for 10 min) expected at ground level from the SRM exhaust from launches of the shuttle at KSC. The species found on Merritt Island are less sensitive than the most sensitive species, thus it is unlikely that the SRM exhaust cloud will have significant adverse effects on natural vegetation and citrus in and around KSC.

1.4. Effects: Insects

Honey bees (Apis mellifera L.) are of considerable economic importance in Florida for pollination of citrus and vegetable crops, and the production of honey. During the last two decades the effects of various insecticides and herbicides on bees have been investigated intensively with the resultant development of methods that are applicable for the subject research. We have adapted these methods to determine the ED_{50} for HCl gas on honey bees, the corn earworm (Heliothus zea Boddie) and the common lacewing (Chrysopa carnea Stephens). We also studied the effects of SRF exhaust on the behavior of honey bee colonies. This research was the thesis research of Louise Romanow. Her thesis is attached as an addendum to this report.

1.4.1. Materials and methods

The HCl exposures for the insect species were conducted in the greenhouse CSTR chambers used for the plant exposures. The SRF exhaust studies on honey bee were conducted in the field burn system using two hives of bee colonies per chamber.

Two bee colonies were used to collect bees for the greenhouse ED_{50} studies. Foragers, the only bees that leave the hive, were exposed to HCl. Groups of 20 foragers each were collected in polyester-screen cages and exposed to HCl (0-160 ppm) for 30 to 480 min. The bees were then moved to observation cages where food was always available. They were checked daily over a 72 hr period to determine mortality. All tests were duplicated and replicated. Data were analyzed using probit and regression analyses; ED_{50}s for mortality were determined. Eggs, several larval stages, and the adults of corn earworm were exposed to HCl. Corn earworm larva were raised on an artificial diet in exposure containers in groups of 10 to 50. They were exposed to HCl doses of 10 to 200 ppm for 30 to 240 min and then returned to holding containers with food. Lacewings were raised to early and late instars and exposed to HCl doses of 15 to 180 ppm for 30 to 240 min. Observations of mortality were made daily for 72 hr. The 48 hr data were analyzed using a probit analysis and the ED_{50}s for mortality were calculated.

For the studies on effects of bee colony behavior from exposure to SRF exhaust, one colony in each of two beehives were used in each of four chambers. The colonies were exposed twice a week over 25 days (11 exposures) to a 0, low (ca. 10 ppm), medium (ca. 20 ppm), and high (ca. 30 ppm) concentration of SRF exhaust, monitored as HCl. All exposures were for 45 to 60 min. The colonies were placed in the chambers on May 11, so the bees could adjust to the move. Exposures occurred between May 19 and June 12. Chamber sides were removed except during exposure. Regular observations were made of hive weight (indicative of honey production), brood area (live strength), pollen collection, daily mortality, and reaction to mouse baits (indicative of aggression). The colonies were observed through August 8th to determine chronic effects of the exhaust stress.

1.4.2. Results and discussion

The honey bee dose-response design with HCl shows that the forager honey bee was not acutely sensitive (50% mortality) to HCl concentrations below 100 ppm at 5 to 20 min exposure durations. The ED_{50} values for mortality of forager bees (Figure 6) are 25 ppm for 8 hr and about 100 ppm for 2 hr. Extrapolation of the curve suggests that the ED_{50} for 30 min would be about 150 ppm. The expected HCl concentration in a shuttle ground cloud is only 5 to 8 ppm for 5 to 20 min. This is below that dose expected to cause 1% mortality (20 ppm for 80 min); thus, acute toxicity effects are not expected around the shuttle site. The ED_{50} values suggest that the concentration factor is three-fold more important than the time factor.

The results for corn earworm show that this species (in the adult stage) is about as tolerant as the honey bee (Table 5). ED_{50} values were developed for several life stages at exposure durations of 60 min. Larvae appear more tolerant than adults; requiring about 200 ppm to kill 50%. The pre-ovipositional adult was the most sensitive but the ED_{50} (102 ppm for 60 min) is well above any expected SRM exhaust cloud concentration.

ED_{50}s for lacewings showed that larvae were about as tolerant as the corn earworm larvae; over 150 ppm HCl was required for one hour to kill 50%.

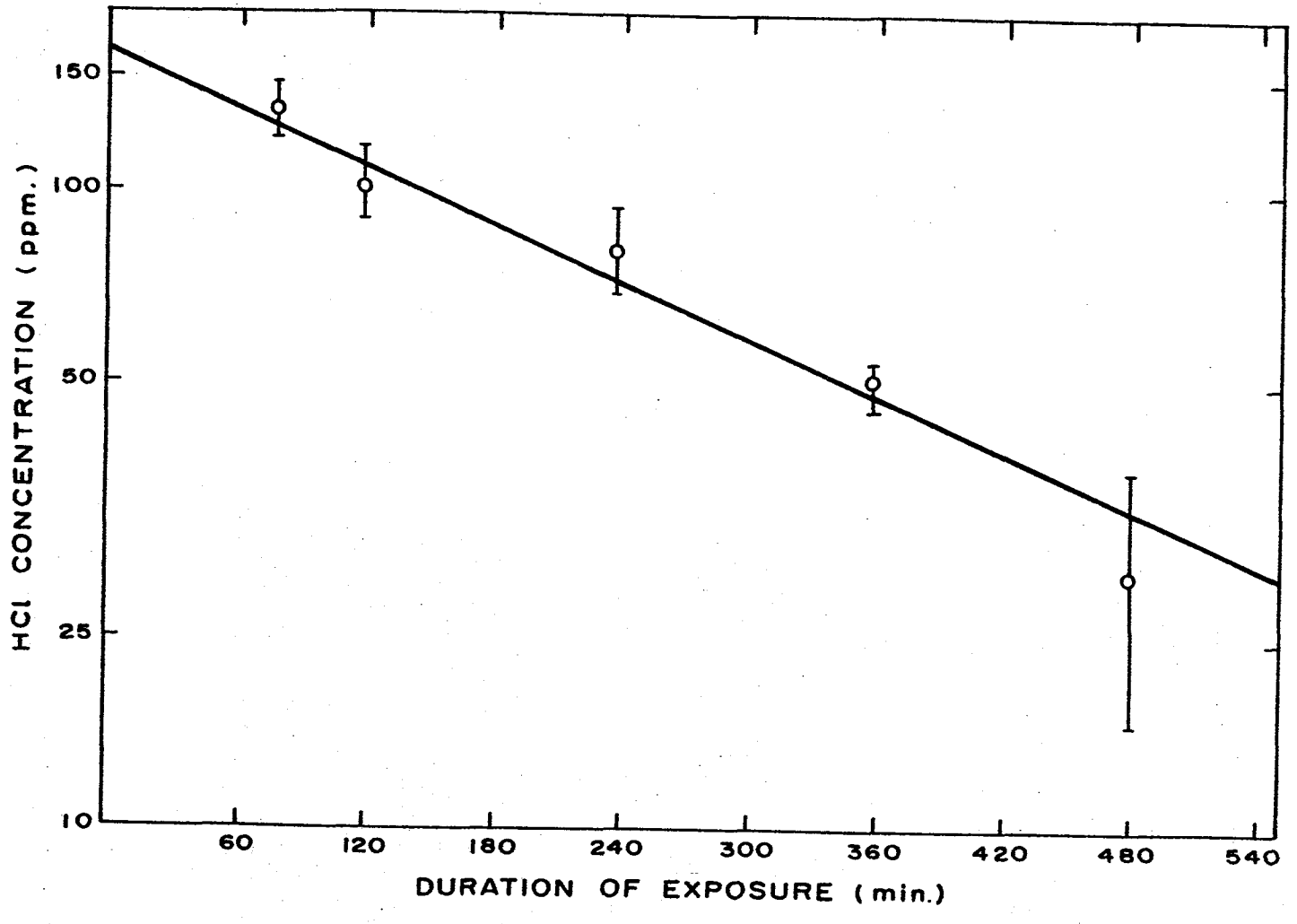

Figure 6. The ED_{50} for HCl mortality of foraging honey bees at different exposure concentrations and durations, probit analysis.

Table 5. The ED_{50} of HCl for mortality of corn earworm at several life stages.[1/]

Life stage		ED_{50} in ppm[2/]
Eggs		240*
Larvae:	1st Instar	152
	5th Instar	275*
Adult:	Pre-ovipositional	102
	Ovipositional (60 min)	153
	Post-ovipositional	188

1/ All exposures were 60 min in duration except as noted.

2/ We were not able to obtain concentrations in excess of 200 ppm, the * are projected concentrations.

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The responses of honey bee colonies to SRF exhaust were documented in several ways. Brood and honey production were measured from May 11 to July 6 over exposure and postexposure time periods. The results for brood production (Figure 7) show effects at all levels of exhaust concentrations although the results were variable. Low exposure colonies (ca. 10 ppm HCl) were able to recover from SRF exhaust effects. One medium exposure (ca. 20 ppm HCl) and one high exposure (ca. 30 ppm HCl) colony was unable to recover from SRF exhaust exposures. The other medium and high exposure colonies would probably not have survived continued exposures. Honey production was depressed or absent in medium and high exposure colonies. Aggression was considerably increased even in the low exposure colonies.

The results of these insect studies suggest that no adverse short-term effects on insects will be found as a result of the shuttle programs.

1.5. Summary

1. A four-chamber greenhouse exposure system was constructed and equipped to dispense HCl gas into each chamber at any concentration from 0 to 100 ppm. The system permitted continuous monitoring of each chamber for real-time concentrations of HCl. One chamber was additionally equipped to dispense and monitor Al_2O_3 at a wide range of concentrations, up to several hundred mg/m^3.

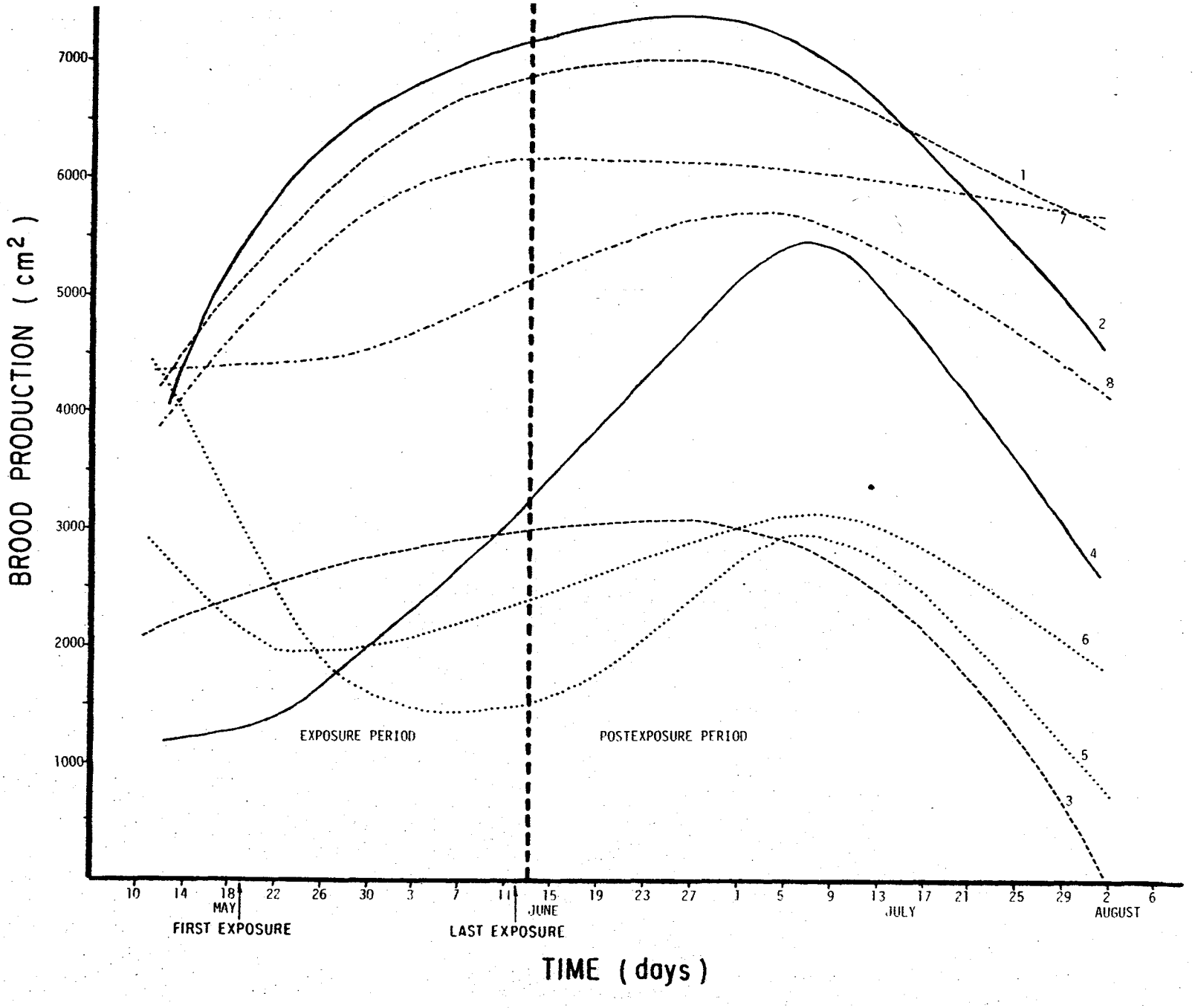

Figure 7. Effects of SRF exhaust on brood production in honey bee colonies. The curves represent 2 sample hives from each of 4 concentrations of the exhaust (control ———; low -·-·-; medium -----; high ·····). These are trinomial regression lines fit over the total observation period.

2. A five-chamber field exposure system was constructed to expose plants to SRF exhaust. The system permitted the burning of SRF and the dispensing of the exhaust to three of the chambers. A monitoring system, similar to that used in the greenhouse chambers, was adapted to this exposure system.

3. Horticultural methodologies for growing 23 native and 12 cultivated plant species were established.

4. Sensitive plants were injured by HCl at from <10 to 20 ppm when the length of exposure was 20 minutes or less and from <5 to 10 ppm when the exposure duration was 40 to 80 minutes. Plants were not injured by Al_2O_3 alone or in combination with HCl. Plants were injured by SRF exhaust at dosages similar to those causing injury by HCl alone.

5. The ED_{50} for mortality of foraging honey bees was about 130 ppm of HCl for 60 minutes; 100 ppm of HCl for 60 minutes for the corn earworm at its most sensitive life stage; and 150 ppm of HCl for 60 minutes for the common lacewing. The SRF exhaust permanently impaired brood production in honey bee colonies when the colonies were exposed to exhaust containing 20 to 30 ppm of HCl for about 60 minutes two times a week for 4 weeks (11 exposures). The exhaust depressed productivity in all hives exposed to SRF exhaust. The low exposures also stimulated aggressive responses of bee colonies.

2. RECOMMENDATION

We consider this section as an opportunity to review our entire project and to make certain recommendations to the project officer that NASA should seriously consider. The nature of our program and the needs of NASA merge in three areas of mutual interest.

(1) Research - we have suggested additional lines of research that would increase NASA's understanding of SRF exhaust effects on biological systems.

(2) Demonstration - we have suggested the maintenance of the field exposure facility for demonstration purposes and for rapid turn around, short-term research projects.

(3) Biological monitoring - we have suggested a biological monitoring program for use with shuttle launches.

2.1. Research

Research is never done but is often terminated for a number of reasons. This project has adequately addressed the major components of SRF exhaust (HCl and Al_2O_3) and their probable impact on a range of native and cultivated plants, and on selected insect species. The basic toxicology is now understood and should suffice for a general understanding of the potential environmental impact of the SRM exhaust from the shuttle flights. One research area not reported in this document and several areas of concern are briefly presented. In addition several lines of research that would more fully document possible effects are discussed.

(1) Acid precipitation - It is essential that NASA understand the possible effects of HCl-acid rain. This will result, if the shuttle exhaust cloud mixes with a rain cloud. Current work is being completed on the effects of simulated acid rain (HCl) on citrus that will be reported by June of 1980. Based on work with gaseous components and on observation of weed species in the vicinity of the simulation experiments, citrus is a relatively resistant species. Thus, NASA should seriously consider some additional work with native species and several of the more sensitive cultivated species.

(2) Other gases - Theoretical calculations have shown both Cl_2 and NO_x (especially NO_2) to be components of SRM exhaust. Preliminary investigations reported in this document suggest that sensitive plants may be injured by Cl_2, if it occurs in the exhaust gas as 10% of the HCl concentration. If this concentration of Cl_2 is expected, additional dose-response designs on selected plants and insects should be initiated. The preliminary investigations suggest that NO_x (NO_2) would be non-toxic at the doses expected in the exhaust cloud.

(3) HCl and Al_2O_3 - These chemicals have been well studied. The importance of humidity and misting of leaves suggests that other environmental parameters may influence the response of

plants to HCl. It would be of value to understand how temperature, light, other atmospheric gases (such as O_3 and SO_2) and soil factors affect the response of selected plants to HCl exposures. Research to date suggests that additional work with Al_2O_3 would be of little value.

(4) SRF exhaust - The experimental designs that were completed were well executed but only involved a selected group of plant species. This system should be used for a more complete characterization of plant response. Additionally, the system as set up would permit a complete characterization of the physical and chemical components of the SRF exhaust. This should be considered since most of the data available to our program resulted from theoretical calculations.

(5) Field exposure system - This system can be used to characterize the exhaust components of any fuel mixture. This could include biological, chemical and physical characterization.

2.2. Demonstration

The field exposure system with the "burn" box, the distribution system and the exposure chambers is a unique facility. It is conceptualized, designed and built as a simple system that would perform a complex function. Funding required inexpensive components and thus a temporary facility. The system performed to expectations with several design flaws that were corrected over the life of the original system. The system should be rebuilt based on our latest design criteria and using materials that are resistant to the heat and the exhaust gases. Such a system should be a demonstration unit for other interested investigators and a research tool for further characterizing exhaust chemistry and physics as well as biological effects.

2.3. Biological monitoring

Plants should be used as a sensitive bioassay for monitoring the shuttle flights. If a biological system were used, it would have the following characteristics:

(1) It would be uniformly sensitive so similar responses would be found under similar exposures.

(2) The system response would not change significantly over time.

(3) It would be possible to correlate the system used with expected effects on native and cultivated plants growing on Merritt Island.

(4) The response would be easily monitored and fairly specific.

(5) The lack of a response would suggest no biologically harmful concentrations.

The plant bioassay will integrate a biological response to all components of the shuttle exhaust. This is not possible with instruments for

chemical and physical detection. The bioassay should not replace physical monitoring but should supplement it. All locations with instrument monitoring should have biomonitors. The latter could also be set out at locations where instrument monitors would not be practical.

We recommend the development of two sensitive species for this program. This should include a cultivated species (radish, 'Comet') and a native species (pennywort). These were the most sensitive plants tested. Under some conditions an intermediately sensitive plant species may be added to the monitoring scheme.

The plants should be grown in containers under prescribed conditions in a greenhouse facility. They should be at a given physiological age at the time of exposure. Plants should be carried to and from the monitoring sites (10 to 15) in panel trucks with adequate cooling. They should be positioned several hours prior to launch so they can equilibrate with the external environment. Special exposure enclosures could be built for cool/cold weather exposures, if desired.

3. FACILITIES

3.1. Introduction

Some type of containment system (chamber) is necessary for exposing plants to air pollutants under laboratory conditions. Chambers permit obtaining real time data by using direct measurement techniques and controlling all possible parameters. Several types of gaseous exposure chamber systems have been used (3, 8, 26, 33, 36, 37, 55, 58, 60). Those systems that use the concepts of a continuous stirred tank reactor (CSTR) in their design best satisfy the requirements for a good plant exposure facility (26, 33, 58). The CSTR provides uniform mixing, a predictable performance over time, a continuous flow rate and yields direct kinetic rate data (58).

HCl was dispensed in past studies by metering vaporized HCl to the exposure chamber system (20, 47, 63). HCl may also be obtained from a tank mixture and metered into the chamber system. Monitoring designs for HCl include (35, 59): 1) bubblers, 2) chemiluminescent detectors (Geomet), 3) coulometers, 4) modified condensation nuclei counters, 5) Millipore filters, 6) copper-coated glass disks, 7) pH papers, and 8) electrets. Bubblers with subsequent chloride titration have been used most extensively for monitoring vegetation exposures (13, 47, 63). Gregory and Storey (22) used the Geomet HCl analyzer to monitor HCl concentrations in rocket exhaust from Titan IIIC and Voyager launches at KSC.

The first quantitative particulate generator was designed by the United States Bureau of Mines in 1939 (73). Particulate dust was placed in a long narrow tube that was slowly raised so that the particulate was sucked into an orifice by an air ejector and blown into a chamber. This system was subsequently modified so that the tube was closed and the particulate was blown out rather than sucked out, and a simple screw-feed device was added. A commercial particulate generator (73) was designed in 1950 to scrape a thin layer from a cake of particulate directly into an air stream. At the University of California, Riverside a commercial Al_2O_3 particulate was dispensed into a plant chamber using a vibrating hopper, brush-fed system (56). Particulate monitoring may be accomplished using: Millipore filters, Nuclepore filters, cascade impactors, rotating vanes, or settling plates. Particulate sizing is possible using a quartz crystal micro-balance (mass monitor), an eight-stage Anderson impact filter (version of a cascade impactor), a serial filtration arrangement with Nuclepore filters, or a scanning electron microscope (SEM). Researchers at the University of California at Riverside (13) monitor Al_2O_3 in their chamber by cascade impactors and Nuclepore membrane filters. They also did size evaluation using SEM. Dobbins and Strand (12) compared the tank collection method to that of spectrophotometric tests in Al_2O_3 sampling of rocket effluents. A specialized Al_2O_3 particulate sampling technique using isokinetic particle sampling probes was used to sample the exhaust plumes of Titan IIIC rockets.

Systems for exposing plants to SRF exhaust have been of the batch type; small lumps of SRF were burned in a chamber. At the University

of Central Florida, a small piece of SRF was burned in a cylinder and the exhaust gases were circulated to a specimen chamber of the same dimensions (66). Another system used at the University of Central Florida consisted of a 35 cf plexiglass chamber with a wire rack for test specimens in the center of the chamber and a pan of sand on the floor of the chamber for burning the SRF (65). Fenton and Purcell (45) fired solid rocket motors into a steel sphere and subsequently exhausted the effluent into a Teflon lined exposure chamber. Granett and Taylor (20) used 0.7 cf rectangular Teflon chambers with impellers for exposing field plants to SRF exhaust. The chamber was placed over the plants, the fuel was burned, and the chamber was removed after 15 min. No continuous burn systems were available that would maintain a given concentration in a controlled environment over some specified time period.

The primary objective of this section was to design, construct and calibrate both a greenhouse and a field exposure system for HCl, Al_2O_3 and SRF exhaust that was compatible with the biological systems to be tested. Specifically, the objectives were:

(1) Develop an exposure system for dispensing and monitoring HCl gas at 1 to 100 ppm in air; and, determine the mixing and flow characteristics in the exposure chambers.

(2) Develop a system for dispensing and monitoring commercial Al_2O_3 particulate that is compatible with the exposure system for HCl; characterize the Al_2O_3 in the air stream.

(3) Develop an exposure system for dispensing and monitoring SRF exhaust from a non-pressurized burn; and, determine the mixing and flow characteristics in the exposure chambers.

(4) Analyze and compare the simulated and actual exhaust Al_2O_3.

This report contains the essential elements of our facilities design, construction and calibration. For additional detail the theses by Sawyer (59) and Tyson (70) should be consulted. The appendix of Sawyer's thesis may be helpful to an investigator wanting to duplicate our experimental system.

3.2. Dispensing, Monitoring, and Instrument Calibration For All Systems

Two systems were designed and developed: a greenhouse exposure chamber system for dispensing and monitoring HCl with an attachment for dispensing Al_2O_3; and, a field exposure chamber system for handling a controlled burn of SRF.

3.2.1. Greenhouse exposure chamber system

The four greenhouse chambers that were used for the HCl exposures are shown in Figure 8. Charcoal filtered air was pulled through the chamber system by a high pressure blower that was attached to a common exhaust manifold (Figure 1). The air containing the gaseous or particulate pollutant passed through a scrubber before it entered the blower

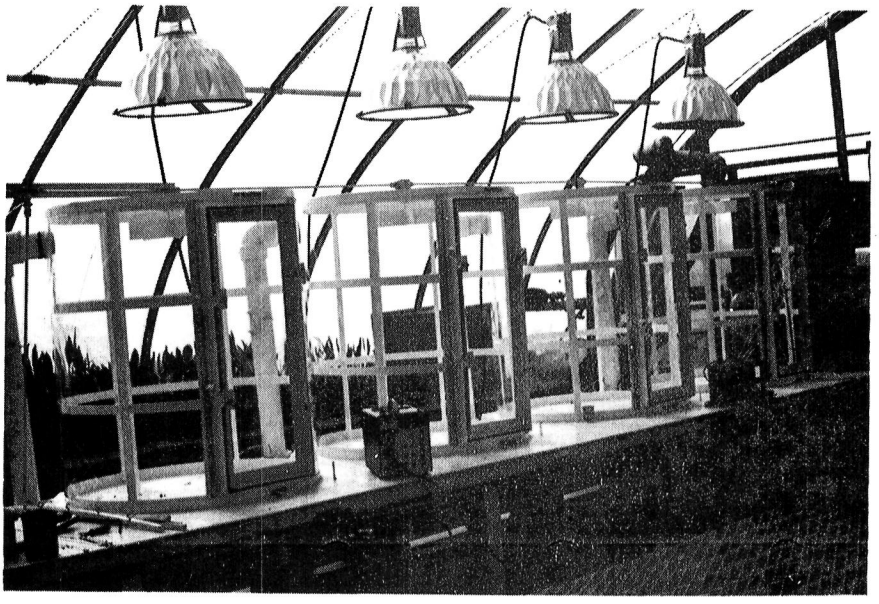

Figure 8. The greenhouse exposure system showing the four exposure chambers with high intensity lamps; two Geomet monitors are by the chambers.

and was exhausted outside the greenhouse. This design maintained the chambers under a slight negative pressure during exposures. The basic design and operational rationale for this system was described in detail by Heck et al. (33). The chambers were modified to accomodate the corrosive HCl gas by constructing the inlet ducts from four-inch PVC pipe and the outlet ducts of two-inch PVC pipe. The inlet duct was equipped with three 60% baffles to ensure uniform mixing of the gases entering the chamber (Figure 9).

3.2.1.1. Hydrogen chloride dispensing and monitoring

Hydrogen chloride gas was dispensed into the inlet duct of each chamber from a cylinder of approximately 25% HCl in dry N_2 (Figures 9 and 10). The HCl cylinder was connected to a cylinder of dry N_2 that was used to purge the flow dispensing system after each exposure. The HCl was carried from the tank in quarter-inch Teflon tubing through one or two flowmeters, arranged in parallel, and then into the inlet duct. The use of two parallel flowmeters permitted HCl flowrates in the range of 5 to 830 cc/min. Based on design calculations, HCl flowrates in this range permitted dispensing HCl gas into the chambers at concentrations within the range of 1 to 50 ppm.

The HCl concentration in each chamber was measured with a Geomet (Model 401B) Hydrogen Chloride Monitor. The hydrogen chloride was drawn through a ceramic tube coated with sodium bromate and bromide. The HCl reacted with the coating to produce hypochlorite, hypobromite and bromine which reacted with luminol to produce visible light (chemiluminescence). The amount of light produced was related to the HCl concentration. Since HCl adsorbed on all surfaces, we inserted the ceramic tube directly into the exposure chamber through a small opening in the chamber wall instead of using a shared-timing monitoring design as originally intended. This required a Geomet for each exposure chamber.

Calibration of the Geomet was usually done by injecting 5 µl of 0.0303 M hydrochloric acid in a methanol solution into the ceramic tube (21). By injecting a known hydrochloric acid concentration and monitoring the Geomet response with a strip chart recorder and an integrator, the Geomet response was adjusted to agree with the HCl ppm/sec input. This method of calibration gave a single calibration point but it provided no information on the response at other concentrations or to the linearity of the Geomet. Thus, a second system was designed and built to provide a continuous calibration method. An aluminum cylinder was used to contain a mixture of HCl gas in dry N_2. This cylinder was carefully calibrated and was found to have 855 ppm HCl by volume. Calibration of the tank was checked each time the Geomet was calibrated to assure tank stability. Tank calibration utilized a bubbler and a specific ion electrode. The tank HCl was then used to calibrate the Geomet over the range of 0 to 50 ppm by using a flow dilution system. This system provided an accurate calibration for various HCl concentration in an ambient air flow (59, 70).

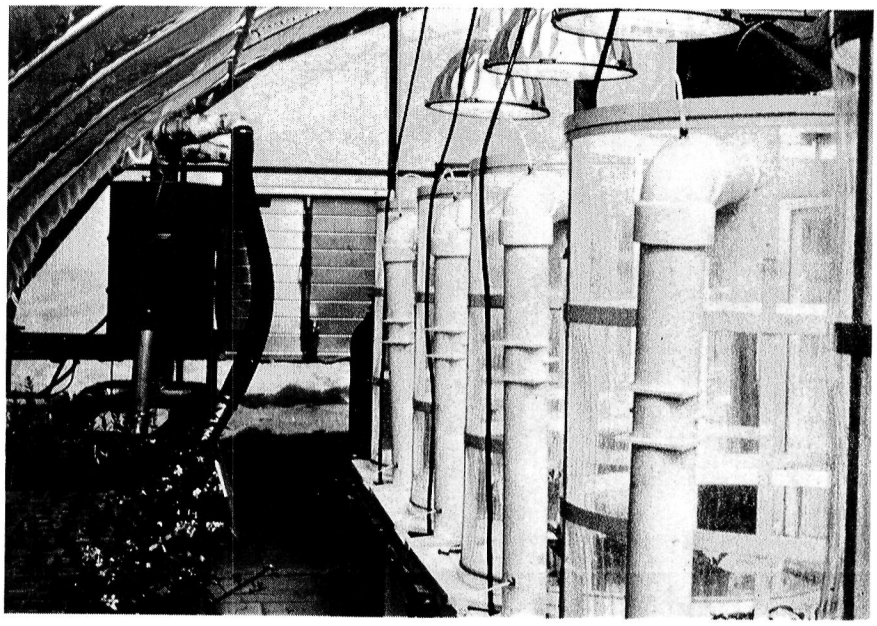

Figure 9. The PVC inlet duct from two greenhouse exposure chambers (bottom to top; HCl dispensing port, 60% baffles, inlet monitoring port).

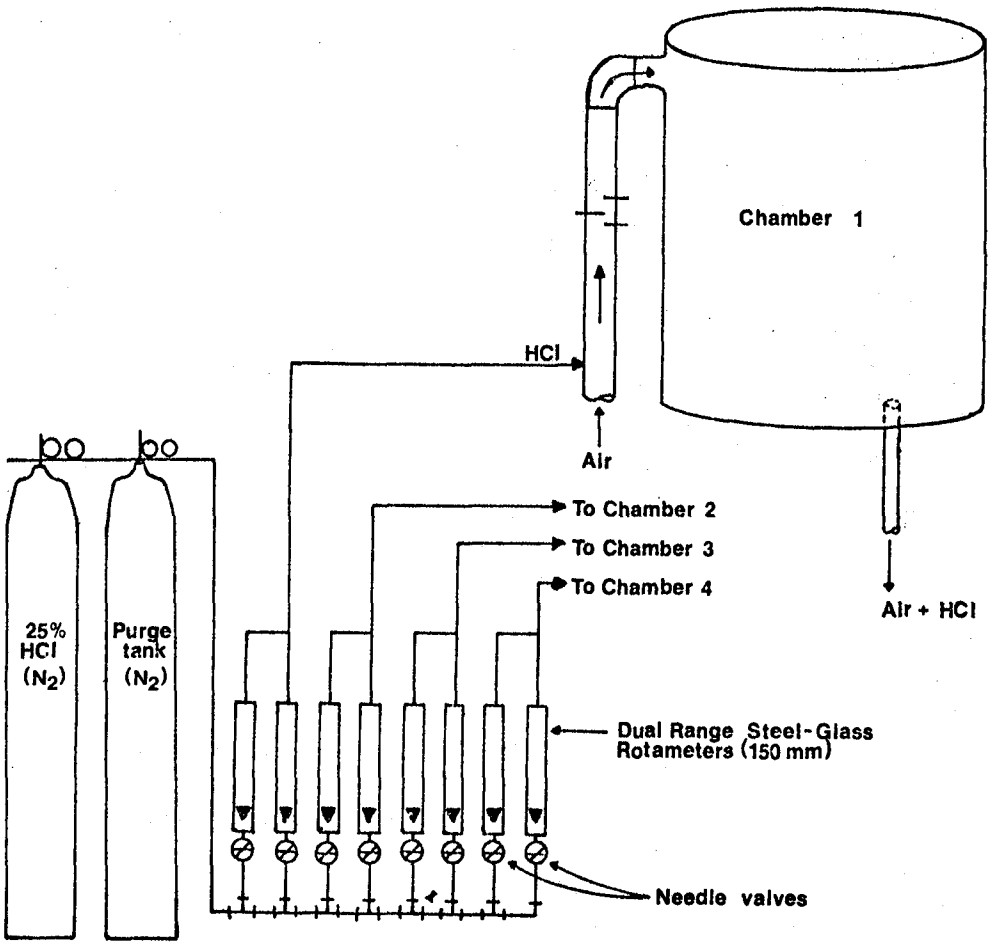

Figure 10. Schematic diagram of HCl dispensing system

3.2.1.2. Aluminum oxide (Al_2O_3) dispensing and monitoring

An Al_2O_3 dispensing system was mounted beside one of the greenhouse exposure chambers for injecting Al_2O_3 into the inlet duct. The system operated on a positive displacement principle (Figures 2, 11). A Teflon cylinder (1.5 in. OD by 0.25 in. ID) was threaded into a rubber collar in the inlet duct. The Teflon cylinder (machined to a lesser wall diam and with a beveled edge) protruded to the center of the inlet duct (Figure 2) so that the Al_2O_3 particulate could be released into the center of the air stream. Sections of 1/4 in. OD Teflon tubing (0.2 in. in length) were stacked in the quarter-inch hole of the Teflon cylinder. The particulate was then loaded with periodic taping of the cylinder assembly to insure uniform filling of the Teflon cylinder. The sections of Teflon tubing were slowly pushed into the inlet duct by a Teflon tipped plunger coupled to an aluminum rod that extended from a Sage syringe pump. The air flowrate through the inlet duct removed the particulate and carried it into the chamber. However, since the airflow did not adequately break up the Al_2O_3, an 0.25 in. OD glass tube was positioned over the Teflon cylinder and a constant airflow was directed on the Al_2O_3 being displaced. This technique permitted a reasonably uniform rate of particulate dispersion into the chamber. The syringe pump could be set at various speeds to drive the particulate at a rate necessary to give the required chamber concentrations of Al_2O_3.

The Al_2O_3 was a mixture of two Al_2O_3 phases: a gamma (γ) phase Al_2O_3 with an average particle diam. of .02μm, and an alpha (α) phase Al_2O_3 with particles from 3-20μm in diam. The γ-phase is considered an active phase and the α-phase a non-reactive phase. The Al_2O_3 mixture was 90% α-phase with 10% γ-phase, by weight. The average surface area for this mixture was 19 m^2/gm and its average density was 0.66 gm/cm^3. The Al_2O_3 mixture was dried and stored in glass jars within a desiccator when not being used.

Nucleopore membrane filters (pre-weighed) were used to collect and monitor the particulates in the chamber. Filters with pore sizes of 0.1, 2, and 10 μm were used to separate the particulate into three sizes. Two filters were used in series in multiple aerosol holders during any single sampling period in one of three sequences: 10 μm over 2 μm, 10 μm over 0.1 μm, and 2 μm over 0.1 μm. The 10 μm over 2 μm arrangement proved to be the best and was used most frequently. Air was drawn through the stacked preweighed filters for a prescribed time period at a constant airflow rate. Airflow was sufficient to maintain particulate suspension. Particles were drawn to the filter surface and collected primarily by diffusion and impaction with electrostatic forces playing a small part. Once collected, the membranes were reweighed and the chamber concentration calculated. The particulates on the membranes were periodically examined with a scanning electron microscope (SEM).

3.2.2. Field exposure chamber system for SRF exhaust

The field exposure chamber system consisted of an air delivery blower, a burn box, three exposure chambers, a scrubbing chamber, and

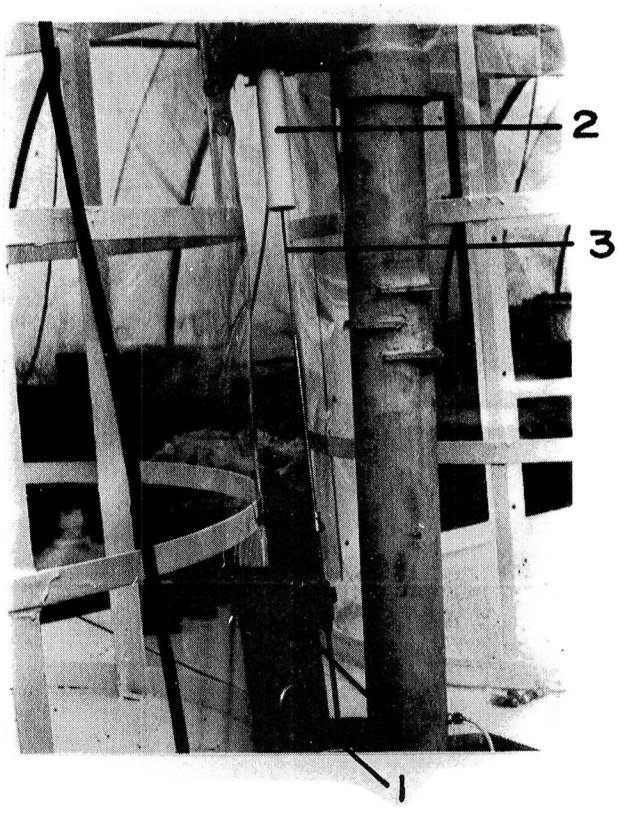

Figure 11. The components and mounting location of the Al_2O_3 dispensing system: 1) syringe pump, 2) Teflon cylinder, 3) plunger.

a control chamber with its own air delivery blower. The five chambers used in this system were 10 ft in diameter and 8 ft in height with a volume of approximately 625 cf. The basic chamber structure followed the design of Heagle et al. (26) except that the lower panels were single layered without perforations. The aluminum frame was covered with an upper and lower Krene (8 mil) panel. A 12 in. diam Krene inlet duct was attached to the top panel and a 12 in. exit duct was attached to the bottom panel for the three exposure chambers and the control chamber. The ducts were 24 in. in diam for the scrubbing chamber. All chambers were covered with a Krene top.

The field exposure system (Figures 3 and 12) had a 3000 cfm constant flow blower that was connected to the burn chamber by a 39 in. diam galvanized tin duct. The burn chamber (Figure 13) was a 30 cf box constructed on angle iron and covered with galvanized tin sheets. Two galvanized baffles were located in the burn chamber at the inlet duct to disperse the airflow through the burn chamber. The exhaust duct was a 24 in. diam by 6 in. long galvanized sleeve for attaching a Krene duct. A galvanized door was hinged to one side of the burn chamber for entry.

Inside the burn chamber, three 0.25 in. thick copper plates (4 ft^2) rested on angle iron mounts (Figures 14 and 15). The copper plates were equally positioned about the center of the burn chamber. The plates were grooved 0.125 in. deep by 0.438 in. wide to hold the SRF in a geometry that permitted 10 ft. of fuel per plate. Removable copper baffles (3 in. wide by 20 in. long) were positioned in smaller grooves between each adjacent groove in the copper plate to prevent flaring of the fuel.

The burn chamber had a firing system that sequenced the SRF burning from plate to plate or allowed the SRF from any one of the plates to be burned independently. The firing system used 14 gauge high temperature wire that was insulated with asbestos. The ignition wire was nichrome and the wiring terminals were ceramic. The firing system operated from a 110 volt AC current through a system of relays and the control components were mounted in an aluminum box on top of the burn chamber. Ignition of the SRF in the first plate was performed manually. The second and third plates of SRF were activated as the burn ended in the preceding plate. The firing system had a safety switch that was activited by opening and closing the access door.

A Krene duct extended from the galvanized sleeve of the burn chamber to a polypropylene flow divider (Figures 13 and 16). The flow divider accepted the exhaust air stream and divided it into three equal streams for transmission to the three exposure chambers. Three 60% baffles were positioned within the flow divider to assure a uniform stream split. Variable area baffles were inserted into each of the three outlet sleeves to permit variable flow into each chamber. Krene ducts connected the exits of the flow dividers with the chambers. Each duct had a variable flow dilution blower prior to entrance into the chambers. The use of the flow divider baffles to regulate flow to each chamber in combination with the variable speed dilution blowers (Figure 13) permitted

Figure 12. View of the field exposure system. The control portion is in the upper left portion; the exposure portion fills the remainder of the picture.

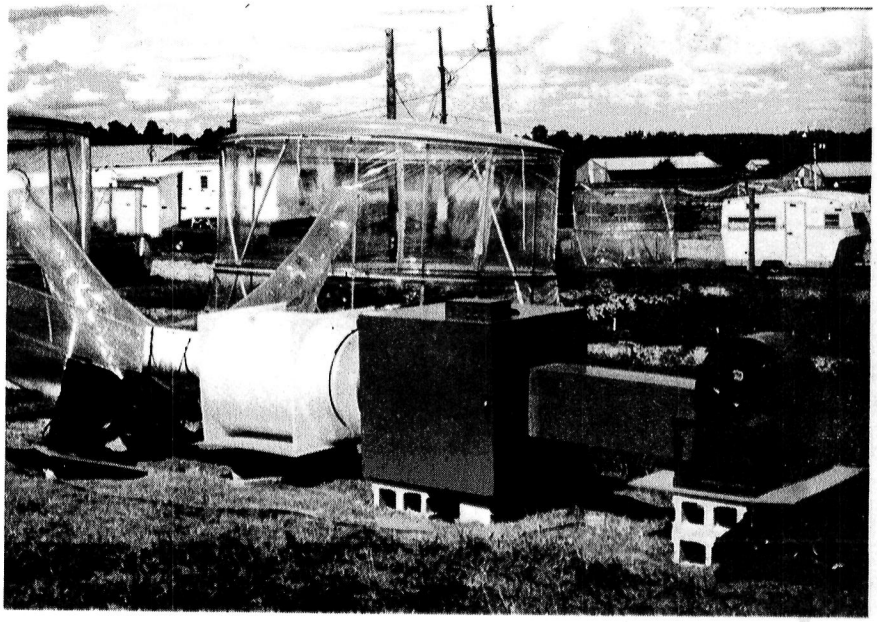

Figure 13. The components of the inlet side of the field exposure chambers are shown: from right to left, the blower, the burn chamber, the flow divider and the dilution blowers.

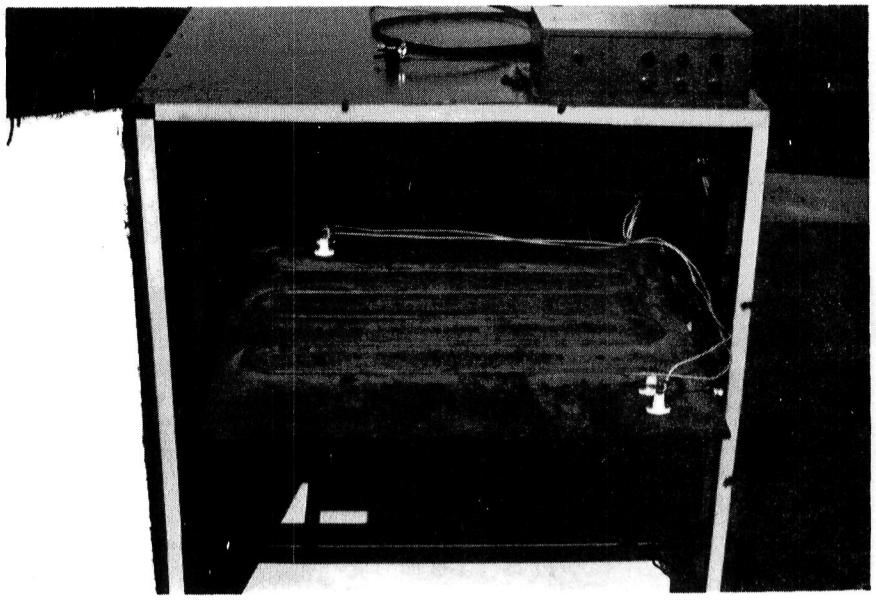

Figure 14. Close-up of the copper burn plate. The inside of the burn chamber is shown with detail given to the copper burn plate, the ceramic terminals are shown, with asbestos wiring leading from the terminals to the sequencing device mounted on top of the burn chamber. The plates have been rotated in actual use.

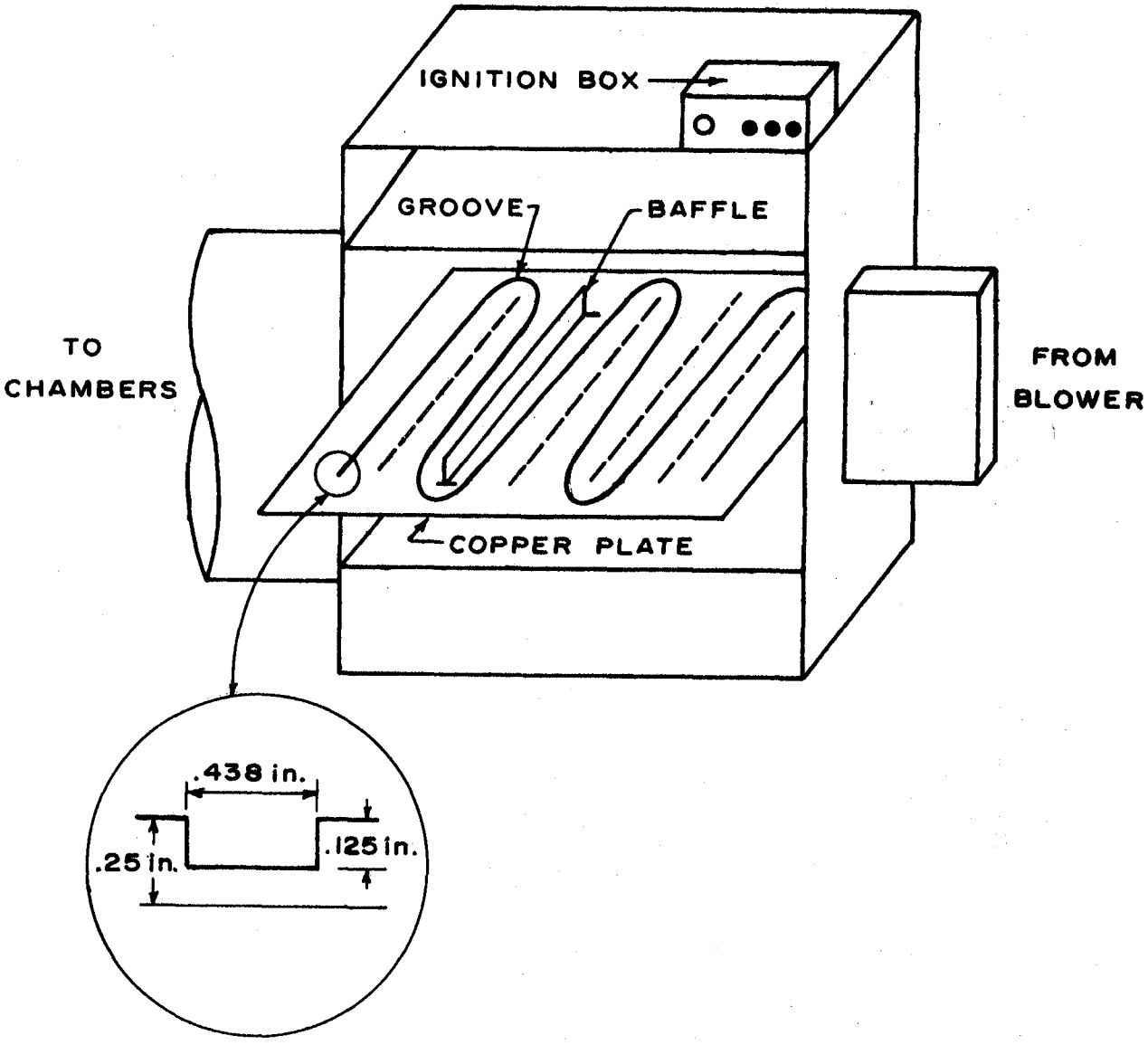

Figure 15. The SRF burn chamber

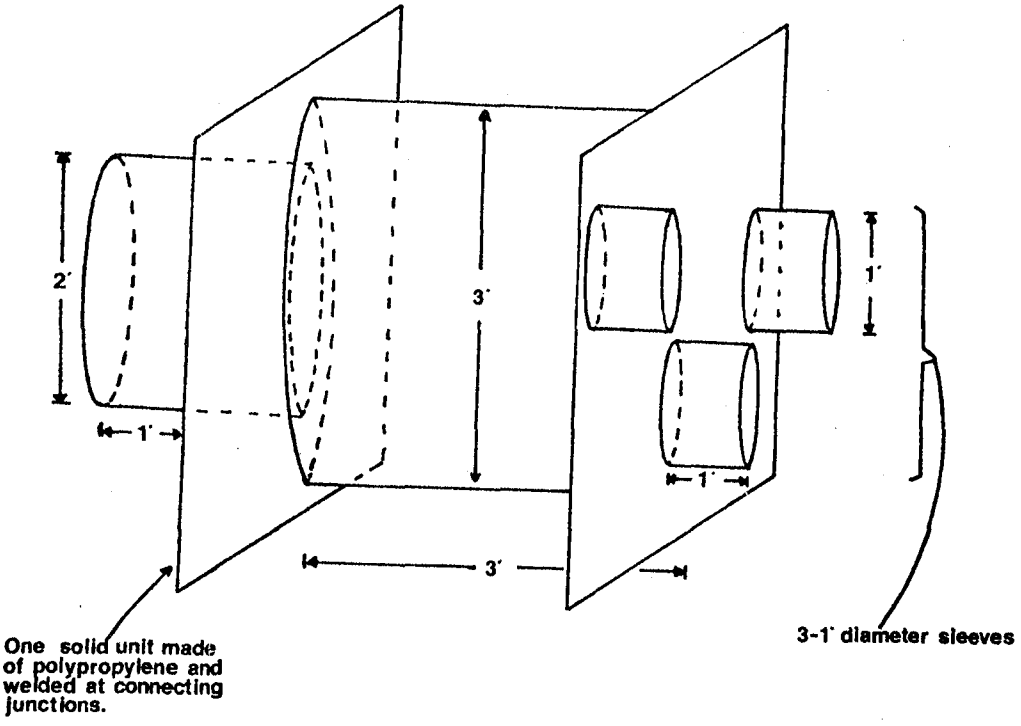

Figure 16. Schematic diagram of the flow divider

maintaining different chamber exhaust concentrations and a fairly uniform total flow through the chambers. The residence time of the exhaust gas in each chamber was approximately 0.63 min based on a chamber flowrate of 1000 cfm. The pressure drop through the field chambers was assumed to be negligible, because they acted as constant volume reservoirs with no density change of the air between the inlets and outlets.

The exhaust gas exited the three field chambers through another flow divider that combined the exit flow into a single air stream that passed into the scrubber chamber (Figures 3 and 17). The scrubber was a field chamber with five water spray nozzles mounted in the top to remove the HCl and Al_2O_3 from the air stream prior to release into the environment.

Each exposure chamber was monitored for HCl with the Geomet by inserting the ceramic tube into the Krene exit duct of each chamber, at the chamber. The Al_2O_3 was monitored by nucleopore filters in the multiple holders described for the greenhouse chamber system.

The solid rocket fuel (SRF) was obtained from Thiokol Corp. through an agreement with NASA. The SRF is a mixture of ammonium perchlorate (70%) and aluminum (16%) held in a binder (14%) of 50% polybutadiene and 50% polyacrylonitrite (PBAN). The SRF was shipped in slabs (0.25 in. thick, 4 in. wide and 6 in. long) and cut into strips of variable width depending upon the maximum HCl chamber concentration desired. Burn rate tests, conducted on the SRF strips, showed an approximate 4 in./min linear burn rate with a much faster surface burn rate. The propellant surface was coated with a burn restrictor (methyl ethyl ketone, 79.1%; tricresyl phosphate, 2.7%; ethyl cellulose, 18.2% by vol.) to stop the surface burn. The coating was applied by dipping the propellant strips into the liquid restrictor and allowing the coating to dry. Four or five coatings were necessary to ensure proper burning.

The field control portion of the system (Figures 12 and 18) consisted of a variable flow blower connected to a field chamber with a 1 ft diam Krene duct. The airflow through this chamber could be varied to match that in the exposure chambers.

3.3. Operational Testing of All Systems

The HCl dispensing and monitoring portions of the greenhouse system were tested. The greenhouse and field chambers were characterized using HCl as a tracer gas. The greenhouse chambers were biologically characterized using plants exposed to HCl.

3.3.1. Testing and characterization of the HCl systems

The greenhouse and field exposure systems were tested to assure that they met design requirements. Tests were performed using the HCl and Al_2O_3 as the test chemicals.

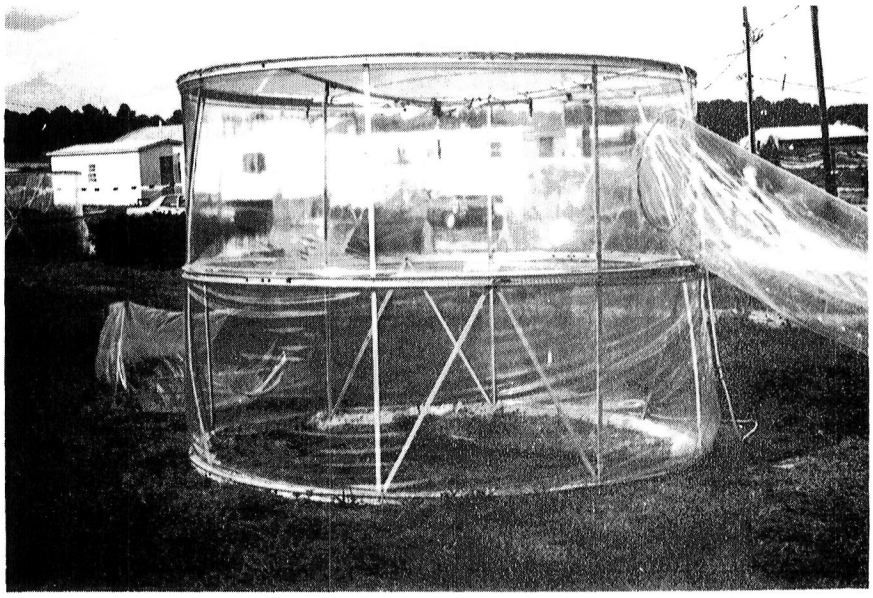

Figure 17. Field chamber scrubber: the spray nozzles are mounted on two aluminum bars that criss-cross the top of the chamber.

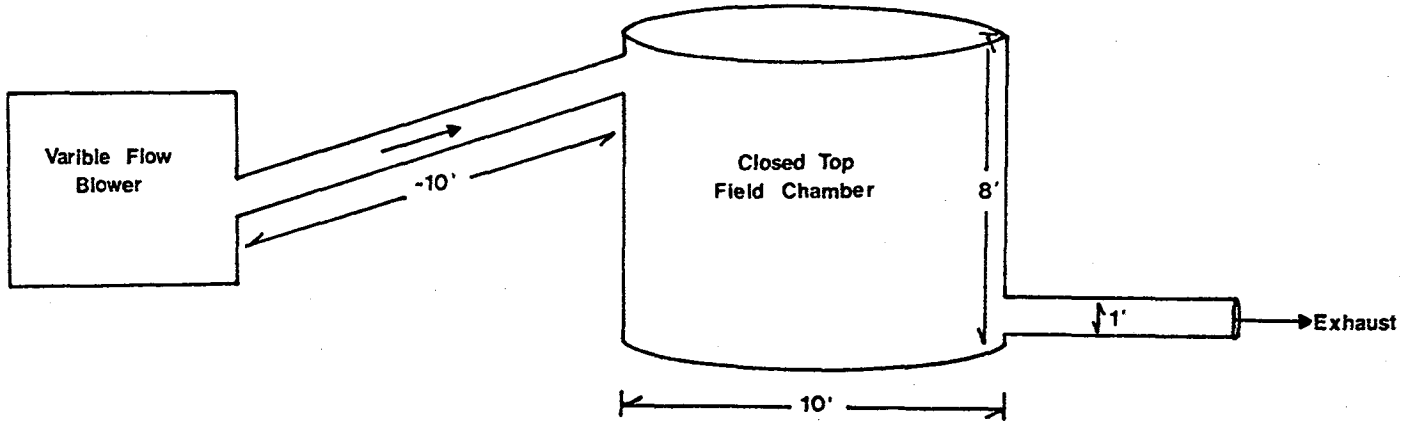

Figure 18. Schematic diagram of control system

3.3.1.1. Physical characterization of the greenhouse and field systems

The HCl dispensing system - This system performed as designed with good comparisons between predicted and measured HCl concentrations. This comparison was made by monitoring chamber concentrations with the Geomet at recorded temperatures and pressures and converting the concentrations to STP conditions. These actual concentrations were then plotted along with the predicted concentrations for various flowmeter settings. Results for chamber 1 are shown in Figure 19.

The greenhouse exposure chamber system - Tracer experiments were used to test the mixing and flow characteristics of the greenhouse chambers. Experimental chamber decay curves were obtained by using HCl as a tracer in the chamber airflow. A constant HCl flow was injected into the air stream of a greenhouse chamber until a steady state chamber concentration was attained. Then, the HCl injection was stopped and negative step changes in the chamber HCl concentration were determined by continuously monitoring the HCl with the Geomet. The experimental decay curve was obtained by plotting the quantity C/C_o as a function of time (where C_o was the steady state concentration and C was the chamber concentration at any given time period after the HCl injection was stopped).

Data for the greenhouse chambers were taken at low and high volumetric flowrates. The decay curve for the greenhouse chambers (Figure 20) with real time as the abscissa, indicates a significant deviation from ideal CSTR behavior for the low volumetric flowrates. This deviation from ideal behavior is shown by the two distinct straight line sections of the log of C/C_o vs t graph (Figure 21). This type of response indicates two flow behavior regions within the chamber that could be explained as a combination of two ideal CSTRs with differing residence times. Therefore, a model was proposed that described the actual flow behavior in the greenhouse chambers as two ideal CSTRs connected in parallel, one with a low residence time (CSTR 1) and the other with a long residence time (CSTR 2). The model gave a good fit to the actual chamber response (Figure 4). The model provides a mathematical expression that describes the chamber as consisting of two regions with differing flowrates. These two regions do not necessarily exist in the chamber but the flow behavior, as derived from tracer analysis, can be viewed this way. The deviation was present but not as pronounced at the higher volumetric flowrates, probably due to greater chamber turbulence and more complete mixing of HCl at this flowrate.

Absolute humidity was also found to have a significant effect on the model parameters for the greenhouse chambers. This effect was mainly in the "tail" of the curve and was described by CSTR 2. Thus, increasing humidity causes a longer residence time for CSTR 2 and results in greater deviations from the ideal CSTR behavior. This humidity effect is also less at the higher volumetric flowrates. The most probably explanation for the humidity effect is that the water on the chamber walls serves as a sink for the HCl gas. As the chamber HCl concentration decreases, desoprtion from the sink occurs resulting in the observed

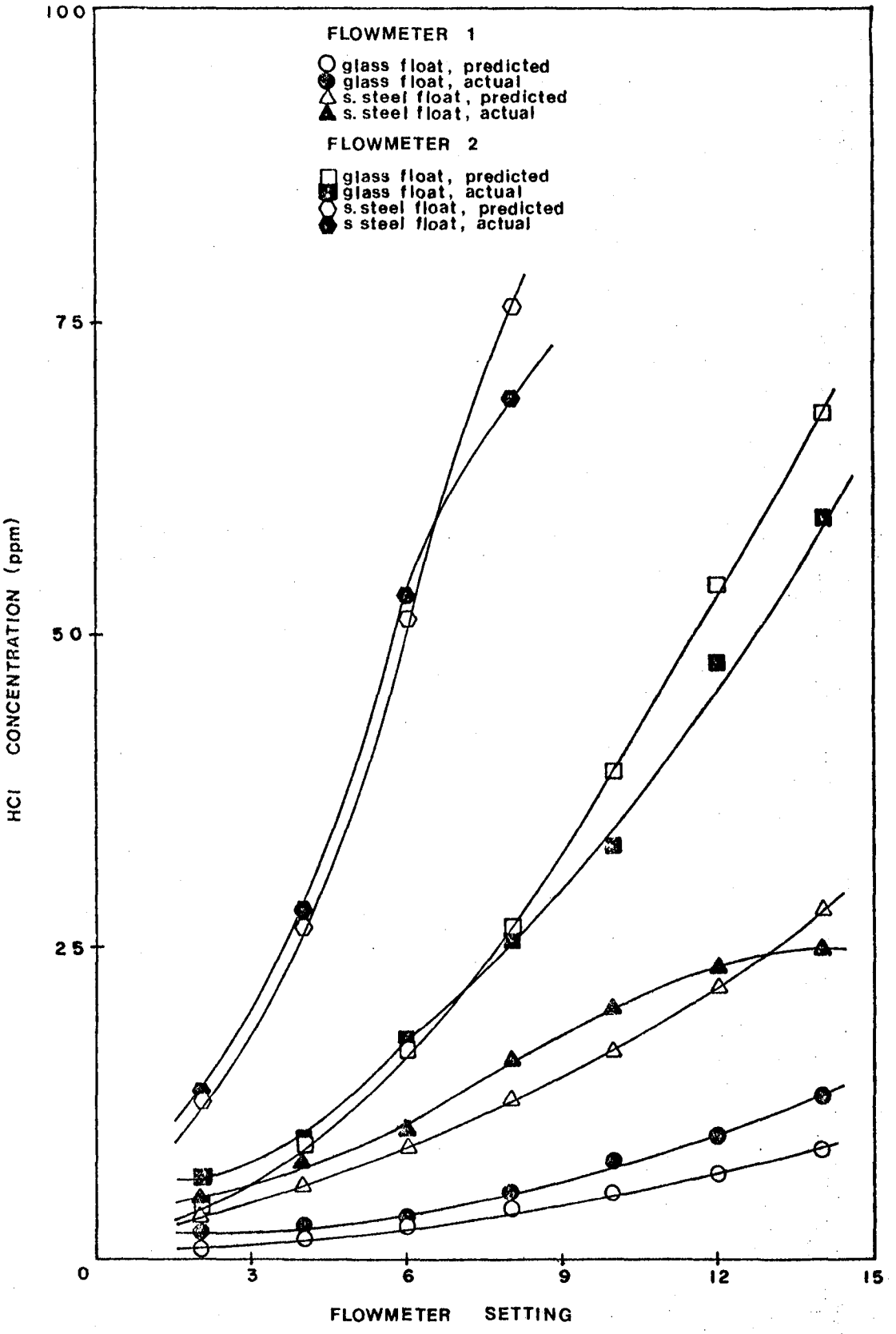

Figure 19. Comparison of predicted and measured HCl concentrations in Chamber 1.

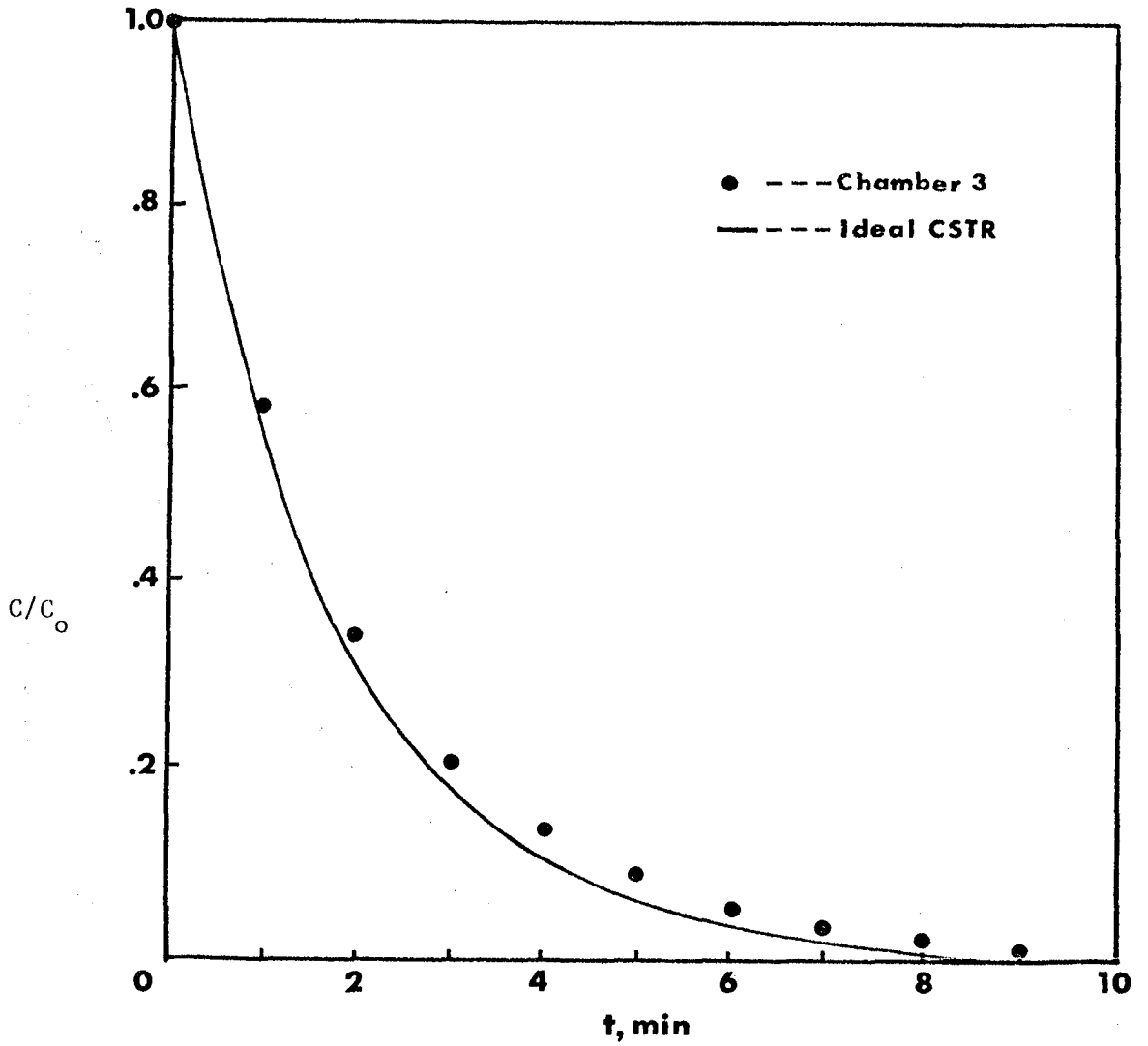

Figure 20. Typical HCl decay curve for a low volumetric flow rate in the greenhouse chambers. C = chamber concentration, C_o = steady state concentration.

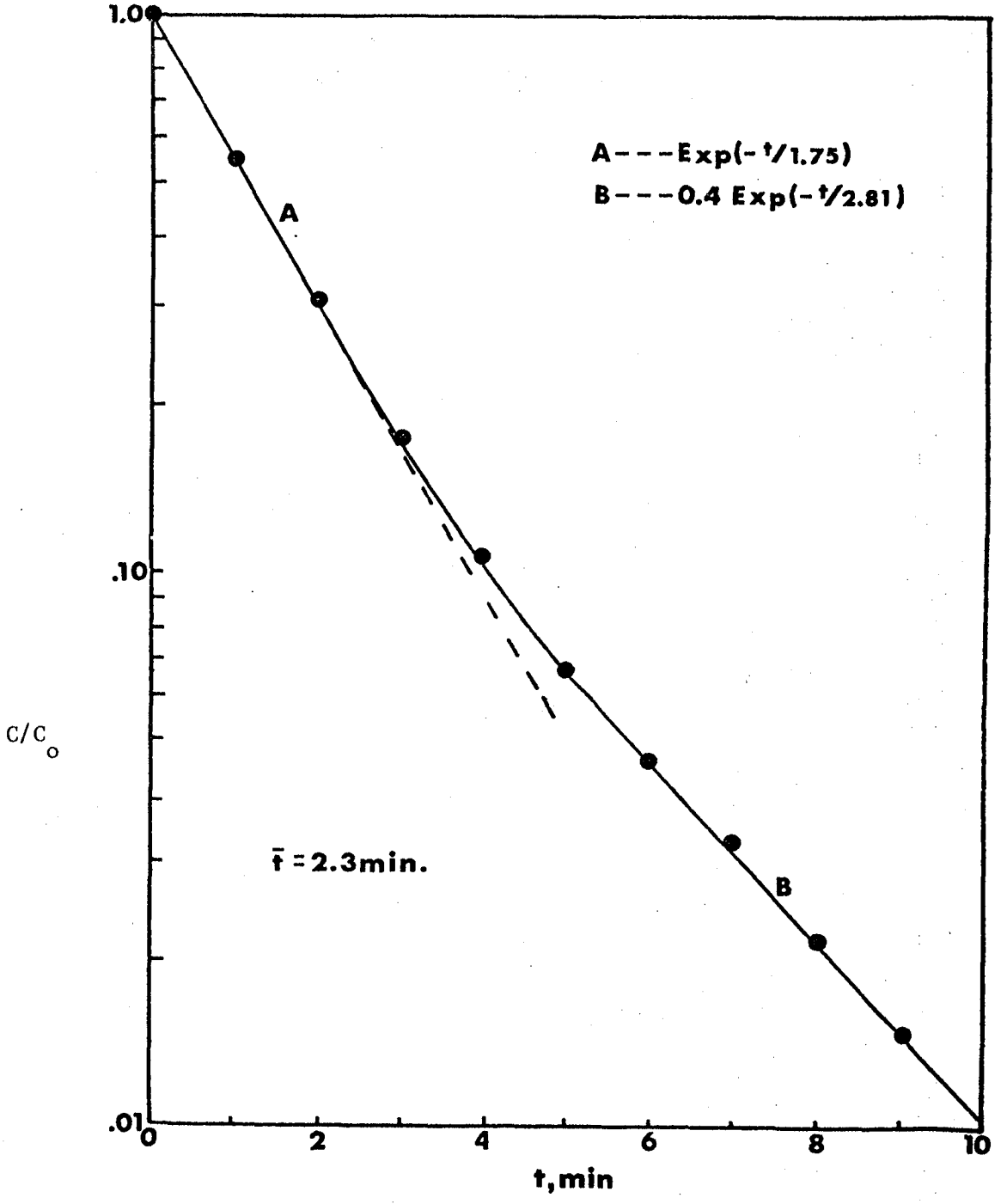

Figure 21. Log of C/C_o against time for greenhouse chamber 3 at the low volumetric flow rate showing two linear curve sections (A and B) - or CSTR 1 and CSTR 2. C = chamber concentration, C_o = steady state concentration.

"tail" section. Operation of the greenhouse chambers usually occurred at lower humidities where changes in the humidity did not severely affect the chamber conditions.

The field exposure chamber system - The components of this system performed as outlined in the design. The burn chamber functioned well but the materials of construction were badly corroded by the exhaust gases. The flow divider gave fairly equal splitting of the 3000 cfm airflow. The chamber equilibration time for given concentration settings was five minutes. Data was taken to compare the actual HCl concentrations to the predicted concentrations based on the equal splitting of the system airflow through the three chambers (Table 6). The results show that the actual concentration in the field chambers was lower than the predicted concentration by 10 to 34%. This was expected due to surface adsorption of HCl, difficulties in measuring airflow rate, and non-uniformity of cross-sectional area in the SRF strips. The differences in HCl concentration in the three chambers suggest uneven mixing of the exhaust within the burn chamber and the flow divider. The channelling favored the center chamber over the other two.

The field exposure chambers were characterized using HCl as a tracer that was generated by burning the SRF. The chambers were equilibrated (brought to steady state) and monitored through the decay process after the burn was completed. The decay of HCl in the chamber was monitored at the exit duct with a Geomet and recorded as a function of time. The experimental decay curves were obtained from the chamber responses and were plotted on semilog paper (Figure 22 gives the results for chamber 2). These curves were essentially linear for all three chambers, indicating ideal CSTR behavior. The mean residence times of chamber 1, 2, and 3 were 0.72, 0.98, and 0.91 min, respectively. These times reflect the addition of baffles in the flow divider and the use of the dilution blowers. These residence times were used to achieve the desired concentration ratio in the 3-chamber system. The ideal CSTR model fits the data well (Figure 5). No humidity effect was found during testing. Since the flow behavior in these chambers was ideal, the necessary condition of uniform concentration over the entire chamber was met.

3.3.1.2. Biological characterization of the greenhouse chambers

The greenhouse exposure chambers were monitored for uniform distribution of the pollutant within and between chambers at several concentrations. Any differences in plant response at various positions within the same chamber were determined and the average response of plants in different chambers was compared. Experiments were conducted using soybeans and radish as the monitoring species. Two experimental designs were used.

Experimental designs - Soybean ('Dare') and radish ('Comet') were grown to 21 and 14 days respectively following methods in section 4.2.1.1. before being exposed in these designs.

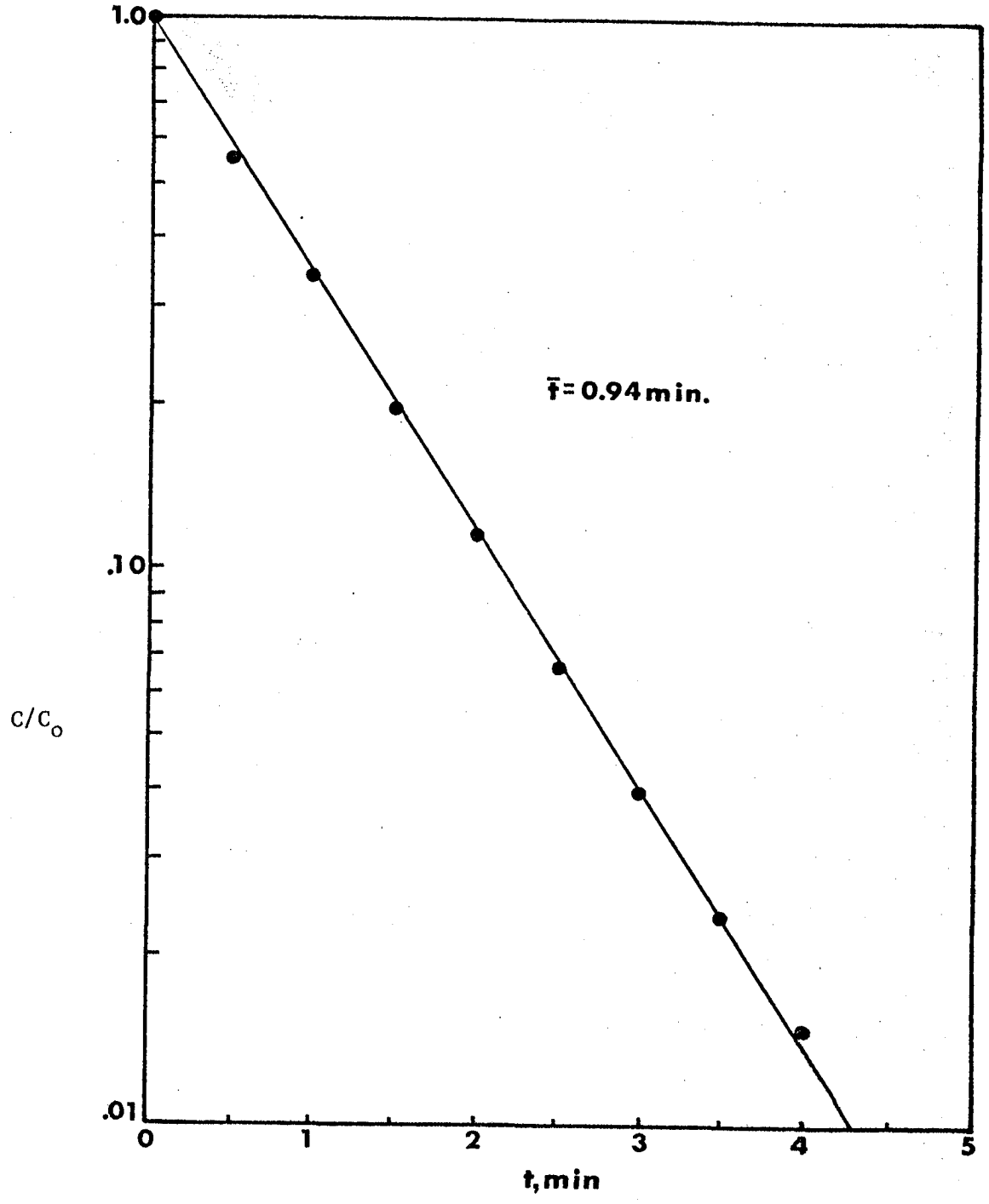

Figure 22. Log of C/C_o against time curve for field chamber 2. C = chamber concentration, C_o = steady state concentration.

Table 6. Comparison of actual and predicted HCl concentrations in the field exposure chambers.[1/]

SRF Strand Area (in.)	Field Chamber	HCl Conc (ppm) Predicted	HCl Conc (ppm) Actual
0.125 x 0.125	1	4.70	3.68
	2	4.70	5.28
	3	4.70	3.86
Ave[2/]			4.27
0.193 x 0.193	1	10.57	7.65
	2	10.57	7.89
	3	10.57	5.48
Ave			7.01
0.25 x 0.25	1	18.78	14.81
	2	18.78	18.64
	3	18.78	14.52
Ave			15.99

[1/] These tests were done before baffles were inserted into the flow divider and without operation of the dilution blowers.

[2/] Assuming equal airflow into the three exposure chambers.

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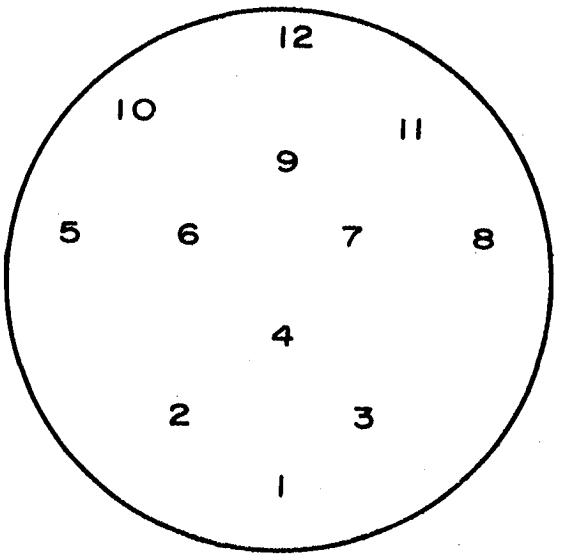
In the first design the four chambers were equilibrated at 10 ppm HCl. Twelve radish plants were placed in each chamber, one plant at each of the 12 positions as diagrammed below (position 1 is at the chamber door).



The plants were exposed for 40 min and then returned to the greenhouse bench. The experiment was replicated 3 times over consecutive days to give 3 plants/chamber position/chamber. The experimental design was repeated for soybean.

In the second experiment, chambers were set at four different HCl concentrations (in the first replication chambers 1 to 4 were set at 0, 5, 10 and 20 ppm respectively). Radish plants were placed in each of the chambers at each of the 12 positions. The plants were exposed 40 min and then returned to the greenhouse bench. The exposures were replicated 4 times over consecutive days with each chamber being set at a different concentration across the four days, in the following fashion:

replication	HCl concentration (ppm)			
	Chamber #			
	#1	#2	#3	#4
1	0	5	10	20
2	20	0	5	10
3	10	20	0	5
4	5	10	20	0

The zero concentrations were not used in the analysis. This experimental design was repeated for soybean.

In both experiments, the plants were rated for % foliar injury of individual leaves in 5% increments (0 to 100% including 1%) 48 to 72 hours after the exposure. Plant values were the average of the individual leaf injury values. Data were analyzed using an analysis of variance with mean separation using LSD at the 0.05 significant level.

Results and Discussion - There were no position by chamber interactions for either species in either experimental design. Thus the simple effects of position and chambers were determined. The results of the 2 experiments were similar across chambers but not across positions for radish. Soybean showed neither chamber nor position effects and thus the results are not shown.

There were no chamber effects for radish in either experimental design (Table 7). This suggested that the chambers were identical and could be used without concern for confounding effects if an experimental design was not completely balanced. A position effect was found in experiment 1 for the radish but not in experiment 2 (Table 8). Over the course of several other position effect experiments occasional position effects were found. However, no consistant effect was identified. Thus, we concluded that under certain conditions positional effects randomly occurred in the exposure chambers. Thus, it was important that all plants be randomly placed in experimental designs,

Table 7. Foliar injury response of radish to the same dose of HCl across the four exposure chambers.[1/]

Exposure Chamber	Foliar Injury (%)
1	35
2	37
3	36
4	35

1/ Data is from experiment 1 (Table 9). Each value is the mean of 36 observations (12 positions by 3 reps). The values are not significantly different. Experiment 2 is not shown but the results were similar with an average injury over the treatments of 38.

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even though position effects usually did not occur. These effects were not due to variations in HCl concentrations within chambers.

The biological tests confirmed the chemical characterization of the greenhouse chambers as being uniform across and within the four chamber system.

3.3.2. Testing and characterization of the Al_2O_3 system

The dispensing and monitoring systems for Al_2O_3 in the greenhouse system worked reasonably well within the limits of the design specifications. Dispensing was not entirely continuous and particulates often entered the chamber in a mildly pulsating manner. Visually, particulate distribution within the chamber was not entirely uniform. The particulate mass tended to compact during dispensing, especially at high humidities. This packing added to the pulsing tendency and thus affected the uniformity of particulate dispensing during an exposure. A possible alternative to this dispensing system might be the Wright Dust Feeder (73). However, for the studies conducted during this investigation, the Al_2O_3 dispensing system, as described, worked satisfactorily.

Nucleopore membrane filters exposed directly to the chamber atmosphere adequately determined the chamber concentration of Al_2O_3. Weight measurements made during exposures confirmed that particulate concentrations within the chamber were close to calculated (predicted) values. The 2-part membrane filters (10 μm plus 2 μm) gave some information on the distribution of particle size. The 0.1 μm membrane was not helpful because most of the γ-phase adhered to the larger α-phase particles. The particulate distribution for the Al_2O_3 particulate mixture (90% by weight α-phase and 10% by weight γ-phase Al_2O_3) was determined from a scanning electron micrograph of a 10 μm Nucleopore membrane subjected to a 50 mg/m^3 Al_2O_3 concentration for an hour, at a sampling rate of 10

Table 8. Foliar injury response of radish to HCl at different positions within the greenhouse chambers.

Chamber position	Foliar Injury and Order of Severity for Each Position[1/]			
	Experiment 1		Experiment 2[2/]	
	Injury (%)	Order	Injury (%)	Order
1	32	9	37	8
2	40	3	36	9
3	35	7	39	5
4	46	1	40	2
5	27	12	40	2
6	38	5	35	12
7	34	8	38	6
8	38	5	38	6
9	40	3	41	1
10	28	11	40	2
11	43	2	36	9
12	30	10	36	9
LSD at 0.05(%)	11		NS	
Average Injury (%)	36		38	

[1/] Each value is the mean of 12 observations (3 reps across 4 chambers). Plants were exposed to 10 ppm HCl for 40 min (Exp. 1) or to 3 HCl concentrations (5, 10, 20 ppm) for 40 min (Exp. 2).

[2/] There were no significant differences in this experiment.

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1/min. The particulate distribution was bimodal and simulated the particulate actually found in the rocket exhaust (67). The bimodal distribution actually was for the α-phase since the γ-phase did not separate. The mean particle size diameter was calculated for each mode. The majority of the particles in the first mode fell into a size range of 2.32 to 6.22 μm, and the majority of the particles in the second mode fell into a size range of 9.22 to 18.32 μm. The data for the particulate distribution are tabulated in Table 9 and are represented graphically in Figure 23. Scanning electron micrographs confirmed the adherence of γ-phase Al_2O_3 to α-phase Al_2O_3 (Figure 24).

Photomicrographs were taken on the simulated particulates from the greenhouse system (Figure 25) and of the real particulates from the combustion of SRF in the field system (Figure 26) using a scanning electron microscope.

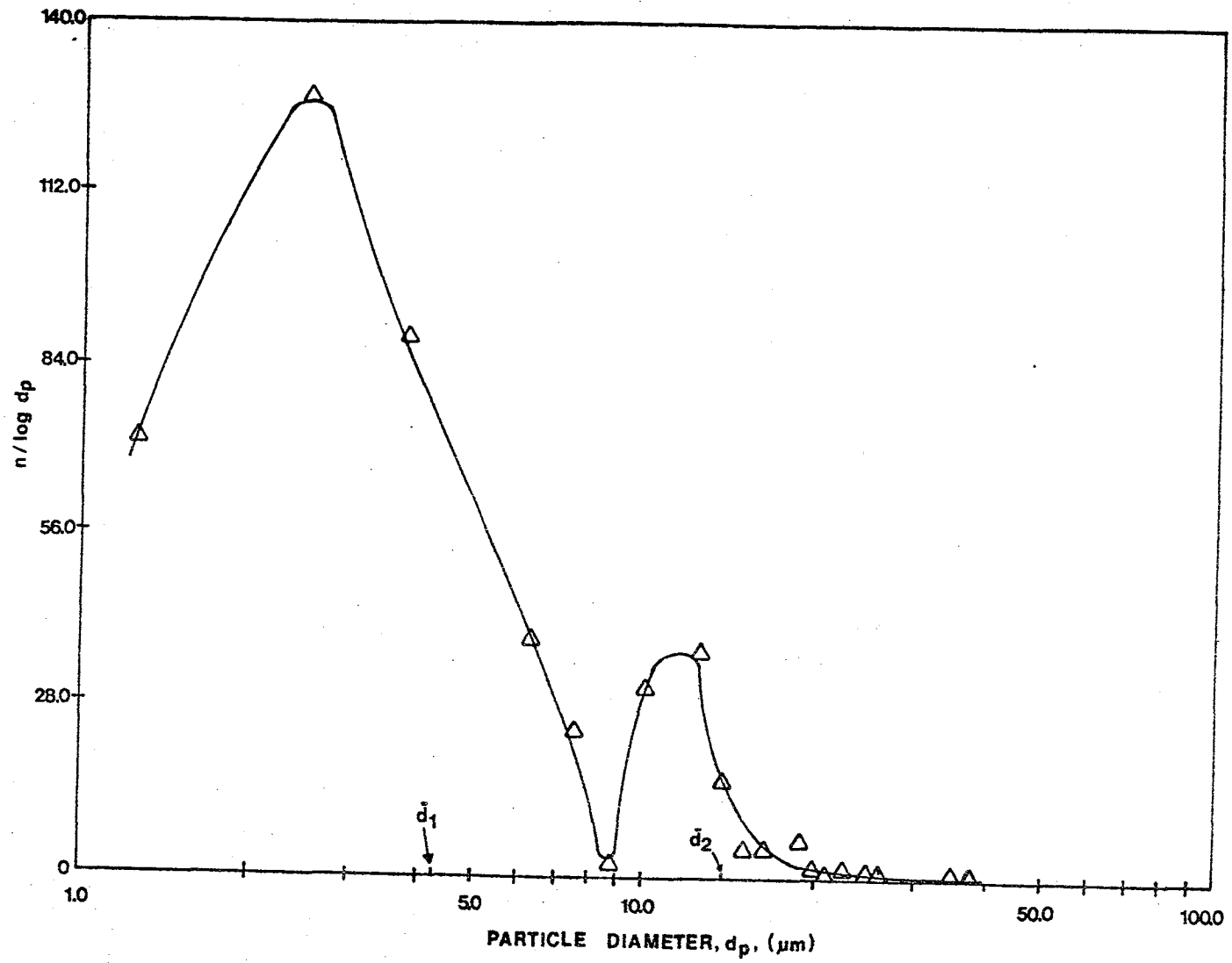


Figure 23. Particle size distribution data, analysis of the simulated Al_2O_3 particle system (Data from Table 9).

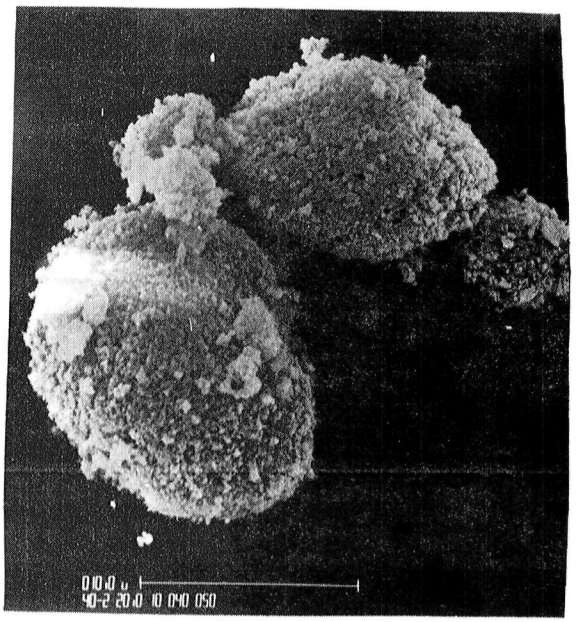
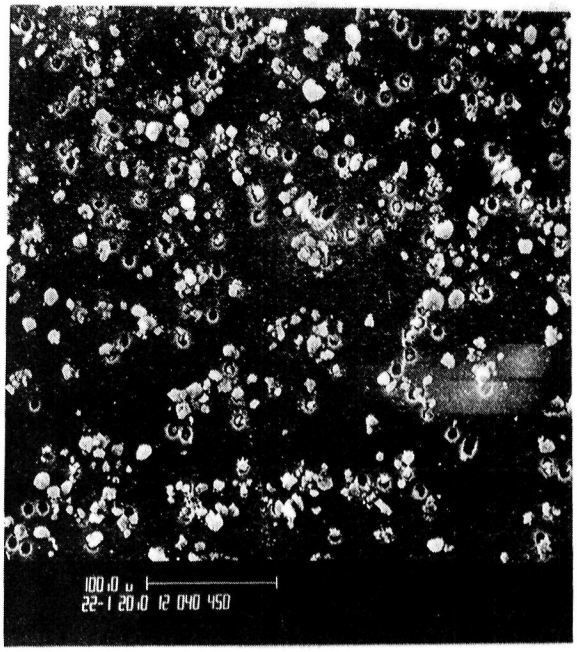
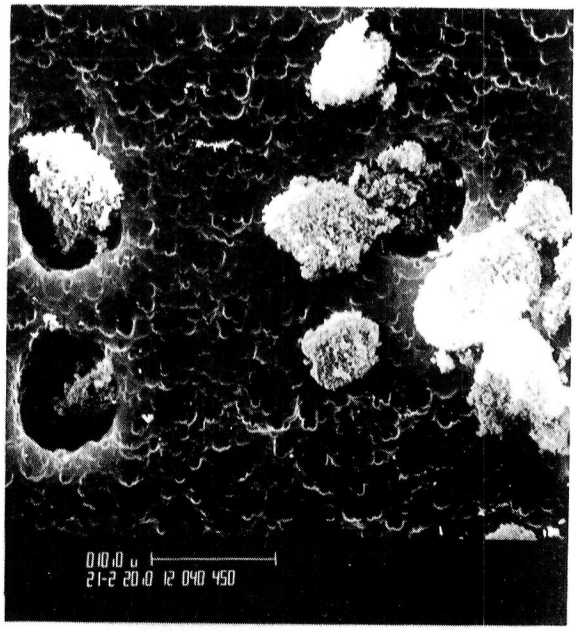


Figure 24. Scanning electron micrograph of simulated Al_2O_3 particulate mixture at a magnification of 4000. It shows fluffy γ-phase Al_2O_3 adhering to large spherical α-phase Al_2O_3 particles.

A. 220 X

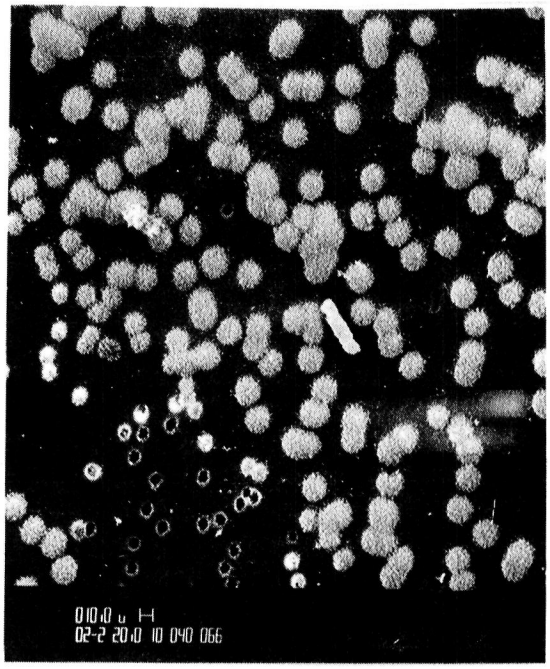


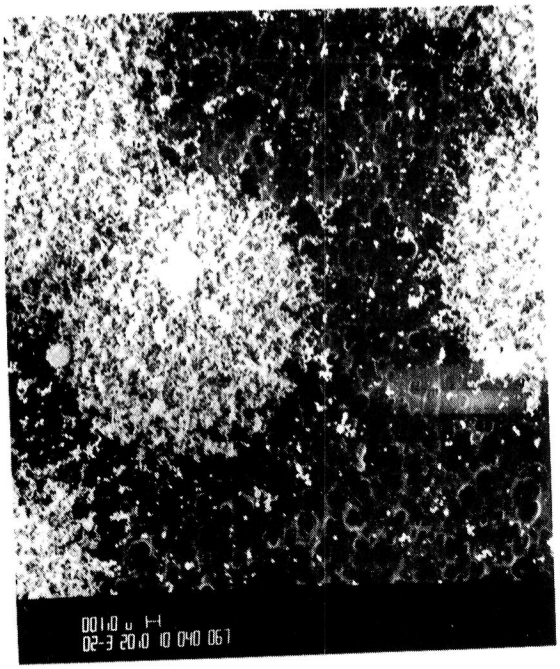


B. 2100 X

Figure 25. Scanning electron micrographs of simulated Al_2O_3 particulates from a 10 μm membrane in the greenhouse chambers, at 2 magnifications.

A. 200 X





B. 2000 X

Figure 26. Scanning electron micrographs of simulated Al_2O_3 particulates from a 10 μm membrane in the field chambers (SRF exhaust), at 2 magnifications; note the lack of very large particles.

Table 9. Particulate size distribution of Al_2O_3 from the simulated Al_2O_3 particulate system (greenhouse).[1/]

Particle Diameter, dp (μm)	Number of Particles, n	log dp	n/log dp
1.25	7	0.10	72.23
2.50	51	0.40	128.16
3.75	51	0.57	88.85
6.25	31	0.80	38.95
7.50	21	0.88	24.00
8.75	2	0.94	2.12
10.00	31	1.00	31.00
12.50	41	1.10	37.38
13.75	18	1.14	15.81
15.00	6	1.18	5.10
16.25	6	1.21	4.96
18.75	8	1.27	6.28
20.00	2	1.30	1.54
21.25	1	1.33	0.75
22.50	2	1.35	1.48
25.00	2	1.40	1.43
26.25	1	1.42	0.70
35.00	1	1.54	0.65
37.50	1	1.57	0.64

[1/] Data are representative of the α-phase Al_2O_3 since most of the γ-phase adhered to the α-phase.

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The photomicrographs of both the open burn (field system) and the simulated particulates (greenhouse system) exhibited a distinctly bimodal character. For the open burn, a more spherical shape and narrower distribution was found with a majority of the particles falling into two size ranges: a large number of very fine particles (<2 μm) and a much smaller number of large particles (>5 μm). The simulated particulates, while exhibiting a bimodal character, appeared quite different from the open burn particulates. Most of the fine γ-phase particles were agglomerated onto the large α-phase particles, producing irregular shaped particles with a wide distribution of sizes. Very little of the more reactive γ-phase particles existed as free particles. The excessive agglomeration of the simulated particulate may be caused by humidity and/or electrostatic conditions, but no work was done to verify this.

4. EFFECTS: VEGETATION

4.1. Introduction

Vegetation in the vicinity of the launch site for the new shuttle vehicle at Kennedy Space Center, Merritt Island, Fla. will be periodically exposed to SRM exhaust from launches of the shuttle. Most of the vegetation within the Merritt Island area is composed of either native coastal plant communities or citrus groves. Stout et al. (65, 66) reported that exposure of important upland vegetation cover types on Merritt Island to SRF exhaust for 10 min had no noticeable impact on the vegetation. They found a decrease in dry weight of English peas after a 10 min exposure to SRF exhaust containing 1000 ppm HCl. Due to the equipment used during these exposures, neither the actual HCl concentration nor the characteristics of the chamber atmosphere during the exposures were described. Similar designs are being conducted at Riverside, California using small exposure chambers and burning preweighed amounts of SRF (20). Thus, a critical evaluation of the effects of SRF exhaust on vegetation is not available.

The two principal components of SRF exhaust are hydrogen chloride gas (HCl) and alumina particulates (Al_2O_3). Hydrogen chloride is known to injure plants (23, 32, 42, 47, 48, 53, 54, 62). Particulates may have deleterious effects on plants (4, 10, 16, 44, 46). Alumina is not known to affect plants by itself but Lerman (45) reported that HCl plus Al_2O_3 in a 1:1 ratio caused slightly more injury on American marigolds than did HCl alone. The effects of these components on plants native to Merritt Island are not known.

The investigation of the effects of HCl and SRF exhaust on vegetation has utilized a single acute exposure (relatively high concentration for a short time period) followed by an evaluation of the results (20, 47, 54, 62, 65). Granett and Taylor (20) conducted a few multiple exposures of selected plants with SRF exhaust and evaluated the effects. However, current information does not indicate whether exposure of a plant to either HCl or SRF exhaust alters the response of the plant to subsequent exposures with the same pollutant.

The influence of environmental factors on the response of plants to air pollutants has been reviewed (27, 61, 68). Increasing relative humidity is considered to increase plant sensitivity to air pollutants (30). Godish (19) observed more injury to 'Bonny Best' tomatoes exposed to 8 to 10 ppm of HCl as relative humidity increased. Free water or dew on the leaf surface may affect the sensitivity of plants to air pollutants. Macdowall (50) reported that dew on the leaves of tobacco did not contribute to the amount of fleck caused by a preceding dose of ozone. Macdowall (49) and Taylor et al. (69) suggested that leaf dew may enhance ozone injury on plants in moisture-deficient soil by increasing the plants' turgor. Brennan et al. (6) found that plants misted before Cl_2 exposures responded in a similar manner to non-misted plants. Guderian and Stratmann (24) observed plant leaves with etched surfaces and acid burns after a rain in an area with high SO_2. The effect of these environmental factors on the response of plants to HCl is not understood.

Time of day and time of year also affect a plant's response to air pollutants. Plant sensitivity generally shows a diurnal pattern over a 24 hour period with the greatest sensitivity centered in the midday to early afternoon (29, 41). A seasonal effect has also been noted in air pollution exposures on plants (27).

The primary objective of this research was to determine the effects of HCl, Al_2O_3, and SRF exhaust at various concentrations and durations of exposure on selected native and cultivated plant species. This included a 60 min screen of all plants that were studied to four concentrations of HCl. More detailed studies on selected plants were done for: HCl dose-response curves, Al_2O_3 screens, HCl + Al_2O_3 mixtures, SRF screens, and SRF dose-response curves. Several secondary objectives were to: 1) determine if a single short-term exposure of a plant to HCl or SRF exhaust altered the plant's sensitivity to the pollutant if it was exposed to the same pollutant a few days later; 2) investigate the effects of time of day, time of year, relative humidity, and leaf surface moisture on the response of radish to HCl; 3) study the uptake of Cl^- by soybean; and, 4) conduct an interlaboratory dose-response comparative study using radish and zinnia.

4.2. Hydrogen Chloride (HCl)

The major objective of the vegetation studies was to understand the effects of HCl as a major and probably the most phytotoxic of the SRF exhaust components. A screen of selected native plant species and cultivated species and cultivars (cv) was first done. This was followed by a dose-response study for a few plant species based on the screening results. The environmental effects and multiple HCl exposures were studied, as time permitted, on several species.

4.2.1. Preliminary HCl screen and dose-response studies

The HCl screen was developed to give a general idea as to the sensitivity of a variety of native and cultivated species of plants. From the screen we assigned plants to several susceptibility levels from very sensitive to tolerant and resistant. The dose-response design permitted a more indepth understanding of the response of selected species as both concentration and duration of exposure were varied. All designs were of short duration to reflect expected shuttle launch conditions.

4.2.1.1. Methods and materials

Studies were carried out in a greenhouse on a research field site south of Raleigh, N. C. Plants (Appendix 7.1) were grown in 4, 5, or 6 in. diam plastic pots filled with a standard soil mixture of sand: sandy loam soil:Metro-Mix 200 A (1:1:1). Horticultural and agronomic plants, except for tomato, were seeded 3 to 5 per pot, then selectively thinned to one plant per pot after one week. Tomatoes were germinated in vermiculite filled flats, then selectively transplanted to pots, one seedling per pot, seven to ten days after germination. Native plants were collected from Merritt Island, shipped bare rooted or as cuttings to Raleigh, N. C., and potted, one plant per pot, in the standard soil

mix. Slash pine (1 year seedlings), citrus (3-5 ft trees) and live oak (2-3 ft trees) were purchased from nurseries in the Merritt Island area and shipped to Raleigh as potted plants. Plants were watered as needed and, except as noted below, were fertilized once a week with 100 ml per pot of a solution containing 10 gm of VHPF (6:25:15 of NPK with micronutrients) in 3.8 liters of water. The trees were fertilized every 6 months with the recommended amount of Agriform (20:10:5 of NPK) time release pellets. They were also treated with the VHPF fertilizer solution, 300 ml per pot, approximately every other month.

Temperature in the greenhouse was controlled during the summer months by two Alpine coolers (evaporative water cooling) and during the winter months by a LP gas heater. Sunlight was augmented on cloudy days by auxiliary 1000 w multivapor halide lamps. The lamps were also used during the shorter winter days to extend the day length to 14 hours. For a complete listing of monthly averages and maximum and minimum temperatures and relative humidities in the greenhouse see Appendix 7.3.

Plants were grown to a certain size, physiological age, or chronological age depending on the plant being tested or the time of year of the exposure. Plants grown from seed were exposed, during warmer weather, at 14, 21, or 28 days after seeding depending on the species. During the cool winter months plants grew from seed at a slower rate; thus, these plants were exposed at a physiological age similar to that obtained by the plants in warmer conditions after 14, 21, or 28 days of growth. Trees and native plants were exposed after they became well established and had added at least 2 in. of new stem and leaf tissue and/or four new nodes on the primary shoots. Plants were selected for growth uniformity before each exposure and then randomly distributed over the treatments.

Plants were exposed in the four chamber, greenhouse exposure system between 1000 and 1400 hrs (3.2.1.). Geomet HCl analyzers were used to monitor the HCl concentration in all chambers. The instruments were calibrated routinely as discussed in 3.2.1.2. Exposure chamber concentrations of HCl were checked occasionally with a bubbler and subsequent chloride titration; the results were compared with the Geomet readings.

Light and humidity were monitored continuously in the control chamber during all exposures and temperature was monitored in all chambers. Metal halide lamps (1000 w), positioned over each chamber, were lighted during all exposures. Table 10 summarizes the chamber conditions by the seasons of the year for all exposures.

Screens to HCl - Plant species (7.1.) were screened to HCl by exposing them to four concentrations of HCl (i.e., 0, 10, 20, 40 ppm) for 60 min. Each screen was replicated 3 times over consecutive days with 2 duplicate plants in each replication (6 plants per treatment per plant species or cv). The experimental design (4 HCl conc) used 24 plants/species or cv. Each plant was graded for percent foliar injury 48 to 72 hr after the exposure.

Table 10. Mean environmental conditions (with standard deviations, SD) in the greenhouse exposure chambers for all exposures conducted during this project.[1/]

Environmental parameter	Average environmental conditions (SD) over time of year[2/]			
	Spring	Summer	Fall	Winter
Temperature, C°	28 (± 4)	32 (± 4)	29 (± 2)	25 (± 3)
Relative humidity, percent	74 (± 15)	74 (± 18)	52 (± 11)	59 (± 10)
Light intensity, microeinsteins/ cm^2/sec	700 (± 200)	754 (± 123)	632 (± 60)	542 (± 104)
N (sample #)	105	102	124	87

[1/] Each value is an average of all exposures conducted during the given months.

[2/] Spring - March through May; Summer - June through August; Fall - September through November; Winter - December through February.

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This was done using either individual leaves and rating them in 5% increments (0 to 100% including 1%) or by rating whole plants in 10% increments (0 to 100% including 5%). Injury was taken on individual leaves for all cultivated plants (except citrus) and for arrowhead and pennywort. All other readings were taken on whole plants.

Dose-response designs for HCl - After the screen, a selected group of species and cvs were exposed to HCl in dose-response designs. Each plant form (species or cv) was exposed to 4 HCl concentrations over 4 times periods (either 15, 30, 60, and 120 min or 10, 20, 40, and 80 min). The HCl concentrations were determined from the results of the screens. The most frequently used concentrations were 0, 4, 8, and 16 ppm; 0, 5, 10, and 20 ppm; or 0, 10, 20, and 40 ppm. Each dose-response design was replicated 3 times over consecutive days with 3 duplicate plants in each replication (9 plants per treatment per plant species or cv). The 4 x 4 factorial design used 144 plants/species or cv. The plants were graded for percent foliar injury 48 to 72 hr after the exposure, as in the HCl screens. In addition, those plants that were assigned a total plant injury value were divided into three segments (top, middle, and bottom) and the percent foliar injury, the range of individual leaf injuries, and the number of leaves injured were determined within each segment. All plants were harvested 7 days after the exposure and shoot dry wt, root dry wt, fresh wt of radish roots, and total plant dry wt of pennywort were measured. In selected experiments with tomato (Betterboy) and snapbean (BBL 290)

flower and fruit number and fruit fresh weight were measured. Plant materials were dried in a forced air oven at 70°C for 72 hr before the dry wt measurements were taken.

Representative results of both the screen and dose-response designs were analyzed for statistical significance by an analysis of variance. Treatment means were separated by use of LSD values at the 0.05 level of significance. Data for each plant type were analyzed separately. Results from other experiments were summarized or placed on computer cards for future analysis.

4.2.1.2. Results

Foliar injury - The primary acute symptom of foliar injury to most plant species exposed to HCl was a bifacial interveinal necrosis. Necrotic areas were generally large and irregular, and white to off-white in color. The first injury to most plants was tip and marginal necrosis with the injury progressing toward the base and midvein of the leaf after prolonged exposure. The necrosis appeared first as water soaked areas when the exposure concentration was high or the duration of exposure was long. This was often followed by chlorotic blotches, and after 24 to 48 hours the bifacial necrosis developed. When injury was slight, it generally occurred as light chlorotic or necrotic spots and/or stipple on the adaxial (upper) leaf surface. Pines showed a distinct, tan colored, tip necrosis of the needles that progressed toward the base with prolonged exposure to high concentrations of HCl. The symptoms of acute injury were similar to those produced by sulfur dioxide (SO_2) and nitrogen dioxide (NO_2).

We found some symptom variation to this general description. Trifoliate leaflets of soybean were first injured in the central region of the leaf blade with the injury progressing toward the margins during prolonged exposures. Young leaves of radish, snap bean and soybean which were expanding at the time of the exposure were often curled at the margins, crinkled across the blade surface, and usually did not obtain maximum expansion (growth). A few species (i.e., zinnia and snap bean) developed abaxial (lower) leaf surface glazing after exposure to lower HCl concentrations. Leaves of marsh elder developed a white glazing on both surfaces of the leaf that appeared to be restricted to the epidermal layer. Leaves of railroad vine developed an interveinal chlorosis and stippling of the upper leaf surface with very few leaves developing bifacial necrotic areas. Wax myrtle leaves developed dark brown necrotic areas, when injured, that were similar to fall senescence. Foliar injury on citrus leaves appeared initially on the lower leaf surface as small to large yellowish necrotic areas. After prolonged exposures to high HCl concentrations, these necrotic areas often progressed through the leaf forming the typical bifacial necrotic areas.

Screens to HCl - These were conducted on 36 plant species (49 plants, including cultivars). Results are shown in Appendix 7.2. Injury threshold (5%) for HCl concentrations for the 60 min exposures ranged from well under 10 ppm in the more sensitive species to >40 ppm in the most resistant. An average of plant injury over the three HCl concentrations used in the screens (control values were all zero and were not

used in this average) ranged from 64% in the most sensitive plant to 0 in the resistant plants. This value was used to help develop the sensitivity categories (Table 2). Horticultural and agronomic plants were generally more sensitive than the native plants with radish ('Comet') being the most sensitive of the group. Arrowhead, pennywort and groundsel were the most sensitive native species while glasswort, sea oats and smooth cordgrass were not injured at any HCl concentration used during the screens.

Plants were grouped into six relative susceptibility categories according to their response to HCl (Table 2). The rankings were arbitrary and were based on a reasonable separation of injury ranges for plants from our screening exposures (Appendix 7.2.) as defined in Table 2. The sensitive category contains only four cultivated species (6 cv). The moderately sensitive category has 10 species; seven are cultivated (12 cv). Four species were intermediate in sensitivity, including three native species. The moderately resistant (7 species) and resistant (11 species) were all native species except for tobacco and citrus. Four species were not injured. Cultivars of four species were listed in two different susceptibility categories.

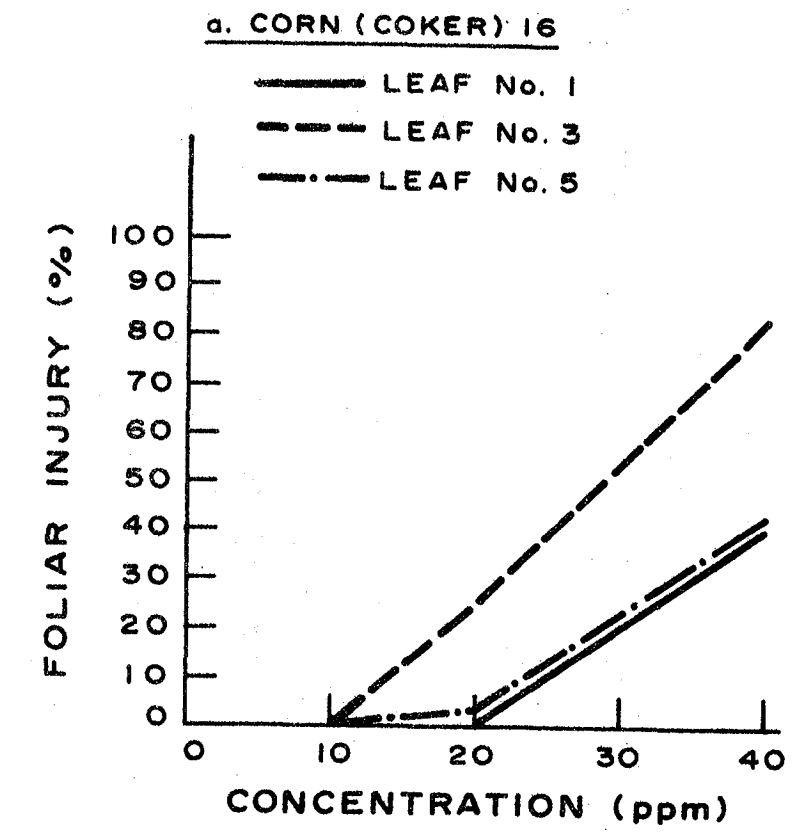
The screens clearly showed the variation in sensitivity of leaves based on leaf maturity (age). This was shown for all plants and results for corn and marsh elder are shown in Figure 27. Generally leaves that had just reached maximum expansion were the most sensitive of HCl. In marsh elder the older leaves tend to retain sensitivity while in most other species the older leaves lost sensitivity.

Other data from the HCl screens is not shown in the body of the report. Selected data is shown in Section 7.4.1. This data compares injury and biomass for tomato and summarizes more data on the variation of sensitivity of plant leaves.

Dose-response designs for HCl - Radish ('Comet') and soybean ('Dare') were the most sensitive to HCl of all the plants exposed in the dose-response designs (Table 11). There was a greater effect on radish root wt than on soybean shoot wt for a given percent foliar injury, but the variability of the radish data was greater than the soybean data. The biomass exposure data is presented as a percent reduction from the average of the combined controls, since the control data was not different for either species.

Similar data were developed for tomato (Betterboy) and corn (Silver Queen) in Table 12. In these species the 4 control (0 HCl) biomass measures were significantly different from each other but they were still averaged for comparative purposes. Essentially no significant biomass reduction was noted except at foliar injury values over 30%.

Similar data was developed for pennywort, arrowhead and marsh elder (Table 13), three of the most sensitive native species. The biomass data for these three species was handled as for the cultivated species. However, the growth rates of both arrowhead and marsh elder were so slow that no dry wt loss would be expected 7 days after an exposure, even with considerable injury. Similar data were obtained for wax myrtle and citrus but biomass changes were not reported since no wt changes occurred regardless of exposure concentration or duration. Injury data is shown for citrus and wax myrtle (Table 14).



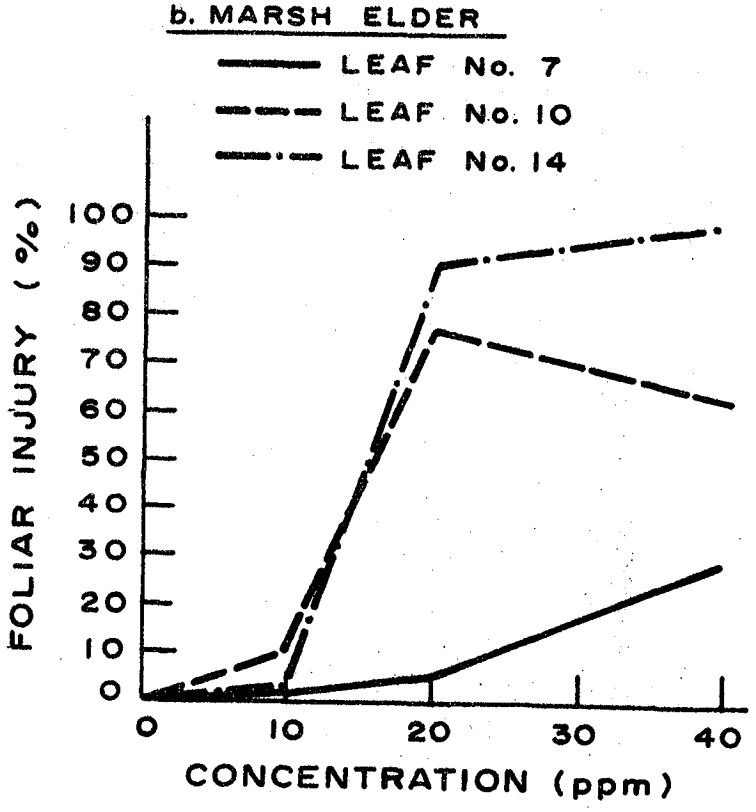


Figure 27. Effects of leaf maturity on leaf responses to a 60 min. exposure to HCl. a) Corn, leaves were counted from base to apex. b) Marsh elder, leaves were counted from apex to base.

Table 11. Foliar injury and biomass responses of radish and soybean as a function of HCl concentration and duration of exposure.[1/]

Exposure duration (min)	Foliar injury (%) at four HCl concentrations (ppm)				Biomass[2/] changes at four HCl concentrations (ppm)			
	0	5	10	20	0	5	10	20
Radish (Comet)					(Root fresh wt)			
10	0	+	6	49	2.65 g	21	28	85
20	0	3	36	66	(±0.25)	2	70	81
40	0	5	49	91		0	77	77
80	0	16	89	98		47	77	92
(LSD - 0.05, 7.7%)					(LSD - 0.05 = 1.5 g, 56.6%)			
Soybean (Dare)					(Shoot dry wt)			
10	0	0	+	9	3.48 g	0	0	14
20	0	0	1	70	(±0.13)	0	0	37
40	0	+	14	76		0	8	40
80	0	6	69	94		0	34	54
(LSD - 0.05, 7.6%)					(LSD - 0.05 = 0.4 g, 11.5%)			

1/ These designs included 3 duplicates and 3 replications for each treatment for a total of 9 plants per treatment and 144 plants per design. Radish were exposed once at 14 days of age, visual injury was determined at 16 days and plants were harvested at 21 days. Soybean were exposed once at 21 days of age, visual injury was determined at 23 days and plants were harvested at 28 days.

2/ The control data were not different for either species. Thus the average control biomass (for the 4 time periods) was used with the standard deviation in ( ). All exposure data is presented as a percent reduction from the average of the combined controls, the 0 values were either the same as or greater than the controls, but not different from the controls.

Table 12. Foliar injury and biomass responses of tomato and corn as a function of HCl concentration and duration of exposure.[1/]

Exposure duration (min)	Foliar injury (%) at four HCl concentrations (ppm)				Biomass[2/] changes at four HCl concentrations (ppm)			
	0	5	10	20	0	5	10	20
Tomato (Betterboy)					(Top fresh wt)			
10	0	0	0	21	15.0 g	5	22	16
20	0	0	3	20	(±1.31)	12	16	4
40	0	1	4	35		15	1	26
80	0	5	10	47		6	4	26
(LSD - 0.05, 4.8%)					(LSD - 0.05 = 2.55g, 17%)			
Corn (Silver Queen)					(Top dry wt)			
10	0	0	+	29	2.45 g	31	2	18
20	0	0	2	35	(±0.40)	18	2	10
40	0	+	3	31		10	0	18
80	0	+	5	67		0	6	39
(LSD - 0.05, 6.4%)					(LSD - 0.05 = 0.4g, 16.3%)			

1/ These designs included 3 duplicates and 3 replicates for each treatment for a total of 9 plants per treatment and 144 plants per design. Tomato plants were exposed once at 28 days of age, visual injury was determined at 30 days and plants were harvested at 35 days. Corn plants were exposed once at 21 days of age, visual injury was determined at 23 days and plants were harvested at 28 days.

2/ The control data were different for both species. However, the average control biomass (for the 4 time periods) was used with the standard deviation in ( ). All exposure data is presented as a percent reduction from the average of the combined controls, the 0 values were either the same as or greater than the controls, but not different from the controls.

Table 13. Foliar injury and biomass responses of three native species as a function of HCl concentration and duration of exposure.[1/]

Response measure	Time (min)	Pennywort				Arrowhead				Marsh Elder			
		HCl Concentration (ppm)											
		0	10	20	40	0	8	16	32	0	8	16	32
Injury (%)	10	0	+	9	23	0	+	1	7	0	0	+	2
	20	0	1	11	72	0	+	1	31	0	0	1	22
	40	0	4	60	94	0	+	2	55	0	+	2	34
	80	0	49	88	98	0	1	26	85	0	1	5	52
LSD at 0.05			8.2%				7.9%				4.2%		
Top dry wt[2/]	10	3.43 g	15	15	7	2.75 g	9	17	16	0.33 g	8	8	8
	20	(±0.15)	15	18	30	(±0.29)	0	16	16	(±0.043)	8	8	38
	40		0	30	30		5	9	16		38	8	38
	80		30	27	36		9	5	20		8	8	38
LSD at 0.05			23%				15%				31%		

[1/] Each design included 3 duplicates and 3 replications for each treatment for a total of 9 plants per treatment and 144 plants per design. Plants were exposed after they became well established (at least 2 in. of new growth and/or four nodes were added to the primary shoot); visual injury was determined 48 to 72 hr after the exposure and plants were harvested seven days after the exposure.

[2/] The control data were not different except for arrowhead (the 40 min control was significantly greater than the others). Thus the average control biomass (for the 4 time periods) was used with the standard deviation ( ). All exposure data is presented as a percent reduction from the average of the combined controls. There were no biologically significant changes in arrowhead or marsh elder.

Table 14. Foliar injury to citrus and wax myrtle as a function of HCl concentration and duration of exposure.[1/]

Exposure duration (min)	Foliar injury (%) at three HCl concentrations (ppm)			Exposure duration (min)	Foliar injury (%) at three HCl concentrations (ppm)		
	40	60	80		10	20	40
Citrus (2 species)							
20	0	+	+	10	+	1	15
40	0	2	5	20	+	3	21
80	1	9	23	40	1	5	53
				80	1	12	45
(LSD - 0.05, 11.7%)				(LSD - 0.05, 10.7%)			

[1/] The citrus design included 2 duplicates (an orange and a grapefruit) for each treatment and was replicated 3 times for a total of 6 plants per treatment and 96 plants in the design. Plants were exposed once at 48 days after the spring flush. Visual injury was determined 48 to 78 hr after the exposure. The two species were combined because no differences in their response was found in the preliminary screen.

The wax myrtle design included 3 duplicates and 3 replications for each treatment for a total of 9 plants per treatment and 144 plants in the design. Plants were exposed after they became well established and visual injury was determined 48 to 72 hr after the exposure.

The + signifies less than 0.5% average injury.

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The injury data for most native species and citrus suggests a real difference in the sensitivity of the upper and middle portions of the plant. Data for wax myrtle was obtained and is shown in Appendix 7.4.2. Variation in the sensitivity of leaves as a function of age was noted for all the cultivated plants. Data for individual leaves of soybean is shown in Appendix 7.4.2.

Tomato (Betterboy) and snap bean (BBL-290) had reduced flower number and fresh fruit weight from control plants when the total foliar injury was greater than 50%. These reductions were not statistically significant due to large variations in the measurements between plants but appeared to be biologically significant. Data on tomato fruit number is found in Appendix 7.4.2.

Considerable dose-response data was developed in this project. The data shown in the body of the report is representative of all data collected. Additional data has been summarized in Appendix 7.4.2.

4.2.1.3. Discussion

Vegetation injury induced by HCl is similar to that caused by sulfur dioxide, NO_2, and ammonia, except that ammonia rarely bleaches white. The symptoms generally are acid-type necrotic lesions. Some variation in symptomology was found, probably due to differences in exposure concentration and/or species. Plants such as railroad vine developed stippling reminiscent of ozone injury. Zinnia and snap bean (to a lesser degree) developed lower surface glazing reminiscent of PAN type injury.

The range of HCl concentrations that injured plants agreed with the range in which similar injurious effects to various plants were reported by Shriner and Lacasse (63), Means and Lacasse (54), Lerman et al. (47), and Heck et al. (32). The concentrations causing injury to even the most sensitive plants exposed during this study were at least an order of magnitude higher than those reported by Guderian (23) in his acute exposures, even for plants he referred to as more resistant. The reason for the discrepancy between Guderian's results and those of the researchers in the U. S. is not presently known. However, the repeatibility of our results suggests that the Germans either miscalculated the HCl concentrations or were not able to accurately monitor their chamber HCl concentrations. We did not do chronic exposure so we have no comparative studies to Guderian's chronic exposures.

The concentration of HCl that is required before injury induction is high compared to the air pollutants of major concern in the U. S. Ozone can cause similar injury severity at concentrations of at least one order of magnitude less than that of HCl. Chlorine and SO_2 are injurious to most plants at concentrations that are 25 and 20%, respectively, that of HCl. Ammonia and nitrogen dioxide are thought to cause injury at about the same concentrations as HCl.

The sensitivity of the various species to HCl was variable. Cultivated plants were generally more sensitive than native species. Trees and woody shrubs such as citrus, live oak, slash pine, and wax myrtle were very resistant to HCl. The variability in response between species was not always predictable from the response of the species to other pollutants. For example, Bel W_3 tobacco is extremely sensitive to ozone but was resistant to HCl. Variability in response was also apparent between certain cultivars of the same species (i.e., tomato and soybean).

An equal dose (concentration x duration of exposure) of HCl did not cause the same amount of injury. Concentration was much more important than time for the times and concentrations used during this study. This response is similar to that observed on plants exposed to other air pollutants (28).

Growth and yield were not significantly affected until the injury was extensive. In most instances growth or yield responses were not statistically significant until injury was at least 30-40% of the total leaf area. The data suggests that injury is a more reliable indicator of an effect than growth responses. This is related to both the specificity of the injury response and the greater variability in all growth measurements. Growth variability is expected but probably masks real plant

responses to HCl. In the dose-response designs, averaging the biomass data for the four control groups (with their SDs) tends to reduce the variability seen but may also suggest real effects when none are present. Growth was not stimulated by HCl in any of the designs.

The most sensitive leaves to HCl were the ones that had just completed full expansion. The mature leaves were less sensitive than the recently expanded leaves but more sensitive than the youngest or expanding leaves, which were the most resistant leaves.

The HCl concentration that caused threshold injury to the most sensitive species (radish) was about the same as the maximum expected at ground level from the SRM exhaust from launches of the shuttle at KSC; HCl concentrations could reach 6 ppm for 10 minutes. This exposure may occasionally injure the most sensitive radish plants. For the most sensitive plant species found on Merritt Island, the injury threshold for HCl could be as high as 15 ppm for 10 min exposures. The HCl concentrations are higher before significant biomass reductions are found on cultivated and native species. However, these effects may be masked because of the variability in biomass responses.

4.2.2. Environmental factors - their effects on the response of radish to HCl

4.2.2.1. Methods and materials

Radish ('Comet') were seeded in 4 in. diam plastic pots and thinned to one per pot five days after seeding. The soil mixture, fertilizer, and horticultural procedures were identical to those used in Section 4.2.1. Plants were exposed 14 days after planting. At this time, they generally had two cotyledons, three fully expanded leaves, and one partially expanded leaf.

Greenhouse conditions are summarized in Appendix 7.3. and chamber conditions in Table 10, unless specified otherwise. Five experiments were conducted in the course of a year to investigate the effects of certain environmental parameters on the response of radish to HCl. The data, when analyzed, were analyzed for statistical significance by an analysis of variance. Treatment means were separated by use of LSD values at the 0.05 level of significance.

Time of day and time of year - Four groups of 14 day radish plants (5 duplicate plants per treatment) were exposed to 0, 5, 10, or 20 ppm of HCl for 60 min. This sequence was repeated with matching groups of plants every other hr over a single 15 hr time period starting at 0600 and finishing at 1600 hrs (8 exposure times) for 32 treatments (160 plants) per day. The design was repeated three times during one year (in August, January, and May). The 1000 hr exposure was repeated three times over consecutive days during each of the three month exposure times.

Relative humidity (RH) - Fourteen day radish plants (6 duplicate plants per treatment and 3 replications) were exposed to 0, 5, 10, or 20 ppm of HCl for 40 min. Two chamber humidities were used [ambient (averaged 69%) and >95%] for 8 treatments and 72 plants in the complete design.

Pre- and post-misting - Fourteen day radish plants (3 duplicate plants per treatment and 3 replications) were exposed to 0, 5, 10, or 20 ppm of HCl for 40 min. Three sets of plants were misted to the drip point with water from an atomizer: the first set was misted before the exposure, the second set was misted after the exposure and the third set was not misted. This gave 12 treatments and 108 plants in the complete design.

Method of generating HCl - Fourteen day radish plants (3 duplicate plants per treatment and 3 replications) were exposed to 5, 10 or 20 ppm of HCl (2 sets) or to 0 ppm of HCl (1 set) for 40 min. The HCl was generated either from the dry 25% HCl in N_2 (set 1) or from a 0.1 molar hydrochloric acid solution (set 2). This gave 7 treatments and 63 plants in the complete design. The acid solution was dispensed in the chamber using a sonic atomizing nozzle to produce an acid aerosol condition. The dry HCl was a gas, at least in its initial generation state. The three replications for the same concentration were run on the same day; the three HCl concentrations were run over three consecutive days.

Soil moisture - Radish plants were grown under identical soil moisture conditions for 14 days. The plants were watered uniformly and divided into 5 groups. Group 1 was not watered again, group 2 was last watered on day 16, group 3 on day 18, group 4 on day 20 and group 5 on day 22, the day of the exposure. Three plants from each soil moisture treatment were exposed to 0, 5, 10, or 20 ppm HCl for 20 min. The design was replicated 3 times for a total of 9 plants per treatment. This gave 20 treatments and 180 plants in the complete design.

In these five experimental designs, the plants were placed into the exposure chambers that were preset to the desired HCl concentration. The plants were removed before the system was turned off and returned to the greenhouse bench. Injury was determined 48 to 72 hr after the exposure (4.2.1.1.). When harvest data was taken, the plants were harvested 7 days after the exposure and top dry wt and/or root fresh wt were measured.

4.2.2.2. Results

The response of 14 day radish plants to HCl was affected by both the time of day and the time of year of the exposure. Plants exposed at 1000 hrs during May and August had a similar percent foliar injury at each HCl concentration. Plants exposed in January had from 34 to 65% less foliar injury than the average injury for the May and August exposures at each HCl concentration (Table 15). A graph of foliar injury vs time of day (Figure 28) for each of the three HCl concentrations shows a single significant deviation (drop) in injury at 1600 hrs. One aspect of these curves that is worth noting is that they do not reflect a diurnal cycle; injury at night was equal to or greater than that which occurred during the day. There were no two or three way interactions across concentration, time of year or time of day.

Radish ('Comet') exposed at two relative humidities (54% and >95%) had significantly more injury at the higher humidity for the 2 highest HCl concentrations (Table 16). Plants exposed to 5 ppm HCl showed the same trend in injury response but the difference was not significant.

Table 15. Foliar injury to 14 day radish ('Comet') exposed to HCl at three different times of the year.[1/]

Time of year	Foliar injury (%) at three HCl concentrations (ppm)		
	5	10	20
January	6	19	61
May	13	56	92
August	11	53	92
LSD - 0.05	4	10	9

[1/] Each value is an average of 15 plants (5 duplicates and 3 replications). The plants were exposed for 60 min starting at 1000 hrs. The replications were on 3 successive days.

Table 16. Foliar injury to 14 day radish ('Comet') exposed to HCl at two levels of relative humidity during exposure.[1/]

Relative humidity (%)	Foliar injury (%) at four HCl concentrations (ppm)			
	0	5	10	20
69 (ambient)	0	1	44	60
>95	0	5	52	69
(LSD - 0.05, 5%)				

[1/] The plants were exposed for 40 min. Each value is an average of 18 plants (6 duplicates and 3 replications).

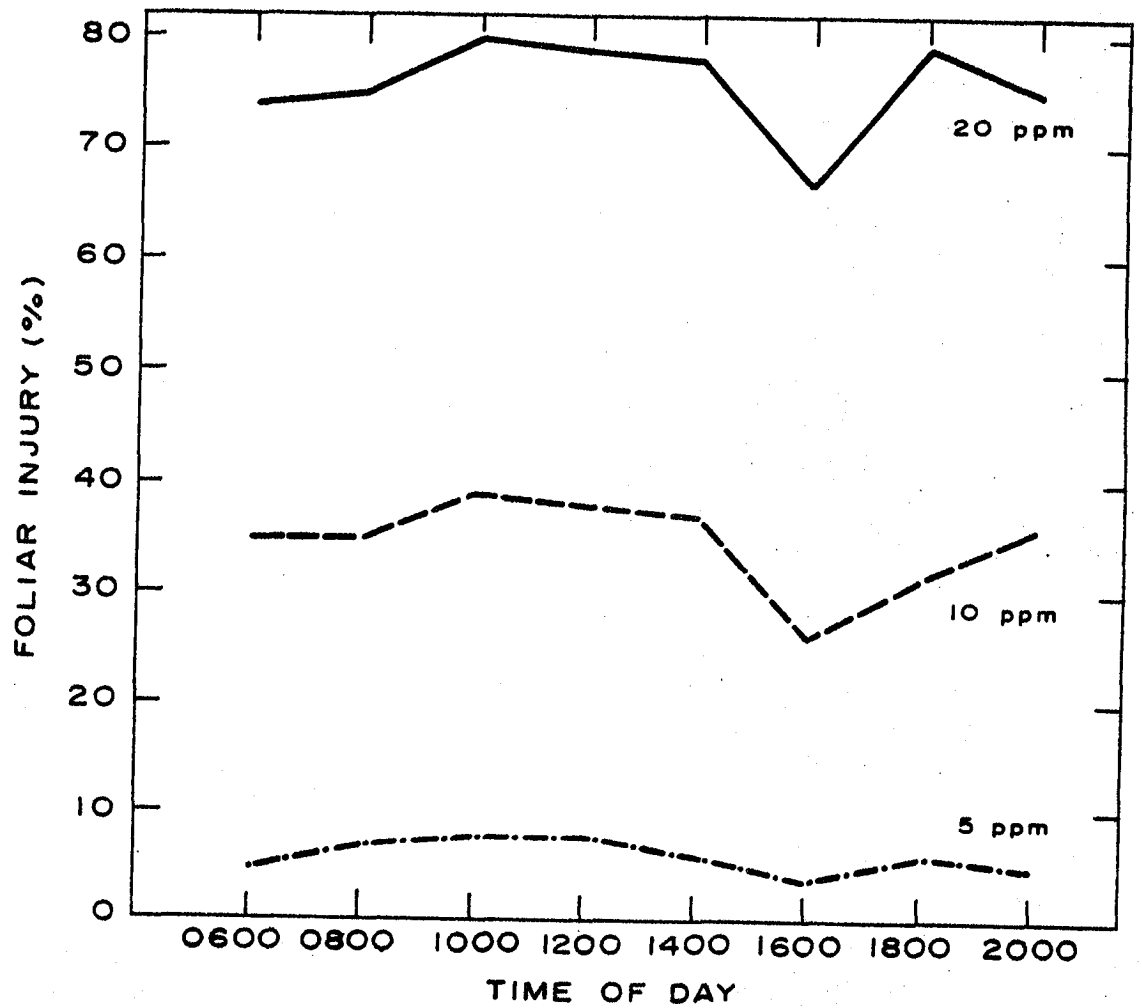

Figure 28. Foliar injury response curves of 14 day radish ('Comet') exposed to three different concentrations of HCl at eight times during the day. The plants were exposed for 60 min at three different times of the year. Each value is an average of 15 plants (5 duplications, 3 times of the year). Each concentration was analyzed separately. The LSD values are: 5 ppm = 4%, 10 ppm = 8%, 20 ppm = 8%.

Radish ('Comet') with their leaf surfaces covered with a fine mist just prior to exposure had significantly more foliar injury after exposure to any of three HCl concentrations than plants not misted prior to the exposure (Table 17). Misting the foliar surface after the exposure did not alter the response of the plants at any of the three HCl concentrations. Changes in fresh wt of the edible radish root were related to the severity of injury from HCl.

Exposure of radish ('Comet') to similar chamber concentrations of HCl generated by different methods produced significant differences in foliar injury (Table 18). Plants exposed to 10 or 20 ppm HCl generated by atomizing an 0.1 molar hydrochloric acid solution were about half as sensitive as plants exposed to HCl generated from dry gas.

Radish ('Comet') watered daily through the day of exposure (22 days of age) were injured more than plants under any of the four soil moisture stress conditions (Figure 29). Plants without added water 2, 4 or 6 days before exposure (16, 18, or 20 days after planting) had similar amounts of foliar injury. However, plants without water for 8 days (14 days after planting) had significantly less injury than plants at any of the other soil moisture treatments. These plants showed signs of wilting at the time of exposure. The results were similar for each of the three HCl concentrations.

Radish ('Comet') exposed under conditions of high humidity or with the foliar surface moist with water or during early morning or evening exposures, developed a red color around the leaf margins and in the area where the petiole is attached to the blade. The red color developed early in the exposure. Reddish-brown to brown necrotic areas appeared on the upper leaf surface without the development of water-soaked areas. Injury development was not marginal or in the midvein area but was more random. Petioles were often severely necrotic and immature partially expanded leaves were just as, if not more, sensitive than the leaves that had just reached their full expansion. Many necrotic areas were obviously associated with points of standing water on the leaf surface. These foliar injury symptoms were identical to those that developed from spraying the plant with hydrochloric acid.

4.2.2.3. Discussion

Exposure of plants to HCl, under conditions conducive to high moisture levels on or around the foliar surface, caused more foliar injury than would be expected on plants exposed under drier conditions. The increased leaf surface moisture could be due to high relative humidity, surface water (i.e., dew) or both. Spraying plant leaves prior to an exposure with HCl caused more foliar injury than when leaves were not sprayed even though the chamber relative humidity was about 50%. Yet the exposure of plants to HCl generated by an acid aerosol resulted in dramatically less foliar injury (and a different type of injury) than in plants exposed to similar HCl concentrations generated from dry gas; when the exposures were conducted under a relative humidity of 30 to 50%. This suggests that the enhanced foliar injury due to elevated moisture levels is probably related to a physiological plant response to the moisture or to the absorption of HCl by the water or the leaf surface, and not to the adsorption of HCl by moisture droplets in the general atmosphere of the chamber.

Table 17. The response of 14 day radish ('Comet') exposed to HCl as affected by foliar misting (pre- and post-exposure).[1/]

HCl concentration (ppm)	Mist treatment					
	Foliar injury (%)			Fresh root wt[2/]		
	No mist	Pre-	Post-	No mist	Pre-	Post-
0	0	0	0	5.62 g (± 0.18)		
5	2	11	2	6	0	5
10	13	54	16	7	35	7
20	77	92	69	46	78	47
LSD - 0.05		8.8			13.9	

[1/] The plants were exposed for 40 minutes. Each value is an average of 9 plants (3 duplicates and 3 replications).

[2/] The 3 sets of control plants were not different. Thus they are averaged and the standard deviation shown ( ). The other values are percent reductions from the average control value. The 0 value was greater than the average control value but was not different from the control.

Table 18. Foliar injury to 14 day radish ('Comet') exposed to similar concentrations of HCl generated by two methods.[1/]

Method of generation	Foliar injury (%) at four HCl concentrations (ppm)			
	0	5	10	20
HCl gas	0	1	30	87
Hydrochloric acid aerosol	0	1	16	49
(LSD - 0.05, 9%)				

[1/] The plants were exposed for 40 min. Each value is an average of 9 plants (3 duplicates and 3 replications). A single set of control plants was run for the two HCl series.

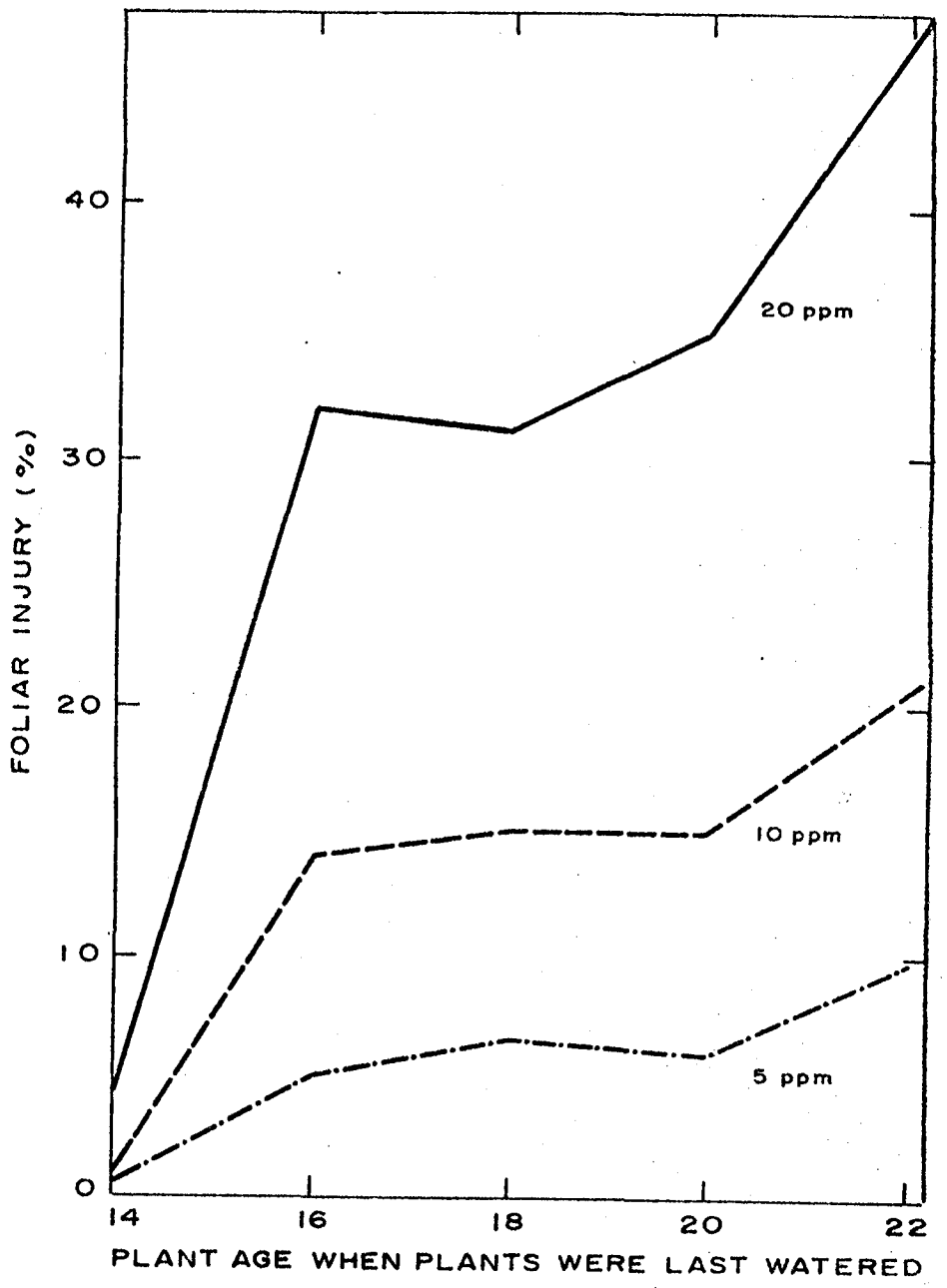

Figure 29. The effects of soil moisture on the response of radish 'Comet' exposed 20 min at three concentrations of HCl. The plants were exposed once at 22 days of age to HCl. There were 3 duplicates and 3 replications for a total of 9 plants per treatments. The treatment LSD at 0.05 was 6%.

The absence of a diurnal rhythm in the response of radish plants to HCl (Figure 28) first suggested the importance of humidity and moisture on plant response to HCl. Granett and Taylor (20) in similar studies did report a diurnal response cycle that was similar to those reported for other pollutants. The normal diurnal cycle is related to stomatal activity of the plant; the absence of such a cycle suggests that some mechanism in addition to stomates may control the entrance of HCl into the leaf tissue. Our data suggests that humidity and leaf moisture may enhance injury and override the stomatal control. The dip in injury in the response curves for time of day occurred at 1600 hrs (Figure 28). The normal closing of stomates at this time of day in addition to the low humidity could explain the days in response. The later evening and early morning exposures are associated with high humidity which evidently overrode the stomatal responses in this series of exposures. Light and temperature do not explain the diurnal response (Figure 28).

The development of foliar injury on plants exposed with moist leaf surfaces differs from that described in earlier HCl exposures (4.2.1). Water on the leaf surface may absorb atmospheric HCl and produce an acid type leaf injury. The water film or high humidity may also enhance cuticular absorption of HCl into the leaf. Both of these responses would be independent of stomatal activity. Thus, HCl may cause foliar injury by three different mechanisms; entrance of the gas through the stomates, a surface acid effect, and cuticular absorption. The foliar necrosis from any one of these mechanisms is practically identical 48 to 72 hours after the exposure.

The importance of these results for KCS is that plants will be injured more severely when their leaves are wet; and, night exposures of plants to HCl may be as injurious as daytime exposure. Plants will also be less sensitive during the winter than at other times of year (Table 15).

4.2.3. Multiple exposures to HCl

4.2.3.1. Methods and materials

Eight species were subjected to multiple exposures of HCl. Plants were seeded or propagated and grown in the greenhouse as described in section 4.2.1.1. Greenhouse conditions are summarized in Appendix 7.3. and exposure chamber conditions are in Table 10, unless specified otherwise.

The species used and their age at the first exposure were: seven day old radish ('Comet'); 14 day old soybean ('Dare'), snap bean ('BBL-290'), and corn ('Silver Queen'); 21 day old tomato ('Betterboy'); and, established pennywort, marsh elder, and sunflower (Section 4.2.1.1.). These plants were exposed to 0, 5, 10, and 20 ppm of HCl for 20 minutes. There were 3 duplicates per treatment and the design was replicated 3 times on the same day (9 plants/treatment/species). Multiple exposures were made seven days apart for a total of 2 or 3 exposures. The complete design included all combinations of exposures over the two (1, 2, 1 + 2) or three (1, 2, 3, 1 + 2, 1 + 3, 2 + 3 and 1 + 2 + 3) exposure times for

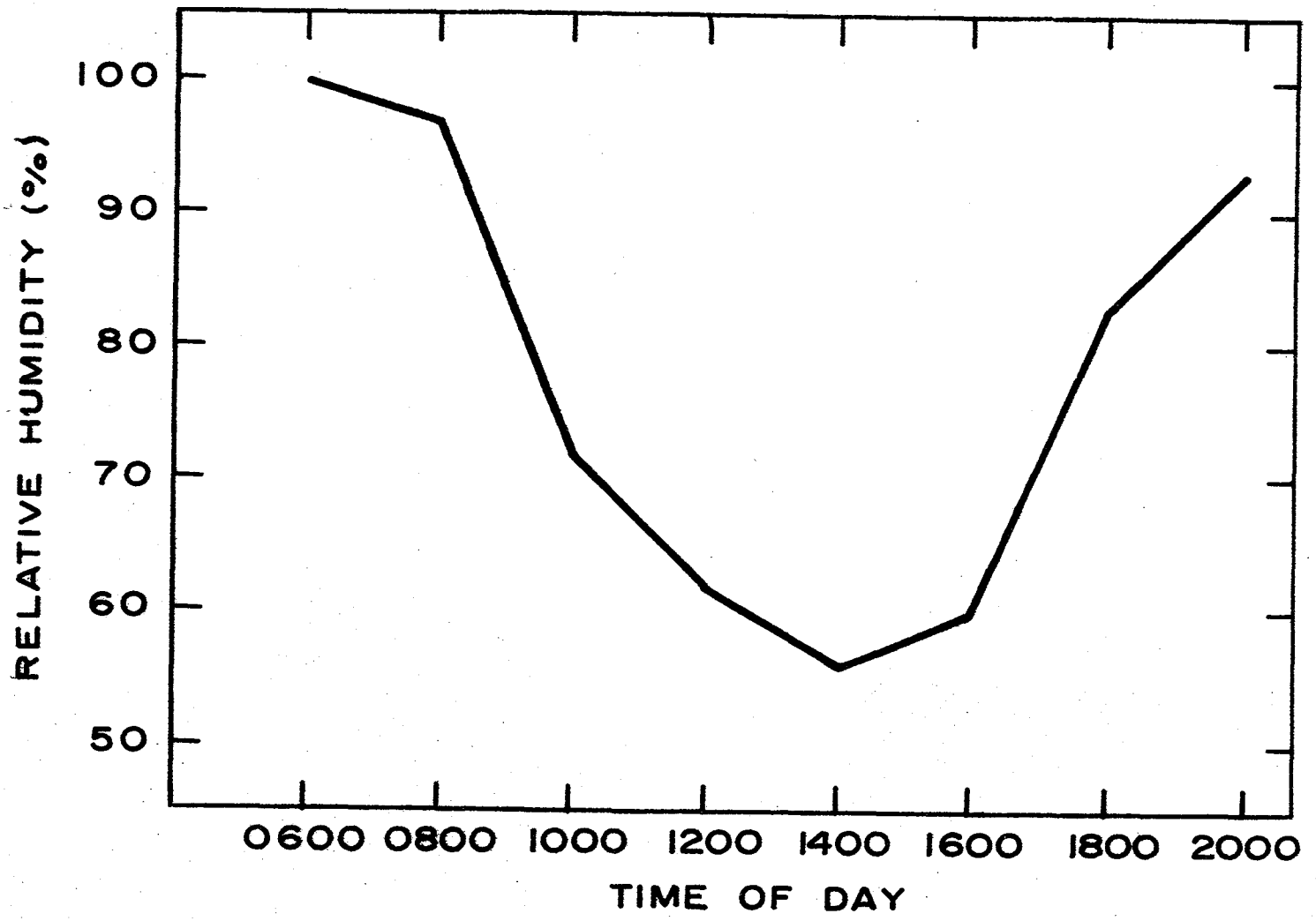

Figure 30. Greenhouse relative humidity (%) as a function of time of day when HCl exposures were conducted (see Figure 28).

three or seven treatments, respectively. Foliar injury was estimated 48 to 72 hrs after each exposure and 48 to 72 hrs after the final exposure. Injury was estimated for individual leaves or for the whole plant (4.2.1.1.). Plants for a given design were harvested 7, 14, 21, or 28 days after the final exposure; top dry wt and/or fruit fresh wt was determined. Statistical analysis (4.2.1.1.) were conducted on injury values taken 48 to 72 hrs after the final exposure and on the harvest measurements.

4.2.3.2. Results

Examples of results obtained are shown in Tables 19 and 20 for four of the cultivated plants using only data from double exposures, since the results for triple exposures were similar. The results, at the most, show an additive effect of multiple exposures from HCl. There was some correlation between foliar injury and loss of biomass in all plants studied.

Shoot dry wt for soybean and snap bean was less than the controls in plants exposed at both 14 days and 14 + 21 days to 10 and 20 ppm HCl (Table 20); for the 21 day exposures only soybean at the 20 ppm HCl was different than the control. Yield of snap bean was reduced in the same manner but the reduction was only significant for the 20 ppm exposure. Radish root wt was reduced by 5 ppm HCl in the double exposure. Dry weights for tomato (harvested at 56 days) are not shown since none of the effects were significant; however, plants exposed twice to 20 ppm were lighter than the controls.

Native plants exposed multiple times to specific HCl concentrations responded similarly to the agronomic species. Total foliar injury on pennywort 48 to 72 hours after the first exposure to 5, 10, and 20 ppm was approximately 2, 10, and 50%, respectively. After the second exposure, the plants which were not exposed again had recovered (Table 21), especially the ones exposed to 20 ppm. Plants exposed during both exposures had total foliar injury approximately equal to the sum of the injury of the single exposed plants (when read at the same time). Shoot dry wt was not statistically less than the controls at any treatment though those exposed to 20 ppm during the second exposure and during both exposures showed loss in weight (Table 21).

Plants exposed 3 times to a selected HCl concentration responded in the same manner as did the plants that were exposed twice. Corn and marsh elder (Table 22) had total foliar injury values after either 2 or 3 consecutive exposures that were approximately equal to a summation of injury recorded for similar plants exposed only once at the three times. Injury values for plants exposed only during the second exposure were similar to those recorded here for exposures 1 or 3. Similar data for a triple exposure of soybean to HCl shows similar results (14).

4.2.3.3. Discussion

Multiple exposures to different concentrations of HCl within a 2 to 3 week period had a simple additive effect on the plants tested. A previous exposure to HCl did not make a plant more sensitive or more resistant to

Table 19. Foliar injury to several plant species after multiple exposures to several concentrations of HCl.[1/]

Plant species	Exposure age (days)	Foliar injury (%) at three HCl concentrations (ppm)		
		5	10	20
Radish ('Comet')	7	2	21	94
	14	1	5	33
	7 + 14	5	30	99
	(LSD at 0.05 = 12.8%)			
Soybean ('Dare')	14	1	30	50
	21	0	1	28
	14 + 21	1	21	66
	(LSD at 0.05 = 7.5%)			
Snap bean ('BBL-290')	14	0	11	54
	21	0	6	34
	14 + 21	0	19	75
	(LSD at 0.05 = 5.4%)			
Tomato ('Betterboy')	21	0	1	12
	28	0	3	37
	21 + 28	0	3	36
	(LSD at 0.05 = 3.2%)			

[1/] Plants were exposed either once or twice for 20 min. Each value is an average of 9 plants (3 replicates and 3 duplicates). The replicates were on the same day. Injury was read at 16 days (radish), 23 days (soybean and snap bean) and 30 days (tomato). Injury values for the plants exposed only the first time reflect some recovery from the injury recorded 48 to 72 hours after that exposure.

Table 20. Biomass changes in several plant species after multiple exposures to several concentrations of HCl.[1/]

Plant species	Exposure age (days)	Weights (g) at four HCl concentrations (ppm)[2/] 0	5	10	20
Radish (Root fresh wt)	7	7.93 g	23	87	99
	14	(±0.45)	3	0	18
	7 + 14		41	72	100
(LSD at 0.05 = 33%)					
Soybean (Shoot dry wt)	14	9.17g	8	24	33
	21	(±0.25)	7	1	18
	14 + 21		5	31	50
(LSD at 0.05 = 13%)					
Snap bean (Shoot dry wt)	14	7.47g	0	17	53
	21	(±0.17)	0	0	16
	14 + 21		5	20	65
(LSD at 0.05 = 16%)					
Snap bean (Pod fresh wt, g)	14	23g	30	18	2
	21	31	26	19	25
	14 + 21	20	27	19	1
(LSD at 0.05 = 9.4 g)					

1/ Plants were exposed either once or twice for 20 min. Each value is an average of 9 plants (3 replications and 3 duplicates). The replicates were on the same day. Radish was harvested at 21 days, soybean at 28 days, and snap bean at 42 days.

2/ The control data were not different for the root and shoot wts. Thus, the average control biomass (for the three exposure ages) was used with the standard deviation in ( ). All exposure data is presented as a percent reduction from the average of the combined controls, the 0 values were either the same as or greater than the controls, not different from the controls. Snap bean fresh wt of pods was not handled in this way.

Table 21. Foliar injury and biomass responses of pennywort after multiple exposures to several concentrations of HCl.[1/]

Plant response	Exposure time[2/]	HCl concentration (ppm)			
		0	5	10	20
Foliar injury (%)	1	0	2	5	16
	2	0	2	4	57
	1 + 2	0	4	8	62
(LSD at 0.05 = 7.2%)					
Shoot dry wt (g)	1	2.6	2.7	2.3	2.4
	2	2.6	2.5	2.4	1.8
	1 + 2	2.6	2.6	2.3	1.6
(not significant)					

1/ Plants were exposed either once or twice for 20 min. Each value is an average of 9 observations (3 replications and 3 duplicates). The replicates were on the same day. Injury was read 48 to 72 hrs after the last exposure and dry wt was taken 7 days after the last exposure. The injury values for the first exposure time (age) reflect some recovery when compared with the injury recorded 48 to 72 hrs after that exposure.

2/ The first exposure was after the plants had reached a certain growth (4.2.1.1.); the second exposure was 7 days later.

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subsequent HCl exposures. Thus, if a plant was subjected to several exposures over several weeks, the additive effects would cause more injury to the plants than a single exposure. However, given a reasonable time between exposures (i.e., length of time would depend on growth rate of plant) plants should recover from a single exposure. Plants exposed very early in their life cycle or closer to fruit development are more likely to show an effect on yield than plants exposed during the mid-portion of their life cycle. Therefore, KSC should not expect significant changes in the response of native vegetation even if an exhaust cloud strikes the same area twice within the same year.

4.3. Al_2O_3 and Al_2O_3 Mixed with HCl

4.3.1. Methods and materials

Five species (radish, soybean, corn, pennywort, and marsh elder) were exposed to Al_2O_3 singly and in combination with HCl in a single

Table 22. Foliar injury to corn and marsh elder after multiple exposures to several concentrations of HCl.[1/]

Plant species	Exposure ages (day)	Foliar injury (%) at three HCl concentrations (ppm)		
		5	10	20
Corn	14	1	1	16
	28	1	1	11
	21 + 28	1	3	20
	14 + 21 + 28	1	6	39
(LSD at 0.05 = 2.5%)				
	Exposure time[2/]			
Marsh Elder	1	1	2	10
	3	1	2	9
	2 + 3	1	3	22
	1 + 2 + 3	1	8	38
(LSD at 0.05 = 3.8%)				

1/ Plants were exposed once, twice, or three times for 20 min. Each value is an average of 9 plants (3 relications and 3 duplicates). The replicates were on the same day. Injury was read 48 to 72 hrs after the last exposure. The injury values for the first and second single exposures reflect some recovery when compared with the injury recorded 48 to 78 hrs after those exposures.

2/ The first exposure was after the plants had reached a certain growth (4.2.1.1.); the other exposures were each separated by 7 days.

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chamber within the greenhouse exposure chamber system. A mixture of Al_2O_3 consisting of 90% by weight of the α-phase and 10% of the γ-phase was used in all exposures (3.2.1.3. and 3.3.2.). The average surface area for this mixture was 19 m^2/gm. The Al_2O_3 mixture was dispensed into the chamber inlet air stream at a pre-determined rate so as to add a constant amount of particulate over time (3.2.1.3.).

Plants to be exposed were grown in the greenhouse in 4 or 5 in. diam pots by methods described in section 4.2.1.1. They were exposed at the same chronological and/or physiological ages used in the HCl screens.

Three plants per species were exposed to 20, 40, and 80 mg/m^3 of Al_2O_3 for 60 min during each screen and three replications were run over time, for 9 plants per treatment. With only one chamber for particulate exposures, the different concentrations were run in sequence, one immediately following the other on the same day. Control plants were placed in an adjacent chamber during the exposure period.

The chamber was monitored during exposures by pulling air from the chamber through preweighed nucleopore membrane filters using 10 and 2 μm pore sizes in series. A known volume of air was sampled, the filters were removed and weighed, and the particulate weight was calculated. The particles on each filter size were examined periodically with a scanning electron microscope to determine particle size and to characterize the particulate (3.3.2.). Greenhouse conditions are summarized in Appendix 7.3. and exposure chamber conditions are in Table 10.

In the HCl plus Al_2O_3 exposures, plants were exposed to 10 or 15 ppm of HCl for 40 min with and without the addition of 20 or 35 mg/m^3 of Al_2O_3, respectively. The HCl concentrations were chosen because they had caused 10 to 30% foliar injury in the test plants in previous exposures. The Al_2O_3 concentrations were chosen to give approximately 2 HCl to 3 Al_2O_3 (weight ratios) of the two pollutants; this is similar to the ratios found in the rocket exhaust.

Four additional experiments were conducted to investigate the effects of Al_2O_3 + HCl mixtures on the sensitive radish, 'Comet'. Three duplicate plants and three replications were used in all four designs. 1) The leaves were covered to 4 different visual densities of particulate Al_2O_3 by hand sprinkling. The lightest particulate condition had the Al_2O_3 slightly visible on the leaf surface and the heaviest had the leaf surfaces totally covered with Al_2O_3. These plants were then exposed to 0, 5, 10, and 20 ppm HCl. 2) Four HCl concentrations (0, 5, 10, 20 ppm) were used with three Al_2O_3 concentrations (10, 20, 40 mg/m^3) in a factorial design. 3) In order to determine if leaves with wet surfaces responded differently to HCl and HCl plus Al_2O_3, the first experimental design (10 or 15 ppm or HCl for 40 min with and without the 20 or 35 mg/m^3 of Al_3Cl_2, respectively) was repeated using three misting treatments (no-mist, mist prior to exposure, mist after exposure). Plants were misted by spraying to drip with a hand atomizer. 4) The objective of this experiment was to determine if HCl, as hydrochloric acid, could interact with Al_2O_3 and cause effects to the exposed plants that would not be expected from either Al_2O_3 in water or the hydrochloric acid alone. Radish ('Comet') and soybean ('Dare') were the test plants. The four test solutions were: hydrochloric acid (pHs of 0.5, 1.0, 2.0 and 4.0), Al_2O_3 in water (0.5, 1.0, 2.0 and 4.0 gm per 100 ml of water), a mixture of each combination of the hydrochloric acid and Al_2O_3, and plain water (a total of 25 treatments). The test plants were sprayed to drip with a hand atomizer in this 5 x 5 factorial design. All solutions were mixed well before the plants were sprayed.

In all experiments, plant injury was taken 48 to 72 hours after the exposure in a manner described in Section 4.2.1. and the plants were harvested 7 days after the exposure. Standard statistical analyses were done on the data as described in 4.2.1.

4.3.2. Results and discussion

The Al_2O_3 alone did not affect any of the species tested even when the leaves had a dense coating. The particulate rested loosely on the leaf surface and was lost by plant movement or during plant watering. Exposures to Al_2O_3 after the plants were sprayed to drip with water, did not injure the leaves; nor, were the plants affected when sprayed with a slurry of water and Al_2O_3.

The Al_2O_3 did not alter the response of any of the tested species to HCl. The results with radish (Table 23) are similar to results found with the other test species. When plants were misted prior to exposure, they were more sensitive to HCl (Section 4.2.4.2.); no interactions were seen between Al_2O_3 and HCl. No Al_2O_3 by HCl interactions were found even when the Al_2O_3 was mixed with HCl and sprayed on the plants. In all cases, plants responded to the mixtures as they did to the HCl alone. Thus, Al_2O_3 from the SRM exhaust should not injure plants in the vicinity of the launch.

4.4. Solid Rocket Fuel (SRF) Exhaust

4.4.1. Dose response

4.4.1.1. Method and materials

Ten plant species were grown in the greenhouse in 5 in. diam plastic pots in the manner described in Section 4.2.1. The exposures were conducted in open top field chambers (26) that had been modified and closed with a top to simulate a CSTR chamber (3.2.2.). Hand cut strands of fuel, usually 0.5 x 0.5 x 6 in. in size were layed end to end within the grooves of the copper plates in the burn box. See Section 3.2.2.3. for a complete description of fuel preparation and usage.

Radish 'Comet' (14 day), soybean 'Dare' (21 day), corn 'Silver Queen' (21 day), citrus (grapefruit and orange) and 6 established native species (arrowhead, sunflower, pennywort, wax myrtle, marsh elder, and slash pine) were exposed to four concentrations of SRF exhaust for 10, 20, or 40 min. The SRF exhaust concentrations were reported in terms of the HCl present in the exhaust. Other exhaust components were presumed to be present in concentrations theoretically calculated for SRF exhaust having the measured HCl concentrations. We planned to use SRF exhaust containing 0, 10, 20 and 30 ppm of HCl but the actual exposures averaged about 0, 12, 24 and 35 ppm of HCl ($\pm$ 5 ppm). The HCl measurements were made at the outlet duct of each chamber using a Geomet HCl analyzer. The Al_2O_3 was occasionally measured during exposures as described in Section 4.3.1. The filters were weighed after the exposure and the particulate concentration calculated. The filters were also viewed with a scanning electron microscope to establish particle characteristics, size and distribution (3.3.2.).

The exhaust (HCl) concentration in the chambers did not remain steady at 12, 24 and 35 ppm. Fluctuations were due to unequal fuel sizes and "flashing" (multiple strand combination or combustion of several strands simultaneously) of the fuel especially around the turns of the copper

Table 23. Foliar injury and biomass changes in radish ('Comet') exposed to HCl and/or Al_2O_3.[1/]

Pollutant treatment[2/]	Misting treatment	Foliar injury (%)	Shoot dry weight (g)
Control plants		0	5.2
HCl	none	13	4.9
	pre-	54	3.7
	post-	16	5.2
HCl + Al_2O_3	none	11	5.1
	pre-	50	4.3
	post-	12	5.7
Al_2O_3	none	0	5.4
	pre-	0	5.2
	post-	0	6.0
LSD at 0.05		7	0.5

[1/] Plants were exposed for 40 min to each of the treatments listed. Each treatment is the average of 9 plants (3 duplicates and 3 replicates, the replicates were run on the same day).

[2/] Plants were exposed to 10 ppm of HCl and/or 20 mg/m^3 of Al_2O_3. Each pollutant treatment contained a pre-mist, a post-mist and a no-mist sub-treatment.

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plates. The two high concentration chambers (24 and 35 ppm HCl) were monitored continuously during the exposures with the Geomet analyzers. The average concentration for these chambers was calculated, using two minute readings, and the maximum and minimum values were obtained. The chambers maintained an average 24 and 35 ppm HCl concentration $\pm$ 5 ppm over approximately 80% of each exposure time. The minimum and maximum values for the 24 ppm chamber were 11 and 34 ppm, respectively, and for the 35 ppm chamber were 23 and 52 ppm, respectively. Since the 12 ppm chamber was read only occasionally (once every 20 min) we assumed that the concentration fluctuated similarly to the other two chambers. The periodic readings did show that the chamber generally maintained the desired exhaust concentration. The maximum and minimum concentrations occurred at most for 30 seconds or less during a given exposure. A similar quantity of fuel was burned during each exposure to insure the same dosage for each exposure.

The plants were placed into the exposure chambers just before ignition and removed immediately after the exposure was over. Temperature was recorded in one of the exposure chambers during each exposure, while light and humidity were measured at the beginning and end of each exposure in the same chamber. Temperature, light, and humidity were measured during the exposures in the area outside the chambers. Chamber temperature averaged 27°C during the exposure, light intensity averaged 520 me/cm^2/sec and relative humidity averaged 62%. The chamber air flow was maintained during the time the plants were in the chambers.

The 10 plant species were exposed one time using a dose-response design of four exhaust concentrations (0, 12, 24 and 35 ppm, as HCl) and three exposure durations (10, 20 and 40 min) for 12 exposure treatments. The four concentrations were run simultaneously and the three exposure durations were run in sequence on the same day starting with the longest time period. This sequence of exposures was replicated over three successive days. For each plant species, 3 duplicates per treatment were exposed during each replication for 9 plants per treatment and 108 plants per species for the experimental design. Plant injury was estimated 48 to 72 hrs after the exposure, as described in Section 4.2.1. Plants were harvested 7 days after exposure for biomass measurements. Data was handled using an analysis of variance with LSD's at the 0.05 level (4.2.1.).

4.4.1.2. Results

All injured plant species typically developed large water soaked areas on sensitive leaves immediately after the exposure. These areas became chlorotic and then formed irregular white to off-white, interveinal, bifacial necrosis. The injury usually formed first on the margins of the leaf apex. As the severity of injury increased, the injury symptoms were found toward the midvein portion of the leaf and then toward the base of the leaf. Most species developed a second symptom, if the dose was not too high, at the 12 and 24 ppm concentrations and at the short exposure for the 35 ppm. This symptom complex showed small necrotic and/or chlorotic foliar lesions; the necrotic spots were usually upper leaf surface but were occasionally bifacial. Several species developed pigmented stippling on the upper surface of a few leaves. Marsh elder developed a white glaze over both leaf surfaces and pine developed a tan needle tip burn on young candles. Citrus typically developed yellowish necrotic areas on the lower leaf surface and some bifacial necrotic areas at the highest HCl concentration.

Radish ('Comet') illustrates the response of the plants to the SRF exhaust. Injury generally increased with increasing exhaust concentration (Table 24). There was no strong relationship between injury and exposure duration for radish. This difference was probably associated with the timing of the exposures (thus environmental differences) or to the variation in concentration during the exposure. Loss in root fresh weight of radish was associated with the high injury responses (Table 24). Yield loss appeared to be directly related to injured foliar surface, with a 35 to 45% foliar loss usually necessary to cause a significant reduction in root growth.

Table 24. Foliar injury and biomass responses of radish ('Comet') as a function of SRF exhaust concentration and duration of exposure.[1/]

Plant response	Exposure duration (min)	SRF exhaust concentration (ppm of HCl)			
		0	12	24	35
Foliar injury (%)	10	0	17	54	62
	20	0	13	42	64
	40	0	34	57	74
(LSD at 0.05 = 8.3%)					
Radish root fresh wt (g)[2/]	10	7.33 g	0	67	66
	20	(±0.54)	11	25	67
	40		48	66	81
(LSD at 0.05 = 53%)					

[1/] This design included 3 duplicates and 3 replicates for each treatment for a total of 9 plants per treatment and 108 plants per design. Plants were exposed once at 14 days of age, visual injury was determined at 16 days and plants were harvested at 21 days.

[2/] The control data were not different. Thus the average control wt (for the three time periods) was used with the standard deviation in ( ). All exposure data is presented as a percent reduction from the average of the combined controls, the 0 value was not different from the control value.

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Soybean, corn, and citrus were less sensitive than radish to the SRF exhaust. These three species show a slight to good relationship between injury and exposure duration and thus reasonable dose-response results for both time and concentration. Usually a similar dose presented at a shorter time caused more injury than the same dose at a longer time (Table 25). The sensitivity order for the native plants is shown in Table 26. A significant shoot dry wt change was noted occasionally for radish, soybean and corn, but the changes were not consistent (7.4.3.). Percentage reductions were related to severity of injury and trends were similar to those shown for HCl. Plant growth was too variable to permit separation of small growth differences in the native species and citrus.

Table 25. Foliar injury to soybean, corn and citrus as a function of SRF exhaust concentration and duration of exposure.[1/]

Plant species	Exposure duration (min)	Foliar injury (%) at three SRF exhaust concentrations (ppm of HCl)		
		12	24	35
Soybean ('Dare')	10	4	21	44
	20	3	25	39
	40	23	41	54
(LSD at 0.05 = 7.9%)				
Corn ('Silver Queen')	10	0	1	5
	20	1	2	4
	40	2	4	11
(LSD at 0.05 = 1.9%)				
Citrus[2/]	10	0(0)	1(0)	0(0)
	20	1(1)	1(0)	3(1)
	40	1(0)	1(0)	8(3)
(LSD at 0.05 = 1.8%)				

1/ These designs included 3 duplicates and 3 replicates for each treatment for a total of 9 plants per treatment and 108 plants per design. Soybean and corn were exposed once at 21 days of age and visual injury was determined at 23 days. Citrus was exposed once during regrowth and injury was determined 48 to 72 hr after exposure.

2/ Values in ( ) are for a second exposure design.

Table 26. Foliar injury to several native species as a function of SRF exhaust concentration and duration of exposure.[1/]

Plant species	Exposure duration (min)	Foliar injury (%) at three SRF exhaust concentrations (ppm of HCl)		
		12	24	35
Sunflower	10	7	3	43
	20	2	18	51
	40	8	14	47
(LSD at 0.05 = 8.0%)				
Pennywort	10	4	11	20
	20	4	13	18
	40	12	14	44
(LSD at 0.05 = 7.0%)				
Arrowhead	10	0	0	7
	20	2	8	25
	40	1	16	35
(LSD at 0.05 = 5.6%)				
Marsh elder[2/]	10	0(2)	0(3)	18(4)
	20	3(2)	9(5)	41(12)
	40	4(4)	17(7)	51(22)
(LSD at 0.05 = 3.1%)				
Wax myrtle[2/]	10	0(1)	0(1)	1(1)
	20	0(1)	2(1)	5(1)
	40	1(1)	6(1)	11(2)
(LSD at 0.05 = 1.2%)				
Slash pine	10	0	0	0
	20	0	0	2
	40	0	1	4
(LSD at 0.05 = 1.2%)				

[1/] These designs included 3 duplicates and 3 replicates for each treatment for a total of 9 plants per treatment and 108 plants per design. Plants were exposed once after they became well established and visual injury was determined 48 to 72 hr after exposure.

[2/] Values in ( ) are for a second exposure design.

The portion of the plant that was most sensitive to the exhaust varied between species. Leaves just reaching full expansion were generally the most sensitive followed by older leaves; the young expanding leaves were least sensitive. Data for sunflower, wax myrtle and marsh elder support these observations and are shown in Appendix 7.4.3.

4.4.1.3. Discussion

The SRF exhaust concentration required to cause significant injury to sensitive species was higher than that expected to be present from the SRM exhaust cloud at ground level. The native plants from Merritt Island were not among the most sensitive species and thus should not be injured by expected ground level concentrations. The results of exposure of plants to SRF exhaust support the hypothesis that HCl is the primary toxicant in the exhaust gas. First, the relative sensitivity of the various species to SRF exhaust was similar to that for the HCl exposures (4.2.1.). Second, injury symptoms after exposure to the SRF exhaust were similar to those induced by HCl (4.2.1.). Third, recently expanded leaves were the most sensitive to both HCl and the SRF exhaust. Fourth, the concentration of HCl measured within the exhaust was similar to the HCl concentrations that caused comparable foliar injury to the test species.

One symptom variation that was noted in most species was an oxidant-like injury. Oxidant-like symptoms were noted on a couple of species after exposure to HCl; however, the symptoms were not as extensive and were only found on a few species. These symptoms may indicate the presence of chlorine (Cl_2) in the SRF exhaust. Whatever the causal agent, percent foliar injury was not different in the HCl or SRF exhaust at similar HCl concentrations.

4.5. Cooperative Project: Univ. of Calif., Riverside and N. C. State Univ. - HCl

We have past associations involving cooperative research with the Univ. of Calif., Riverside. They had an Army contract to study HCl effects on western plant species. Thus we felt it would be valuable to compare exposure systems and techniques using the same test species. This comparison was set up by W. M. Knott (N. C. State Univ. - NCSU) in cooperation with A. L. Granett (Univ. of Calif., Riverside - UCR) during May and early June of 1977.

4.5.1. Materials and methods

A complete dose-response design was run four times in this cooperative project. The two test species were radish ('Comet') and zinnia ('White Gem'). During the first week the design was run concurrently at NCSU (run 1) and UCR (run 2); the following week the design was run at NCSU (run 3) with both investigators present; the third week both investigators ran the design at UCR (run 4). Data were collected in a similar way during each run. Foliar injury (%) and biomass were the responses used and all data were recorded and analyzed in the same way.

Plants were seeded in 4 in. diam plastic pots and thinned to the one most uniform plant per pot 5 days after seeding for radish and 10 days for zinnia. Horticultural techniques were similar at both locations; they were standard for the respective laboratories. The NCSU methods are described in Section 4.2.1. The UCR methods differed in that: they used a sandy loam-redwood chip potting medium, and fertilized plants once a day with a 1/2 strength modified Hoagland's solution. Fourteen day radish and 21 day zinnia were exposed to 0, 5, 10, and 20 ppm HCl for 10, 20, 40, and 80 min (4.2.1.1.). Two duplicates and 3 replicates, over 3 consecutive days, were used for a total of 6 plants per treatment and 96 plants per species per run. The exposure chambers were preset for a specific concentration; plants were added at specific times to generate the required exposure durations (each exposure duration less than 80 min took the center portion of the 80 min exposure).

Plant injury was determined 48 to 72 hr after exposure by each researcher using both the NCSU and UCR methods of assessment. The NCSU method determined percent foliar injury to each leaf in 5% increments (0 to 100%, including 1%). The UCR method assigned each leaf a single numerical value from 0 to 4. This value was converted to a percentage by letting 1=12.5%, 2=37.5%, 3=62.5% and 4=87.5%. Foliar injury for a whole plant was the average of all leaves. Plants were harvested 7 days after the exposures; radish root fresh weight, and radish and zinnia shoot dry wts were taken.

4.5.2. Results

The investigators, who assessed injury at NCSU and UCR, showed general agreement in their rating whether using the NCSU method (Figure 31) or the UCR method (Figure 32). The data for zinnia suggested that the NCSU method gave slightly lower injury values when injury was less severe and slightly higher values when injury was more severe (Table 27). The results for zinnia also suggest that the NCSU investigator generally rated injury lower than the UCR investigator. When radish was used as the experimental plant, there was good agreement between the two methods of assessment, when used by the same investigator (Figure 33). A comparison of the injury developed on radish under the different conditions of the two locations showed that at both 10 and 20 ppm of HCl the plants were more severely injured at UCR (Figure 34).

4.5.3. Discussion

There were no major differences between rators, assessment methods or research locations as seen from the results of four identical experimental runs using radish and zinnia as test species. This was somewhat surprising since the investigators had not worked together prior to this experimental series and had not used each others method of assessing injury. However, we had hypothesized that these results might occur since the two laboratories have done collaborative research in the past.

The consistently higher injury readings by the UCR investigator for zinnia were due to the way he assessed the abaxial (under leaf surface) glazing which was normally found in zinnia and seldom found in radish.

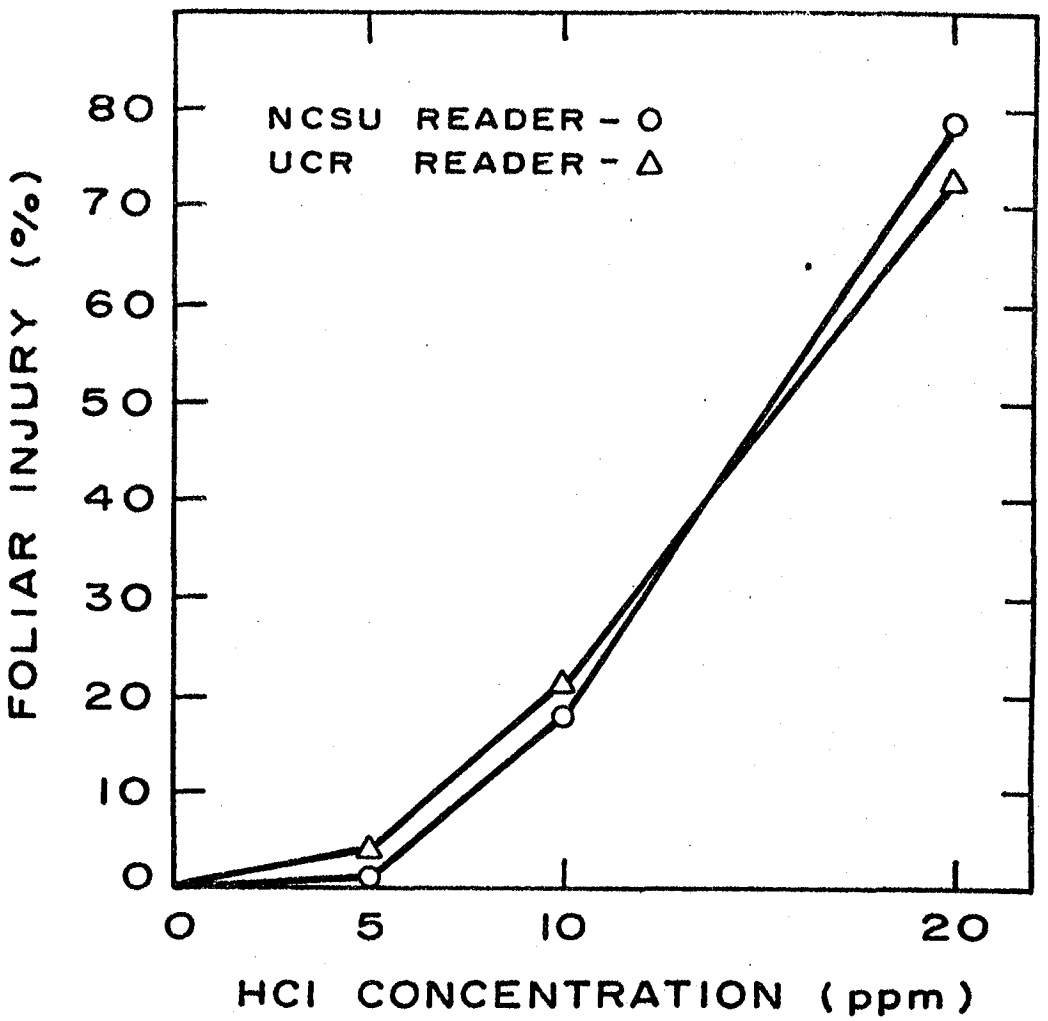

Figure 31. Estimates of radish foliar injury made by two researchers using the NCSU method on the NCSU campus. Data came from radishes exposed for 40 min during an experiment at NCSU, run 3.

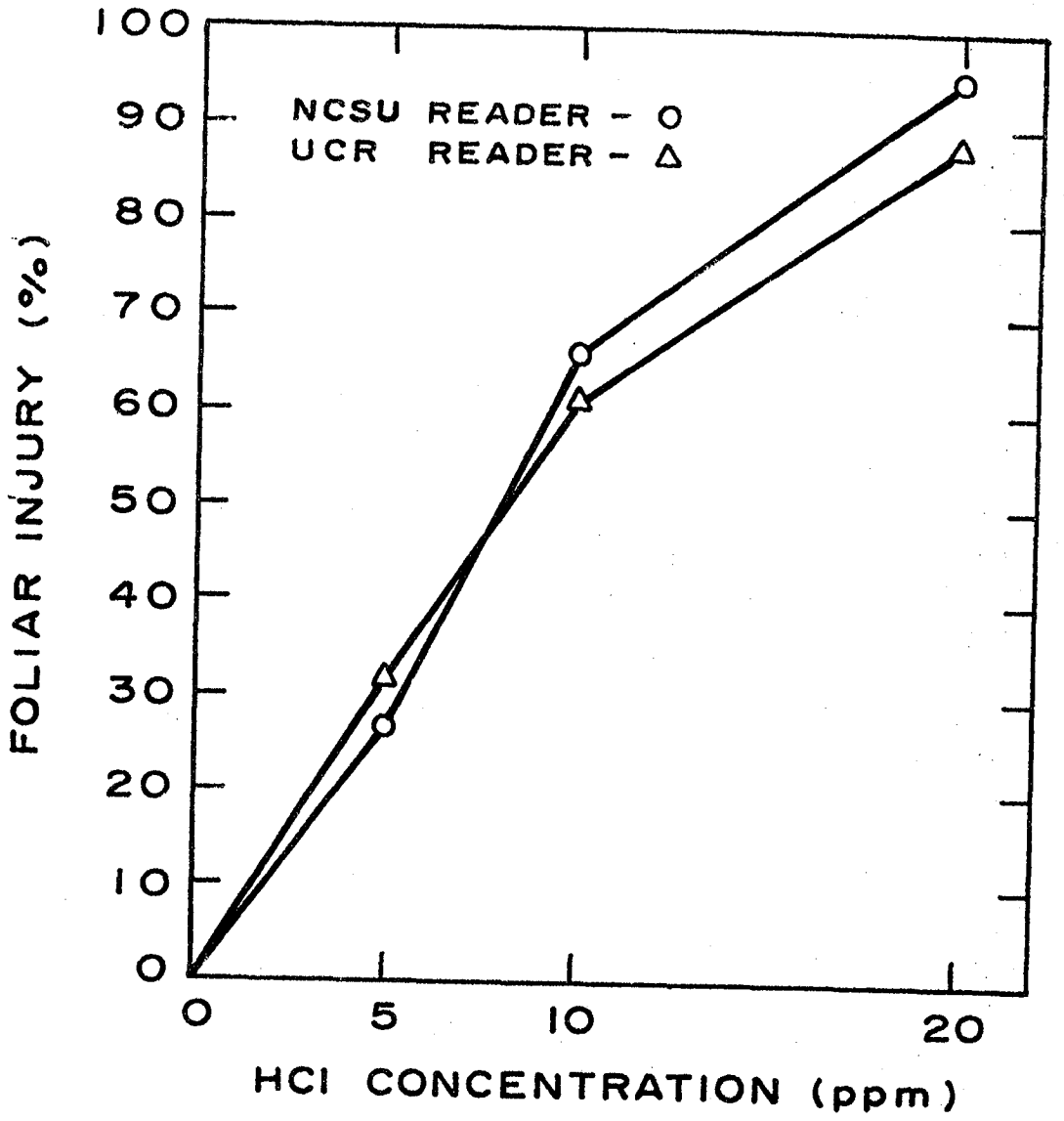

Figure 32. Estimates of radish foliar injury made by two researchers using the UCR method on the UCR campus. Data came from radishes exposed for 80 min during an experiment at UCR, run 4.

Table 27. Comparison of foliar injury assessment methods and investigators; zinnia exposed to HCl.[1/]

HCl dose		Foliar injury (%)[2/]			
		UCR method		NCSU method	
Concentration (ppm)	Duration (min)	UCR rator	NCSU rator	UCR rator	NCSU rator
5	10	+	+	0	0
5	20	+	+	0	0
5	40	6	2	+	+
5	80	10	6	2	2
10	10	1	1	+	+
10	20	11	7	1	1
10	40	26	15	9	9
10	80	35	22	17	13
20	10	30	16	16	10
20	20	52	43	46	40
20	40	67	63	74	67
20	80	70	63	77	68

[1/] Data is averaged over an experimental design at NCSU and one at UCR (runs 3 and 4). Each value is the average of 2 duplicate plants, 3 replications and 2 runs for 12 plants per treatment.

[2/] The NCSU method is a direct estimate of percent foliar injury. The UCR method uses a 0-4 injury index transplanted to percent injury.

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Differences between injury taken by the two methods could be attributed to the greater rounding-off of values by the UCR method and thus a lack of discrimination with this method when compared to the NCSU method. This difference was especially apparent when injury was not severe or was very severe. The UCR method tended to exaggerate light injury and under read severe injury. Differences between the two locations could be attributed to differences in plant sensitivities; probably due to the cloudy weather that occurred during the exposures at NCSU (Figure 34).

This series of experiments confirms that the results from HCl exposures made by the two laboratories are comparable.

4.6. Nitrogen Dioxide and Chlorine

The SRF exhaust contains both chlorine (Cl_2) and nitrogen dioxide (NO_2) at about 10% of the HCl concentration. Previous experimental work with Cl_2 (6, 7, 42) suggests that it might be more than 10 times as toxic to vegetation as HCl. Previous results with NO_2 (51) suggests that it is comparable to HCl in toxicity. Although research with these two phytotoxicants was not part of our responsibility, results from the SRF

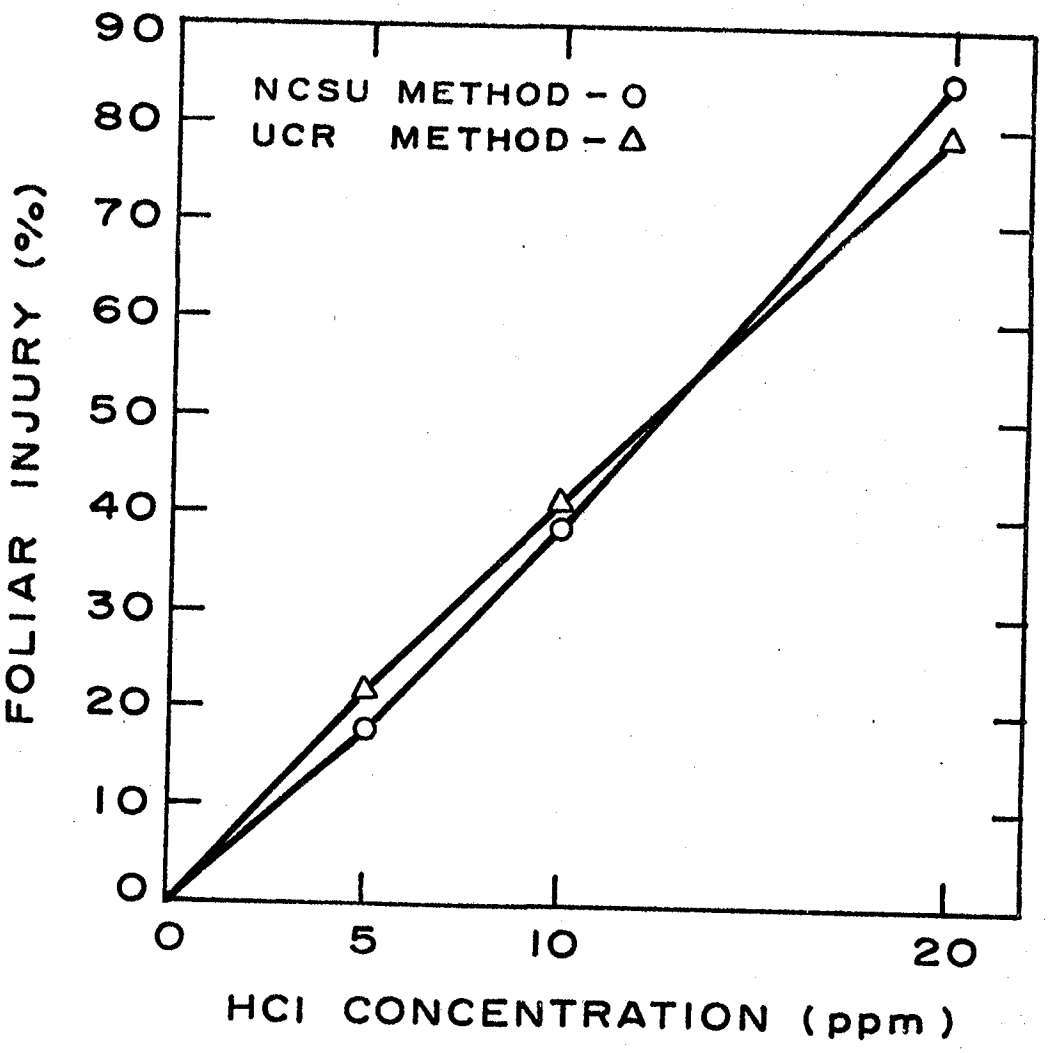

Figure 33. Comparison of the NCSU and UCR methods for assessing foliar injury, made during an experiment at NCSU (run 1) by the NCSU rator, data came from radish exposed for 80 min.

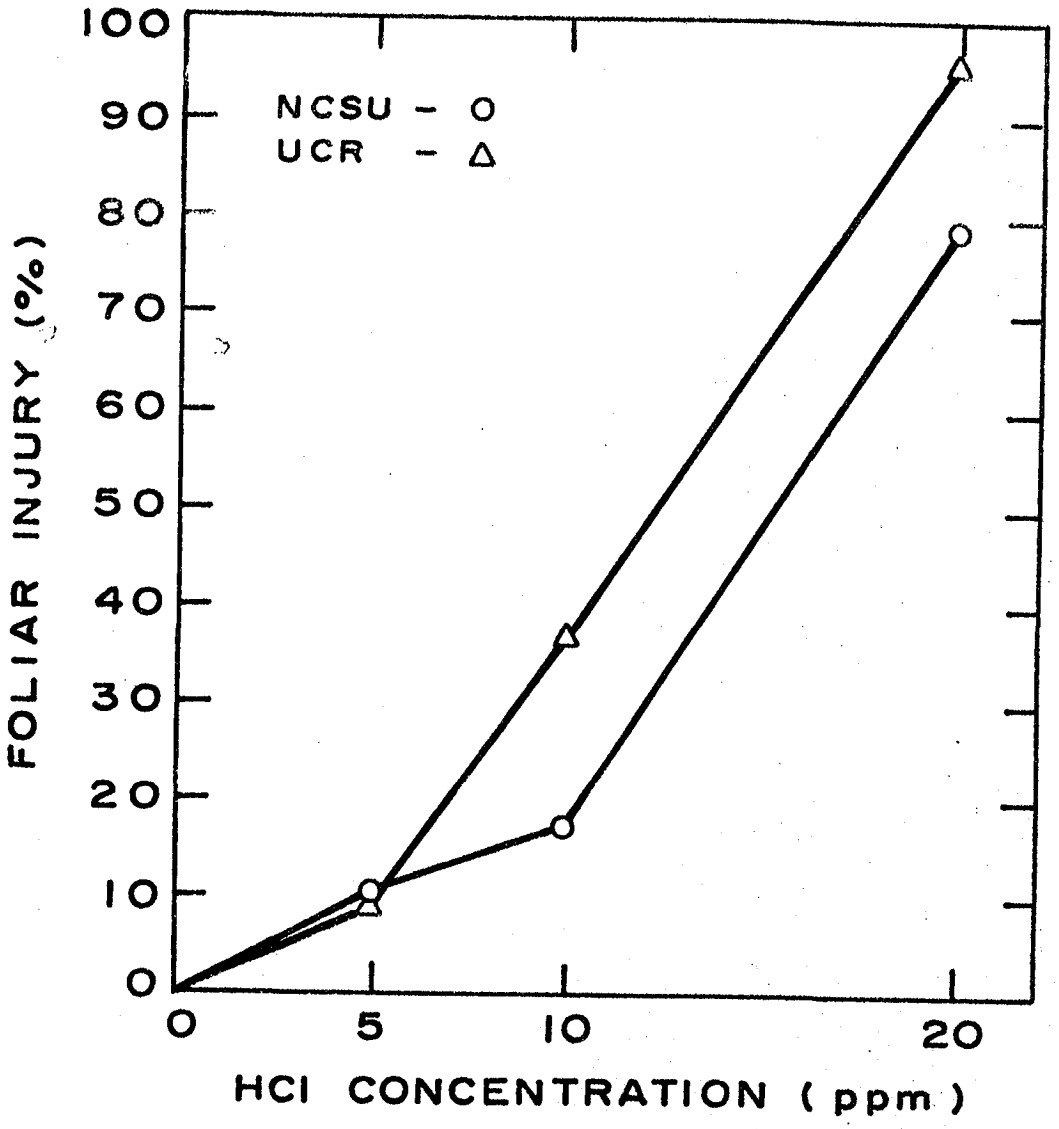

Figure 34. Comparison of foliar injury readings between the NCSU and UCR locations taken by the NCSU investigator and using the NCSU method. Data came from radish exposed for 80 min during the 3rd and 4th runs at NCSU and UCR, respectively. The weather was cloudy when the run was made at NCSU.

exposures suggested the presence of an oxidant in the mixture. Thus, we decided to run a preliminary dose-response design with both Cl_2 and NO_2. This was done to determine whether additional research should be recommended to NASA.

4.6.1. Methods and materials

Four plant species were used in these experimental designs (radish, 'Comet'; soybean, 'Dare'; pennywort; and marsh elder). Plants were seeded and grown as in the HCl exposures (4.2.1.). The exposures were conducted in four CSTR exposure chambers in a glass greenhouse. The system design was identical to that used for the HCl exposures. The gas concentrations were calculated using tank gas concentrations, gas flow rate and air flow rate into the exposure chambers. The NO_2 chamber concentrations were checked, using a dilution system, with a Monitor Laboratory chemiluminescent NO_x instrument; the Cl_2 chamber concentrations were checked with the Geomet. The actual concentrations should be within $\pm$ 10% of those shown.

The NO_2 exposures included four concentrations (0, 4, 8 and 16 ppm) and three time periods (20, 40 and 80 min). The Cl_2 exposures included five concentrations (0, 1, 2, 4 and 8 ppm) and three time periods (20, 40 and 80 min). The designs included 2 duplicates for each treatment and they were replicated 3 times for a total of 6 plants per treatment. The NO_2 design used a total of 72 plants and the Cl_2 design used 90 plants. Plants were exposed once at 21 days (radish), 28 days (soybean), or when regrowth had occurred (pennywort and marsh elder). Visual injury (4.2.1.) was determined 48 to 72 hrs after exposure and the plants were harvested seven days after exposure. Data were analyzed using an analysis of variance and treatment means were separated at the 0.05 level of significance using LSD's.

4.6.2. Results

The cultivated plants were more sensitive to NO_2 than the native species with radish being the most sensitive and marsh elder the least sensitive (Table 28). There was no evidence of weight changes in soybean as a result of NO_2 exposures (Table 29).

The cultivated plants were more sensitive to Cl_2 than the native species; radish and marsh elder were the most and least sensitive, respectively (Table 30). All plants were severely injured at the highest Cl_2 concentrations and the injury generally increased with increasing duration of exposure. Biomass changes in soybean were generally associated with foliar injury above 40% (Table 29).

4.6.3. Discussion

The species (cultivars) tested were generally 2 to 4 times less sensitive to NO_2 than to HCl and 4 to 20 times more sensitive to Cl_2. The results suggest that the trace of NO_2 in the SRF exhaust will not affect sensitive vegetation. However, there could be injury from Cl_2 under some conditions where injury from HCl is also found. Chlorine

Table 28. Foliar injury to selected plant species as a function of NO_2 concentration and time.[1/]

Exposure time (min)	Foliar injury (%) at three NO_2 concentrations (ppm)			Exposure time (min)	Foliar injury (%) at three NO_2 concentrations (ppm)		
	4	8	16		4	8	16
Radish ('Comet')				Marsh elder			
20	+	1	7	20	+	1	+
40	1	1	17	40	1	1	5
80	1	1	63	80	1	2	11
(LSD at 0.05 = 16.8%)				(LSD at 0.05 = 1.7%)			
Soybean ('Dare')				Pennywort			
20	+	1	2	20	1	1	2
40	+	1	7	40	+	2	2
80	1	1	24	80	1	2	17
(LSD at 0.05 = 4.7%)				(LSD at 0.05 = 2.3%)			

[1/] All values are average foliar injury to test plants taken 48 to 72 hrs after exposure. The injury covers a 0 to 100% injury range in 5 or 10% increments and are averaged over 6 plants (2 duplicates and 3 replicates). Data were analyzed by analysis of variance and treatment means were separated by LSD (0.05). The + signifies less than 0.5% foliar injury.

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injury from an acute exposure would resemble the HCl injury (Section 4.4.1.3.). Concentrations of Cl_2 and NO_2 expected in the SRM exhaust should not be injurious to plants in the vicinity of the shuttle launch.

4.7. Chloride Accumulation in Soybean

4.7.1. Introduction

Accumulation of chloride in plant tissue as a result of exposure to HCl under controlled conditions as well as under natural situations has been reported (4, 11, 17, 23, 38, 62, 63). Attempts to relate the amount of injury from HCl exposures to chloride accumulation have been

Table 29. Biomass of soybean ('Dare') as a function of Cl_2 or NO_2 concentration and time.[1/]

Exposure time (min)	Cl_2 concentration (ppm) 0	1	2	4	8
Root fresh wt (g)					
20	7.5g	+22	+12	+9	+2
40	(±0.29)	+6	2	7	43
80		+22	8	34	24
	(LSD at 0.05 = 21%)				
Shoot fresh wt (g)					
20	13.0g	+11	+12	2	25
40	(±1.26)	+3	0	18	45
80		+8	15	32	35
	(LSD at 0.05 = 19%)				

Exposure time (min)	NO_2 concentration (ppm) 0	4	8	16
Root fresh wt (g)				
20	8.9g	+24	+2	+16
40	(+1.11)	+14	+8	6
80		+5	+7	12
	(LSD at 0.05 = 39%)			
Shoot fresh wt (g)				
20	14.1g	+6	13	4
40	(±.02)	6	4	10
80		4	11	1
	(LSD at 0.05 = 23%)			

[1/] Data are averaged over 6 test plants (2 duplicates and 3 replicates). Biomass was taken seven days after exposure. Data was analyzed by analysis of variance and treatment means were separated by LSD (0.05). This control data were not different. Thus the average control cost (for the three time periods) was used with the standard deviation in ( ). All exposure data is presented as a percent reduction or increase (+) from the average of the combined controls.

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mostly unsuccessful (5, 11, 47, 62). Wood (72) showed a positive correlation between foliar chloride levels and the degree of defoliation of black cherry. Van Haut and Guderian (in 23) demonstrated that excess chloride in clover taken up from the soil or air inhibited growth without causing injury, but a direct relationship between accumulated chloride and growth was not demonstrated. Chloride accumulation in plants from exposure to equivalent doses of HCl under different concentrations and exposure durations has not been investigated.

Shriner (62) examined the sites of chloride accumulation in tomato and chrysanthemum plants exposed to acute doses of HCl. The greatest increase in chloride content was found in the immature secondary leaves of the tomato plants and in the upper leaves of the chrysanthemum plants. Increase in the chloride content of all exposed portions of the test plants was found; however, the movement of chloride over time was not

Table 30. Foliar injury to selected plant species as a function of Cl_2 concentration and time.[1/]

Exposure time (min)	Foliar injury (%) at four Cl_2 concentrations (ppm) 1	2	4	8	Exposure time (min)	Foliar injury (%) at four Cl_2 concentrations (ppm) 1	2	4	8
Radish ('Comet')					Marsh elder				
20	25	79	79	90	20	1	3	5	42
40	51	88	87	95	40	1	5	14	53
80	73	83	92	93	80	1	18	27	68
(LSD at 0.05 = 8.9%)					(LSD at 0.05 = 9.7%)				
Soybean ('Dare')					Pennywort				
10	-	-	-	57					
20	5	15	38	60	20	+	11	25	77
40	6	24	61	71	40	3	18	56	80
80	11	43	63	69	80	3	39	67	88
(LSD at 0.05 = 6.9%)					(LSD at 0.05 = 8.4%)				

[1/] All values are average foliar injury to test plants taken 48 to 72 hrs after exposure. The injury covers a 0 to 100% injury range in 5 or 10% increments and are averaged over 6 plants (2 duplicates and 3 replicates). Data were analyzed by analysis of variance and treatment means were separated by LSD (0.05). The + signifies less than 0.5% foliar injury.

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examined. The chloride content of the distal portions of tomato and chrysanthemum leaves exposed to HCl was higher than that of the proximal portions of these leaves. Guderian (23) showed that grape leaves exposed to 0.46 ppm of HCl for 80 hrs contained less chloride in the leaf margins than in the rest of the leaf lamina. Two weeks after exposure this difference was no longer apparent. However, the chloride content of lilac was distributed evenly throughout the leaves.

The amount of chloride in specific cells after exposure to HCl has not been examined. In studies of cellular sites of fluoride accumulation in fir needles after exposure to hydrogen fluoride gas, Garrec and Lhoste (18) found more fluoride in the spongy mesophyll cells than in the pallisade cells, some accumulation in the epidermal layers, and very little fluoride in the hypodermis. It would be of interest to know whether chloride accumulates in different cells or tissue.

The present study was initiated with soybean to: 1) determine whether HCl dose was related to chloride accumulation or whether either HCl concentration or exposure duration affected accumulation; 2) determine if foliar injury is correlated with chloride accumulation; 3) determine the relationship between HCl concentration, exposure duration, foliar injury, dry weight, and chloride accumulation; 4) examine chloride distribution, movement, and retention within exposed plant parts; 5) examine the effects of an HCl exposure on subsequent exposures to HCl; and 6) determine the chloride content of mesophyll and epidermal cells of leaves exposed to HCl. These were the primary objectives of the Master's thesis that Madeleine Engel completed as part of this project (14). This thesis is attached as an addendum to this report.

The first two listed objectives were of primary interest to this project and are thus included in this report. It is of interest for NASA to know whether chloride does accumulate after HCl exposures and, if so, is it related to foliar injury.

4.7.2. Materials and methods

Soybean [Glycine max (L.) Merr. 'Dare'] were planted in 5 in. plastic pots (4 seed/pot) and subsequently grown as described in Section 4.1.1. Uniform plants were exposed to HCl when their third or fourth trifoliate leaves were expanding (21 or 28 days from seed). The HCl exposure system and monitoring procedures are described in Sections 3.2.1.1. and 4.2.1. Temperatures, relative humidities, and light intensities in the chambers during exposure were 24 to 37°C, 19 to 100% RH, and 27 to 39 klux, respectively.

Plants were exposed to 0, 4, 8, or 16 ppm of HCl for 15, 30, 60, or 120 min. Two replications were run (plants were 21 days old for the first replication and 28 days for the second) with 6 duplicates for 12 plants per treatment and 192 plants in the design.

Plants were returned to the greenhouse benches after exposure and the percentage of necrotic area of the primary and trifoliate leaves arising from the main stem were estimated (4.2.1.) 3 days after exposure. Plants were harvested at the same time and dried in a forced air oven at 70°C for 3 days for dry wt measurements. The dried tissue was ground to 40-mesh size and prepared for chloride analysis as described by Adriano et al. (1). The nitric acid extracts were analyzed with a Buchler Digital Chloridometer, Model No. 42500. Data were analyzed by analyses of variance, and LSD values and correlation coefficients were determined.

4.7.3. Results

Injury to soybean leaves was as described in Section 4.2.1.2. The HCl concentrations and exposure durations used in this experiment resulted in a wide range in injury severity. The effects of concentration and duration of exposure on the percent foliar injury and the differences in leaf sensitivity were similar to those reported earlier (4.2.1.2.).

Foliar injury was slight at 4 and 8 ppm HCl and moderate to severe at 16 ppm depending on the duration of exposure (Figure 35). At equal doses, the concentration was far more important than duration of exposure in determining the percent of foliar injury. Chloride accumulation was also directly related to the dose with the concentration more of a factor than exposure duration (Figure 36). Significant chloride accumulation occurred at 4 and 8 ppm for some exposure durations even though foliar injury was slight or absent. The overall correlation between foliar injury and foliar chloride content was high ($r = 0.92$). The correlation between HCl concentration and percent chloride in the leaf tissue was also significant ($r = 0.72$).

4.7.4. Discussion

The effects of HCl concentration and time on the percent of foliar injury was discussed in Section 4.2.1.3. The results in this section support the conclusions made in that discussion (4.2.1.3.). At equal doses (HCl concentration x exposure duration) foliar injury was more severe on plants exposed to high concentrations for short durations than that on plants exposed to low concentrations for longer durations. This same concept held for chloride accumulation. A decrease in the dry weight of soybean shoots and roots was directly related to severity of injury.

Chloride did accumulate in plants exposed to HCl and the amounts were dependent on concentration and duration of exposure. Chloride in plant tissue will reduce growth (23) when in excess and will stimulate growth (39, 71) when added to nutrient substrates that are low in chloride. However, results from this study did not indicate that the chloride taken up by the plant tissue during the HCl exposures affected the growth of the plants directly (i.e., injury and/or growth were not a result of chloride ion concentration within the tissue).

Even though we found good correlations between foliar injury, foliar accumulation of chloride and HCl concentration, investigators should be cautious in their attempts to relate chloride in plant tissues to HCl exposures. Our experimental designs were done using controlled soil and nutrient additions. Thus, our results only prove that correlations can exist. For the area of Merritt Island, a major study of chloride content in native and managed plants would be necessary before we could suggest possible correlations between plant chloride concentrations and HCl from SRF exhaust. Our personal assessment would be that such correlations would be found but the increases of chloride would be less than we have shown and the increase would be present only for a brief time.

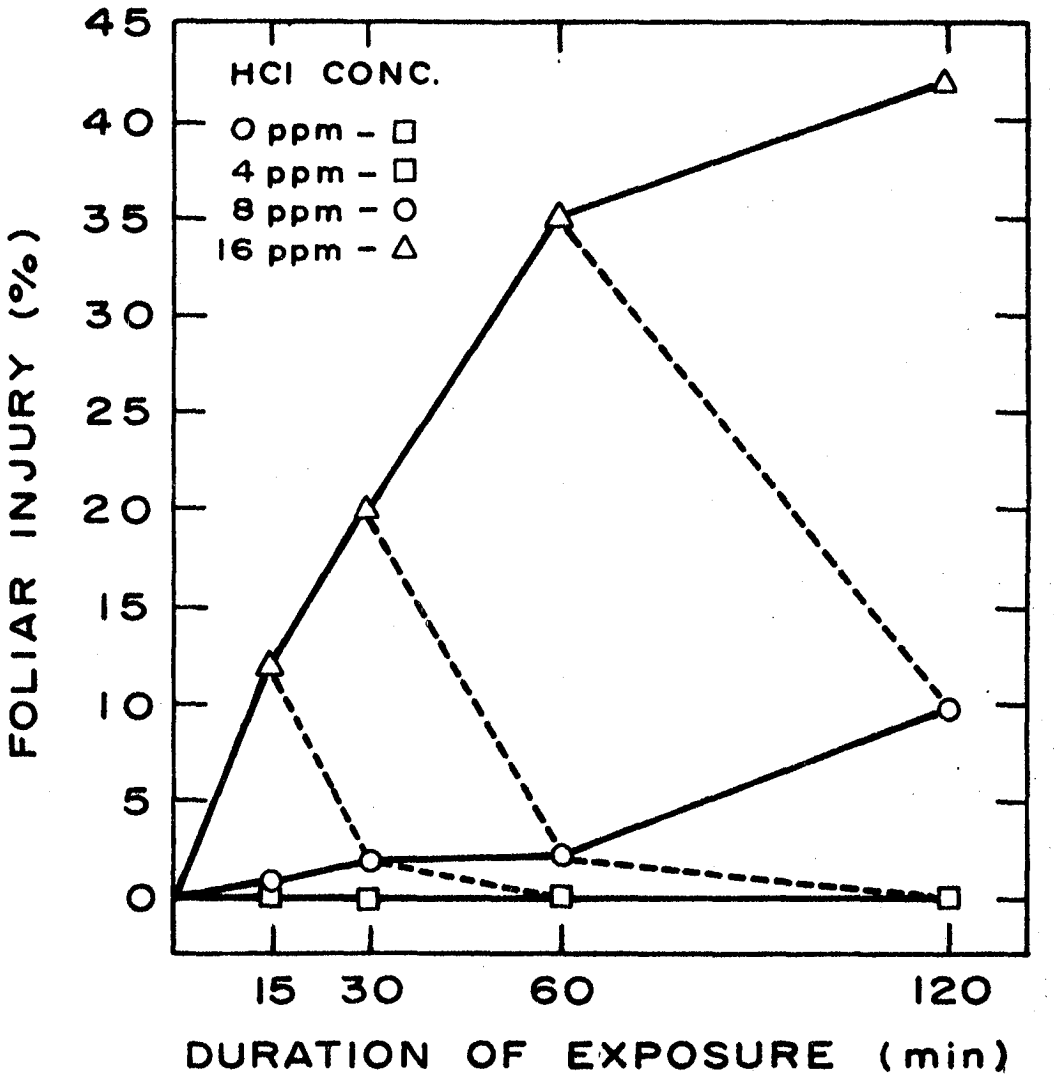

Figure 35. The effect of HCl concentration and exposure duration on foliar injury of soybean, 'Davis'. Solid lines connect points of equal exposure concentration. The dashed lines connect points of equal dose (concentration x duration). An average injury per plant (primary and first, second, and third trifoliate leaves included) was determined. Each point is the mean of 12 plants (6 plants per replication).

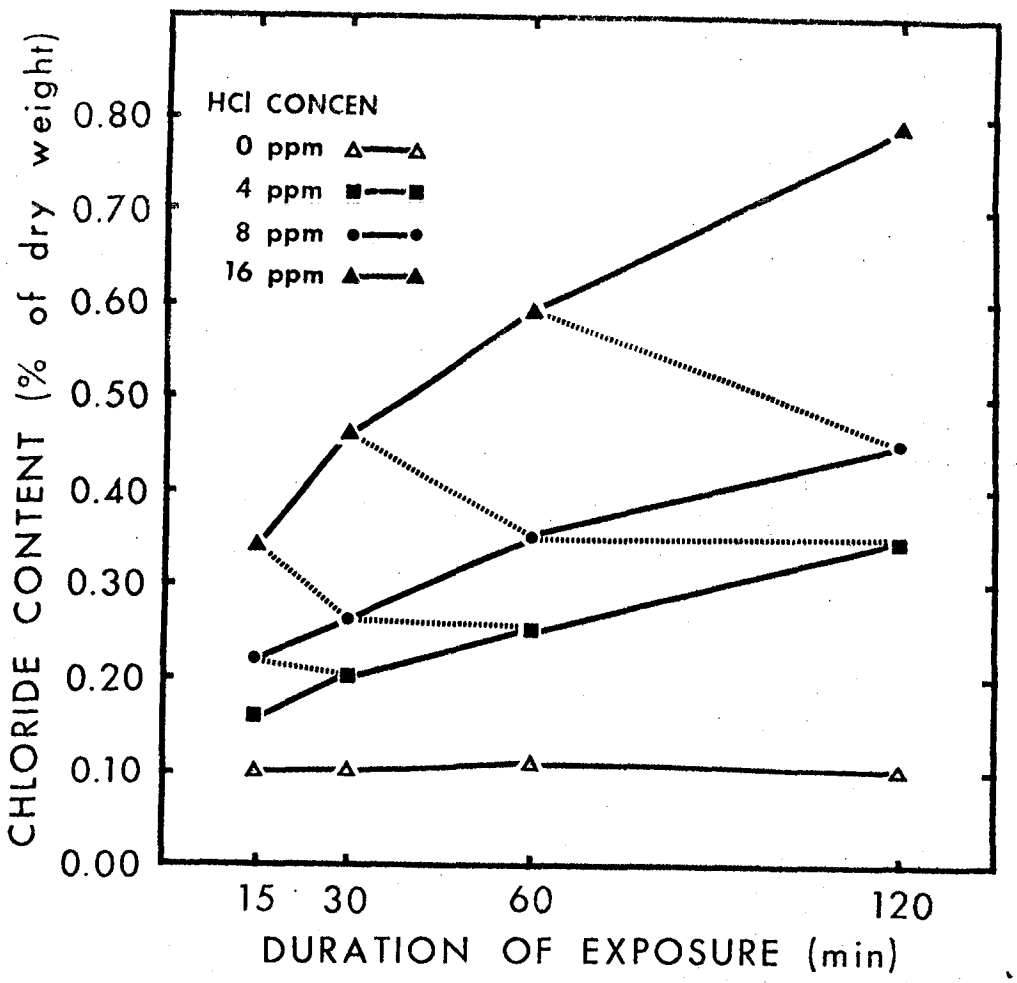

Figure 36. Relation between the HCl concentration and exposure duration on the chloride content of soybean shoots. Solid lines connect points of equal exposure concentration. The dashed lines connect points of equal dose (concentration x duration). Each point is the mean of 12 plants (6 plants per replication). (LSD at 0.05 = 0.10).

5. EFFECTS: INSECTS

5.1. Introduction

Air pollution emissions from combustion and industry often disturb forest and agricultural systems as well as damage human health. Although insects are an important part of all ecosystems, they have been largely overlooked in air pollution studies. For this work, three representative insect species were chosen to study the acute and chronic effects of SRF exhaust: a pollinator, the honey bee (Apis mellifera L.); a predator, the common green lacewing (Chrysopa carnea Stephens); and an ubiquitous pest, the corn earworm (Heliothis zea Boddie). These insects are all found on Merritt Island in Brevard County, Florida.

The honey bee is an important pollinator of millions of dollars of crops, including citrus, every year in the U. S. Honey is an important agricultural product in Brevard County where a beekeeping industry of 2600 colonies of bees annually produces approximately 140,000 pounds of honey. Honey bee larvae and young adults remain in the hive while the older adults (workers) forage, collecting nectar and pollen, and pollinating a number of plants.

Citrus is an important agricultural crop in Brevard County. Lacewing larvae prey on a number of citrus pests, especially aphids and scale insects. Lacewings pupate above ground in cocoons on slender stalks suspended from vegetation. Adults feed on insects to a lesser degree than do larvae, then lay their eggs on vegetation.

The larvae of the corn earworm substantially damage many field and garden crops. Adult corn earworms feed on nectar and lay eggs on exposed portions of vegetation. Larvae on soybeans and some other crops may remain exposed throughout their larval life. As fifth instars they enter the soil to pupate, emerging as adults about 15 to 20 days later.

The primary objective of this study was to determine the concentrations of hydrogen chloride (HCl) gas, at different durations of exposure, that cause 50% of the individuals exposed to cease movement (the effectively lethal dose, ED_{50}). Acute toxicity of HCl gas was determined for the honey bee, the common green lacewing, and the corn earworm. Only those developmental stages that might come in contact with an air pollutant were tested. Corn earworm and lacewing eggs, larvae, and adults are usually found unprotected while only foraging honey bees normally venture outside the hive. By exposing a number of life stages of the corn earworm and lacewing to HCl, the effects of age and developmental stage on susceptibility to HCl were determined. Finally, the effects of chronic exposures of honey bee colonies to various concentrations of SRF exhaust were studied.

5.2. Methods and Materials

Exposures of insects to HCl gas and SRF exhaust were conducted in a manner similar to that of the plant species examined in this project (4.2.1.).

The Geomet was used to determine concentrations less than or equal to 120 ppm of HCl. Concentrations were monitored every 15 min during exposures up to one hr, every 30 min during exposures from 1 to 3 hr, and every 50 min during exposures of 4 hr or longer. Exposures to HCl above 120 ppm were monitored using the bubbler system. The Geomet was within ± 15% of the bubbler HCl concentrations between 80 and 120 ppm. The SRF exhaust studies were conducted in the same field chambers used for the vegetation studies (4.4.).

The acute toxicity of HCl on foraging (adult) honey bees and a number of developmental stages for each of two other insects was studied. These tests included eggs, first, third, and fifth instars and young, mature and old adults of the corn earworm; and eggs, and early and late instars of the lacewing (Table 31). Honey bee colonies (in hives) were also exposed to SRF exhaust.

A rearing room in the NCSU Entomology Department was used to raise H. zea and C. carnea at 26° C, 14 hr photoperiod. Groups of individuals were then transported to the experimental farm for exposure to HCl. The insects were kept in a small laboratory at the farm site where they were maintained at 26° C, 14 hr photoperiod. Insects were transferred to special containers for exposure to HCl and then returned to the laboratory after exposure.

5.2.1. Honey bee

Two similar 2-year old colonies of Italian honey bees, each with two supers partially filled with honey, were moved to the field site in June. The hives were placed in an air-conditioned trailer with their landing boards facing south. The following spring the hives were opened and the full honey supers were replaced with empty ones.

For each set of exposures, foraging bees were collected from the entrance of one of the hives shortly before exposure. Bees were collected alternately from each hive which provided variability in the sample population since worker bees in each have were 3/4 identical. To facilitate bee collection, a 20 cm long funnelling device was made of 3 mm mesh hardware cloth. The large end was rectangular and fit the entrance of the hive. The opposite end of the device was reduced to a 3 cm diam spout that fit the openings of the exposure cages.

Sixteen cylindrical exposure cages (13 cm diam by 13 cm high) were made of PeCap^{R} monofilament polyester screen (.18 cm mesh opening, 61% open area) that was sewn together with polyester thread. The open conical tops were fitted with #7 rubber stoppers.

Bees were collected and exposed when weather conditions encouraged flying activity by the bees (June through October). Sixteen cages, each with 20 to 25 bees, were usually collected for each set of exposures, although occasionally a smaller multiple of four cages was used. The cages were then brought into the controlled temperature and light insect handling laboratory and fed a 1:1 honey:water solution, 1.5 ml per cage. Within an hour after collection, the cages of bees were suspended on a bar about mid-height in the CSTR chamber (3.2.).

Table 31. Summary of acute toxicity exposures for the three insect species

Duration of Exposure (min)	HCl Concentration (ppm)																
	0	10	15	20	30	40	50	60	80	100	120	150	160	170	180	185	200
30	a,d,f, h,j	a,g	d	a,g,	d	a	j	a,d	a,d,h	j	d,h	j	d,h	h			
45	g					g											
60	a,b,d, f,h,i, j,k	a	d	a,h	d,i, j	a,b,h	j	a,d, i,j,	a,b,f, h,i,k	j	a,b,f, h,i,j, k	j	a,f	h		f	i,k
70	c	g		g					c		c				c		
80	a								a		a		a				
85	g					g											
90	f								f		f					f	
120	a,b,c, d,e,f, g,h,i, k		d	a,h	d	a,c,e, f,h		a,d, e,g	a,b,c, e,f,g, h,i,k	a,e, g	b,c,e, f,h,i, k		b,h	h	c	f	i,k
160									h								
180	a,d,h		d	a	d	a		a,d	h		h			h			
240	a,b,e, f			a		a,b,e, f		a,f	a,b, e,f	f	b,e,f						
270	c								c		c				c		
360	a			a		a		a									
480	a			a				a									

1/ The above dose response designs were selectively used for the insect studies. The code for the insects is:

a - A. mellifera foraging bee
b - C. carnea - egg
c - C. carnea - early instar
d - C. carnea - late instar
e - H. zea - egg
f - H. zea - first instar
g - H. zea - third instar
h - H. zea - fifth instar
i - H. zea preovipositional adult
j - H. zea mature adult
k - H. zea postovipositional adult

The basic experimental design utilized all four CSTR chambers: one as a control and three held at various predetermined concentrations of HCl. Four duplicate bee cages were normally used in each chamber. Temperature, relative humidity, and duration of exposure were recorded. The HCl concentrations were monitored 15 min after introduction of the bees to verify the steady state HCl concentrations and periodically after that depending upon exposure duration. All exposures were made during the day without supplemental light, except for the 8-hour exposures where supplemental light was provided by 1000 W metal halide lamps. Exposure duration ranged from 30 to 480 min; HCl concentration ranged from 10 to 160 ppm.

After exposure the bees were placed in the field laboratory under controlled conditions; the number of immobile bees was determined and the bees were transferred to holding cages (13 x 13 x 13 cm) made of 3 cm mesh hardware cloth with Velcro^{R} closure fastenings. The transfer was made using an aspirator that sucked the bees from each exposure cage into a corresponding holding cage through a 1.27 cm diam clear plastic hose inserted through a small hole in the holding cage (Figure 37).

The holding cages were set on trays and 1.5 ml pipettes with 1:1 honey:water solution were positioned on each cage and replenished as necessary. Initially empty brood comb was placed in some holding cages to determine if the comb had any effect on bee longevity (79). This was discontinued when no significant difference in mortality between bees with and without comb was found. Initially bee immobility was observed every 15 min for the first hr, then hourly for the next four hr and then at 24 hr intervals through 72 hr. After a number of tests, it was determined that 48 hr after the exposure the apparent mortality rate of control groups equalled that of exposed groups. Thereafter, observation of bee immobility was made immediately, 24 hr and 48 hr after exposure. Final determination of the acute toxic effects of HCl on honey bee was based on the 48 hr immobility data.

5.2.2. Corn earworm

Corn earworm larvae were reared in batches of 350 from stock cultures maintained at the NCSU Entomology insect rearing facilities. First instars were individually placed into 60 ml plastic cups that were half filled with a corn-soy-milk blend diet (78). Larvae were reared separately because they are cannibalistic. The selective pressures of laboratory rearing often produce weak strains of corn earworm that are less resistant to various stresses than wild strains. Therefore note was made of the generation of individuals exposed to HCl and a comparison of responses was made that showed no differences in susceptibility. Thus all data were analyzed as an unit.

When larvae pupated they were transferred to one pint, cylindrical, cardboard, cheesecloth covered containers with a 2 to 3 cm layer of damp vermiculite. When adults emerged they were supplied a 1:1 sucrose:water solution in 30 ml plastic cups with a tissue to facilitate feeding and to prevent drowning.

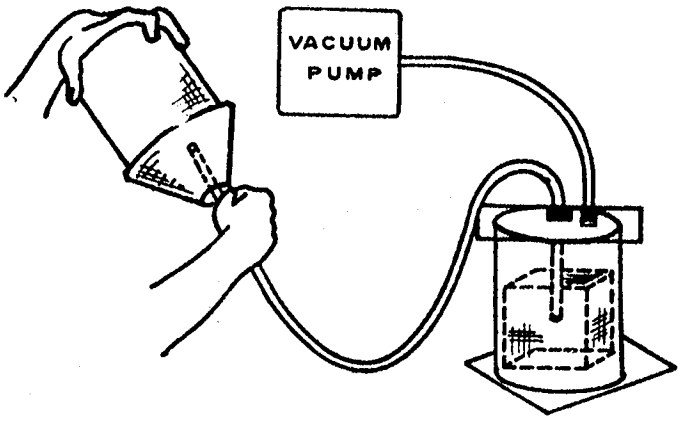

Figure 37. Aspirator for transferring bees from exposure to holding cages.

Eggs; first, third and fifth instars; and young (pre-ovipositional), mature, and old (post-ovipositional) adults were exposed to HCl. Initially, eggs were exposed in groups of about 25 in open glass petri dishes or on 10 x 10 cm pieces of white paper. To test whether HCl would cause differential pupation or emergence rates, individual eggs were placed in half-filled diet cups and raised through adult emergence. These studies showed that the effects of HCl were observable 48 hr after exposure and that there were no latent effects. In subsequent exposures, eggs were placed on a thin layer of diet in disposable petri dishes and observations of number hatched and number moving were made 24, 48 and 72 hr after exposure, before cannibalism became a mortality factor.

First instars were treated in groups (cannibalism is rare before larvae mature to third instar). The larvae were exposed in petri dishes covered with a fine (2 mm mesh opening, 44% open area) PeCap^{R} monofilament polyester screen secured with rubber cement. Many larvae stuck to the rubber cement thereby being removed from the test population; these losses were compensated for by using large numbers of larvae (40 to 50) in each duplicate. Usually 15 to 20 larvae comprised each test group. Separately contained individuals were observed through adult emergence until it was determined that any toxic effects were manifested 48 hr after exposure.

Larvae that were exposed as third or fifth instars or as adults were set into half-filled diet cups as soon as they hatched. To prevent ingestion of HCl due to cannibalism, third and fifth instars were handled and exposed individually in bundles of exposure tubes [20 glass tubes (1.3 cm diam and 4 cm long) glued together] one individual per tube. PeCap^{R} fine mesh screen was glued with rubber cement to the ends of the tube groups and they were hung horizontally

in the exposure chambers. HCl concentrations within the tubes were the same as in the chambers. Two sets (tube bundles), 10 to 20 individuals each were used in each exposure. After exposure, larvae were returned to the diet cups and observations were made 24, 48 and 72 hr after exposure.

Groups of individuals were reared to adults and separated by post-emergence age [0 to 2 day (young or pre-ovipositional) adults, 2 to 4 day (mature) adults and 4 to 6 day (post-ovipositional) adults]. These adults were exposed to HCl to determine if age affected susceptibility. The adults were first anesthetized with a 10 to 15 second exposure to CO_2 and placed, in groups of 10, into polyester screen cages. After exposure, they were returned to pint cardboard containers and fed sugar solution for 72 hr after exposure.

Exposure duration ranged from 30 to 240 min and HCl concentration ranged from 10 to 200 ppm (Table 31). Final determination of the acute toxic effects of HCl was based on 48 hr immobility data.

5.2.3. Common lacewing

Lacewings were supplied from cultures maintained by the USDA Cotton Insect Laboratory, College Station, Texas. Lacewing eggs and eggs of a prey species (Sitotroga cerealella Olivier, the Angoumois grain moth) were mailed on a regular schedule or on request. Lacewings were raised by the method developed by Morrison (77). Conditions for rearing were 26° C, 14 hr photoperiod. Adults did not emerge in sufficient number to run exposures of similarly aged individuals, so acute toxicity tests of adults were not done.

Eggs and early instar larvae, which are cannabalistic, were exposed to HCl. Eggs were exposed to HCl in open glass petri dishes, and larvae were exposed in the glass tube containers designed for H. zea exposures. High numbers were exposed because many larvae stuck to the rubber cement. After exposure, the larvae or eggs were returned to the Verticel^{R} apparatus. Due to the small size and fragility of lacewing larvae, 30 to 40% of the individuals in all groups were lost during post-exposure manipulations of larvae. Each replicate was examined 48 hr after exposure to determine the number of individuals immobilized.

Exposure duration ranged from 30 to 270 min and HCl concentration ranged from 15 to 180 ppm (Table 31).

5.2.4. Exposure of bee colonies to SRF exhaust

Eight healthy colonies (hives) of Italian bees were moved from Bladen County, NC to the field test site. The queen in each hive was marked for identification with white nail polish on the dorso-thorax. Two hives were placed in each field chamber. The lower panels on the field chambers were dropped, except during exposure, to allow the bees free foraging movement. Colony variation was minimized by pairing the weaker with the stronger as determined by the number of combs with brood. The most extreme variations (the weakest with strongest colonies) were

used as controls (#'s 2 and 4). The low exposure colonies (12 ppm) were #'s 7 and 8, the medium exposure colonies (24 ppm) were #'s 1 and 3, and the high exposure colonies (35 ppm) were #'s 5 and 6. The hives with more brood tended to have less honey.

The colonies were not disturbed for one week after which a series of exposures to SRF exhaust was initiated. During exposure the chambers were closed. Each exposure was approximately 1 hr in duration and concentrations were approximately 0, 12, 24 and 35 ppm of HCl. The colonies were exposed twice each week for a total of 8 exposures. Brood area was determined twice a week, after each SRF exhaust exposure. The following measurements were also made regularly: 1) hive weight was recorded at 1700 hr every one or two days; 2) the number of dead workers, drones, worker pupae, drone pupae, and larvae in the containers of dead bee traps was determined at 1700 hr every other day; 3) pollen was collected over the 24 hr period following each SRF exhaust exposure; and 4) aggressiveness was quantified by the response of bees to a "mouse" bait on the days of exposure. Results of these latter four measurements are found in the thesis by Romanow (80), but are not included in this report. Hives were left undisturbed between exposures.

Two hours after each exposure the hives were opened for inspection. The area of eggs, uncapped brood, and capped brood were traced on a clear sheet of acetate, drawing both sides of each frame. These areas were determined with a Compensating Polar Planimeter. Eggs, larval, and pupal production were estimated separately and summed as total brood production for each hive.

The eight SRF exhaust exposures were made over the 25 day test period (May 19 to June 12). Time between exposures was 3 to 4 days. The burns were started between 1100 and 1300 hr and lasted 40 to 65 min. At the time of exposure, the exposure chambers were closed by raising the lower side panels. The Geomet HCl instruments periodically monitored the exhaust concentration leaving each chamber.

Periodic (2 to 5 times/burn) flare-ups (5 to 10 sec each) raised HCl concentrations briefly by a factor of 2 or 3. After each exposure, the lower wall of each chamber was dropped and the bees were left undisturbed for approximately two hr. One exposure was aborted after a large flare-up in the burn box that gave each treatment at least 90 ppm HCl for two min.

After the exposure series all hives were removed from the chambers and taken to the NCSU apiculture facilities. During the next four weeks brood area was traced four times at seven-day intervals. One month later the hives were examined and brood area was traced.

During brood examination the incidence of disease or apparent pesticide poisoning was observed. Terramyacin^{R} was added in patty form to each hive a week after exposures started when early signs of European foulbrood were discovered in hive 5. Subsequently no disease symptoms found in hives were treated.

5.2.5. Data analysis

5.2.5.1. Acute toxicity studies

The data recorded from the acute toxicity studies included: the insect stage, the number of insects per group, the 48 hr mortality and two environmental conditions (relative humidity and temperature). These data were analyzed to determine the importance of HCl concentration and duration of gas exposure. The effects of relative humidity and temperature during exposure on response of insects to HCl were also determined, but are not included in this report (80).

Immobility was used as the response to determine the effective dose for 50% of the individuals (ED_{50}). No emergence was considered the effective response for H. zea and C. carnea eggs. Probit analyses were performed to determine the regression line of response to concentration for each duration. To determine the ED_{50} of egg exposures, the dose-effect analysis of Litchfield and Wilcoxon (76) was used.

Percent immobility results were manipulated by probit transformation; further analyses of variance and linear regressions determined the importance of concentration, duration, relative humidity, and temperature on the ED_{50}. These data are not included in this report.

5.2.5.2. Chronic exposures of bee colonies

Correlations were determined for variables recorded during the exposure and post-exposure periods. Brood production was correlated with treatment concentrations during the exposure, post-exposure, and total observation periods. Brood production, indicative of hive strength, was regressed over time using linear and cubic formulas for each hive; the results were plotted. Duncan's multiple range test was performed on an analysis of variance of brood production over treatments and hive. Within each treatment, brood production was compared between the two hives.

5.3. Results

5.3.1. Acute toxicity of honey bee to HCl

Thirty min exposures of bees to HCl from 0 to 80 ppm did not injure the bees. The sixty min exposures from 0 to 160 ppm HCl did show a response but it was not sufficient to determine an ED_{50}. The ED_{50}'s were calculated for the 80 to 480 min exposure durations and are shown with 95% confidence limits in Figure 6. The slope of the line of ED_{50} over duration has a slope of -.23 ppm/min.

Using ED_{50} data, manipulated by a probit transformation, an analysis of variance determining the significance of concentration, duration of exposure, and their joint effect (dose) showed that concentration was more important than duration of exposure (comparative F values of 64.61 and 20.16) and more important than dose (concentration x duration) although all three variables were highly significant.

5.3.2. Acute toxicity of corn earworms to HCl

ED_{50}s for corn earworm eggs exposed to HCl for 120 and 240 min are shown in Table 32. An analysis of variance of the probit transformed data showed that concentration and duration were significant.

The graph for ED_{50}s for first instar larvae of corn earworms exposed to HCl (Figure 38) showed that the slope of ED_{50} over exposure duration was -0.27 ppm/min. Analysis of these data showed that concentration, duration, and dose (concentration x duration) were highly significant.

Probit analysis was not performed on the third instar larvae data.

The graph of ED_{50}s for fifth instar larvae of corn earworm exposed to HCl (Figure 38) showed that the slope of ED_{50} over exposure duration was -0.72 ppm/min. The values are also shown in Table 32. Analyzing the combined results of all exposures of fifth instar larvae showed that concentration and dose (concentration x duration) were significant.

Results of probit and regression analyses of exposures of preovipositional adults are in Table 32. Analysis of variance of all exposures showed that concentration and duration of exposure were significant.

Results of exposures of mature adults were analyzed by regression analysis and showed that concentration was highly significant (Table 32).

The results of analyses of exposures of postovipositional adults are summarized in Table 32. Analysis of variance showed that concentration, duration of exposure and dose were highly significant.

Analysis of variance of the probit transformed results of 60 exposures of first instar, fifth instar, preovipositional, mature and postovipositional adults showed that stage of development and concentration were highly significant. The effects of stage (all but mature adults exposed) and concentration were highly significant in the 120 min exposures. An analysis of variance of stage and dose showed that both factors were highly significant. Analysis of variance of all larval exposures to HCl gas showed a significant difference in the response of first and third instars exposed for 60 min, and all larval stages exposed for 120 min. Analysis of variance showed a significant difference of probit response to 60 min HCl exposures between adult stages (Figure 39).

5.3.3. Acute toxicity of the common lacewing to HCl

The common lacewing eggs exposed to HCl gas experienced high mortality associated with handling. Short duration exposures were not analyzable for effects of HCl gas. Analysis of variance of the 240 min exposures showed that concentration was highly significant. Analysis of variance of all egg exposures showed that concentration, duration and dose were highly significant.

The results for the early instar showed an ED_{50} of 152 ppm HCl for the 70 min exposures and an ED_{50} of 142 for the 120 min exposures. Regression analysis of 270 min exposures showed that concentration explained

Table 32. ED_{50} values for various developmental stages of corn earworm exposed to HCl for several exposure durations.[1/]

Developmental Stage	Exposure Duration (min)	ED_{50} Values (HCl Conc in ppm)	95% Confidence Limits
eggs	60	240	
	120	120	
	240	60	
first instar	60	152	142, 163
	120	132	119, 146
	240	92	62, 102
third instar			
fifth instar	60	275	199, 355
	120	251	188, 318
	180	179	143, 214
preovipositional adults	60	102	84, 121
ovipositional adults	60	153	121, 415
postovipositional adults	60	188	

[1/] Data was developed using probit analysis.

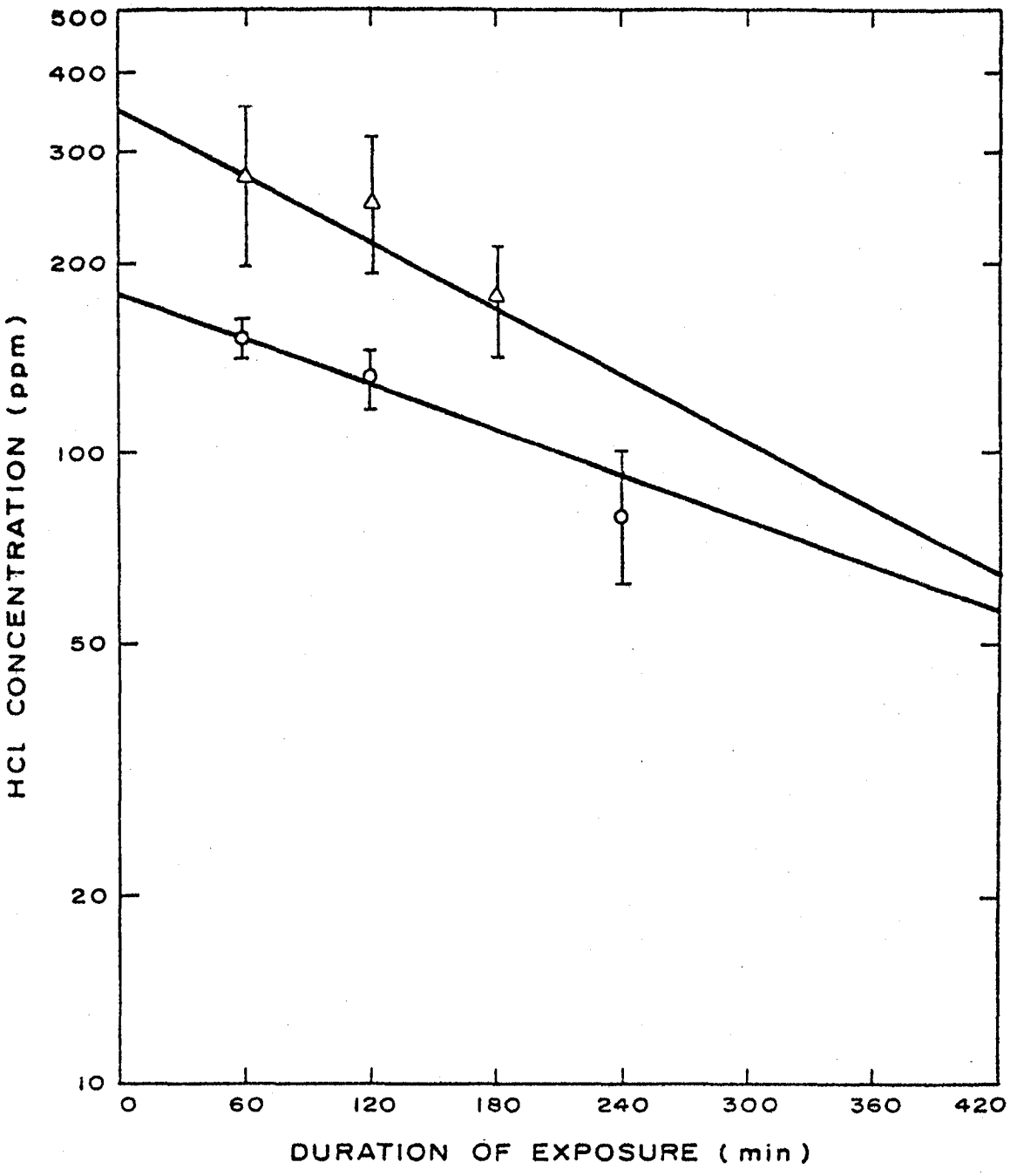

Figure 38. ED_{50} probit values for first and fifth instar larva of corn earworm; the values reflect larva immobility 48 hr after exposure to HCl for several exposure durations (o = first instar, Δ = fifth instar).

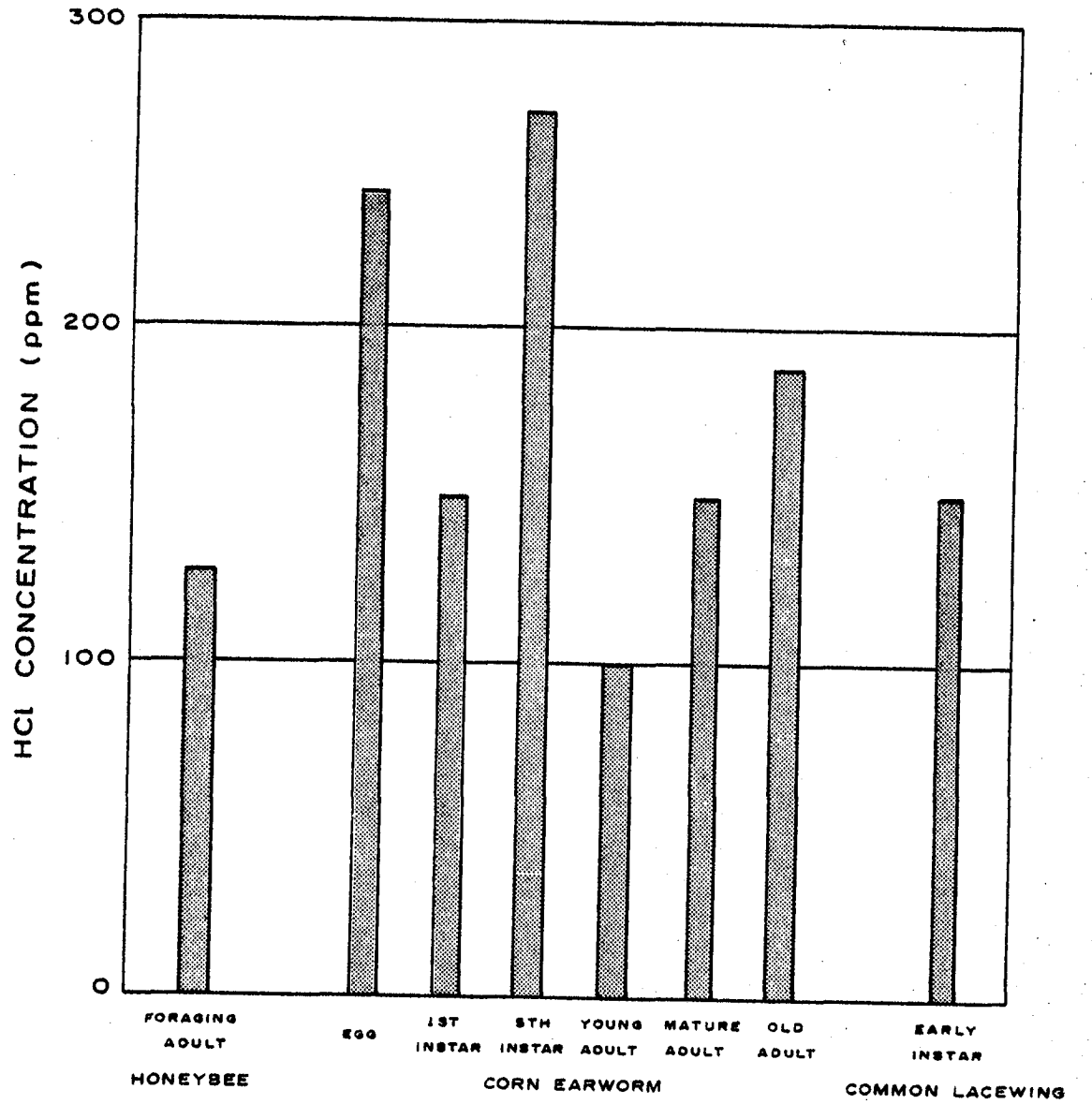

Figure 39. Comparison of 60 min ED_{50}s

most of the variation in the data. Analysis of variance of all exposures of early instars showed that concentration and duration but not dose were highly significant.

Late instars, exposed in small groups, experience high mortality due to handling. No probit analyses were performed on these data and thus no ED_{50}s were calculated. Analysis of variance of the 60 min exposure tests of eggs and late instars showed both developmental stages (egg and late instar) and concentration of HCl were highly significant. Analysis of variance of 120 min exposures of egg, early instar and late instar showed the stage and concentration were highly significant.

5.3.4. Comparison of ED_{50}s for the three species

Several stages of the corn earworm, the honey bee adults and the early instar of the common lacewing were exposed to HCl gas for the same duration of exposure (60 min). The ED_{50}s for 60 min are illustrated in Figure 39.

5.3.5. Chronic toxicity of SRF exhaust to honey bee - brood production

Both control colonies (2 and 4) grew over the duration of the exposure (Figure 7, Table 33). In the post exposure period, colony two lost brood production over time, while colony four's brood production slowed at a lesser rate (Table 33).

The low exposure colonies (7 and 8) also grew in brood area over the total observation period (Figure 7, Table 33), however at lesser absolute and relative rates than the control colonies. During the post exposure period colony seven's brood production declined slightly, while colony eight lost brood area at a slighly higher rate.

Colony one, a medium exposure colony, grew over the total observation period, while colony three, the other medium exposure colony, lost brood area over the observation period (Figure 7, Table 33).

Colonies five and six, the high exposure colonies, lost brood area over the total observation period (Figure 7, Table 33).

Using Duncan's multiple range test to compare brood area among treatments (Table 34), a difference in strength between the control and the low and medium exposure colonies was found for the exposure period but not for the post-exposure period. Comparing all colonies, the difference in brood area caused by exposures remained throughout the observation period (Table 35). Brood area in high exposure colonies (5 and 6) and medium exposure colony three, remained significantly less than that of colonies in other treatment groups.

Comparing colonies at each treatment level, the control colonies (2 and 4) had a significantly different brood area during the entire observation period and during the exposure period (Table 35). However, in the post exposure period there was no significant difference in brood area in colonies two and four. The low exposure colonies (7 and 8) were statistically similar in brood area over the entire observation period and during the exposure and post exposure periods. The medium exposure

Table 33. Effects of SRF exhaust exposure on brood production in honey bee colonies.[1/]

SRF exhaust treatment (ppm HCl)	Hive number	11 May brood area (cm^2)	Growth rate (cm^2/day) Total observation period	Exposure period	Post exposure period	6 July brood area (cm^2)	11 August brood area (cm^2)
0	2	3198	7.46	92.99	-39.91	8982	4223
	4	983	35.45	74.32	- 5.49	5830	2688
12	7	2840	21.22	60.47	- 5.05	7669	5654
	8	4088	8.01	26.86	-12.39	6232	4091
24	1	3136	14.52	78.13	-20.30	7987	5460
	3	1821	-17.02	26.57	-52.48	3244	0
35	5	3409	-19.01	-107.78	-27.03	2651	616
	6	2838	-0.01	-11.99	- 8.59	3417	1714

[1/] Trinomial regression curves fit to data are shown in Figure 7.

Table 34. Duncan's multiple range test comparing brood areas between treatments

Treatment HCl concentration (ppm)	Average brood area (cm^2)[1/] Total observation period	Exposure period	Post exposure period
0	4530.[a]	4356.[b]	5221.[a]
10	4422.[a]	5010.[a]	5754.[a]
20	5296.[a]	4098.[b]	4529.[a]
30	2271.[c]	2220.[c]	2352.[b]

[1/] Means with the same letter are not significantly different (comparisons only within each time period).

Table 35. Duncan's multiple range test comparing mean brood area between hives.

Treatment HCl concentration (ppm)	Hive number	Average brood area (cm^2) 1/ Total observation period	Exposure period	Post exposure period
0	2	6254.[a]	6066.[a]	6553.[a]
	4	2807.[c]	2130.[c]	3889.[bc]
10	7	5689.[ab]	5316.[ab]	6287.[a]
	8	4904.[b]	4705.[b]	5221.[ab]
20	1	6284.[a]	6017.[a]	6711.[a]
	3	2561.[c]	2694.[c]	2347.[c]
30	5	2179.[c]	2165.[c]	2201.[c]
	6	2363.[c]	2275.[c]	2503.[c]

1/ Means with the same letter are not significantly different (comparisons only within each time period).

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colonies (1 and 3) were not significantly different in area of brood production for any period, while the high exposure colonies (5 and 6) were not significantly different for any time period.

5.4. Discussion

5.4.1. HCl - acute toxicity studies

Eighty min exposures of honey bees to HCl were the lowest duration exposures where an ED_{50} value could be determined. High constant concentrations of HCl (over 120 ppm) were difficult to maintain in the exposure chambers, even for short durations; long (over 120 min) constant concentrations were also difficult to maintain. Thus, there is a tendency to underestimate the ED_{50} concentrations. From probit analysis of the results of 80 min exposures, 20 ppm HCl is predicted to affect 1% of honey bees in an exposed population. This effect is so low that it would not be perceptible in a natural situation. Extrapolating from the results of ED_{50} analyses, one can predict that concentrations of over 150 ppm HCl would have to be encountered for 30 min to cause 50% of the population to be immobilized. The data suggest that expected HCl concentrations in shuttle exhaust clouds would not harm honey bees.

Acute toxicity tests of various toxins have been performed in a different manner than those used by E. L. Atkins (2) for honey bee. His methods include collecting only young adults from comb in the hive. This reduces the variability in age and fitness of the test populations, and reduces natural mortality. Collections in our tests were of all bees leaving the entrance of the hive. The bees collected tended to be foragers which normally spend time outside the hive. Occasionally young bees leaving the hive, to evacuate themselves, were also collected. Foraging adults are older; they may die naturally during the period of tests and observation. The variability within the test population is therefore increased by this method of collection, but the test population more closely approximates a real situation than would a population of young adult bees.

Mortality of control groups of corn earworm eggs was so high (around 50%) that it was difficult to assess the effects of HCl. This mortality in the control groups was associated with a lack of knowledge on egg viability and the loss of the fragile eggs during handling. However, the probit analysis of the results of exposures of corn earworm eggs indicated that 480 ppm of HCl for 30 min was needed to acutely affect eggs.

Cannibalism was an important factor in reducing the test population size in corn earworm. Thus, since eggs are generally laid singly in the field, we separated the larva for these studies. These results indicated that first instars were much more sensitive to HCl than third or fifth instars. This may be related to the greater activity of the first instars since the third and fifth instars were confined in glass tubes. Also, many of the fifth instars were approaching pupation and were in a particularly quiescent stage at the time of the tests. Even the first instars were not sensitive to HCl since the ED_{50} for 30 min was 175 ppm.

Corn earworm adults were examined in three different groups, based on their reproductive status. These stages were also characterized by different degrees of activity; preovipositional adults were the most active, followed by mature and then postovipositional adults. Here, as with the larval stages, the younger adult was more sensitive to HCl. The ED_{50} for 60 min was 102 ppm for the young adult.

The results suggest that no stage of corn earworm should be sensitive to the SRM exhaust from the Shuttle launch.

Probit analysis of the 240 min exposures of the common lacewing indicated that eggs were highly resistant to HCl and that larvae were more sensitive to HCl than eggs; likely there was no difference in response of early and late instar larvae. The sensitivity of the larvae was similar to that of the first instar of corn earworm. The common lacewing is known to be tolerant to many chemicals (74); this may be due to avoidance behavior.

The correlation of activity level of corn earworm adults and their susceptibility to HCl indicated that the mode of entry of the gas was probably through the respiratory system. Similarily, in comparing the ED_{50}s of adults with those of larvae, we suggest that activity level was a significant determinant of response to exposure. The gradation of

resistance to HCl gas was correlated with activity level for all the insects tested. Permeability of insect integument (a consideration in pesticide studies where the toxin enters the cuticle by passive diffusion) does not appear to be an important consideration in HCl toxicity.

5.4.2. Bee colony analysis

The amount of brood in a colony has often been used as an indication of hive strength. Changes in brood production are a reliable indicator of the health of the bees, the suitability of the environment, and the availability of food. Environmental stress is often manifested as a decrease in brood production. As a unit, a colony's survival depends on sufficient brood production to maintain food supplies, to feed larval bees, and to cluster to survive low temperatures in winter. Under normal conditions brood area increases in times of ample food supplies (through mid-summer), then decreases gradually during the hot period of summer and through the fall and winter when food resources are reduced and environmental stress causes bees to attend to other needs of the colony. Colony weight, which is mainly due to honey stores, usually follows a similar cycle. Weight is increased substantially during the spring and summer followed by a slow depletion of stores with decreased weight through the remainder of the year.

The control colonies, 2 and 4, at the beginning of these tests had a large difference in brood area. This may have been due to a difference in queen viability in the early spring or to environmental conditions. When placed under the same conditions the larger colony, 2, increased brood area at a lesser rate (2.9% per day) than did the smaller colony, 4 (7.6% per day). This is probably due to the tendency of a colony to respond in the most advantageous way to its environment. In the postexposure period, colony four brood production continued to increase at a higher rate than colony two (3.2% per day compared to 1.1% per day) during that same period. In the last month of observation both colonies decreased brood production at the same rate (1.5% per day). Although they had started with substantially different brood areas, they were fairly close in strength by the end of the observation period. The response of these two colonies over the season was used for comparison when examining the growth and productivity of colonies exposed to SRF exhaust.

During the exposure period the brood area of the low exposure (12 ppm) colonies, 7 and 8, grew at reduced rates (2.1% and 1.1% per day, respectively) than did the control hives. This suppression of growth rate indicates that the bees were under some stress. In the 25 days following exposures colonies seven and eight brood area grew at about the same rate as the control colonies (1.4% and 1.7% per day, respectively). Brood production was not permanently affected by one month of SRF exhaust exposures to 12 ppm of HCl. Prolonged exposure may have prevented the resurgence of these colonies. If brood production had not increased after the exposure this depression would have eventually become obvious in decreased honey production, as fewer workers would have been available to collect and store nectar.

The medium exposure (24 ppm) colonies, 1 and 3, grew at rates similar to those of the low exposure colonies, but significantly less than those of the control colonies during exposure. The larger colony, 1, maintained brood production during the 25 days after exposure that was similar to that of the control and low exposure colonies. The small colony, 3, showed the stress of exposures during the 25 days following the exposure period. Brood area was maintained until 6 July but no brood was evident on August 11. Probably the colony was unable to return to the levels of honey and brood production necessary to offset the losses sustained during the exposure period. The response of these colonies indicates that a one hr exposure to 24 ppm of SRF exhaust twice a week will likely cause significant damage to bee colonies. If the colony is strong before exposure it may withstand the stress of the exposures.

The highest exposure (35 ppm) colonies, 5 and 6, lost brood production (about 3.2% and 0.4%, respectively) during the exposure period. Colony five was unable to recover from the effects of the exposures. Although it gained brood area over the 25 days following exposure, it lost area after that and by August 11 the brood area was only 616 cm^2; this hive was lost. Colony six was able to increase honey stores after exposure while maintaining the same level of brood production. This difference in colony response suggests that colony six survived the month of SRF exhaust exposures because it was able to reestablish honey stores quickly, after exposure, to support brood production. Had exposures continued, colony six would probably not have survived.

Results suggest that, if exposures were continued for an extended period of time, both the medium and high exposure colonies would have been lost. Colonies exposed to low concentrations of SRF exhaust (12 ppm, as HCl) are likely to grow at a lower rate than nonexposed colonies, but these colonies may not be lost.

The results of these insect studies suggest that no direct observable acute effects on insects will be found as a result of the shuttle program. However, as shown in a study of the effects of selenium ash on honey bees (81) the effects of SRF exhaust on honey bees and other insects may be due to destruction of food sources (e.g., plants) more than the direct effects on the insects.

Detrimental effects to the pollination and honey industry are possible due to increased susceptibility of exposed colonies to stress-related diseases with a subsequent decrease in honey production and pollination efficiency.

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7. APPENDICES

The appendices contain information of value to all serious research workers. However, the data is not necessary for understanding the basic results presented in the body of the report. The authors have used their own discretion in developing these appendices. Much supplementary material is not included because it is found in theses (14, 59, 70) developed as part of this project. These theses are available from the NASA project coordinator.

7.1. Plant and Insect Species

All species are alphabetized by their common names. Cultivars are shown for the cultivated plant species.

Common Name	Scientific Name
Native Plant Species (24)	
arrowhead	Sagittaria lancifolia L.
boston fern	Neprolepis cordifolia (L.) Presl.
camphor weed	Heterotheca subaxillaris Britt. and Rus.
cattail	Typha latifolia L.
croton	Croton punctatus Jacq.
fetterbush	Lyonia lucidia (Lam.) K. Koch
glassworth	Salicornia virginiana L.
grape (muscadine)	Vitis rotundifolia Michx.
groundsel	Baccharis halimifolia L.
live oak	Quercus virginiana Mill.
marsh elder	Iva frutescens L.
Paspalum	Paspalum vaginatum sw.
pennywort	Hydrocotyle umbellata L.
primrose	Oenothera laciniata Hill
railroad vine	Ipomoea pes-caprae (L.) R. Brown
sea grape	Coccoloba urifera (L.) L.
sea lavender	Limonium carolinianum Britt.
sea oats	Uniola paniculata L.
sea ox-eye	Borrichia frutescens (L.) OC.
slash pine	Pinus elliottii Engelm.
smooth cordgrass	Spartina alterniflora Loisel.
sunflower	Helianthus debilis Nutt.
switch grass	Panicum virgatum L.
wax myrtle	Myrica cerifera L.
Agronomic plant species (3) (9 cultivars)	
corn	Zea mays L. 'Coker 16', 'Silver Queen'
soybean	Glycine max (L.) Merr. 'Dare', 'Scott', 'Hood', 'Lee'

tobacco	Nicotiana tabacum L. 'Bel B', 'Bel W_3', 'Florida'

Horticultural plant species (9) (16 cultivars)

celery	Apium graveolans L.
grapefruit	Citrus maxima L.
lettuce	Lactuca sativa L. 'Grand Rapids'
lima bean	Phaseolus limensis Macf.
orange	Citrus sinensis Osbeck. 'Valencia'
radish	Raphanus sativus L. 'Cherry Belle', 'Comet'
snap bean	Phaseolus vulgaris L. 'BBL-290', 'Burbee dark'
tomato	Lycopersicon esculentum Mill. 'Betterboy', 'Fantastic', 'Heinz', 'Roma', 'Small Yellow Pear', 'Tiny Tim'
zinnia	Zinnia elegans Lorenz. 'White gem'

Insect species (3)

common lacewing	Chrysopa carnea Stephens
corn earworm	Heliothis zea Boddie
honey bee	Apis mellifera L.

7.2. The Effects of the HCl Screen on Foliar Injury to the Test Plants

Results from the major screen of 36 plant species (49 different plant selections) are shown here. Additional preliminary screens were done initially and some of this data is shown in Section 7.4.1.

Plant Species[1]	Average Foliar Injury To Test Plants (%) at Three HCl Concentrations (ppm) 10	20	40	Ave Injury[2] (%)
1. Native Species (24)				
arrowhead	4	53	77	45
pennywort	12	39	69	40
groundsel	8	5	94	36
marsh elder	1	22	58	27
switch grass	4	16	50	23
sea lavender	1	12	52	22
railroad vine	3	12	43	19
wax myrtle	1	15	37	18
croton	0	10	42	17
sunflower	1	1	48	17
cattail	0	10	38	16
sea ox-eye	0	7	31	13
muscadine	0	3	29	11

slash pine	0	3	12	5
camphor weed	0	2	9	4
Paspalum	0	0	10	3
fetterbush	0	2	4	2
primrose	0	0	3	1
live oak	0	0	3	1
boston fern	0	0	2	1
sea grape	0	0	1	+
glasswort	0	0	0	0
sea oats	0	0	0	0
smooth cordgrass	0	0	0	0
2. Horticultural Species (9)				
radish, 'Comet'	23	71	98	64
celery	23	62	90	58
tomato, 'Yellow pear'	18	41	94	51
tomato, 'Betterboy'	13	40	91	48
snap bean, 'BBL-290'	2	45	97	48
tomato, 'Roma'	14	37	88	46
tomato, 'Heinz'	14	36	88	46
tomato, 'Fantastic'	11	40	88	46
lettuce, 'Grand Rapids'	15	45	75	45
radish, 'Cherry Belle'	11	33	70	38
lima bean	1	24	90	38
zinnia, 'White gem'	14	27	61	34
tomato, 'Tiny Tim'	8	20	71	33
snap bean, 'Burbee dark'	1	32	64	32
grapefruit	0	0	3	1
orange	0	0	2	1
3. Agronomic Species (3)				
soybean, 'Dare'	15	67	98	60
soybean, 'Scott'	11	64	91	55
soybean, 'Lee'	12	61	93	52
soybean, 'Hood'	9	52	79	47
corn, 'Silver Queen'	0	15	74	30
corn, 'Coker 16'	0	12	70	24
tobacco, 'Bel W_3'	0	0	2	1
tobacco, 'Bel B'	0	0	0	0
tobacco, 'Florida'	0	0	0	0

1/ In each plant category the species and cultivars are listed from most sensitive to most resistant. All exposures were for a 60 min duration. The control plants are not shown because no injury was observed.

2/ Average injury (over three HCl concentrations) was determined only to aid in developing the sensitivity categories (Table 2).

7.3. Summary of Greenhouse Environmental Conditions

The following table summarizes the temperature (°C) and relative humidity (% RH) values obtained in the experimental greenhouse during the course of these investigations. The minimum and maximum values are 2 hr averages for the month in question. The 100% RH is common as a night RH under greenhouse conditions. The temperature and humidity averages were obtained from 12 2-hr estimates for each day of the month [e.g. in January 372 values went into the averages and the calculation of the Standard Deviation (SD)].

Dates	Temperature (°C)			Relative Humidity (%)		
1977	Ave (SD)	Min.	Max.	Ave (SD)	Min.	Max.
January	16.4 (± 4.3)	4	30	74.8 (± 11.4)	44	100
February	18.8 (± 5.6)	9	34	78.9 (± 18.3)	31	100
March	21.1 (± 5.4)	12	34	76.2 (± 21.4)	30	100
April	22.6 (± 5.5)	12	37	72.3 (± 22.6)	29	98
May	25.0 (± 5.9)	11	39	73.8 (± 23.2)	29	98
June	27.5 (± 7.7)	12	45	70.4 (± 25.1)	32	100
July	30.0 (± 5.9)	17	48	83.4 (± 18.3)	39	100
August	26.7 (± 4.8)	18	38	90.1 (± 12.9)	55	100
September	24.6 (± 5.3)	13	37	88.4 (± 18.7)	51	100
October	21.5 (± 4.9)	9	35	80.8 (± 19.0)	34	100
November	19.3 (± 3.4)	12	31	90.0 (± 16.2)	34	100
December	17.8 (± 2.3)	14	29	91.7 (± 12.6)	37	100
1978						
January	16.7 (± 3.4)	9	31	88.8 (± 12.4)	44	100
February	16.8 (± 5.3)	1	34	85.7 (± 15.9)	46	100
March	18.7 (± 5.4)	7	35	87.0 (± 18.9)	30	100
April	20.8 (± 6.9)	8	38	84.9 (± 20.5)	37	100
May	22.8 (± 5.7)	7	35	91.8 (± 13.9)	47	100
June	25.6 (± 5.6)	13	41	93.4 (± 11.8)	53	100
July	27.5 (± 6.0)	17	43	85.4 (± 20.0)	36	100
August	28.0 (± 6.6)	18	43	83.7 (± 20.7)	38	100
September	25.0 (± 7.2)	12	44	80.9 (± 23.3)	30	100
October	21.7 (± 5.8)	7	37	71.0 (± 23.9)	27	100

7.4. Supplemental Data on the Response of Plant Species and Cultivars to HCl, Al_2O_3 and SRF Exhaust

Sufficient data was included in Section 4 to document the basic results and support the conclusions drawn in this report. The data presented in this section is meant to supplement data presented in Section 4, since some investigators might be interested in the additional

data. Data is presented in the same order as for Section 4 and in tabular form. We have made the tables complete by using footnotes. Basic methods are as detailed in Section 4. Much of the data presented was not analyzed but mean separations are probably similar to those data that were analyzed.

7.4.1. HCl screens

7.4.1.1. Comparison of injury and biomass

Table 36. Effect of HCl concentration on foliar injury and top dry wt of tomato, 'Tiny Tim'.[1/]

HCl Concentration (ppm)	Injury (%)	Top Dry wt[2/]
0	0	2.98g
10	9	+1
20	30	26
40	69	53

1/ Exposures were 60 min in CSTR chambers. The design was replicated 3 times with 2 duplicate plants in each replication for 6 plants per treatment. Plants were exposed at 28 days from seed, foliar injury was determined at 31 days and plants were harvested at 35 days.

2/ Control wt is shown, the other values are % increase (+) or decrease from the control.

7.4.1.2. Variation of leaf response of test plants to HCl

Table 37. Comparison of whole plant foliar injury and injury to the 4 most sensitive leaves of 6 native species exposed to HCl.[1/]

Plant Species	Foliar Injury (ave of all leaves) at three HCl concentrations (ppm)			Foliar Injury (ave of 4 most sensitive leaves) at three HCl concentrations (ppm)		
	10	20	40	10	20	40
Marsh Elder	1	27	67	5	75	99
Arrowhead[2/]	0	15	62	0	21	93
Sunflower	1	2	48	1	7	68
Pennywort	2	8	40	12	25	99
Wax Myrtle	1	2	6	1	3	80
Railroad Vine	1	1	7	1	2	10

Table 37. continued

1/ The data was not statistically analyzed. The injury covers a 0 to 100% injury range estimated in 5% increments and averaged over 6 test plants (2 duplicates and 3 replicates). Exposures were 60 min in CSTR chambers. Plants were exposed after they became well established and foliar injury was determined 48 to 72 hrs after exposure.

2/ The second data set was averaged over two leaves instead of four.

7.4.2. HCl dose-response designs

7.4.2.1. Basic dose response injury designs

Table 38. Foliar injury to selected species (cv) as a function of HCl concentration and exposure duration.1/

Exposure Duration (min)	Foliar Injury at Three HCl Concentrations (ppm)			Foliar Injury at Three HCl Concentrations (ppm)			Foliar Injury at Three HCl Concentrations (ppm)		
	Soybean (Dare)*			Snap bean (BBL-290)*			Zinnia (White Gem)*		
	4	8	16	10	20	40	10	20	40
15	0	2	24	1	1	5	0	1	4
30	0	6	30	1	2	5	0	1	27
60	1	5	23	1	2	6	1	6	43
120	1	7	35	1	5	3	1	10	67
	(LSD - 0.05, 7%)			(LSD - 0.05, 3%)			(LSD - 0.05, 18%)		
	*Ave, 2 prim. lv.			*Ave, 2 prim. lv.			*Ave, 4 lv.		
	Tomato (Fantastic)*			Tomato (Tiny Tim)*			Corn (Coker 16)*		
	10	20	40	10	20	40	10	20	40
15	1	3	11	0	1	3	1	1	36
30	1	2	27	1	3	38	1	2	67
60	1	4	34	1	8	32	1	2	74
120	1	10	40	1	7	42	1	2	84
	(LSD-not determined)			(LSD-not determined)			(LSD-not determined)		
	*Ave, lvs. 2 to 5 (4)			*Ave, lvs. 3 to 5 (3)			*Ave, lvs. 2 to 4 (3)		
	Radish (Cherry Belle)*			Radish (Comet)*			Tomato (Better Boy)*2/		
	5	10	20	5	10	20	8	16	32
15	1	2	2	1	1	3	0	2	6
30	1	2	6	1	3	15	0	3	12

Table 38. continued

60	2	5	41	3	6	52	2	2	45
120	6	10	85	3	9	85	1	8	60
	(LSD-not determined) *Ave, lvs 3 to 4 (2)			(LSD-not determined) *Ave, lvs 3 to 4 (2)			(LSD-0.05, 12.7%) *Ave, all leaves		

	Sunflower*			Slash Pine*			Citrus (both species)*		
	8	16	32	10	20	40	40	60	80
10	0	0	5	0	0	0			
20	0	+	34	0	0	0	0	1	1
40	+	+	43	0	0	2	0	3	9
80	0	2	52	0	0	21	1	11	28
	(LSD-not determined) *Ave, all leaves			(LSD-0.06, 7.7%) *Whole plant ave.			(LSD-not determined) *Whole plant ave.		

1/ Foliar injury was estimated from 0 to 100% in 5% increments except for the pine and citrus where 10% increments were used. Data was averaged over 6 test plants (2 duplicates and 3 replicates) - the duplicate citrus plants represented the 2 species. Plants were exposed at specific ages or development and foliar injury was determined 48 to 72 hr after exposure. Where data was analyzed, an analysis of variance was used and treatments means were separated by LSD (0.05). The + signifies less than 0.5% average injury. Controls are not shown because they were not injured.

2/ The exposure durations for tomato were 10, 20, 40 and 80 min and not 15, 30, 60 and 120 min.

7.4.2.2. Comparison of sensitivity in leaves or groups of leaves

Table 39. Foliar injury to soybean (Dare) and its individual leaves as a function of HCl concentration and exposure duration.1/

Exposure Duration (min)	Foliar Injury (%) at three HCl Concentrations (ppm)			Foliar Injury (%) at three HCl Concentrations (ppm)		
	5	10	20	5	10	20
	Whole Plant			Primary 1		
10	0	+	9	0	0	4
20	0	1	70	0	1	70

Table 39. continued

40	+	14	76	0	20	90
80	6	69	94	5	66	94
	(LSD - 0.05, 7.6%)			(LSD - 0.05, 15%)		
	Trifoliate 1			Primary 2		
10	0	+	5	0	0	3
20	0	2	88	0	1	68
40	+	25	92	0	20	82
80	8	86	95	3	67	93
	(LSD - 0.05, 5.0%)			(LSD - 0.05, 16%)		
	Trifoliate 2			Trifoliate 3		
10	0	+	28	0	0	5
20	0	2	92	0	0	33
40	1	3	74	0	1	40
80	8	93	94	5	34	93
	(LSD - 0.05, 6.0%)			(LSD - 0.05, 7.9%)		

1/ The design included 3 duplicates for each treatment and was replicated 3 times for a total of 9 plants per treatment and 144 plants in the design. Plants were exposed once at 21 days of age and visual injury was determined at 23 days. Trifoliate leaves are counted from the oldest to the youngest. Data were analyzed by an analysis of variance and treatment means were separated by LSD (0.05). The + signifies less than 0.5% average injury. Controls are not shown because there was no injury in the controls.

Table 40. Foliar injury to wax myrtle and to 3 groups of leaves as a function of HCl concentration and exposure duration.1/

Exposure Duration (min)	Foliar Injury (%) at three HCl Concentrations (ppm)			Foliar Injury (%) at three HCl Concentrations (ppm)		
	10	20	40	10	20	40
	Whole Plant			Lower Third		
10	+	1	15	+	4	10
20	+	3	21	0	4	12
40	1	5	53	1	3	55
80	1	12	45	2	20	62
	(LSD - 0.05, 10.7%)			(LSD - 0.05, 26%)		

Table 40. continued

	Middle Third			Upper Third		
10	+	+	25	0	+	11
20	0	6	37	+	+	14
40	+	12	57	+	1	48
80	2	15	43	1	2	31
	(LSD - not determined)			(LSD - 0.05, 15.3%)		

1/ The design included 3 duplicates for each treatment and was replicated 3 times for a total of 9 plants per treatment and 144 plants in the design. Plants were exposed after they became well established and visual injury was determined 48 to 72 hr after the exposure. Data were analyzed by an analysis of variance and treatment means were separated by LSD (0.05). The + signifies less than 0.5% average injury. Controls were not shown because there was no injury in the controls.

7.4.2.3. Comparison of injury with other plant responses

Table 41. Foliar injury and biomass response of radish, soybean, corn and pennywort as a function of HCl concentration and duration of exposure.1/

Exposure Duration (min)	Foliar Injury at three HCl Concentrations (ppm) 2/			Biomass Changes at Four HCl Concentrations (ppm) 3/			
Radish (Comet)				Root fresh wt			
	5	10	20	0	5	10	20
10	+	+	2	0.69g	7	6	13
20	1	2	44	(±0.05)	7	14	64
40	1	18	79		0	0	75
80	2	39	85		4	62	86
Pennywort				Top dry wt			
	10	20	40	0	10	20	40
10	1	8	24	3.31g	11	21	4
20	2	18	68	(±0.29)	11	13	27
40	6	63	88		0	27	27
80	51	87	99		27	24	34

Radish (Comet)[3/]

	5	10	20
10	+	6	49
20	3	36	66
40	5	49	91
80	16	89	98

(LSD - 0.05, 7.7%)

Top dry wt

0	5	10	20
0.4g	0	25	25
(all the	25	25	50
same)	0	25	50
	0	25	50

(LSD - 0.05, 50%)

Soybean (Dare)[3/]

	5	10	20
10	0	+	9
20	0	1	70
40	+	14	76
80	6	69	94

(LSD - 0.05, 7.6%)

Root dry wt

0	5	10	20
1.35g	41	41	48
(±0.21)	33	11	41
	48	48	56
	26	63	56

(LSD - 0.05, 52%)

Tomato (Better Boy)[3/]

	5	10	20
10	0	0	21
20	0	3	20
40	1	4	35
80	5	10	47

(LSD - 0.05, 4.8%)

Fruit number

0	5	10	20
1.0	1.3	1.8	1.3
0.7	2.2	2.0	1.5
1.3	1.5	1.3	1.0
0.8	1.5	1.5	0.3

(LSD - not determined)

Corn (Coker 16)

	7.5	15	30
10	0	+	18
20	0	1	27
40	+	1	24
80	+	3	43

(LSD - 0.05, 5.0%)

Top dry wt

0	7.5	15	30
3.18g	0	0	18
(±0.04)	9	0	12
	0	6	18
	2	9	28

(LSD - 0.05, 19%)

1/ When data were analyzed, an analysis of variance with LSD separations was used. Data was averaged over 9 test plants (3 duplicates and 3 replicates).

2/ Foliar injury was estimated from 0 to 100% in 5% increments (pennywort was in 10% increments). The + signifies less than 0.5% average injury.

3/ The mean control wt is shown with the standard deviation in ( ). All other values are in percent reduction from the average control wt. This was not done for fruit number in tomato. All values are fruit numbers. The zero values indicated that values are the same or greater than the average control value but not significantly greater.

4/ Injury data is used in Tables 11 and 12.

7.4.3. SRF exhaust designs

7.4.3.1. Comparison of injury to different sections of plants

Table 42. Foliar injury to sunflower, wax myrtle and marsh elder as a function of SRF exhaust concentration and duration of exposure. 1/

Plant Section	Exposure Duration (min)	Foliar Injury (%) at Two SRF Exhaust Concentrations (ppm HCl)					
		Sunflower		Wax Myrtle		Marsh Elder	
		24	35	24	36	24	35
Whole Plant	10	3	43	0	1	0	18
	20	18	51	2	5	9	41
	40	14	47	6	11	17	51
(LSD at 0.05)		8.0%		1.2%		6.7%	
Upper Third	10	1	30	0	0	0	7
	20	12	39	0	1	4	21
	40	7	21	0	1	8	22
(LSD at 0.05)		4.6%		NS		7.5%	
Middle Third	10	4	56	0	1	0	23
	20	22	62	2	11	16	49
	40	23	61	9	22	28	66
(LSD at 0.05)		9.6%		2.5%		8.1%	
Lower Third	10	3	43	0	1	0	22
	20	19	53	5	3	11	42
	40	12	59	8	10	22	61
(LSD at 0.05)		7.6%		4.2%		9.2%	

1/ Each design included 3 duplicates and 3 replications for each treatment for a total of 9 plants per treatment and 108 plants per design. Plants were exposed once after they became well established and foliar injury was read 48 to 72 hr after exposure. The control and 12 pphm exhaust are not shown because of no injury or very little injury to the plants.

7.4.3.2. Effects of SRF exhaust on biomass

Table 43. Biomass responses of several plant species as a function of SRF exhaust concentration and duration of exposure.[1/]

Plant Species	Exposure Duration (min)	Shoot Dry Wt (g) at Four SRF Exhaust Concentrations (ppm of HCl)[2/]			
		0	12	24	35
Radish ('Comet')	10	0.70g	4	30	40
	20	(±0.03)	13	31	44
	40		10	23	61
(LSD at 0.05 = 30%)					
Soybean ('Dare')	10	3.45g	15	13	25
	20	(±0.02)	8	10	21
	40		19	21	27
(LSD at 0.05 = 20%)					
Corn ('Silver Queen')	10	4.64g	14	16	4
	20	(±0.43)	2	0	21
	40		5	13	16
(LSD at 0.05 = 18%)					

1/ Each design included 3 duplicates and 3 replications for each treatment for a total of 9 plants per treatment and 108 plants per design. Plants were exposed once after they became well established and were harvested seven days after exposure.

2/ The control data were not different. Thus the average control wt (for the 3 time periods) was used with the standard deviation in ( ). All exposure data is presented as a percent reduction from the average control value.

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15. Abstract

With Space Shuttle launches from the Kennedy Space Center scheduled for late 1980 or early 1981, NASA asked the USDA-SEA at North Carolina State University to investigate the effects of solid rocket motor fuel exhaust on selected plant and insect species in the Merritt Island, Florida area. The purpose of the investigation was to determine if the exhaust clouds generated by Shuttle launches would adversely affect the native plants of the Merritt Island Wildlife Refuge, the citrus production, or the beekeeping industry of the island. Conditions were simulated in greenhouse exposure chambers and field chambers constructed to model the ideal continuous stirred tank reactor. A plant exposure system was developed for dispensing and monitoring the two major chemicals in SRF exhaust, HCL and AL_2O_3, and for dispensing and monitoring SRF exhaust (controlled fuel burns). Plants native to Merritt Island, Florida were grown and used as test species. Dose-response relationships were determined for short-term exposures of selected plant species to HCL, AL_2O_3, and mixtures of the two to SRF exhaust. The effects of HCL, AL_2O_3, and mixtures of the two on honey bees were also studied, as were the effects of HCL on corn earworm and common lacewing, and the effects of SRF exhaust on honey bee colonies.

16. Key Words

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