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THE PHYTOTOXICITY OF DESIGNATED POLLUTANTS

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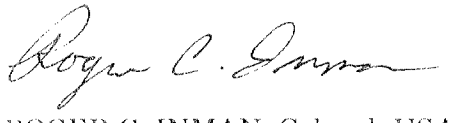

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The phytotoxicity of short exposures of gaseous hydrogen fluoride (HF) and of drenches of JP4 jet fuel were examined. Germination and growth of radish and tomato seeds were reduced when planting was delayed one or six hours after 20-minute exposures of the seeds to HF gas. Tomato seeds were more sensitive than radish. Barley, bean, and zinnia plants were exposed once to hF gas when six to 28 days old. Sensitivity of barley plants to injury from HF increased with age; beans were most sensitive when six or eight days old; zinnia sensitivity was not significantly affected by age at exposure. Plants were all harvested when 35

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days old; weight and other biomass measures correlated well with plant age at exposure. Plants exposed at an early age never developed well, whereas plants exposed when older were not greatly reduced.

Jet fuel applied around the base of 15 plant species caused injury ranging from no symptoms to death. Sensitivity to fuel varied with species and was similar to the toxicity of fuel sprays and vapors. Movement of fuel in soil was traced through vertical soil columns. Some microorganisms responded to the presence of jet fuel; growth and sporulation was delayed for two fungi growing in fuel-contaminated media. Fuel drenches inhibited nitrogen-fixing Rhizobium spp. on roots of beans.

A sorghum seed assay was developed to evaluate toxicity of fuel-contaminated soil and to measure its recovery. Diluting fuel concentrations by adding amendments reduced toxic conditions, particularly if soil was aerated and dried before planting. In vitro evaporation of fuel produced residues more toxic than the original fuel.

Tests designed to compare the toxicity of petroleum-derived fuel (JP4-P) with shale-derived JP4-S were inconclusive, but JP4-S was often the more toxic of the two.

PREFACE

This report covers work performed by members of the Statewide Air Pollution Research Center at the University of California, Riverside, during the period from July 1, 1981 to July 31, 1982. The project was funded by United States Air Force Contract F-33615-80C-0512 administered by the University of California, Irvine. The authors acknowledge the cooperation of Air Force Contract Monitor Major James M. Livingston, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. The comments and suggestions of Drs. T. T. Crocker, H. Hodge, J. D. McEwen, and D. T. Tingey of the Scientific Advisory Board have been useful and were appreciated. We acknowledge the technical assistance and expertise of H. E. Stone and E. C. Smith; M. A. Lardner and M. Vest, University of California students, who aided during portions of the project. Mr. Thomas S. Fisher of the Statewide Air Pollution Research Center performed gas chromatography on our jet fuel samples.

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SUMMARY

Investigations during the second contract year concerned the phytotoxicity of hydrogen fluoride (HF) gas and liquid jet fuel. Hydrogen fluoride, as a gaseous exhaust product of certain solid fuel rockets, might impact vegetation near launch sites. A continuous stirred tank reactor (CSTR) chamber was used to expose seeds or plants to 20-minute doses of HF at appropriate elevated levels. Following exosure, seed germination was reduced and subsequent seedlings were shorter than unexposed control seeds. Soil appeared to neutralize residual acid on the exposed seeds and least phytotoxic response occurred when seeds were transferred to soil with the shortest delay after exposure. Other seedlings exposed to HF gas when six to 28 days old suffered injury that could be related to the number of leaves exposed. The younger the plants when exposed, the greater was the reduction in plant growth at harvest when plants were 35 days old.

JP4 fuel was applied to soil in a series of tests. When mixed into the soil, the fuel caused more plant injury and greater reduction in germination than when the same amount of toxicant was applied to the soil surface. Most plants responded similarly to jet fuel in the soil although some species, such as carrot, were more tolerant. Injury was more likely with a fuel drench than with sprays or vapors.

The reaction of several microorganisms to the presence of jet fuel was tested. Growth of two fungus species grown in artificial media was limited or delayed when fuel was present. One of these fungi, Penicillium rubrum, produced a characteristic red pigment only if fuel concentration in the media was less than 1% (v/v). Rhizobium spp., a nitrogen-fixing bacteria, was inhibited when soil was contaminated with fuel.

Sorghum seeds sown on fuel-contaminated soil suffered reduced germination with stunting of surviving seedlings and thus provided a useful bioassay with which to study fuel movement through soil and soil recovery attempts. The fuel and its phytotoxic fraction penetrated soil to a certain depth or traveled a certain distance depending chiefly on the amount of fuel applied. The toxic fraction could not be forced further into the soil matrix by applying water.

The bioassay technique was used to study the reclamation of soil contaminated with fuel. Diluting contaminated soil with relatively inert materials or with uncontaminated soil lessens total fuel concentration and therefore decreases toxicity. Increasing the nutrient levels in the soil through liquid fertilizers did not decrease toxicity significantly. Aging fuel in vitro by evaporation did not lessen toxicity; many of the phytotoxic components merely became more concentrated. When fuel volumes were reduced up to 70% by evaporation, toxicity increased. Leaving contaminated fuel sit or age effected limited recovery and spreading the soil out to aerate and dry further reduced detectable toxicity.

INTRODUCTION

This project is a continuation of Air Force-sponsored research under contract F-33615-80C-0512 in which our group studied the effects of Air Force-related pollutants on terrestrial vegetation.

In earlier work on this project, the phytotoxicity of gaseous HCl and aluminum oxide particulates have been investigated (Granett and Taylor, 1976, 1977, 1978, 1979, 1980a, 1980b, 1981a, 1981b). These materials are the chief exhaust products of solid rocket engines and are released in prodigious amounts in a ground cloud which may impact plants in the vicinity of the launch (Dawburn and Kinslow, 1976; Nadler, 1976). Hydrogen fluoride gas is produced by rocket engines more powerful than the ones releasing HCl. Hydrogen fluoride is considerably more toxic than HCl so it is important to consider the phytotoxic effects of such a ground cloud. Investigations carried out last year (Granett and Taylor, 1981a) compared the effect of short exposures of HF gas on different species with similar exposure to HCl. Tests conducted this year concerned the effect HF gas had on seeds and on seedlings at different ages.

A major thrust of research conducted this year concerned jet fuel. Jet fuel can impact the environment in numerous ways through accidental or emergency releases during flight, transfer, and transport (Baker, 1970; Clewell, 1980, Van Overbeek and Blondeau, 1954). Last year we investigated the effects of fuel sprays and vapors, whereas this year soil drenches were studied. Soil contaminated with jet fuel poses problems for both the owner of impacted land in terms of possibly reduced plant growth, and for the Air Force due to legal responsibilities. The investigation undertaken during the current year concerned fuel movement and soil recovery. Seed germination and growth was tested as a convenient bioassay of jet fuel. Pure cultures of several microorganism species were surveyed to determine relative sensitivities. Microorganisms might be used as sensitive assays for hydrocarbon fuel. In addition, populations of beneficial microorganisms may decrease under the influence of fuel while other organisms may utilize soil-bound fuel as a nutrient source. Jet fuels from two sources were routinely compared; petroleum-derived fuel (JP4-P) is the standard available, whereas JP4-S is from shale sources but performs in engines identically to JP4-P. We tried to document biological differences.

Fuels are complex mixtures of numerous compounds, some of which have greater phytotoxicity than others. Some of our investigations concerned whether fractions derived by simple evaporation of fuel had toxicities differing from unevaporated fuel.

MATERIALS AND METHODS

EXPOSURE EQUIPMENT

HF Exposure Chambers

Two metal-framed, continuous-stirred tank reactors (CSTR) chambers were used to expose plants and seeds to HF gas. These units have been previously described by Granett and Taylor (1978, 1981a).

HF Generating System

The gas generating equipment, also previously described by Granett and Taylor (1981a), consisted of Teflon syringes which injected a small but continuous stream of HF acid into Teflon tubing. The acid vaporized when it met a 12-liter-per-minute stream of dried and pre-heated air. The acidic vapor was diluted as it entered the air inlet of the CSTR chambers. An oven, aluminum conduit, insulation, and electronically regulated heat cable prevented condensation of the vapor. The system produced chamber concentrations of 0.5 to 10 mg HF m^{-3}.

Equipment for Applying Jet Fuel to Plants

Although previous work with jet fuel as vapors and sprays required the use of special equipment, current investigations were limited to soil drenches. In most cases, the fuel was pipetted onto the surface of soil already in pots or columns or onto preweighed amounts of soil in 30 x 30 x 20-cm plastic wash basins. The soil in the basins was stirred to thoroughly mix the fuel into the matrix before 100-cm diameter plastic pots were filled.

TOXICANTS AND SUPPLIES

Hydrogen Fluoride

Hydrogen fluoride gas was generated by injecting 10% hydrofluoric acid solutions into the described system. The solution was prepared by diluting stock, 52% hydrofluoric acid to 10% with distilled deionized water (ddw).

Jet Fuel

The Air Force Aerospace Medical Research Laboratory supplied two formulations of JP4 fuel. A standard jet fuel, derived in the conventional manner from petroleum, was designated JP4-P. The other fuel was derived from material extracted from shale rock and was designated JP4-S and was from Air Force batch 15B. The fuels were delivered in one- or five-gallon drums. Single one-gallon cans of each of the fuels were kept in a laboratory hood while the remaining supply was stored in an outside storage facility. Both JP4-P and JP4-S have the same additives, comparable chemical composition, and aviation characteristics.

Soil

UC Soil Mix II (Table 1) is a modification of Mixture B described by Matkin and Chandler (1972). Agriculture Operations Department of the University prepared and sterilized the mix, and we stored it up to four months in protected bins. Care was taken to prevent contamination by using clean utensils and by covering benches or treating them with copper-naphalate. Soil additives used in one study were commercially available materials and will be further discussed in the appropriate section.

TABLE 1
COMPOSITION OF GLASSHOUSE SOIL MIX

Components	Amounts
Soil (Oakley sand)	0.40 m^3 (14 ft^3)
Canadian peat moss	0.20 m^3 (7 ft^3)
Redwood shavings or fir bark	0.20 m^3 (7 ft^3)
Single super phosphate [$Ca(H_2PO_4)_2$]	1.13 kg (2.5 lbs)
Potassium nitrate [KNO_3]	0.11 kg (4 oz)
Potassium sulfate [K_2SO_4]	0.11 kg (4 oz)
Dolomite limestone	1.70 kg (3.75 lbs)
Oyster shell lime	0.68 kg (1.5 lb)
Micronutrients	
Cu	30 ppm (dry basis)
Zn	10 ppm (dry basis)
Mn	15 ppm (dry basis)
Fe	15 ppm (dry basis)

Field soil was obtained directly from the top 15 cm of a UCR field. The soil is a decomposed granite with a high sand component.

Plant Nutrient Solution

Plants were regularly fertilized with a nutrient solution described by Hoagland and Arnon (1950). Composition of the solutions was increased for nutrient enrichment treatments (Table 2).

Microbial Culture Medium

Microbes were grown in a standard potato dextrose medium (Table 3). Potato dextrose agar (PDA) consisted of potato dextrose broth (PDB) with 2% agar. In some cases a yeast-PDB broth was prepared (Table 3). Jet fuel was added in various ways.

BIOLOGICAL MATERIALS

Plant Species

Plant species chosen for the HF and fuel exposures included monocots and dicots representative of common field, vegetable, and garden varieties (Table 4). Species exposed to jet fuel drenches included those varieties previously tested for sensitivity to fuel vapors and sprays (Granett and Taylor, 1981a). For other tests ease of growth, horticultural type, and physical characteristics such as size, shape, and number of leaves dictated

TABLE 2
NUTRIENT SOLUTION FOR PLANTS

Material	Normal single strength (low)	Triple strength (high)
K_2HPO_4	1.37 g	4.11 g
KH_2PO_4	2.04 g	6.12 g
KNO_3	10.10 g	30.30 g
$Ca(NO_3)_2$	23.60 g	70.80 g
$MgSO_4$	9.86 g	29.58 g
Iron chelate	1.00 g	3.00 g
Micronutrients	19 ml	57 ml
Water to make	10 gal	10 gal

TABLE 3
POTATO DEXTROSE MICROORGANISM CULTURE MEDIA

Potato Dextrose Nutrient Broth (PDB)

Potatoes	250 g
Dextrose	20 g

1. Cover diced potatoes with water and boil 1 hour until soft
2. Add dextrose to decanted potato water
3. Add distilled water to make 1 liter
4. Autoclave

Potato Dextrose Agar (PDA)

PDB	
Difco agar	15 g

1. Prepare PDB
2. Add agar at same time as dextrose

TABLE 4
PLANT SPECIES USED IN HF AND FUEL TESTS

Species	Name	Variety
Alfalfa	Medicago sativa L.	CUF101
Barley	Hordeum vulgare L.	CM67
Bean	Phaseolus vulgaris L.	Pinto
Carrot	Daucus carota L. subsp. sativus (Huffm.) Arcang.	Red-cored Chantenay
Corn	Zea mays L. subsp. mays	Golden-cross Bantam
Cotton	Gossypium hirustum L.	SJ4
Lettuce	Lactuca sativa L.	Black-seeded Simpson
Marigold	Tagetes patula L.	Goldie
Radish	Raphanus sativus L.	Cherry Belle
Sorghum	Sorghum sudanensis (Piper) Stapf.	Piper
Squash	Cucurbita moschata Duchesne	Early Prolific Straight-neck
Sunflower	Helianthus annuus L.	Mammoth
Tomato	Lycopersicon esculentum Mill.	Tiny Tim
Wheat	Triticum aestivum L.	Yecora Rato
Zinnia	Zinnia elegans Jacq.	Scarlet Queen

species of choice. Barley, bean, and zinnia seedlings provided a range of reactions when studying the effect of plant age on HF sensitivity. Likewise, tomato and radish seeds were useful for the direct exposure of HF. They had been used in previous HF and HCl work where tomato seeds had been sensitive and radish seeds were tolerant (Granett and Taylor, 1980b, 1981a). Sorghum proved useful as a bioassay for jet fuel.

Plant Production

All plants were grown in glasshouses supplied with charcoal-filtered air and evaporative coolers. A large (6 x 30 m) glasshouse was equipped with steam heat, whereas two small (4.6 x 6 m) houses relied on mild winter conditions, plastic film insulation, and heat cables to protect plants from low temperature extremes. For the HF studies, plants were grown from seed in 350-ml pierced styrofoam coffee cups containing standard soil mix. Sturdier 10- and 25-cm diameter plastic pots were used for most fuel drench experiments. Plastic flats (43 x 43 cm) and 7.6-cm diameter, 60-cm long PVC columns were used to detect horizontal and vertical movement of fuel, respectively. Unless otherwise outlined, plants were regularly watered with liquid fertilizer.

Seed Growth

Seed bioassays were conducted in 10-cm diameter pots of soil or on filter paper disks enclosed in 9-cm diameter glass or plastic Petri dishes. Pots were incubated in a glasshouse, whereas the Petri dishes were incubated in the dark in the laboratory at 22 to 25°C.

Microorganisms

Pure cultures of nine fungi and one bacteria were obtained from collections at the University of California (Table 5). All grew adequately on PDA or PDB at room temperature (22 to 25°C) under standard fluorescent laboratory lighting. Colonies were checked visually and microscopically. Fuel was added directly to the surface of Petri dishes of agar, to suspensions of spores during transfer to agar or broth, or to the broth cultures before inoculum spores were added. For some tests, cultures were merely observed, whereas for others the liquid broth and fungi were drawn through a Buchner filter onto a preweighed filter paper disk using vacuum (Figure 1). The disks and mycelium were dried in a Precision Thelco model 18 laboratory oven at 70°C for 72 hours and were weighed on a Mettler model H16 electronic balance.

EXPOSURE TO TOXICANTS

Exposure of Plants to HF Gas

Plants exposed as seedlings were transported to fumigation facilities on the morning of the exposure. Gas was generated into the chambers for about an hour before plants were inserted. Two samples of chamber air were collected in bubblers before plant exposure and five times during the 20-minute exposure period. Plants were removed from the chamber and returned to glasshouse benches for subsequent grading and harvest. After the last fumigation, syringes and lines were flushed with distilled water and the chamber was flushed with glasshouse air for 30 minutes.

Seeds were exposed to HF gas while on filter paper in open Petri dishes. Seeds were transferred to unexposed filter paper and dishes or to soil after 20-minute exposures. The Petri dishes were covered and incubated in the dark in the laboratory.

Exposure of Plants to Fuel

All fuel exposures were conducted in well-ventilated areas, usually within a glasshouse reserved for such work. Personnel wore gas masks and rubber gloves during the operation. The glasshouse remained open for two hours after application to rid the air of fuel fumes. Untreated plants in the same glasshouse were not visibly affected under these conditions.

By visually dividing the pot surface into quarters, the plant could be judged to occupy one of the quarters if it was not at the center (Figure 2). Fuel was then applied to the middle of each of the three quarters and

TABLE 5
MICROORGANISM SPECIES USED IN TESTS

Class	Order	Species	Nature
Fungi			
Oömycetes	Peronosporales	*Phytophthora parasitica*	parasitic
Oömycetes	Peronosporales	*Pythium vexans*	parasitic
Ascomycetes	Pezziales	*Peziza ostrichoderma*	saprophytic
Basidiomycetes	Agracales	*Armillaria mellea*	parasitic
Imperfecti	Moniliales	*Aspergillus flavus*	pathogenic
Imperfecti	Moniliales	*Fusarium solani*	parasitic
Imperfecti	Moniliales	*Penicillium rubrum*	saprophytic
Imperfecti	Moniliales	*Thielaviopsis basicola*	saprophytic
Imperfecti	Mycelia sterilia	*Rhizoctonia solani*	parasitic
Imperfecti	Mycelia sterilia	*Sclerotinia rolfsii*	parasitic
Bacteria			
Eubacteria	Actinomycetes	*Streptomyces* spp.	saprophytic
Eubacteria	Pseudomonads	*Pseudomonis putida*	saprophytic

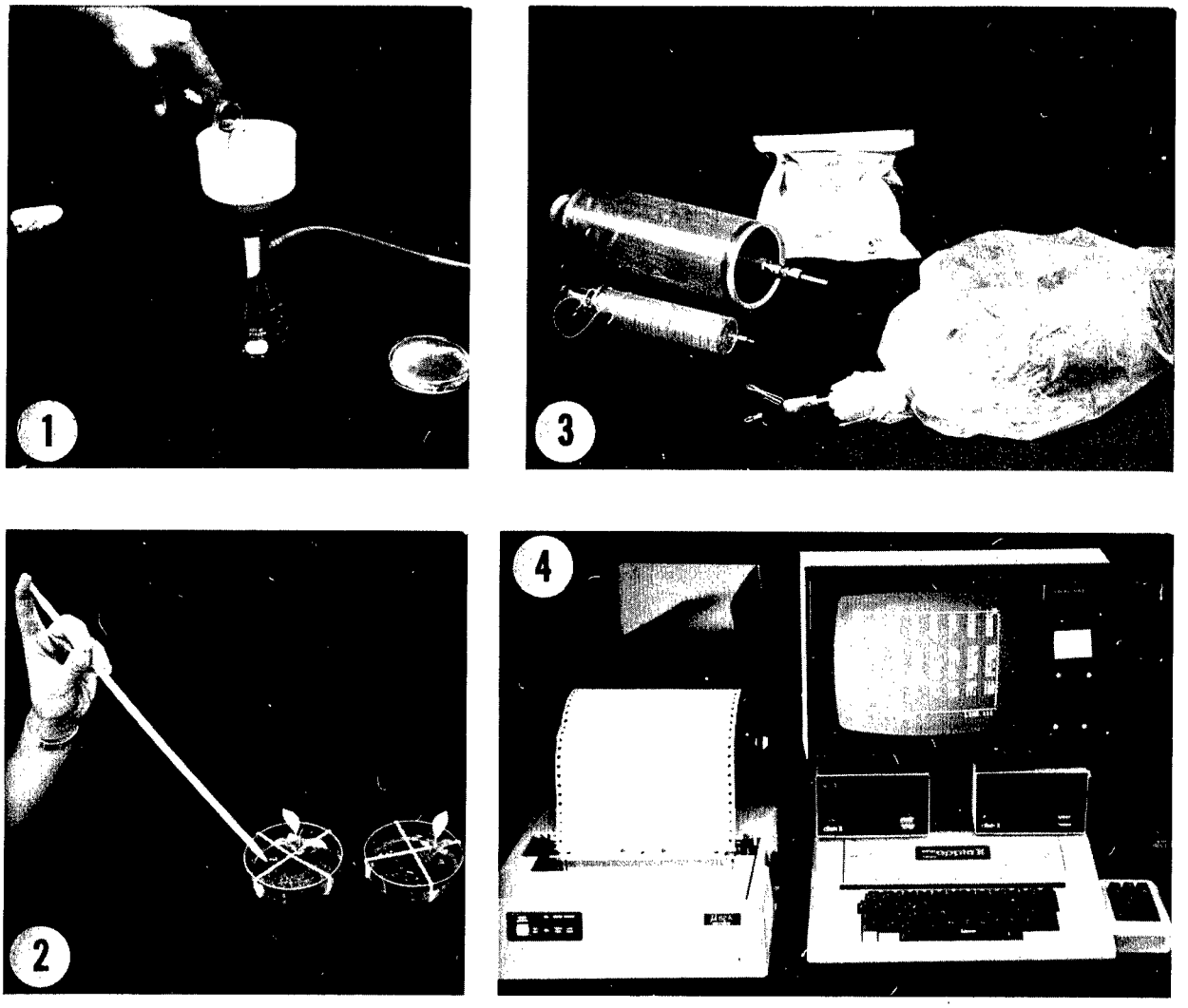
to the center or to the middle of the four quarters. This procedure helped to evenly distribute the fuel over the soil without direct application to the plant stem, which would cause certain injury.

Experiments involving fuel movement in soil were conducted within flats or columns and will be further detailed under the appropriate investigation. Contaminating flats and columns with fuel usually took place in the glasshouse.

Soil Recovery

Soil recovery studies included aging fuel-contaminated soil. In one test, aging was conducted outside the glasshouse in soil columns protected from precipitation and direct sunlight. In other tests, one-gallon pots of contaminated soil were placed within larger two-gallon pots packed with perlite to help insulate the smaller pots from direct sun.

Columns of soil used in experiments dealing with fuel movement or aging were 50 cm long and 7.6 cm diameter (Granett and Taylor, 1981a). They were extracted from PVC tubes using a plunger and cut into 5 cm wide sections. Each section contained ca. 228 cm^3 and weighed 348 ± 49 g. Soil in each section was placed in a 10-cm diameter pot and mixed before bioassay seeds were sown.



EXPERIMENTAL EQUIPMENT

Figure 1. Fungal harvest system. Growth media and fungi are poured into filter where liquid is removed by vacuum.

Figure 2. Fuel drench procedure. Placement of plant is determined and fuel is pipetted into four quarters or into three quarters and center such that contact with plant is avoided.

Figure 3. Nitrogenase activity detection apparatus. Soil and root ball are placed in plastic bag which is evacuated and filled with acetylene/air mixture.

Figure 4. Apple II Plus microcomputer system. Apple II console, two disk drives, monitor, Okidata printer, and acoustic coupler.

To further test methods which might facilitate soil reclamation, uncontaminated soil, sand, clay, charcoal, vermiculite, or perlite were mixed with the contaminated soil prior to potting.

Fuel Evaporation

Fuel was evaporated to test the toxicity of different fractions. Under a hood, the fuel was placed in 100-ml beakers on a magnetic stirrer. Fuel remaining after certain periods was measured with a graduated cylinder. Once evaporated to the desired amount, residues were kept in glass-stoppered volumetric flasks. Temperatures other than ambient (22 to 25°C) were accomplished with ice baths (4°C) or with a Corning model PC-351-RC hot plate-stirrer. The prepared residues were pipetted and mixed into preweighed glasshouse soil and the contaminated soil was potted at 420 g per pot. Potted soil, sown with sorghum seeds, was incubated in the glasshouse for two weeks.

MEASUREMENTS

Injury

Plants were examined for injury 24 hours after exposure to HF gas to allow time for recovery from transient wilting and for development of permanent symptoms. Plants were graded for percent leaf area injured using a one to twelve rating scale detailed in Granett (1982) and Granett and Taylor (1981).

Plants treated with soil drenches of jet fuel were graded for injury on the basis of death or wilting and partial collapse, since injury was not discrete leaf necrosis.

Yield

Plants in some experiments were harvested a certain time after treatment. Harvests consisted of making one or more measurements to determine the biomass or yield of the plant. Maximum length of plant shoots and roots were measured with rulers to nearest millimeter. Plants were weighed with an Arbor model 80 balance accurate to 0.1 g. Leaves were individually measured with a Li-Cor model L13000 or L13100 leaf area meter (Lambda Instruments) to closest 0.01 cm^2. Plants or plant parts, dried in an oven at 70°C for 72 hours, were measured on an Ainsworth model 10 electronic balance accurate to 0.001 g. In one experiment, tomato fruit were counted and weighed fresh.

Seed Tests

Injury to seeds exposed to either HF gas or jet fuel could be assayed by recording the number of germinating seeds or emerging seedlings and by comparing treated and control seedling lengths. In most tests, soil was washed from seeds and seedlings at certain times after exposure to facilitate measuring root and shoot or total lengths. Emerged seedlings could be accurately estimated by counting seedlings with shoot lengths longer than 10

mm. Summing the lengths of all seedlings per pot was sometimes a convenient measure of growth that avoided the statistical problem of zero-mm lengths for non-germinated seeds.

Pollutant Measurements

Hydrogen fluoride gas was measured by bubbling the exposure chamber atmosphere through 0.1 N nitric acid and measuring the F-ion in the resulting solution using an Orion model 901 ionanalyzer as described by Granett and Taylor (1981a).

Jet fuel was dispensed by pipetting the required amounts. The composition of evaporated residues of fuel was analyzed in a brief test using gas chromatography.

Microbial concentrations were estimated by making a 10-fold dilution of the standard inoculum and plating the dilution series onto individual PDA plates. Colonies could be counted at one of the dilutions.

Nitrogenase Activity

Rhizobium spp. growing in nodules on the roots of certain plants produce nitrogenase, the enzyme responsible for fixing atmospheric nitrogen as ammonia. Nitrogenase activity was used as a measure of the effect of pollutants on nodulation and nitrogen fixation. Activity was measured by decapitating plants and putting the intact unwashed root ball in a small air-tight bag (Figure 3). Acetylene was added to the bag and, after a 30- to 60-minute period in the dark, a sample of the chamber was withdrawn and analyzed for ethylene using a five-foot Porapak-N column on a gas chromatograph. This technique, based on the work of Fishbeck et al. (1973) and Hogsett et al. (1980), depends on nitrogenase activity efficiently breaking the triple bond of acetylene to produce ethylene.

Environmental Parameters

Temperature was measured with mercury thermometers and recording thermographs. Relative humidity was measured with a sling psychrometer.

DATA COLLECTION AND ANALYSIS

Data were collected on forms which facilitated hand entry into an Apple II-Plus microcomputer (Figure 4). The information was stored on magnetic floppy disks and could be retrieved and manipulated at will. Most analyses involved a one-way analysis of variance (ANOVA) and subsequent separation of means by Duncan's New Multiple Range Test (DNMR) (1955) or by the SNK or Student Newman Kuel's range test (in Zar, 1974). When multiple factor ANOVA were required, the data could be transferred to the PRIME 400 system from the Apple using an acoustic coupler. Simple graphs were produced using the Apple or a Tektronics model 4052 computer with model 4662 plotter.

PHYTOTOXICITY OF GASEOUS HYDROGEN FLUORIDE

DELAYED PLANTING OF EXPOSED SEEDS

Glasshouse-exposed Controls

Previous work indicated that seeds exposed to HF gas under certain conditions had reduced germination or emergence rates and that surviving seedlings had lengths which were shorter than unexposed controls (Granett and Taylor, 1981a). Seeds were more sensitive when wet or moist than when dry. Soil buffered or neutralized the acid gas to some degree. Seeds incubated in closed Petri dishes were more susceptible to gas injury than when incubated in soil. These findings suggested that HF gas might become an acid and enter the seed over a period of time if neutralizing soil was not present. To test this, radish and tomato seeds were exposed to 4.4 ± 0.8 mg m^{-3} HF gas for 20 minutes in open Petri dishes and were planted in pots of soil 0, 1, or 6 hours later. Six pots of five seeds each were prepared. Equal numbers of seeds left in open Petri dishes in the glasshouse during fumigations were planted as controls at the same time as the treated seeds.

Seedlings were harvested 28 days after treatment, and root and shoot lengths were measured. Tomato seeds were more sensitive to HF gas than radish based on germination rates (Table 6). As the delay between exposure and planting increased, the germination rates of either species decreased.

TABLE 6
GERMINATION OF SEEDS EXPOSED TO HF GAS FOR 20 MINUTES WITH DELAY BEFORE PLANTING

Species	Delay (hours)	HF treatment		% control
		Fumigated	Control	
Radish	0	90 ± 11[1]	47 ± 30	> 100[2]
	1	57 ± 23	87 ± 24	65
	6	12 ± 11	63 ± 27	19
Tomato	0	37 ± 34	50 ± 17	73
	1	3 ± 8	43 ± 20	8
	6	0	43 ± 32	0

[1] %-number of seeds germinated 28 days after treatments, mean and standard deviation of 30 seeds

[2] %-control = (germination fumigated seeds/germination control seeds) x 100%

Seedling lengths did not seem consistent with treatments (Table 7). Tomato seedlings were very sensitive to HF and a delay in planting of one or six hours prevented most germination. With no delay before planting, root lengths were significantly ($P < 0.05$) decreased compared to controls, but shoots were not. Radish was less sensitive. Root lengths of surviving fumigated radish seeds planted six hours after exposure were shorter than roots of seedlings from seeds delayed zero or one hour before planting or of control seedlings. Radish shoots remained unaffected by the treatments. This data indicates that longer delays before planting caused greater seed dysfunction. The delays also suggested that soil moderated the effects of HF and that shoots were not greatly affected.

Chamber-exposed Controls

The delayed-planting experiment was repeated with more pots of seeds per treatment and with the control seeds being exposed to no gas (0 mg HF m^{-3}) in the fumigation chamber. The chamber controls allowed all seeds to receive the same amount of drying air. The 20-minute exposures were at 4.4 ± 0.6 mg HF m^{-3} for the treated seeds and 0.4 ± 0.2 mg HF m^{-3} (equal to background with our detection equipment) for the controls. As in the earlier trial, seeds were transferred from open Petri dishes to soil at 0, 1, and 6 hours after treatment.

Unexposed or control radish seedlings emerged about five days after planting seed and tomatoes emerged at 10 days under glasshouse conditions. Seeds treated with HF took longer, about six and 14 days, for the two species, respectively (Table 8). For radish seeds, the extra time was only for seeds planted six hours after treatment. For tomato, many treated seeds failed to germinate even after 27 days and the six-hour delay group took longest to germinate.

Seed germination rates were calculated based on the number of seedlings present at harvest (Table 9). Germination data were transformed to create normal distributions by using the arc sin transform described by Anscombe (1948):

$$X_t = \sqrt{n + 0.5}\ \sin^{-1}\left(\sqrt{\frac{f + 0.375}{n + 0.75}}\right) \qquad (1)$$

where X_t = transformed value and f = number seeds germinated of n (=5) seeds sown.

After analysis, the means were converted back to percents:

$$G\% = 115\ [\sin(X_t/2.35)]^2 - 7.5 \qquad (2)$$

No statistical differences were found among the radish seeds regardless of gas treatment, delay before planting, or replicates. Germination rate of the more sensitive tomato seeds was decreased by the gas treatment. Lowest rates were associated with the longest (six-hour) delay before the seeds were planted in soil. One-hour delay reduced the germination, but this reduction was not significantly different from the 0-hour delay at $p < 0.05$. The statistics tests also indicated that the germination of tomato

TABLE 7
LENGTH OF SEEDLINGS 28 DAYS AFTER SEEDS WERE EXPOSED TO HF GAS

Species	Delay (hours)	Roots		Shoots	
		Fumigated	Control	Fumigated	Control
Radish	0	267[1]	178	41	45
	1	252	251	38	40
	6	168	246	45	45
Tomato	0	164	245	92	105
	1	382	194	126	105
	6	-	280	-	120

[1]Length of seedlings in mm, mean of up to 30 seeds; "-" indicates no seeds germinated

TABLE 8
DAYS FOR HF EXPOSED SEEDS TO EMERGE

Species	Treatment[1]	Delay in planting (hours)			Average[3]
		0	1	6	
Radish	HF	4.8 ± 1.0[2]	4.5 ± 0.9	7.5 ± 2.2	5.6 ± 2.0
	Air	4.1 ± 0.4	4.2 ± 0.9	6.1 ± 3.7	4.8 ± 2.4
Tomato	HF	12.5 ± 5.6[4]	13.5 ± 8.4[5]	14.5 ± 3.0[4]	13.5 ± 5.3
	Air	8.8 ± 2.4	11.6 ± 3.7	8.2 ± 1.3	9.5 ± 3.0

[1]Treatment was 20-minute seed exposure at 0 or 4.4 mg HF m^{-3}

[2]Number of days for maximum number of seeds to emerge, mean and standard deviation for eight pots, except where noted

[3]Average number of days for emergence, mean and standard deviation of up to 24 pots

[4]Mean for six pots (seeds in two of eight pots had no emergence)

[5]Mean for four pots (seeds in four of eight pots had no emergence)

TABLE 9
GERMINATION OF RADISH AND TOMATO SEEDS PLANTED UP TO SIX HOURS AFTER EXPOSURE TO HF GAS

Species	Exposure gas	Delay in planting (hours) 0	1	6	Average
Radish	HF	92[1] a[2]	93 a	89 a	91.6[4]
	Air	96 a	93 a	100 a	96.6
	(% of air control)[3]	(96)	(100)	(89)	
Tomato	HF	60 xy	28 yz	4 z	28.3
	Air	87 wx	94 w	91 w	90.7
	(% of air control)	(69)	(30)	(4)	

[1]%-Number of seeds germinated 27 days after treatments, mean of eight pots sown with a total of 40 seeds; arc sin transformed and reconverted to percent after analysis

[2]Means followed by same letter(s) not significantly different at $P < 0.05$ by Duncan's new multiple range (DNMR) test, species analyzed separately

[3]% of air control = (germinated HF-exposed seeds/germinated air-exposed seeds) x 100%

[4]Seed germination for all delay treatments, mean of 24 pots

seeds exposed to HF and sown directly onto soil (60%) was not significantly different from the control seeds exposed to air and also directly-sown (87%). Germination of tomato seeds exposed to HF was significantly reduced with 30% reduction with a one-hour delay and 4% reduction with a six-hour delay in planting. Replicates were not different.

Seedling root and shoot lengths were measured after soil was washed from roots (Tables 10 and 11). Radish seedling lengths showed no differences between means for gas or delay treatments nor for replicates; shoots were always shorter than roots, and no differences existed between shoot/root ratios.

Analysis of tomato lengths revealed significant differences between treatments. All seeds exposed to HF gas had shorter roots and shoots than the seeds exposed to air. Roots of tomato seeds planted immediately after HF exposure were not statistically different from seeds exposed to air and planted immediately or those exposed to HF and delayed one hour. The one- and six-hour delays in planting HF-exposed seeds seemed responsible for significant reduction in root lengths compared to air controls. Means of seedling shoot lengths were significantly different for each of the three delay treatments.

TABLE 10
LENGTH OF RADISH SEEDLINGS 27 DAYS AFTER SEEDS
WERE EXPOSED TO HF GAS AND PLANTED IN SOIL

Measure	Exposure gas	Delay in planting (hours) 0	1	6
Roots	HF	238[1] a[2]	236 a	236 a
	Air	271 a	256 a	275 a
	(% of air control)[3]	(88)	(92)	(86)
Shoot	HF	22 a	23 a	22 a
	Air	25 a	24 a	24 a
	(% of air control)	(88)	(96)	(92)
Shoot/root ratio	HF	0.09	0.10	0.09
	Air	0.09	0.09	0.09

[1]Length of seedlings in mm, mean of up to 40 seedlings (if 100% germinated)

[2]Means of same measure group (i.e., roots) followed by the same letter(s) are not significantly different at $P < 0.05$ by DNMR test

[3](HF length)/(air length) x 100%

TABLE 11
LENGTH OF TOMATO SEEDLINGS 27 DAYS AFTER SEEDS
WERE EXPOSED TO HF GAS AND PLANTED IN SOIL

Measure	Exposure Gas	Delay in planting (hours) 0	1	6
Roots	HF	116[1] xy[2]	55 yz	5 z
	Air	170 wx	234 vw	258 v
	(% of air control)[3]	(68)	(24)	(2)
Shoots	HF	32 y	16 z	3 z
	Air	51 x	62 x	62 x
	(% of air control)	(63)	(26)	(5)
Shoot/root ratio	HF	0.28	0.29	0.60
	Air	0.30	0.26	0.24

[1,2,3]See notes for Table 10

Summary of HF Effect on Seeds

Exposing seeds to HF gas for 20 minutes could affect germination, emergence, and early seedling development, particularly if seeds were exposed unprotected on filter paper. For radish seeds, the effect was apparently limited to a short delay in emergence, particularly if transfer of exposed seeds to soil was not immediate. Neither radish seedling germination rate nor length was adversely affected by the HF gas treatment. Seed germination rate and seedling length indicated that tomato seeds were sensitive to HF gas. Delaying transfer to soil for one or six hours further reduced tomato seedling germination rate and length.

PLANT AGE AND SENSITIVITY TO HF

Background and Experimental Procedures

Plants are more susceptible to foliar injury caused by many pollutant gases at certain ages in their life cycles (Ting and Heath, 1975; Ting and Mukerji, 1971). When HF gas is a pollutant, it is typically present at low concentrations for long periods (Treshow and Pack, 1970). Gas, particularly HF, entering plant leaves can be translocated and accumulates until injury occurs, often at the leaf margins (Jacobson et al., 1966).

Plants exposed to high HF gas concentrations for short periods undergo rapid burn and suffer necrotic injury (Granett and Taylor, 1981a). Since exposure and injury are very rapid with such exposures, plant age may not be important in estimating plant sensitivity. This experiment was set up to test whether the age of barley, bean, and zinnia plants at time of exposure to a concentrated dose of HF gas was a critical factor in immediate plant injury or subsequent plant yield.

Plants were exposed to HF gas on even-numbered days, 6 to 28 days after seeds were sown. Plantings were staggered such that exposures occurred on the same dates. Three replicates of all ages took place on three separate days. Plants were exposed to an average of 4.3 ± 1.6 mg HF m^{-3} once for 20 minutes. Injury was assessed 24 hours after exposure. An estimate was made of the injury on each leaf using a pre-transformed scale described by Granett and Taylor (1982). Harvests of exposed plants were staggered, taking place when each plant was 35 days old. Stem lengths, number of leaves, leaf area, and top dry weights were measured for each plant.

Effect of Age on Plant Injury

All plants were injured by the fumigations. The exposure conditions on the three days were not identical. Some compensations for differences were made by making all exposures between 1000 and 1300 hours on the three days. Cloudy skies and cool temperatures, however, prevailed during one of the three days; lower fumigation concentrations on that day resulted in reduced injury. Data were recorded on the first five leafsets of each plant, but all five were not present on very young plants. Initial analyses compared only the first leafset. Averaging the data for all fumigations showed that age had only a limited effect on plant sensitivity (Table 12).

TABLE 12
INJURY ON FIRST LEAFSET OF THREE SPECIES EXPOSED TO HF GAS

Plant age (days)	Barley	Bean	Zinnia
6	6.1[1]	4.3	8.1
8	6.2	10.0	8.1
10	8.6	10.6	8.3
12	9.2	10.7	7.9
14	8.3	10.7	8.0
16	10.0	9.7	8.0
18	9.9	8.6	6.6
20	9.2	8.9	6.0
22	9.1	6.1	6.8
24	9.3	7.1	6.8
26	8.8	5.0	5.7
28	9.2	6.4	5.9

[1]Leaf injury estimated on 1-12 scale (12 = 100% necrosis), average of first leafset on nine plants

Leaves on the youngest barley plants (six and eight days old) were more resistant than leaves on older plants. For zinnia seedlings, some separations occurred, with sensitivity of the first leafset decreasing with age; seedlings 26 and 28 days old had significantly less leaf injury than younger plants. Pinto bean primary leaves were most sensitive to HF gas when plants were between eight and 20 days of age. When the low (2.6 mg m^{-3}) HF concentration was considered (one of the three replicates), the range of sensitive ages became narrower, from eight to 15 days (Figure 5). This compares well with the sensitive age period of bean primary leaves exposed to HCl gas (Endress et al., 1979).

By averaging the injury found on up to five leafsets present on a plant, a measure of total plant injury was made (Table 13). Barley plants showed increased sensitivity with age. Beans were most sensitive when six and eight days old, but generally sensitivity remained constant with age. Sensitivity of zinnia plants to HF did not seem to change with age.

Effect of Age on Plant Growth and Yield

Plants were harvested on staggered schedules. At 35 days of age, seven to 29 days after exposure, maximum shoot height, number of leaves, total leaf area, and dry weight of shoots were measured for each plant. Each parameter increased with seedling age at time of exposure. This might be expected, since the vegetative area destroyed when the plant was young could

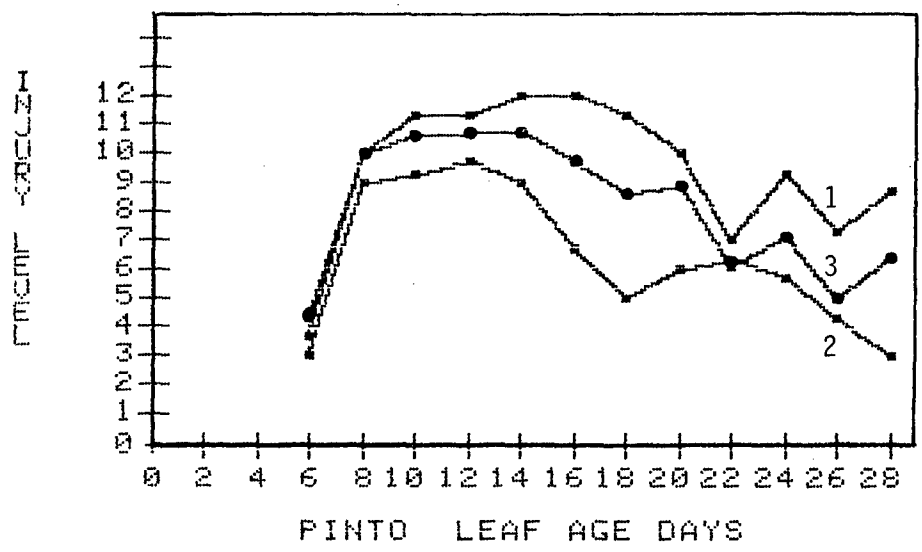


Figure 5. Injury (1-12 scale) on first leafsets of 6- to 28-day-old bean seedlings exposed to HF gas at (Line 1) 5.6 ± 0.5 or (Line 2) 2.6 ± 0.4 mg m^{-3} compared to (Line 3) average exposure of three replicates, 4.3 ± 1.6 mg HF m^{-3}.

TABLE 13
AVERAGE WHOLE PLANT INJURY
OF SIX- TO 28-DAY-OLD SEEDLINGS EXPOSED TO HF GAS

Age (Days)	Species		
	Barley	Bean	Zinnia
6	3.1 ± 4.2[1]	4.2 ± 0.1	4.8 ± 4.6
8	4.0 ± 3.0	10.0 ± 0.1	6.9 ± 1.7
10	7.3 ± 1.8	10.6 ± 0.1	5.0 ± 3.7
12	5.4 ± 4.1	8.8 ± 3.4	6.6 ± 1.9
14	6.4 ± 2.8	8.3 ± 3.8	4.4 ± 3.7
16	6.6 ± 4.2	7.4 ± 4.0	5.5 ± 3.4
18	6.9 ± 3.9	7.5 ± 2.6	5.1 ± 2.6
20	8.4 ± 2.2	9.1 ± 2.0	5.3 ± 2.8
22	7.3 ± 2.6	8.8 ± 2.4	4.3 ± 3.1
24	7.5 ± 2.4	9.1 ± 1.6	5.3 ± 2.4
26	7.2 ± 1.6	8.0 ± 2.5	5.3 ± 1.7
28	8.4 ± 1.6	8.3 ± 6.0	6.4 ± 0.6

[1]Estimate of injury on 1-12 scale (12 = 100% necrosis), mean and standard deviation of nine to 45 leaves (one to five leaves per plant)

only partially regenerate in the period before harvest; comparatively older plants lost less biomass.

Figures 6, 7, and 8 illustrate the effect of HF exposure on shoot length and dry weight for each species. In each case, seedlings exposed on the 28th day after sowing were significantly larger and heavier than plants exposed when only six days old. With all three species, age at exposure correlated significantly with weight at harvest ($r = 0.801$, 0.907, and 0.760 for barley, bean, and zinnia, respectively), but no correlation existed between average injury and weight. Number of leaves per plant separated into at least two groups, depending on when exposure took place (Table 14). Early exposures yielded plants with fewer leaves.

Leaf area per leaf, weight per leaf area, and weight per shoot length have been calculated for each of the three species (Tables 15, 16, and 17). The largest of each of these derived measurements were usually associated with seedlings exposed when 28 days old. Plant development appeared more affected by HF exposures causing injury which took place earlier in the life of the plant than later, but no consistent statistical relationship could be found between age exposed and the calculated measurements.

Summary of HF Sensitivity and Age Tests

The three species exposed to HF gas responded with considerable injury to 20-minute exposures to HF regardless of age. Some ages or ranges of ages were more sensitive, although this depended on species. Harvesting the plants at a fixed age produced evidence that a single HF exposure greatly

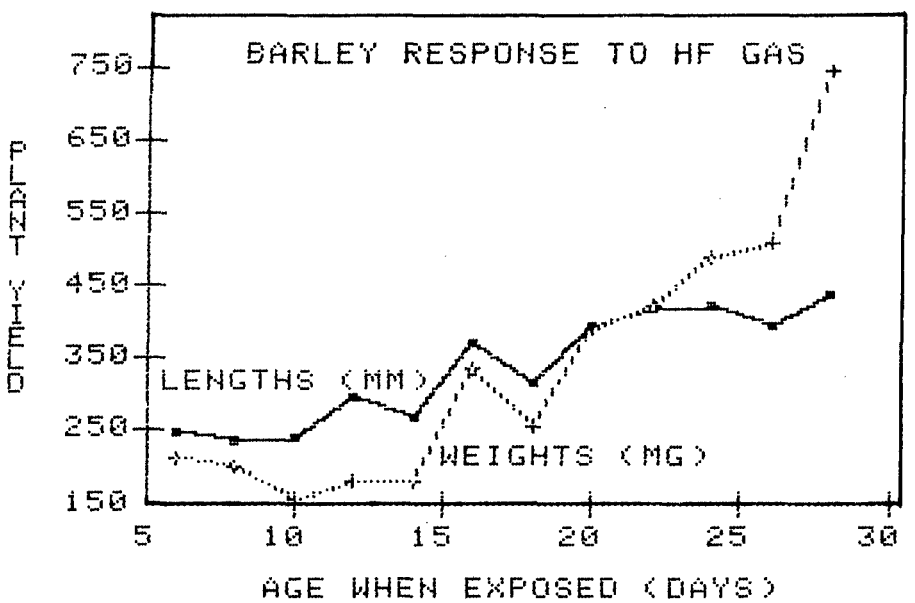


Figure 6. Response of barley exposed to HF gas, when six to 28 days old. Seedlings were exposed once then harvested when 35 days old. Data points for shoot lengths in mm and top weights in mg are averages of nine plants.

TABLE 14
NUMBERS OF LEAVES PER PLANT FOR SEEDLINGS EXPOSED TO HF GAS AND HARVESTED WHEN 35 DAYS OLD

Age when exposed (days)	Species		
	Barley	Pinto bean	Zinnia
6	19 ± 4[1] c[2]	13 ± 3 b	10 ± 2 d
8	25 ± 11 bc	12 ± 0 b	11 ± 3 cd
10	16 ± 4 c	11 ± 1 b	13 ± 2 bcd
12	18 ± 2 c	12 ± 1 b	13 ± 2 bcd
14	18 ± 1 c	12 ± 1 b	13 ± 2 bcd
16	18 ± 1 c	15 ± 3 ab	15 ± 3 abcd
18	23 ± 2 bc	13 ± 1 b	13 ± 1 bcd
20	25 ± 4 bc	14 ± 3 ab	14 ± 2 bcd
22	25 ± 10 bc	17 ± 5 ab	16 ± 4 abc
24	32 ± 3 b	18 ± 2 ab	17 ± 2 ab
26	33 ± 5 a	20 ± 2 ab	18 ± 0 ab
28	43 ± 2 a	21 ± 7 a	19 ± 2 a

[1]Number of leaves per plant, mean and standard deviation for nine plants

[2]Means in columns (for species) followed by the same letter(s) are not significantly different at $P < 0.05$ by Student Newman Keul's range test (SNK)

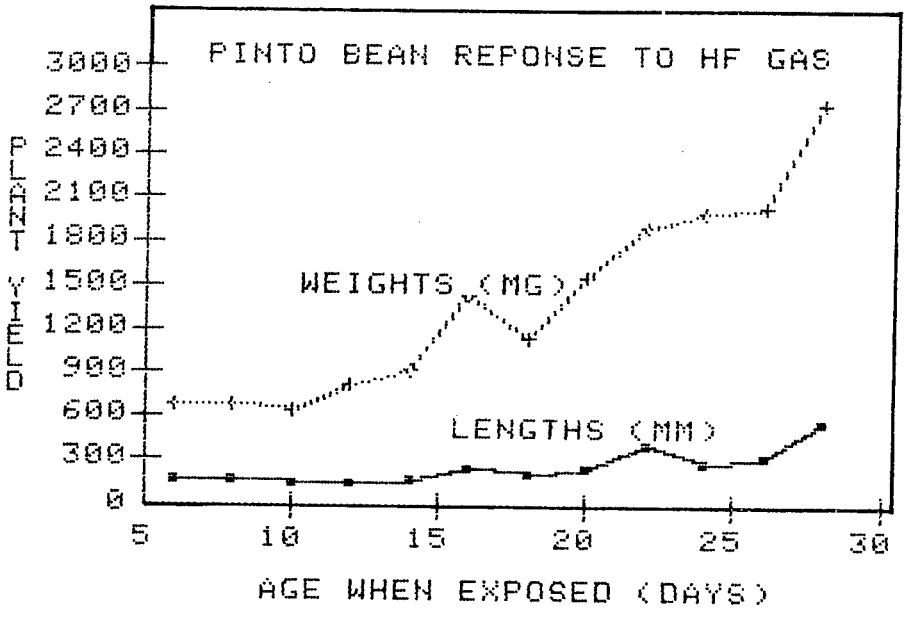


Figure 7. Response of pinto beans exposed to HF gas when six to 28 days old. Seedlings were exposed once then harvested when 35 days old. Data points for shoot length in mm and top weight in mg are means of nine plants.

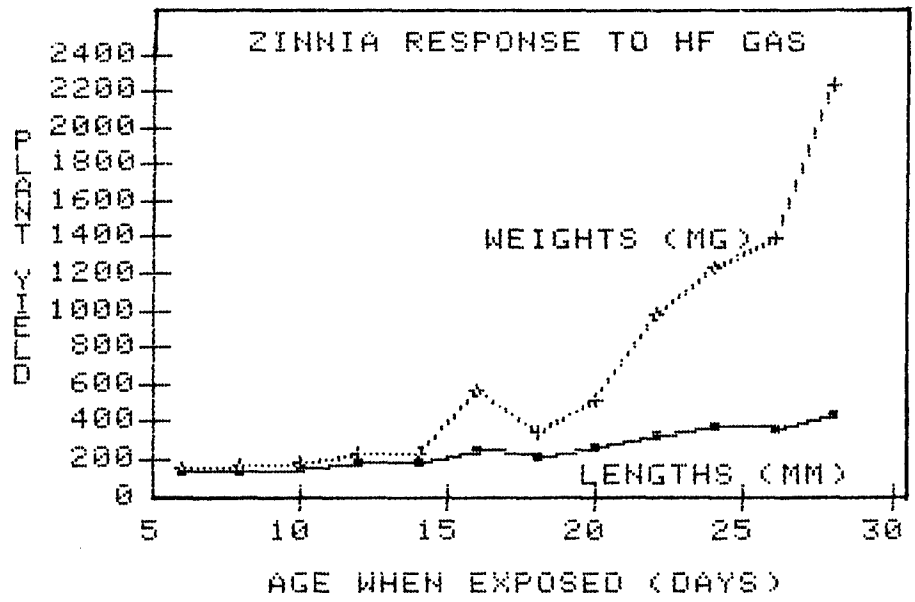


Figure 8. Response of zinnia exposed to HF gas. Seedlings were exposed once then harvested when 35 days old. Data points for shoot length in mm and top weight in mg are means of nine plants.

TABLE 15
GROWTH OF BARLEY SEEDLINGS EXPOSED TO HF GAS AND HARVESTED WHEN 35 DAYS OLD

Age when exposed (days)	Mean area per leaf (cm^2)	Mean weight per leaf area (mg/cm^2)	Mean weight per shoot length (mg/mm)
6	3.8[1]	57.0	0.86
8	3.5	58.8	0.85
10	5.0	31.2	0.65
12	4.6	43.4	0.60
14	4.5	38.7	0.66
16	7.0	46.9	0.90
18	5.0	51.2	0.81
20	6.7	58.9	0.74
22	7.3	57.8	1.01
24	7.3	67.0	1.16
26	7.7	66.4	1.29
28	7.5	99.2	1.70

[1]Mean of up to 45 plants

TABLE 16
GROWTH OF BEAN SEEDLINGS EXPOSED TO HF GAS AND HARVESTED WHEN 35 DAYS OLD

Age when exposed (days)	Mean area per leaf (cm^2)	Mean weight per leaf area (mg/cm^2)	Mean weight per shoot length (mg/mm)
6	24.5[1]	27.1	4.21
8	24.5	27.6	4.05
10	20.5	30.7	4.41
12	23.8	34.7	5.34
14	25.6	35.5	5.21
16	30.8	46.6	5.92
18	25.5	45.8	5.53
20	27.7	56.0	6.53
22	35.1	54.3	4.59
24	26.3	76.9	6.90
26	23.0	88.9	6.07
28	26.3	106.0	4.89

[1]Mean of up to 45 plants

TABLE 17
GROWTH OF ZINNIA SEEDLINGS EXPOSED TO HF GAS AND HARVESTED WHEN 35 DAYS OLD

Age when exposed (days)	Mean area per leaf (cm^2)	Mean weight per leaf area (mg/cm^2)	Mean weight per shoot length (mg/mm)
6	7.2[1]	36.9	1.06
8	7.6	35.5	1.21
10	7.0	37.8	1.23
12	8.8	38.2	1.33
14	8.6	37.6	1.29
16	14.4	55.0	2.27
18	12.4	40.0	1.59
20	14.4	48.7	1.93
22	14.4	54.6	2.98
24	29.6	62.9	3.26
26	52.8	64.9	3.82
28	41.4	86.2	5.03

[1]Mean of up to 45 plants

limited further growth of the plant; plants exposed when young remained stunted at harvest. This differed from plant response to HCl gas where biomass reduction from a single short exposure was considerable, but plant losses were rapidly recovered by subsequent growth (Granett and Taylor, 1979, 1980a, 1981a).

JET FUEL STUDIES

PHYTOTOXICITY OF JET FUEL

Fuel Tested on Tomato Seedlings

Tiny Tim tomato seedlings, 39 days old and ca. 5 cm tall, were grown in groups of three in one-gallon pots. Petroleum- or shale-derived JP4 fuel was surface-applied at 0.07 ml/cm^2 or 13 ml per pot of 800 gm soil. All plants in treated soil collapsed within 24 hours of fuel treatment and died within seven days. When fuel was observed to contact plant stems, collapse was hastened. No differences between shale and petroleum fuels were noted.

Effect of Fuel Soil Drench on Plant Species

Fifteen species were grown from seed until five or six weeks old (Table 18). The soil surface around the plant was contaminated with 4 ml JP4-P or JP4-S pipetted in 1-ml portions or was left as an untreated control. Each treatment contained 10 seedlings, one per 10-cm pot.

Plant injury was assessed one week after treatment by visual observations (Table 19), by a 1-to-3 injury comparison scale (Table 20), and by total (root plus shoot) fresh weight (Table 20).

In all cases, the average weight of plants which had received no fuel was greater than corresponding fuel treated plants. Differences were significant at $P < 0.05$ for all species except cotton, tomato, and zinnia, whose treated weights did not differ from control weights. Plant weights for most species did not differ with fuel type (petroleum or shale). JP4-S-treated lettuce plants, however, exhibited significantly less injury ($P < 0.05$) than plants exposed to petroleum-derived fuel.

On the basis of injury symptoms, carrot, marigold, sunflower, wheat, and zinnia plants treated with fuel could not be distinguished from controls. Obviously some of these were affected, since weight differences were found. A slow-rising, reddish discoloration was visible on fuel-treated cotton stems. Despite this obvious symptom, fresh weight of treated and control plants was the same.

Fuel applied to soil injured plants but the extent of injury was somewhat dependent on plant species. In previous tests, carrots were very tolerant to jet fuel vapors and could be sprayed with liquid JP4 with no harmful effects (Granett and Taylor, 1981a; Granett, Boudoin, and Stone, 1982). In this work, when fuel was applied to soil, carrot seedling weight was reduced, although carrot tops exhibited no injury. We did not determine

TABLE 18
PLANT SPECIES IN FUEL DRENCH TEST

Species	Variety	Age[1]
Alfalfa	CUF101	35
Barley	CM67	35
Bean	Pinto	35
Carrot	Red-cored Chantenay	35
Corn	Golden-cross Bantam	35
Cotton	SJ4	35
Lettuce	Black-seeded Simpson	35
Marigold	Goldie	35
Radish	Cherry Belle	35
Sorghum	Piper	35
Squash	Early Prolific Straight-neck	35
Sunflower	Mammoth	35
Tomato	Tiny Tim	42
Wheat	Yecora Rato	35
Zinnia	Scarlet Queen	35

[1]Age of plants when treated with fuel, in days

whether seedlings took up fuel and ceased growth or whether weight loss was due to water leakage from injured cells. Water loss seemed responsible for shrivelled radish roots.

The study was repeated after six weeks with the same 15 species at the same ages and with the same amount of fuel. Plants were observed for injury (Table 21) and harvested one week after treatment. Fresh weight of whole plants was recorded, then seedlings were oven dried and reweighed (Table 22).

The results were similar to the findings of the first test (compare visible symptoms in Tables 19 and 21). Barley and zinnia plants incurred more injury in the second study, but weights of treated zinnia were still not significantly greater than controls (Table 22). All the carrot plants were very small. Only in alfalfa and tomato plants could fresh weight differences be shown between the shale and petroleum forms of JP4; petroleum was more toxic to alfalfa, while shale was more toxic to tomato.

Root nodules observed on washed alfalfa and bean roots indicated the presence of nodules of nitrogen-fixing bacteria. When cut with a razor, nodules with living organisms were red, while dead nodules were brown. The fuel treatment reduced the number of plants with active nodules (Table 23).

TABLE 19
VISIBLE INJURY ON PLANTS ONE WEEK AFTER JET FUEL SOIL DRENCH: FIRST STUDY

Species	Observations
Alfalfa	Purple pigment on abaxial leaf surface; root mass smaller; nodules without red pigmentation; necrosis at base of leaflet
Barley	Collapse of stems with necrosis; yellowing of older leaves; less root mass
Bean	Senescence of older leaves; collapse of hypocotyl; discoloration of hypocotyl greater after JP4-P treatment
Carrot	Tops all look healthy; roots of treated white, not orange like control
Corn	Browning of younger leaves; stem collapse
Cotton	Collapse of new growth; JP4-P also caused hypocotyl collapse; reddish stem discoloration
Lettuce	Lower leaves of rosette dead; JP4-P caused collapse at soil while JP4-S did not
Marigold	Only margins of older leaves were necrotic
Radish	Globe-shaped root collapsed and withered; sensescent leaves
Sorghum	Complete collapse of plant; stem necrosis
Squash	Collapse at soil with partial stem necrosis
Sunflower	Wilting, some bending over
Tomato	Yellowing of all but youngest leaves similar to nitrogen deficiency symptoms
Wheat	Many plants fell over
Zinnia	No injury or damage observed

This study confirmed that hydrocarbon fuel drenches on soil can injure plants, cause weight loss effects, and are somewhat species-dependent. Differences in the phytotoxicity of shale- and petroleum-derived fuels, JP4-S and JP4-P, were not usually signficant.

SENSITIVITY OF MICROORGANISMS TO JET FUEL

Cultures and Maintenance

Microorganisms for study were obtained and conditions for growth were standardized. Species were maintained on potato dextrose agar (PDA) in glass Petri dishes or test tubes and grown in the laboratory at 22 to 25°C.

TABLE 20
RESPONSE OF PLANTS ONE WEEK AFTER JET FUEL SOIL DRENCH: FIRST STUDY

Species	Symptoms[1]			Weights[2] (g)		
	No fuel	Shale	Petrol	No fuel	Shale	Petrol
Alfalfa	3.0	1.6	1.3	2.7 ± 0.7 a[3]	1.7 ± 0.8 b	1.6 ± 0.7 b
Barley	3.0	1.6	1.5	23.9 ± 1.6 a	18.2 ± 6.1 b	14.8 ± 4.3 b
Bean	3.0	2.2	1.6	26.4 ± 2.9 a	19.8 ± 2.5 b	19.4 ± 2.2 b
Carrot	3.0	3.0	3.0	30.3 ± 0.9 a	14.8 ± 0.5 b	15.5 ± 0.8 b
Corn	3.0	1.7	1.4	31.3 ± 7.6 a	22.6 ± 6.7 b	22.0 ± 5.0 b
Cotton	3.0	1.9	1.7	6.3 ± 1.2 a	6.0 ± 1.1 a	5.8 ± 1.0 a
Lettuce	3.0	2.4	1.1	17.3 ± 2.3 a	4.7 ± 1.2 c	8.7 ± 4.0 b
Marigold	3.0	3.0	2.4	12.5 ± 1.5 a	8.2 ± 2.3 b	9.3 ± 1.4 b
Radish	3.0	1.2	1.0	22.0 ± 5.1 a	8.5 ± 2.0 b	10.3 ± 1.9 b
Sorghum	3.0	1.0	1.0	11.2 ± 2.0 a	4.8 ± 1.0 b	4.1 ± 0.8 b
Squash	3.0	1.1	1.5	35.4 ± 6.1 a	23.4 ± 8.6 b	26.0 ± 1.2 b
Sunflower	3.0	3.0	2.8	51.2 ± 9.1 a	43.1 ± 8.2 b	39.6 ± 5.4 b
Tomato	3.0	2.0	2.0	15.4 ± 1.8 a	13.2 ± 1.9 ab	12.5 ± 3.4 b
Wheat	3.0	2.9	2.9	17.1 ± 2.9 a	14.0 ± 3.5 b	13.8 ± 2.4 b
Zinnia	3.0	3.0	3.0	16.5 ± 1.3 a	15.7 ± 2.9 a	16.9 ± 5.3 a

[1]Injury rated as 1 = Fatal injury
2 = Non-fatal injury to stem and/or leaves
3 = Control or healthy appearance
Mean of 10 plants; second study results were comparable

[2]Whole plant fresh weight in g, mean and standard deviation of 10 plants
[3]Means for species treatments followed by same letter(s) are not significantly different at $P < 0.05$ by DNMR test

General Effects of Jet Fuels

Groups of five Petri dishes containing PDA media were inoculated with small agar plugs of an actively growing fungal species. One dish in each group was inoculated after 0.01 ml JP4 fuel was spread over the agar surface. Another dish in each group was treated by placing a sterile 6.5-mm diameter filter paper disk saturated with fuel on the media about 22.5 mm from the inoculum plug. The rest of the dishes contained no fuel and served as controls.

After 48 hours, growth was measured as mm of mycelium extending beyond the edge of the inoculum plug (Table 24). Normal growth rate varied depending on the fungal species and the treatment. Spreading fuel on the media inhibited growth in most cases, although Phytophthora did not seem affected. Fuel-saturated disks did not inhibit growth as much as spreading the fuel. In some cases, growth was too slow or contamination took place (e.g., Armillaria).

TABLE 21
VISIBLE INJURY ON PLANTS ONE WEEK AFTER JET FUEL SOIL DRENCH: SECOND STUDY

Species	Observations
Alfalfa	Discolored leaves, some stem collapse
Barley	One of 20 treated plants collapsed at soil level, others not affected
Bean	Yellowing, reminiscent of nitrogen deficiency, was more severe on older leaves
Carrot	No visible injury
Corn	Collapse at soil line, sometimes plants had chlorotic younger leaves
Cotton	Necrosis on very young leaves and apical meristem partial necrosis of older leaves near petiole
Lettuce	Necrosis of outer rosette leaves
Marigold	Red discoloration on older leaf margins
Radish	Complete collapse of globe-shaped root
Sorghum	Collapse of plant at crown
Squash	Collapse of hypocotyl; treated roots darker than white controls
Sunflower	Dry necrotic cotyledons, yellowing of first leaves
Tomato	Stem tissue collapse at soil level
Wheat	Stem tissue collapse at soil level
Zinnia	Weakened stems, some stem collapse between 1st and 3rd leaves; chlorosis in youngest leaves

With the saturated disk treatment, fungal mycelial growth may have been insufficient to encounter diffusing fuel.

Another survey study was conducted using seven fungi and one bacterial culture. These particular species were chosen because (1) they were available, (2) they grew easily and rapidly, and (3) they were known soil saprophytes and/or plant parasites. The microbes were maintained on PDA and inoculated into 20 ml PDB in flasks. Half the flasks contained 2% (0.4 ml) JP4-P fuel.

The cultures were harvested after eight days growth by adding 100 ml water, autoclaving to inactivate the microbes, and collecting the hyphal mat on preweighed filter paper. The fungal mat and paper were oven dried, cooled, and weighed (Table 25).

P. ostrichoderma, T. basicola, and T. viride cultures appeared to grow less when fuel was present. Although P. rubrum growth was not reduced by the fuel, the culture in the no-fuel media had sporulated and had produced a red pigment, whereas this was not the case when fuel was present. Pseudomonas

TABLE 22
BIOMASS OF PLANTS ONE WEEK AFTER SOIL DRENCH OF PETROLEUM- OR SHALE-DERIVED JET FUEL

Species	Fresh weight (g)[1]			Dry weight (mg)[1]		
	No fuel	Petrol	Shale	No fuel	Petrol	Shale
Alfalfa	3.7 a[2]	1.5 c	2.7 b	540 a[2]	340 b	430 ab
Barley	16.4 a	12.5 b	12.7 b	1980 a	1670 b	1690 b
Bean	20.4 a	15.8 b	14.9 b	3100 a	2450 b	2440 b
Carrot	0.5 a	0.3 b	0.4 ab	50 a	40 a	50 a
Corn	19.9 a	10.5 b	8.6 b	2110 a	1220 b	1150 b
Cotton	7.4 a	7.8 a	6.6 b	990 a	1060 ab	860 b
Lettuce	11.2 a	2.1 b	3.1 b	790 a	430 b	770 a
Marigold	6.9 a	5.2 b	6.2 ab	700 a	570 a	670 a
Radish	15.3 a	2.4 b	3.1 b	1100 a	640 b	650 b
Sorghum	6.9 a	3.9 b	2.8 b	960 a	670 b	610 b
Squash	30.2 a	17.6 b	17.1 b	2580 a	1950 b	2100 b
Sunflower	23.3 a	16.3 b	17.8 b	2260 a	1980 a	2000 a
Tomato	9.7 a	5.7 b	3.4 c	1050 a	680 b	550 c
Wheat	6.7 a	4.9 a	5.0 a	1330 a	990 a	1060 a
Zinnia	11.2 a	10.2 a	10.3 a	1330 a	1119 a	1210 a

[1]Weight in g or mg, mean of 10 plants

[2]Same letters following control, petrol and shale means for each species indicate no significant difference at $P < 0.05$ by SNK test

TABLE 23
BACTERIAL NODULES ON ALFALFA AND BEAN ROOTS AFTER SOIL TREATMENTS WITH FUEL

Species	Treatment	Number of plants with nodules[1]		
		Living	Dead	Absent
Alfalfa	No fuel	6	1	3
	JP4-P	0	2	8
	JP4-S	0	10	0
Bean	No fuel	8	2	0
	JP4-P	3	7	0
	JP4-S	2	8	0

[1]Ten plants per treatment were observed for nodules; one or two nodules per plant were cut, if red then categorized as living, if dark then nodule assumed to be dead

TABLE 24
GROWTH OF FUNGAL ISOLATES IN PRESENCE OF JET FUEL

Fungus	Maximum growth (mm) from inoculum plug edge		
	Control[1]	Fuel spread	Fuel on disk
Armillaria	0.0	0.0	0.0
Aspergillus	4.3	0.0	3.0
Fusarium	4.0	2.0	2.0
Phytophthora	10.0	9.0	10.0
Pythium	16.3	4.0	15.0
Rhizoctonia	29.3	7.0	18.0
Thielaviopsis	22.0	0.0	19.0
Trichoderma	3.3	0.0	1.0

[1]Control data are averages of two or three dishes, others are growth on only one dish

TABLE 25
YIELD OF CULTURES OF MICROORGANISMS AFTER EIGHT DAYS
IN LIQUID BROTH WITH JET FUEL

Organism[1]	Yield (mg)[2]	
	No fuel	2% fuel
Aspergillus flavus	28.4	31.6
Fusarium solani	41.7	46.7
Penicillium rubrum[3]	43.8	45.7
Peziza ostrichoderma	23.1	2.2
Phytophthora parasitica[4]	11.6	12.6
Thielaviopsis basicola	23.6	13.8
Trichoderma viride	50.7	46.3
Pseudomonas putida	5.3	2.8
Fuel check	not applicable	1.1

[1]All are fungi except *P. putida*, a bacterium

[2]Dry weight in mg of microbial growth

[3]Produced spores and red pigmentation in fuel-free culture only

[4]Seven days growth

putida, the only bacteria tested, produced turbid cultures regardless of whether fuel was present in the media or not.

Culture identification was verified prior to harvest by inoculating opposite halves of a Petri plate containing potato dextrose agar with material growing in fuel-enriched and fuel-free media. All cultures were shown to be identical to original organisms by growth characteristics and by microscopy.

Since only one culture flask was prepared for each treatment, conclusions were limited to the observation that P. ostrichoderma, T. basicola, T. viride, and P. putida responded to the presence of fuel with reduced biomass yield and P. rubrum responded with a lack of normal red pigmentation.

Several fungi were retested using four flasks per treatment. Treatment with 2% JP4-P was achieved by adding 0.4 ml fuel to 20 ml potato dextrose broth. The fungi were harvested by collecting the mycelial mat on filter paper after eight days growth. The mats were oven dried and weighed (Table 26). Analysis indicated that significant differences existed at $P < 0.05$ among the fuel and no-fuel treatments for P. rubrum, S. rolfsii, and T. viride. P. rubrum growth was similar to earlier observations; red pigment formed only in media containing no fuel.

Viability of Trichoderma viride Spores Exposed to Fuel

The fungus Trichoderma viride, a soil saprophyte which grew and sporulated readily under our conditions, was sensitive to the presence of fuel and was selected for further tests. T. viride spores were exposed to concentrations of jet fuel in an aqueous environment by preparing dilutions of the fuel and the spores. Spores were collected in sterile distilled water (SDW) from PDA slant cultures and a stock suspension was then serially diluted from 10^{0} to 10^{-7} ml suspension per ml SDW. Fuel dilutions consisted of 0, 1, 10, 10^{2}, and 10^{3} µl JP4-P fuel per 9 ml SDW. A matrix was constructed by diluting 1 ml of selected spore suspension dilutions with 9 ml of the fuel dilutions. Final fuel concentration was 0, 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} ml JP4 per ml media and final spore concentrations ranged from 10^{-4} to 10^{-7} of the original spore suspension. Samples of 100 µl were removed from each dilution and streaked on PDA in Petri dishes. Dishes were incubated in a small oven at 37°C and growth was visible after 72 hours. Counting was impossible in most cases because colonies coalesced (Table 28).

The results indicated that germination of T. viride spores was not effectively retarded at these fuel exposures.

Since T. viride was not inhibited when spores briefly encountered fuel in an aqueous environment, the same fungus was inoculated into liquid PDB containing fuel. Fifty ml of sterile media were placed in 250-ml flasks and 1 ml JP4-P fuel was added to one set of flasks to create a 2% (v/v) fuel concentration. No-fungus and no-fuel flasks served as controls. One replicate was shaken during growth using a reciprocating platform.

After five days, abundant mycelial growth was present in the inoculated flasks containing no fuel (Table 28). Growth was reduced in flasks containing fuel and sporulation was delayed. Shake cultures promoted somewhat more

TABLE 26
DRY WEIGHTS OF FUNGAL CULTURES GROWN 8 DAYS IN PRESENCE OF JP4-P FUEL

Species	No fuel control	2% Fuel	Significance[2]
Penicillium rubrum	87.0 ± 6.0[1]	59.4 ± 3.9	**
Peziza ostricoderma	35.4 ± 18.1	20.1 ± 6.2	n.s.
Sclerotinia rolfsii	192.3 ± 23.6	30.8 ± 23.5	*
Thielaviopsis basicola	17.6 ± 7.0	9.8 ± 11.4	n.s.
Trichoderma viride	76.9 ± 4.3	66.7 ± 6.9	*

[1]Hyphal dry weight in mg, mean and standard deviation of up to four cultures

[2]ANOVA test: n.s. = fuel and control means not significantly different; * = difference at $P < 0.05$ ** = difference at $P < 0.01$

TABLE 27
GROWTH ON AGAR OF TRICHODERMA VIRIDE SPORES EXPOSED TO JP4-P FUEL

Spore dilution	Fuel concentration (ml fuel/ml suspension)				
	0	10^{-4}	10^{-3}	10^{-2}	10^{-1}
0	0[1]	0	0	0	0
10^{-4}	+++	+++	+++	+++	+++
10^{-5}	++	24+	44+	++	++
10^{-6}	13.2 ± 2.0	16.2 ± 2.9	8.0 ± 1.4	15+	12+
10^{-7}	0	0	0	0	0

[1]Relative numbers of colonies:
+++ = colonies coalescing over entire dish
++ = colonies coalescing over most of dish
24+ = discrete colonies in some areas of dish, mean and standard deviation when all colonies were discrete
0 = no colonies on dish

TABLE 28
GROWTH OF TRICHODERMA VIRIDE IN LIQUID CULTURES CONTAINING JET FUEL

Treatment	Culture			
	Shake		Still	
	Rep 1	Rep 2	Rep 1	Rep 2
Fungus, 1 ml fuel	+[1]	+	+	+
Fungus no fuel	++++	++++	+++	+++
No fungus, 1 ml fuel	-	-	-	-
No fungus, no fuel	-	-	-	-

[1]Relative amounts of fungal growth, - is no growth; + is much less mycelial growth than +++

growth than still cultures, particularly when no fuel was present. No growth in non-inoculated controls with or without fuel indicated that neither media nor fuel was contaminated.

T. viride developed in liquid media, but growth was diminished when jet fuel was present. This was in contrast with the previous experiment where growth was uninhibited by the fuel. In that study, spores contacted fuel diluted in an aqueous environment for a brief period (20 to 60 minutes) before being transferred to PDA. Little or no fuel was present on the agar with the spores.

The effects of jet fuel on the growth of T. viride were further investigated by inoculating a series of flasks with cultures of the fungi and then harvesting flasks during the next 10 days. Fuel treatments of 2% JP4-P were prepared by adding 0.4 ml fuel to 20 ml of PDB. Inoculum consisted of 100 µl spore suspension containing 1.85×10^6 colony producing units (cpu). Two fuel and two non-fuel flasks with fungal growth were sampled by adding 100 ml distilled water, autoclaving, filtering, drying, and weighing oven-dried hyphal mats (Table 29). Growth was plotted as straight lines by converting the time scale to $\log_{10}$ values (Figure 9). Linear regression analyses revealed that each line had a high correlation coefficient, r (Table 30). The regression lines had slopes which were not significantly different, indicating that rate of growth was equal for fuel and no-fuel treatments. Final yield (the y-intercept) was significantly larger ($P < 0.05$) for cultures without fuel. Initial growth of T. viride was delayed by 2% (v/v) fuel and maximum biomass yield was reduced.

Response of Penicillium rubrum to Jet Fuel

Red pigmentation developed as P. rubrum grew in initial tests but was absent when fuel was present. To further test the effect of fuel on this fungus, a time-course study was prepared.

TABLE 29
GROWTH OF T. VIRIDE IN NUTRIENT BROTH CONTAINING JET FUEL

Days after inoculating flasks	2% fuel	No fuel
1	2.34 ± 0.11[1]	3.42 ± 0.70
2	19.67 ± 0.24	28.39 ± 0.38
3	15.79 ± 0.47	59.78 ± 1.78
6	63.61 ± 3.66	75.18 ± 8.03
7	64.74 ± 3.85	86.71 ± 2.21
8	69.46 ± 4.21	79.82 ± 0.25
9	67.68 ± 1.51	81.22 ± 2.67
10	64.91 ± 3.96	80.73 ± 0.63

[1]Dry weight yield of fungal mats in mg, mean and standard deviation of two cultures

Flasks of PDB nutrient media containing 0 or 2% (v/v) petroleum-derived jet fuel (JP4-P) were inoculated with spores scraped from a prepared agar culture of P. rubrum. At 3, 4, 5, 6, 7, 9, and 10 days after inoculation, four culture flasks per treatment were harvested by filtering the cultures and weighing the dried residue (Table 31). The analyzed data reveal that both fuel and no-fuel treatments fit linear equations with high correlation coefficients (Figure 10). The fuel and no-fuel lines representing Penicillium growth (Figure 10) were similar to the Trichoderma fuel and no-fuel lines (Figure 9) in that slopes of each pair were not significantly different ($P < 0.05$). Thus, rate of growth in fuel and no-fuel media was equal. Differences in y-intercepts indicated that onset of fungal growth was delayed and maximum yield was reduced in fuel-enriched medium.

The time-course study was repeated with three harvests made on each of 10 successive days. Inoculum was prepared by suspending spores in distilled water and 100 µl of the suspension was added to 40 ml PDB in each flask. Serial dilution plating of the suspension onto PDA determined that inoculum contained 3.8×10^6 cpu. The dry weight of the mycelial mat indicated standard microbial growth (Table 32). When the log of weight was plotted against time, a growth curve was obtained (Figure 11). With fuel in the media, fungal growth lagged behind growth in no-fuel control cultures, but log-phase growth was similar for both treatments (ca. 10 mg/day for no-fuel and 6 mg/day for fuel). Maximum final yield for the fungus averaged 100 mg/flask whether fuel was present or not. Fuel delayed fungal growth and, as demonstrated here and in previous experiments, prevented the formation of a characteristic red pigment. The fuel apparently did not limit total biomass.

Fuel-free media changed from pale yellow to deep red about four days after inoculation with P. rubrum. The color did not change after 14 days growth with 2% (v/v) fuel in the media.

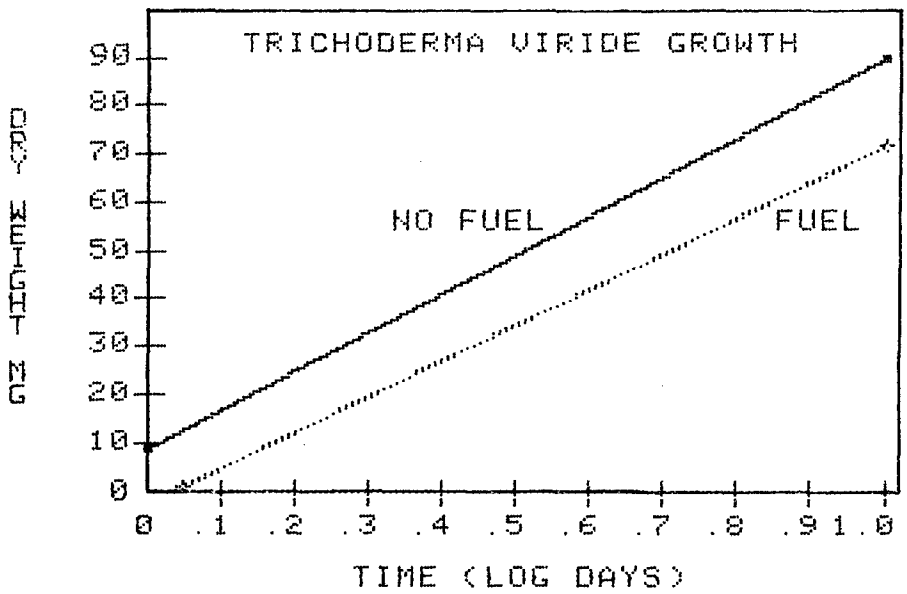


Figure 9. Growth of Trichoderma viride in potato dextrose broth with or without jet fuel.

TABLE 30
STATISTICS OF GROWTH OF T. VIRIDE IN CULTURES SAMPLED OVER 10 DAYS

Statistic	No fuel control	2% fuel
Slope	81.14	74.78
Intercept	8.57	-3.13
r-coefficient	0.967	0.956
Equation of line[1],	y = 81.14(x) + 8.57	74.78(x)-3.13

[1] y is growth as hyphal dry weight in mg; x is $\log_{10}$ of time in days

TABLE 31
FIRST TIME COURSE STUDY *P. RUBRUM* GROWTH IN PRESENCE OF JET FUEL

Harvest (Days after inoculation)	Yield (mg dry weight) No fuel	2% fuel
3	43.4 ± 3.6[1]	6.7 ± 2.3
4	62.8 ± 15.7	25.7 ± 3.5
5	78.6 ± 5.6	35.7 ± 6.2
6	93.2 ± 4.5	47.6 ± 7.9
7	92.8 ± 11.5	63.3 ± 1.1
9	157.1 ± 11.8	100.2 ± 5.2
10	153.5 ± 13.3	133.4 ± 11.9

[1]Dry weight in mg of mycelial mat of *P. rubrum* growing on potato dextrose broth, mean and standard deviation of four cultures

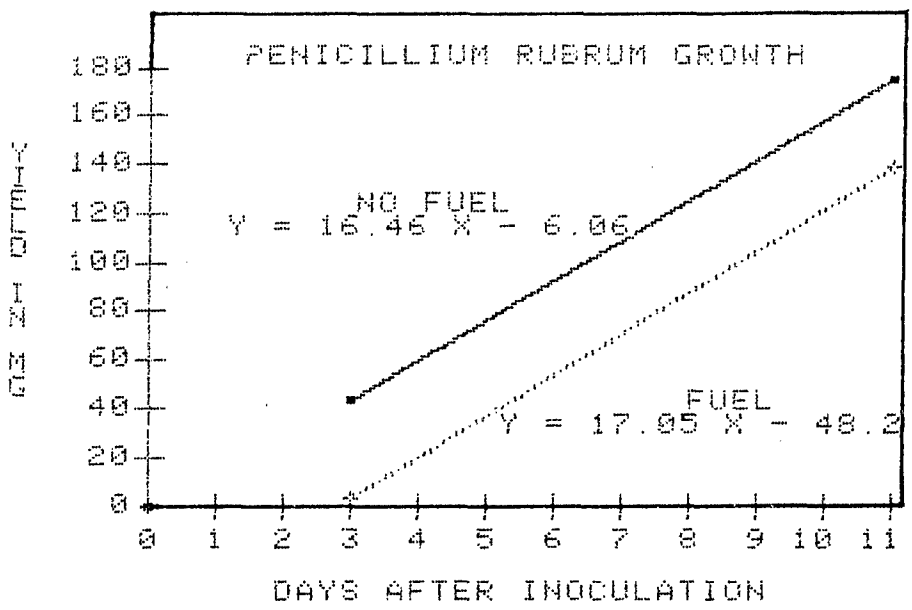


Figure 10. Growth of *Penicillium rubrum* in potato dextrose broth with or without jet fuel.

TABLE 32

SECOND TIME COURSE STUDY OF P. RUBRUM GROWTH IN PRESENCE OF JET FUEL

Growth period (days)	Yield (mg dry weight)	
	No fuel	2% fuel
1	8 ± 4	5 ± 5
2	8 ± 2	3 ± 1
3	84 ± 12	5 ± 1
4	107 ± 23	34 ± 16
5	140 ± 3	91 ± 5
6	137 ± 15	111 ± 33
7	139 ± 17	140 ± 5
8	149 ± 25	141 ± 18
9	132 ± 36	134 ± 25
10	163 ± 31	166 ± 13

[1]Dry weight of mycelial mat of P. rubrum growing on PDB, mean and standard deviation of three cultures

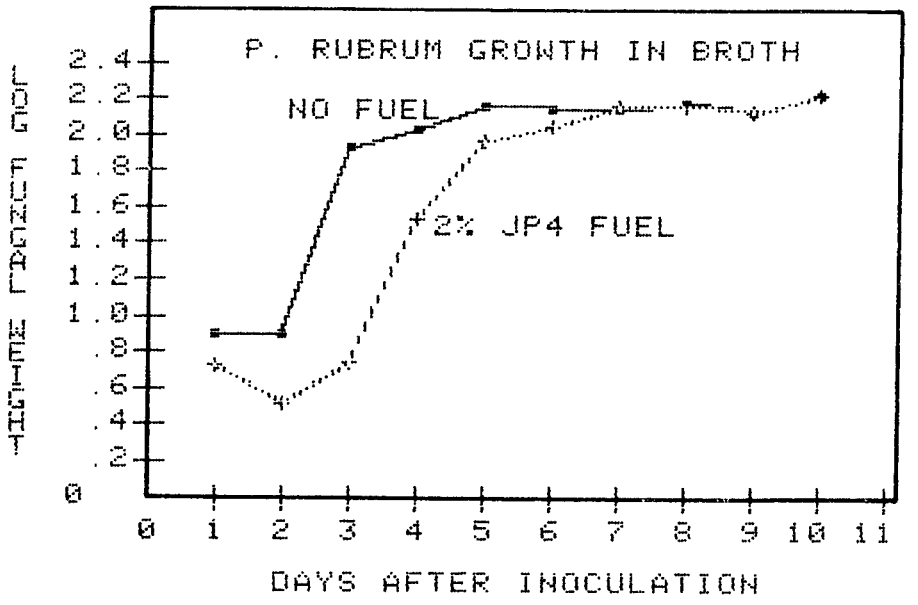


Figure 11. Growth of Penicillium rubrum in liquid cultures. Yield is the log of the mycelial mat weight in mg.

The red color was due to a pigment produced by P. rubrum (Roberts and Warren, 1955) and was probably not a simple pH change, since the pH of the media only decreased from 5.2 to 4.1 after 10 days (Table 33). In addition, no color change occurred when PDB was titrated with HCl. The optical absorption of culture fluid containing the red pigment measured on a Beckman ACTA CII spectrophotometer had a broad range from 370 to 640 nm with peaks at 420 and 500 nm.

The response of P. rubrum in media enriched with increasing concentrations of jet fuel was studied by adding JP4-P to flasks of nutrient broth at the rate of 0, 50, 100, 200, 400, 800, 1600, and 3200 µl per 40 ml of media, or 0, 0.13, 0.25, 0.5, 1, 2, 4, and 8% (v/v) fuel, respectively. Observations were made to determine first appearance of mycelial growth, red pigment, yellow hyphal pigmentation, and sporulation on hyphal surface (Table 34). After 14 days, flasks were harvested to compare hyphal dry weights (Table 34).

One percent was the minimum amount of fuel that could be detected using P. rubrum as a bioassay when fuel was added to liquid media. The appearance of red pigment, a characteristic of normal P. rubrum cultures, was delayed when fuel was present. Delay could be predicted somewhat ($r = 0.961$) by equation (3).

$$D = 5.235 + 0.599\ (F) \qquad (3)$$

where D is the delay in red pigment appearance in days and F is the fuel concentration in the media in percent. The equation was based on up to 8% fuel. Both sporulation and yellow-pigmented hyphae (a precursor of sporulation) were absent from P. rubrum cultures containing 1 to 8% (v/v) jet fuel. The start of mycelial growth was delayed about a day by the highest amount of fuel tested. The amount of mycelium produced after 14 days was reduced significantly ($P < 0.05$) in cultures containing 8% fuel. Less fuel did not significantly reduce final biomass, but achieving maximum weight may have been delayed.

Effect of Jet Fuel on Rhizobium spp.

Rhizobium spp. are bacteria which invade the roots of certain plants, form visible nodules, and fix nitrogen in a usable form (Stainer et al., 1963). We noted in earlier drench experiments that bean and alfalfa roots became naturally infected but that with Rhizobium spp. nodules died after fuel was applied to the soil. The effect of jet fuel on Rhizobium spp. nodulation was tested.

Pinto bean seeds were coated with 'Nirgain', a commercial rhizobium inoculum before sowing in sterile soil. An assay system suggested by a report by Fishbeck et al. (1973) and developed by Hogsett and Tingey (1980) measured the ability of Rhizobium spp. to reduce acetylene to ethylene and this, in turn, estimated the ability to reduce atmospheric nitrogen to ammonia. In our procedure, the plant was detopped, depotted, and the root ball was placed inside a 38 x 50 cm polyethylene bag. The bag was sealed to a short length of 3/4-inch PVC pipe and cap. The cap fitted with 1/4-inch copper tubing and a length of 1/4-inch thick walled latex tubing which could be restricted with a clamp (Figure 2). The bag with root mass was evacuated with a small vacuum

TABLE 33
pH OF CULTURE MEDIA SUPPORTING *PENICILLIUM RUBRUM* GROWTH

Growth period (time after inoculation in days)	pH
2	4.8
3	4.7
4	4.7
5	4.4
6	4.3
7	4.2
8	4.3
9	4.2
10	4.1

TABLE 34
GROWTH RESPONSE OF *P. RUBRUM* IN MEDIA ENRICHED WITH JP4 FUEL

Treatment (% fuel)	Rapidity of growth (days)				Mycelial weight (mg)
	Mycelial growth visible	Red pigment in media	Yellow pigment in hyphae	Sporulation	
0	2.8[1]	4.8	4.0	5.8	91.6 ± 5.6[3] c[4]
0.13	2.8	4.9	4.8	6.6	106.0 ± 7.6 bc
0.25	2.8	5.3	5.8	7.4	127.0 ± 9.1 a
0.5	2.8	5.8	7.4	10.0	116.1 ± 8.5 ab
1	3.3	6.2	N[2]	N	113.2 ± 5.5 ab
2	3.6	6.8	N	N	103.3 ± 2.0 bc
4	3.8	7.8	N	N	91.9 ± 5.2 c
8	3.8	9.8	N	N	68.1 ± 25.2 d

[1]Number of days from inoculation to observed growth characteristic, average of five cultures

[2]N = characteristic not observed by 14 days

[3]Weight of mycelial mat in mg, mean and standard deviation of five weights

[4]Means followed by the same letter(s) are not significantly different at $p < 0.05$ by DNMR test

pump. A mixture of 90% air and 10% acetylene was achieved in the bag by adding 3 ℓ of air, 0.5 ℓ of welding acetylene, and finally 1.5 ℓ additional air. The access tube was sealed and the bags were incubated in the dark for one hour. Samples were removed from the bag by connecting the access tubing to the inlet valve of an evacuated mylar gas sample bag and transferring ca. 1.5 ℓ of gas. The gas was injected into a Varian 1400 gas chromatograph fitted with a Parapak N column with 80/100 mesh.

To determine the effect of fuel on Rhizobium spp., inoculated 35-day-old bean plants were prepared by autoclaving one group, leaving one group untreated, and drenching the soil of one group with 6 ml JP4-P fuel per pot three days prior to sampling. Three plants were in each group. After measuring nitrogenase activity, roots were washed to note and record actual nodulation (Table 35).

This study showed that strong doses of fuel reduced nitrogenase activity and suggested impaired nitrogen fixation. The condition of the nodules on roots having reduced nitrogenase function confirmed the deleterious effect of jet fuel on Rhizobium spp.

BIOASSAY OF JET FUEL WITH SEEDS

It was useful to devise a rapid technique to determine the presence of jet fuel and its toxicity. The use of seeds for detecting various pollutants has been documented (Houston and Dochinger, 1977; Granett and Taylor, 1980b).

Sorghum plants had shown a sensitivity for jet fuel in vapor, spray, and liquid drench forms (Granett and Taylor, 1981a; above in this report) so sorghum seeds were used in initial studies. A 90-mm diameter filter paper disk was moistened with jet fuel and placed in a glass Petri dish. Two-hundred fifty sorghum seeds were spread over the disk surface and were overlain with filter paper which had been moistened with distilled water. Four Petri dishes of seeds were prepared for each of 10 fuel doses. Dishes were covered and incubated in the dark in a sealed container. After 72 hours, both germinated and non-germinated seeds were counted (Table 36). Percent germination decreased with fuel dose and could be estimated reasonably well ($r = 0.909$) with linear equation (4).

$$G = 71.2 - 107\ (Fd) \qquad (4)$$

where G is the percent germination rate and Fd is the fuel dose in ml.

Several factors were unrealistic in this test, particularly the closed container which might trap toxic fumes and the large number of seeds. Pots of soil were used next in the study. Our first aim was to achieve satisfactory seed germination without fuel under winter conditions. Five pots of soil, each containing 10 sorghum seeds, were placed (1) on benches in an unheated glasshouse, (2) in the same glasshouse but with soil-warming heat cables, and (3) in a heated glasshouse (Table 37).

TABLE 35
EFFECT OF JET FUEL ON RHIZOBIUM ACTIVITY OF BEAN PLANTS

Treatment	Nitrogenase activity (ppm ethylene)	Nodulation
Blanks	9.6 ± 0.07[1]	-
Normal, no-fuel plants[2]	13.7 ± 2.75	++ healthy nodules
Autoclaved plants	10.0 ± 0.55	dead nodules
Jet fuel on plant soil	9.7 ± 0.25	brown, mushy nodules

[1]Ethylene detected in ppm, mean and standard deviation of three plants

[2]In other trials ethylene values of over 100 ppm were recorded for no-fuel controls; wet roots for these plants may have lowered activity

TABLE 36
GERMINATION OF SORGHUM SEEDS IN CONTACT WITH JET FUEL

Fuel dose (ml)	Number of seeds		%-Germination
	Non-germinated	Germinated	
0.00	46 ± 3[1]	204 ± 3	81.7 ± 1.2[2]
0.05	70 ± 12	280 ± 12	72.2 ± 4.9
0.08	83 ± 8	167 ± 8	66.9 ± 3.3
0.10	102 ± 16	148 ± 15	59.3 ± 6.1
0.15	114 ± 32	136 ± 32	54.4 ± 12.8
0.20	140 ± 13	110 ± 13	44.2 ± 5.1
0.30	178 ± 14	72 ± 14	29.0 ± 5.6
0.40	210 ± 9	40 ± 9	16.2 ± 3.8

[1]Seeds germinated or not germinated of 250 possible, mean and standard deviation of four plates

[2]Germination (%) = (number germinated/250) x 100%

Emergence was not detected until nine days after sowing and was checked again at harvest at 14 days (Table 38). Greatest germination rate occurred in the heated glasshouse, but the heat cable allowed germination in the cooler environment to be nearly as great. Total lengths of seedlings measured 14 days after sowing (Table 38) were longest for those seedlings growing in the heated glasshouse, although shoot lengths were longer where the soil was warmed.

TABLE 37
TEMPERATURES FOR GROWING SORGHUM UNDER DIFFERENT CONDITIONS

Environment	Air temperature[1]		Soil temperature[1]	
	Low	High	Low	High
Unheated glasshouse	11 ± 2	31 ± 6	4 ± 2	32 ± 10
Unheated glasshouse with heat cables	11 ± 2	34 ± 6	10 ± 2	36 ± 10
Heated glasshouse	17 ± 4	26 ± 3	11 ± 1	24 ± 4

[1]Average daily temperature extremes in °C over the 14-day growing period, each value is mean and standard deviation of 14 observations

TABLE 38
EMERGENCE AND LENGTH OF SORGHUM SEEDLINGS GROWN AT DIFFERENT SOIL TEMPERATURES

Treatment	Emergence[1]		Length[2]		Sum of[3] total length
	9 days	14 days	Shoot	Root	
Unheated glasshouse	28 b[3]	46 b	44 b	92 c	629 b
Unheated glasshouse with heat cable	56 a	66 ab	75 a	130 b	1368 a
Heated glasshouse	64 a	72 a	68 a	168 a	1704 a

[1]Emergence (%) of sorghum seedlings after nine and 14 days, mean of five pots of 10 seeds each

[2]Shoot and root length averages in mm for 14-day old sorghum seedlings, means of 23, 36, and 36 seedlings for the three treatments, respectively

[3]Means in each column followed by the same letters were not significantly different at $P < 0.05$ by DNMR test

This work indicated that an unheated glasshouse could adequately be used for germinating seeds, particularly if inexpensive heat cables were used to increase soil temperatures at night.

A dose response study was prepared in which 0 to 16 ml JP4-P fuel was pipetted on the soil surface. Sorghum seeds were planted in the contaminated soil, 10 per pot with five pots per dose treatment. After two weeks, plants were measured (Table 39). A good linear correlation (r = 0.930) existed between dose and germination [equation (5)]:

$$G = 80.1 - 3.2\ (Fd), \qquad (5)$$

where G is the percent germination and Fd is the fuel dose in ml. A linear function also expressed the harvested total length of the seedlings (r = 0.837)

$$SL = 337 - 14\ (Fd), \qquad (6)$$

where SL is the seedling total length in mm and Fd is the fuel dose in ml.

Germination of sorghum seeds and surviving seedling length were deemed an adequate measurement for detecting fuel-contaminated soil. Seed tests were used to assay the effects of various fuel mixtures, soil and environment conditions, fuel movement, and soil recovery techniques.

MOVEMENT OF JET FUEL IN SOIL

Horizontal Movement of Fuel

Granett and Taylor (1981a) reported that fuel applied to soil moved outward from the point of application and its movement could be seen visually. The fuel or a phytotoxic component associated with the fuel reduced seed germination. Radial movement was further tested by preparing flats of soil (43 x 43 cm) with a 12 x 18 hole pattern as previously described (Granett and Taylor, 1981a). Two sorghum seeds were planted in each hole and the 12 x 18 grid was divided into four 6 x 9-hole blocks. The central 2 x 2 hole grid was treated with 5, 10, 20, or 40 ml JP4-P fuel. Seedling emergence was recorded after six days (Table 40). Seeds within the area of soil "wetted" by the fuel did not emerge, and fuel volumes greater than 5 ml produced phytotoxicity extending to seeds in block boundaries.

The chief problem of using seeds as an effective bioassay technique for measuring fuel movement and toxicity was that seed spacing precluded detection of movement less than 1 cm. Fuel absorbed into the soil to produce a wet appearance which remained visible for several hours after application. Seeds within the wet area did not germinate; as fuel applications increased in volume, so did the area of seed inhibition. The zone could be estimated with various equations (Table 41).

TABLE 39
GERMINATION AND GROWTH OF SORGHUM SEEDS IN POTS OF FUEL-CONTAMINATED SOIL

Dose (ml)	Germination (%)	Total length (mm)
0	80.1	377.4
0.5	78.5	366.6
1	76.9	323.8
2	73.7	278.1
4	67.3	227.1
8	54.6	194.6
16	29.1	134.1

TABLE 40
CHARACTERIZATION OF HORIZONTAL MOVEMENT OF JET FUEL IN SOIL

Fuel applied (ml)	Fuel wet spot		Seed bioassay			
				Non-emerging seedlings		
	Radius (cm)	Area (cm^2)	Number seedlings emerging	Percent of total	Radius of zone (cm)	Area of zone (cm^2)
5	1.50	7.1	33.5 ± 1.9[1]	38.0	2.8 ± 0.3[2]	24.2
10	1.88	11.0	29.5 ± 3.1	45.4	3.9 ± 1.0	47.8
20	2.63	21.6	18.2 ± 4.9	66.2	5.1 ± 0.7	81.7
40	3.21	35.8	11.8 ± 5.6	78.2	6.4 ± 0.8	127.7

[1]Number of seedlings emerging per flat, mean and standard deviation of four replicates of 54 seeds each

[2]Approximate radius in cm of zone where seeds did not emerge, mean and standard deviation of four replicates

Vertical Movement of Fuel Toxicity

Previously reported work by Granett and Taylor (1981a) discussed the movement of fuel into columns of soil and the subsequent bioassay for fuel movement and toxicity by cutting the soil column into 5-cm wide, 350 g sections which were potted and planted with seeds. In those tests, length of seedlings grown in soil sections from the top half of the treated column were shorter than seedlings grown in soil removed from the same depth in untreated

TABLE 41
EQUATIONS RELATING AREA (cm^2) OF SEED INHIBITION ZONE
TO AMOUNT OF FUEL APPLIED

Equation[1]	Type	Coefficient of determination (r^2)	Correlation coefficient (r)
$AI = 17.66 + 2.88\ (Fd)$	Linear	0.976	0.988
$AI = 7.37\ (Fd)^{0.79}$	Exponential	0.986	0.993
$AI = \frac{(Fd)}{0.002\ Fd + 0.19}$	Hyperbolic	0.995	0.998

[1]AI = Area of Inhibition and Fd = fuel dose in ml

columns. Emergence of seeds in the top 5 cm of soil, however, was not reduced relative to controls. Vaporization of fuel from the upper layer and subsequent loss of a toxic agent was speculated, and this hypothesis was tested.

Eight columns were packed with soil. No fuel was applied to two control columns. The six remaining columns each received 45 ml of JP4-P fuel and the tops of three of the columns were sealed with duct tape after fuel application. After standing in a shaded area of the glasshouse for seven days, all columns were cut into sections, stirred, placed in pots, and sown with 15 sorghum seeds. Emergent seedlings were measured 14 days after sowing.

More seedlings emerged in the top 5-cm section of the unsealed fuel-contaminated column than in the layer beneath it (Table 42). Fewer seedlings emerged in soil from the top layer of the sealed column than in the corresponding soil layer from the open column. Percent emergence of seedlings growing in soil sections below 15 cm (third section) was high for all columns, and soil from fuel treatments did not differ from controls at these levels.

Length of seedlings growing in soil from treated columns were reduced in the top five sections (Table 43). Seedlings growing in the sixth soil section (25 to 30 cm) of the sealed column were shorter than seedlings from control or unsealed columns.

Fuel and toxicity associated with fuel moved downward through a soil column. Sealing the column prevented evaporation of the fuel and apparently allowed fuel to move slightly further down the column.

TABLE 42
EMERGENCE OF SORGHUM SEEDLINGS IN SOIL
REMOVED FROM COLUMNS LAYERED WITH JET FUEL

Section depth (cm from top)	Treatment		
	Control (No fuel)	45 ml fuel applied to top	
		Open[1]	Sealed
5	77[2]a[3]	38 b	13 c
10	83 a	9 b	4 b
15	60 a	27 b	11 b
20	83 a	22 b	24 b
25	80	78	71
30	87	71	82
35	90	71	80
40	83	89	87
45	83	85	80
50	80	80	78

[1]Top of column was left open or was sealed with duct tape

[2]Percent emergence of 15 seedlings per pot, mean of 2 (Control) or 3 (Open and Sealed) pots per section depth

[3]Means followed by the same letter or no letter in each row (section) are not significantly different at $P < 0.05$ by SNK test

Influence of Water on Vertical Movement of Fuel

It was important to know if water passing through soil contaminated with jet fuel would move, elute, or transport fuel or associated toxic substance. We had earlier found that drenching soil with massive volumes of water failed to remove the toxic substance, but only destroyed soil structure. A more controlled water rinsing trial was undertaken. Columns packed with soil were layered with 45 ml JP4-P fuel. Water was then applied to the surface in three 45-ml doses on 0, 2, and 5 days after the fuel was applied. Control columns received no fuel or water. Four replicate columns were prepared for each treatment. After seven days, or two days after the last water application, soil was removed from the columns in sections. Each section was potted and sown with 15 sorghum seeds. Seedling emergence and length were recorded seven days after sowing (Tables 44-48).

Most seeds (81.8%) emerged in soil from control treatment columns having no fuel (Table 44). Percent emergence was less for seedlings in the treatments containing fuel, but standard deviations were large and differences between the water and no-water treatment means were not significant at $P < 0.05$.

TABLE 43
TOTAL LENGTH OF SORGHUM SEEDLINGS GROWN IN SOIL
REMOVED FROM COLUMNS LAYERED WITH JET FUEL

Section depth (cm from top)	Treatment		
	Control (No fuel)	45 ml fuel applied to top	
		Open[1]	Sealed
5	344[2]a[3]	123 b	101 b
10	380 a	94 b	84 b
15	371 a	122 b	130 b
20	340 a	138 b	175 b
25	374 a	341 b	291 c
30	357 a	365 a	314 b
35	330	311	339
40	332	378	359
45	329	352	334
50	346	329	312

[1,3]See Table 42

[2]Length of seedlings in mm, mean of up to 27 (Control) or 40 (Open or Sealed) seedlings per level

TABLE 44
EMERGENCE OF SEEDLINGS IN SOIL TREATED WITH FUEL AND WATER
PRIOR TO PLANTING SORGHUM SEEDS

Treatments		Emergence[1] (%)
Fuel (ml)	Water (ml)	
0	0	81.8 ± 5.1
0	135	81.1 ± 8.8
45	0	71.7 ± 18.1
45	135	62.5 ± 27.6

[1]Emergence rate in percent of sorghum seeds in soil, mean and standard deviation of 40 pots of 15 seeds each

TABLE 45
EMERGENCE OF SORGHUM SEEDLINGS IN SOIL FROM COLUMNS TREATED WITH FUEL AND WATER

Depth of soil section (cm)	Treatments			
	No fuel No water	No fuel Water	Fuel No water	Fuel Water
5	83[1]	58	33	17
10	92	77	65	25
15	85	82	52	38
20	82	85	65	48
25	83	90	88	80
30	78	87	88	83
35	75	85	75	83
40	80	82	83	85
45	85	80	85	77
50	75	85	82	88

[1]Percent emergence of sorghum seeds, mean of four pots of 15 seeds each

Soil depth in the column during fuel treatment influenced subsequent growth (Table 45). Soil from the lower 20 to 50 cm of all columns supported fairly uniform growth (average of 82.5 ± 4.3% emergence for all treatments) compared to the lower emergence and larger standard deviation range (64.2 ± 22.8% emergence) for the seedlings growing in soil from the top 20 cm of the columns. Water applied after the fuel treatment appeared to reduce the emergence of seeds compared to no-water controls. The reduction was significant ($P < 0.05$) for seeds in soil from the same three or four sections (10 to 20 cm) that had reduced emergence in the fuel no-water columns. Reduction was less severe when no water was present. On the basis of seedling emergence, the water increased fuel toxicity and did not transport fuel downward.

Shoot and total lengths were summarized and analyzed. Non-emergent seeds were treated as seedlings with 0-mm lengths. Results paralleled emergence; a reduction of growth occurred when fuel was applied (Table 46). Shoots appeared equally stressed since root/shoot ratios were all about 0.50 (Table 46). When depth of soil was considered (Tables 47 and 48), lengths were shorter in seedlings growing in the upper 20 cm of columns treated with fuel. Length reduction was greatest when water was added to fuel than when either fuel or water were applied alone.

Analysis of seedling lengths presented a problem since not all seeds germinated and 0-mm lengths were not true measurements. The data were analyzed with the average seedling length per treatment substituted for the 0-mm

TABLE 46
LENGTH OF SORGHUM SEEDLINGS FROM SOIL TREATED WITH JET FUEL AND WATER

Treatment	Lengths (mm)[1] Total	Lengths (mm)[1] Shoot	Shoot/root ratio
No fuel, no water	186 ± 108 X[2]	62 ± 35 X	0.50
No fuel, water	183 ± 107 X	60 ± 34 X	0.49
Fuel, no water	140 ± 106 Y	48 ± 35 Y	0.52
Fuel, water	126 ± 111 Z	40 ± 35 Z	0.47

[1]Length of seedlings, mean and standard deviation of 40 pots of 15 seeds each; non-emerging seedlings treated as 0 mm lengths

[2]Means in columns followed by same letters (or no letters) are not significantly different at $P < 0.01$ level by DNMR test

data or with the sum of all lengths per pot. In neither case did the results differ from those presented.

In summary, fuel on a soil column decreases soil fertility to a certain depth. Soil from greater depths within the column supported more normal seed growth given the same fuel dose. Water did not extend the phytotoxic effects of the fuel more than 5 cm further into the soil profile. This finding could be important in localizing detrimental effects of fuel spills, but also demonstrated the problems of simple flushing or flooding of fuel-contaminated areas as part of reclamation.

Dose Response of Fuel Movement

In the above tests, 45 ml of fuel (1 ml fuel per cm^2 of column cross-section) were applied to the columns. Theoretically, higher volumes of fuel should travel further into the soil. Before testing different volumes, the fuel-holding capacity of the soil was determined. To one column of soil, 360 ml distilled water was applied while an equal amount of JP4-P fuel was added to a second column. Within 45 minutes all fuel had entered the soil, but 65 minutes were needed for the same amount of water to be absorbed. After about 16 hours, fuel had wet the screen surface on the basal end of the column, yet water had not passed that far through the other column. Jet fuel was slowly applied to the surface of a third soil column until

TABLE 47
TOTAL LENGTH OF SORGHUM SEEDLINGS GROWN IN SOIL TAKEN FROM 50-cm COLUMN TREATED WITH JET FUEL AND WATER

Depth of soil section (cm)	Treatments[1]				
	No fuel No water	No fuel Water	Fuel No water	Fuel Water	Average[2]
5	169 V	117 WX	34 YZ	9 Z	82 C
10	207 V	169 V	67 Y	29 YZ	118 B
15	199 V	184 V	59 Y	45 YZ	122 B
20	183 V	194 V	112 X	61 Y	138 B
25	185 V	207 V	208 V	172 V	193 A
30	195 V	191 V	198 V	193 V	194 A
35	178 V	200 V	176 V	208 V	190 A
40	178 V	189 V	179 V	188 V	184 A
45	209 V	179 V	182 V	163 V	183 A
50	159 VWX	197 V	187 V	189 V	183 A

[1]Total length in mm, mean of 60 seedlings; analysis of all depths and treatments: means followed by same letters are not statistically different at $P < 0.01$ by DNMR test

[2]Average of lengths by depths, mean of 240 lengths; analysis by DNMR test at $P < 0.01$

720 ml had been added. After sitting for 24 hours, 125 ml was collected from the base of the column. Therefore, the void volume or volume of fuel needed to fill the column was 595 ml.

To test the movement of different doses of fuel, 30 PVC columns were packed with soil and 0, 45, 90, 180, or 360 ml of JP4-P or JP4-S fuel was applied. These doses corresponded to 0, 1, 2, 4, or 8 ml fuel cm^{-2}, respectively. Each treatment consisted of two replicates. After seven days in the glasshouse, soil columns were divided into sections which were individually potted and sown with 10 sorghum seeds. Seedlings were measured nine days after planting when controls (0 ml fuel) had grown several centimeters.

Fuel moved completely through the columns at the 180 and 360 ml dose rates and some collected beneath the base after seven days. Less bright winter light, cool temperatures (13 to 37°C), and fluctuating relative humidity (20 to 83%) increased the time for any of the seeds to germinate.

Emergence rates, as a percent of controls, were calculated by dividing number of seedlings emerging from fuel-treated column soil by number of seedlings growing in untreated-column soil (Tables 49 and 50). Analyses of variance were performed after arc sin transformation of the original data ($T = \sin^{-1} \sqrt{x\%}$). Analyzed means were returned to percentages [$x\% = (\sin T)^2$].

TABLE 48
SHOOT LENGTH OF SORGHUM SEEDLINGS GROWN IN SOIL TAKEN FROM 50-cm COLUMN TREATED WITH JET FUEL AND WATER

Depth of soil section (cm)	Treatments[1]				Average[2]
	No fuel No water	No fuel Water	Fuel No water	Fuel Water	
5	62	39	34	3	29
10	70	56	30	10	42
15	65	59	24	15	41
20	60	64	42	22	47
25	61	67	66	53	62
30	62	64	65	60	63
35	59	66	57	65	62
40	60	63	61	60	61
45	68	58	60	52	60
50	57	68	60	63	62

[1]Shoot lengths in mm, mean of up to 60 seedlings

[2]Average shoot lengths by depths, mean of up to 240 seedlings

Significant differences at $P < 0.01$ existed between fuel types. Shale-derived JP4 was more toxic than the petroleum product which seemed more erratic in its effects (Table 49). With either fuel, 45 ml fuel affected emergence only in soil from the first 10 to 15 cm of the column. Doubling the dose to 90 ml increased the depth of contamined soil from 20 to 25 cm for JP4-P and 25 to 30 cm for JP4-S. At the 180 ml dose of JP4-P, soil in the whole column was contaminated, although the first 15 cm supported greater emergence compared to the lower doses. In the column to which 360 ml fuel had been applied, emergence was reduced to less than 50%. With shale fuel, few seedlings emerged in soil treated with 180 or 360 ml fuel, and none emerged in soil taken from below 30 cm of the column treated with 360 ml fuel.

Seedling shoot and total lengths were measured and found to be greatly influenced by fuel dose (Table 51). Dose determined how far fuel traveled into the soil. The depth data revealed three response groups within the 10 sections. Greatest reductions in lengths were for seedlings from soil in the first 15 cm (Z group, Table 51). The next two depths, 20 and 25 cm, yielded seedlings with longer lengths (Y group, Table 51), while longest lengths were associated with seedlings growing in soil taken 30 cm or more from the column surface (X group, Table 51). Fuel type was also important, at least for total lengths data. JP4-S was significantly more inhibitory than JP4-P at $P < 0.01$. Shoot lengths showed no difference between fuel type ($P < 0.05$).

TABLE 49
EMERGENCE OF SEEDS IN SOIL FROM COLUMNS TREATED WITH TWO JET FUELS

Depth of soil section (cm)	JP4-Petroleum (ml)				JP4-Shale (ml)			
	45[1]	90	180	360	45	90	180	360
5	19[2]	50	63	44	0	15	8	8
10	07	60	40	47	16	21	21	37
15	50	61	67	50	55	15	05	30
20	94	50	44	33	82	12	18	12
25	100	76	41	41	93	29	7	14
30	100	94	50	13	89	78	0	0
35	76	100	53	12	100	100	6	0
40	100	100	20	13	100	100	64	0
45	100	93	0	0	94	94	6	0
50	89	100	17	0	100	94	13	0

[1]ml jet fuel applied per column

[2]Percent-emergence of seedlings determined by comparing number emerged in treated and untreated soil from same depth, average of two pots or 20 seeds total

Separating dose and depth effects further clarified the biological patterns (Table 52). When 45 ml fuel was applied, only soil from the surface to 15 cm inhibited growth, whereas a 90 ml drench pushed the zone of growth reduction to 25 cm. Both the 180 and 360 ml fuel applications saturated the column of soil and inhibited growth in the entire 50-cm of soil.

RECOVERY OF FUEL-CONTAMINATED SOIL

General Aims

Soil drenched with jet fuel becomes contaminated depending on how much fuel is applied. Contaminated soil can inhibit seed germination, reduce growth of seedlings, or kill established plants. Several investigations were conducted to determine whether soil contamination could be lessened or the effects of contamination be decreased by certain additives or actions.

Nutrients and Soil Recovery

Naturally occurring soil-inhibiting microorganisms may degrade fuel, but in the process these organisms may deplete the nutrient supply. Likewise, a soil with a larger nutrient reserve should provide a healthier

TABLE 50
EMERGENCE OF SORGHUM SEEDS IN SOIL FROM COLUMNS TREATED WITH JET FUEL

Depth of soil section (cm)	Jet fuel applied to column (ml) 45	90	180	360	Average[2]
5	10[1]	33	36	26	21.4 Z
10	12	41	31	42	29.7 YZ
15	53	38	36	40	39.8 XYZ
20	88	31	31	23	43.0 WXYZ
25	97	53	24	23	52.0 WXY
30	95	86	25	7	49.8 WXY
35	88	100	30	6	57.5 WX
40	100	100	42	7	66.7 W
45	97	94	3	0	42.3 WXYZ
50	95	97	15	0	50.1 WXY
Average[3]	78.0 A	72.2 A	22.8 B	11.5 C	

[1]Percent-emergence obtained by averaging results of the two fuel treatments in Table 49, mean of up to 40 seeds sown per treatment

[2]Average percent-emergence for soil depths after arc sin transformations and analysis (see text), means followed by same letter are not significantly different at $P < 0.01$ by DNMR test

[3]Average percent-emergence for fuel dose after arc sin transformations and analysis, means followed by same letter are not significantly different at $P < 0.01$ level by DNMR test

environment for plant growth. Studies aimed at the effect of soil nutrient levels on fuel contamination were undertaken. Pots of soil contaminated with 4 ml JP4-P fuel were kept on trays in the glasshouse and watered with single- (low) or triple- (high) strength nutrient solution (Table 2) at the rate of 25 ml three times per week for three weeks. Controls received no fuel or were treated with only water or with no water. After three weeks of nutrient treatment, the pots of soil were sown with 10 sorghum seeds each and tap water was supplied as needed. Seedlings were harvested two weeks later. Emergence rate was calculated (Table 53), and seedling length and weight were measured (Table 54).

Emergence did not appear affected by the fuel regardless of the nutrient supplements. Even the pots of control soil receiving fuel plus water or no water had high emergence rates.

Shoot lengths and fresh weights of seedlings from water and no-water treatments were significantly reduced in comparison to the two nutrient

TABLE 51
EFFECT OF DOSE, FUEL TYPE, AND DEPTH ON LENGTHS OF SEEDLINGS GROWING IN SOIL TAKEN FROM 50-cm COLUMNS DRENCHED WITH JET FUEL

Measure	Lengths (mm)	
	Shoot	Total
Dose[1] (ml)		
0	34.7[2]W[3]	124.7[2]W[3]
45	23.0 X	81.7 X
90	17.7 Y	63.7 Y
180	2.0 Z	7.8 Z
360	1.7 Z	6.6 Z
Fuel type		
Petroleum	16.2[4] A	60.1 A[3]
Shale	15.5 A	53.7 B
Depth of soil section (cm)		
5[5]	7.4 Z[3]	28.1 Z[3]
10	9.2 Z	35.2 Z
15	10.4 Z	37.2 Z
20	14.7 Y	53.5 Y
25	14.1 Y	51.9 Y
30	20.1 X	69.8 X
35	21.0 X	75.6 X
40	21.1 X	74.3 X
45	19.5 X	71.2 X
50	20.7 X	72.0 X

[1]Amount of fuel in ml applied to top surface of 50-cm soil column

[2]Length in mm, average of 400 seedlings

[3]Means followed by same letter not significantly different at $P < 0.01$ by DNMR test

[4]Length in mm, average of 1000 seedlings

[5]Length in mm, mean length of 200 seedlings

treatments, but this reduction was regardless of fuel treatment. Fuel appeared to affect (at $P < 0.05$) only the seedlings growing in soil which had the high nutrient treatment. Analysis of total lengths (shoot plus root) indicated results similar to the shoot and fresh weight data.

In this study, nutrients added to the soil after the fuel treatment tended to aid the development of subsequent seedlings, whereas under most conditions fuel did not affect plant development. Other tests have

TABLE 52
TOTAL SEEDLING LENGTH (in mm) FOR SEEDS PLANTED IN SOIL FROM 50-cm COLUMNS DRENCHED WITH 0-360 ml FUEL

Depth of soil section (cm)	Dose (ml fuel)				
	0	45	90	180	360
5	116	1	7	8	8
10	133	2	12	12	17
15	118	23	15	10	20
20	129	111	13	7	7
25	118	107	19	6	9
30	129	111	100	6	2
35	131	107	130	8	2
40	119	116	121	15	1
45	125	125	106	0	0
50	129	114	113	4	0

TABLE 53
EMERGENCE OF SORGHUM SEEDS IN JET FUEL-CONTAMINATED SOIL TREATED FOR THREE WEEKS PRIOR TO SOWING SEEDS

Treatment solutions[1]	No fuel	Fuel
High nutrient	80 ± 12[2]ab[3]	72 ± 19 b
Low nutrient	82 ± 8 ab	82 ± 4 ab
Water	96 ± 8 a	84 ± 9 ab
No water	86 ± 9 ab	88 ± 11 ab

[1]25 ml treatments onto soil three times per week for three weeks prior to seeding

[2]Emergence in percent of sorghum seedlings, mean and standard deviation of five pots sown with 10 seeds each

[3]Means of treatments followed by the same letters are not significantly different at $P < 0.05$ by SNK test

indicated that 4 ml fuel per pot has been sufficient to inhibit seed development. The fuel may have lost much of its phytotoxicity after three weeks, and this loss of toxicity over time was addressed next.

TABLE 54
DEVELOPMENT OF SORGHUM SEEDLINGS GROWN ON JET FUEL-CONTAMINATED SOIL TREATED FOR THREE WEEKS PRIOR TO SEEDING

Treatment	Shoot lengths (mm)[1]		Fresh weight (mg)[3]	
	No fuel	Fuel	No fuel	Fuel
High nutrient	225 ± 55 a[2]	177 ± 54 c	320 ± 130 a[2]	250 ± 110 b
Low nutrient	202 ± 47 b	187 ± 41 bc	280 ± 90 b	250 ± 130 b
Water	91 ± 15 d	81 ± 20 d	100 ± 30 c	100 ± 40 c
No water	91 ± 20 d	82 ± 16 d	120 ± 30 c	90 ± 30 c

[1]Lengths in mm of two-week-old sorghum seedlings, mean and standard deviations of up to 50 seedlings (five pots of 10 seedlings each)

[2]Means of length or fresh weight followed by the same letter(s) were not significantly different at $P < 0.05$ by SNK test

[3]Fresh weight in mg of seedlings, mean and standard deviation of up to 50 seedlings

Only at the high nutrient level application did fuel result in decreased growth. A possible interaction between fuel and nutrients was further investigated in an experiment consisting of three treatments. One was 0 ml of fuel; the other two treatment groups received 7 ml JP4-P fuel per pot applied either at one time or during a three-week schedule of applications of 5, 1, and 1 ml. Nutrient treatments consisted of thrice weekly applications of triple-strength solution, water, or no water. Applications were sufficient (25 to 50 ml) to saturate the soil and were not supplemented by tray watering. Nutrient solution was applied several hours prior to the 1-ml fuel applications. Ten sorghum seeds were sown in each pot after fuel and nutrient applications. Seedlings were harvested two weeks later.

An interaction between fuel and nutrients was confirmed with this test. Emergence of seedlings was high in nutrient-treated soil in which no fuel had been applied (Table 55), whereas when fuel was applied, significantly lower emergence occurred. No differences were noted between one or three nutrient doses. Fuel did not effectively inhibit emergence when soil was treated with the water or no-water alternatives to nutrients. Seedling emergence following fuel and no-water soil treatment was as great ($P < 0.05$) as the emergence for seedlings after no fuel-nutrient treatment.

Similar results were obtained when seedling lengths and weights were considered (Table 56). Nutrients plus fuel together seemed to inhibit growth. Seedlings were significantly ($P < 0.05$) smaller and lighter than controls when nutrient applications followed fuel treatment. No difference was noted in seedling growth when fuel was applied in three doses rather

TABLE 55
EMERGENCE OF SORGHUM SEEDLINGS GROWING IN FUEL CONTAMINATED SOIL

Soil treatment	Fuel-treatment		
	No fuel	Single dose[1]	Three doses[2]
Nutrients[3]	80 ± 14[4]a[5]	42 ± 16 c	46 ± 5 bc
Water	65 ± 10 abc	72 ± 8 ab	56 ± 5 abc
No water	66 ± 13 abc	72 ± 26 ab	84 ± 21 a

[1]7 ml JP4-P at start of three-week period

[2]5 ml JP4-P at start of period, 1 ml for next two weeks, 7 ml total

[3]Triple strength nutrient solution, soil saturated (ca. 25 ml) three times a week

[4]Emergence rate in percent of number seedlings with shoots > 10 mm per number seeds sown per pot, mean and standard deviation of five pots of 10 seeds sown per pot

[5]Pot means followed by the same letters are not significantly different at $P < 0.05$ by SNK test

TABLE 56
DEVELOPMENT OF SORGHUM SEEDLINGS GROWN IN SOIL TREATED WITH JET FUEL AND NUTRIENTS

Soil treatment[1]	Shoot length (mm)			Fresh weight (mg)		
	0[2]	1	3	0	1	3
Nutrients	59[3]ab[4]	15 d	16 d	86[3]ab[4]	49 e	54 e
Water	64 a	41 c	40 c	100 a	88 ab	80 b
No water	53 b	36 c	35 c	86 ab	71 bcd	72 bc

[1]Liquid treatment applied to soil three times a week for three weeks after first fuel application; nutrient treatment is triple strength solution

[2]Fuel treatment: 0 = no fuel, 1 = one 7 ml dose, 3 = 5 ml dose first week, and 1 ml on each of next two weeks

[3]Shoot length or fresh weight of germinated seedlings, mean and standard deviation of 20 to 40 seedlings

[4]Means for each variable (length or weight) followed by the same letter(s) are not statistically different at $P < 0.05$ by SNK test

than one dose, but any of the fuel treatments caused a reduction in yield compared to no-fuel controls.

Seedling development after nutrient treatment of the soil was reduced compared to either the water or no-water treatments.

The presence of nutrients in the soil may increase the deleterious effects of fuel by (1) binding with the fuel, (2) stimulating increased toxic fuel uptake, or (3) providing nutrients for microbes that use fuel as an energy source. It is speculated that nutrients become less available to the plant in the presence of fuel.

Diluting Fuel-Contaminated Soil

The above experiment indicated that nutrients and fuel interacted to produce less favorable conditions for growth than when fuel alone was applied. Contaminated soils might be rendered less toxic if diluted with inert materials. Glasshouse soil mixed first with JP4-P to achieve 2 ml fuel per 220 g soil or about 1% (v/w) fuel. The soil was then mixed with clean, uncontaminated soil or with charcoal, sand, clay, peat moss, vermiculite, or perlite.

Clean soil mixed with 1% fuel-contaminated soil resulted in final fuel contaminations of 0, 0.25, 0.50, 0.75, 0.90, and 1.00% (v/w). Previous work had shown that 1% contamination (4 ml fuel per 400 g soil) was phytotoxic to developing sorghum. Ten seeds were sown per pot of soil and pots were replicated five times. After two weeks, seedlings were harvested, shoot and root lengths were measured, and fresh weights were recorded.

Germination of the seeds was not greatly affected by the fuel or dilutions (Table 57). One analysis considered the treatments as a continuous function of percent-clean soil. The mean separation by the SNK test indicated that longer shoots and roots and heavier fresh weights occurred when seedlings were grown in greater soil dilutions (50 and 75%) than in more contaminated soils. A strong linear relationship did not exist; coefficients for linear equations were $r = 0.812$, 0.648, and 0.819 for shoots, roots, and weights, respectively. Response could be arranged into three groups based on the ratios of clean soil; 0 and 10%, 25 and 50%, and 75 and 100% mixtures produced small, medium, and large yield reductions, respectively. In summary, when fuel present in the soil was diluted, toxicity was similarly reduced.

Fuel-contaminated soil was mixed 1:1 with relatively inert materials. Sorghum seeds were sown in pots of the prepared mixtures or in similar mixtures prepared with uncontaminated soil. After 14 days, seedlings were harvested and weight and length measurements were made.

The substances themselves seemed to stimulate or retard growth of sorghum seedlings when no-fuel controls were considered (Table 58). Charcoal, in particular, appeared to retard growth; perlite and vermiculite seemed to belong to a second group that supported growth, but less satisfactorily than clay, sand, peat moss, or soil.

TABLE 57
GROWTH OF SORGHUM SEEDS PLANTED IN SOIL "DILUTED" WITH UNCONTAMINATED SOIL

Soil mixture (% clean soil)	% Fuel	Germination[1] and emergence rates (%)	Length (mm)		Fresh weight (mg)
			Shoots	Roots	
0	1.00	81 (70)[2]	85 ± 38[3]c[4]	112 ± 33 c	174 ± 72 c
10	0.90	82 (82)	79 ± 50 c	107 ± 42 c	179 ± 82 c
25	0.75	80 (80)	119 ± 47 b	110 ± 28 c	241 ± 102 b
50	0.50	90 (90)	124 ± 38 b	124 ± 42 bc	247 ± 81 b
75	0.25	80 (80)	159 ± 34 a	173 ± 51 a	342 ± 98 a
100	0	83 (78)	145 ± 43 a	136 ± 53 b	302 ± 118 a

[1]Percent of those seeds planted which germinated (or emerged), mean of possible 50 seeds except for 0 and 100% for which 200 seeds were sown

[2]Emergent seedlings developed shoot lengths greater than 10 mm

[3]Length or weight of sorghum seedlings, mean and standard deviation of up to 50 seedlings (200 for 0 and 100%)

[4]Mean separation by SNK test at $P < 0.05$ for columns; same letter(s) imply no significant differences between means

Germination rates of seeds in fuel and fuel-free amended soils did not significantly differ (Table 59). The charcoal pellet treatment was an exception where seed germination was much less when fuel was present. A probable cause of the reduced productivity of charcoal, perlite, and vermiculite was that these materials might remove nutrients from the soil matrix when no fuel was present and remove nutrients more readily than fuel when soil was contaminated.

Certain other relationships emerged when fuel-contaminated soil was compared to controls (Tables 60 and 61). Seedlings from fuel treatments with soil, sand, clay, or charcoal in the medium had significantly shorter shoot lengths and fresh weights than when fuel was not present (Tables 60 and 61). Fuel had no significant effect (at $P < 0.05$) on root lengths or on seedlings growing in peat moss, perlite, or vermiculite. When fuel was mixed with these substances, seedling lengths were not reduced at $P < 0.05$ to lengths shorter than the no-fuel soil controls. In summary, peat moss, perlite, and vermiculite could be used to dilute, absorb, or neutralize fuel contamination of soil.

TABLE 58
GROWTH OF SORGHUM SEEDLINGS GROWING IN UNCONTAMINATED SOIL AMENDED WITH VARIOUS SUBSTANCES

Substance	Length (mm)		Fresh weight (mg)
	Shoot	Root	
Soil control	145[1]a[2]	136 b[2]	302 a[2]
Sand	166 a	166 b	353 a
Clay	153 a	128 b	319 a
Peat moss	149 a	184 a	300 a
Perlite	140 a	151 bc	250 b
Vermiculite	109 b	135 c	201 bc
Charcoal	82 c	85 d	117 c

[1]Length or weight data, mean of up to 50 sorghum seedlings growing in amended soil

[2]Means in columns followed by the same letter(s) are not significantly different at $P < 0.05$ by SNK test

TABLE 59
GERMINATION OF SORGHUM SEED IN FUEL-CONTAMINATED SOIL AMENDED WITH VARIOUS SUBSTANCES

Substance	JP4 fuel	
	2 ml per pot	0 ml per pot
Soil control	90[1]	83
Sand	76	74
Clay	78	90
Peat moss	78	86
Perlite	84	84
Vermiculite	86	80
Charcoal	58	44

[1]Percent-germination of sorghum seeds, mean of five pots

TABLE 60
SHOOT LENGTH OF SORGHUM SEEDLINGS GROWN IN
FUEL-CONTAMINATED SOIL AMENDED WITH VARIOUS SUBSTANCES

Substances[1]	JP4-P fuel		Significance[4]
	2 ml per pot	0 ml per pot	
Soil control	124 ± 38[2]cd[3]	137 ± 41 bcd	NS
Sand	121 ± 45 cd	166 ± 57 a	*
Clay	95 ± 45 ef	153 ± 51 ab	*
Peat moss	130 ± 51 bcd	149 ± 52 abc	NS
Perlite	133 ± 38 bcd	140 ± 40 abcd	NS
Vermiculite	111 ± 41 de	109 ± 32 de	NS
Charcoal	59 ± 29 g	82 ± 53 f	*

[1]Material mixed 1:1 with contaminated or fuel-free soil

[2]Shoot lengths of germinated seeds in mm, mean and standard deviation of up to 50 seeds planted per treatment

[3]Means followed by the same letter(s) are not significantly different at $P < 0.05$, all 14 means were included in the SNK test

[4]Fuel and no-fuel means compared for same substance; * = means are significantly different at $P < 0.05$ by SNK test; NS = not significant

TABLE 61
FRESH WEIGHT OF SORGHUM SEEDLINGS GROWN IN
FUEL-CONTAMINATED SOIL AMENDED WITH VARIOUS SUBSTANCES

Substances[1]	JP4-P fuel		Significance[4]
	2 ml/pot	0 ml/pot	
Soil control	247 ± 81[2]cdf	263 ± 110 bcd	NS
Sand	237 ± 112 cdef	353 ± 138 a	*
Clay	193 ± 86 def	319 ± 149 ab	*
Peat moss	262 ± 116 bcde	300 ± 131 abc	NS
Perlite	245 ± 85 cde	250 ± 91 cde	NS
Vermiculite	215 ± 87 def	201 ± 65 def	NS
Charcoal	124 ± 54 fg	177 ± 101 df	*

[1-4]Same notes as for Table 60 but data represents fresh weight in mg

Aging Soil to Reduce Fuel Toxicity

Aging fuel in pots of soil -

Evidence is available (Allen, 1981) that fields accidentally contaminated with fuel regain productivity after a fallow period. In an initial test, productivity of soil was tested at 0, 4, 7, and 14 days after being contaminated with ca. 1% fuel (v/w) per pot (4 ml/550 g soil). Pots of soil remained in the glasshouse and were watered whether seeds had been sown yet or not. Fifteen sorghum seeds were sown per pot and seedlings were harvested and measured 14 days later.

Two types of uncontaminated controls were prepared: (1) soil set out 14 days prior to sowing of seeds, and (2) pots prepared at the time of sowing.

Seedlings growing in treated pots were inhibited compared to those in the control soil, particularly when lengths were considered (Table 62). The soil-aging treatments affected seedling development, although emergence was not as sensitive a parameter as seedling length. Seedlings emerged in greater numbers and grew longer when seeds were sown in soil left unplanted for seven to 14 days after fuel treatment than when planted directly after fuel treatment.

TABLE 62
EMERGENCE AND GROWTH OF SEEDLINGS SOWN
UP TO TWO WEEKS AFTER CONTAMINATION OF SOIL WITH JET FUEL

Treatment		Emergence		Growth	
Day[1]	Fuel (ml)	Number	Percent (%)	Length (mm)	Percent of 0-day control
0	0	12.4[2] ab[3]	83	366 ± 74[4] a	100
14	0	12.6 a	84	328 ± 82 b	90
14	4	11.6 ab	77	256 ± 53 d	70
7	4	12.0 ab	80	284 ± 80 c	78
4	4	10.9 b	73	216 ± 29 e	59
0	4	10.5 b	70	187 ± 39 f	51

[1]Number of days between placing soil in pots to sowing seeds

[2]Number of seedlings emerging of 15 sown, mean of 10 pots counted 14 days after sowing

[3]Means in a column followed by the same letter(s) are not significantly different at $P < 0.05$ by DNMR test

[4]Total length of emerging seedlings 14 days after sowing, mean and standard deviation of up to 126 seedlings per treatment

Greatest inhibition of seedling emergence (70%) occurred when seeds were sown immediately and emergence rose to 73%, 80%, and 77% when soil was left 4, 7, or 14 days, respectively. Fuel-free soil supported 83.5% emergence. Differences between the emergence means were seen but were not statistically significant at $P < 0.05$. Growth, as measured by total seedling length in mm, showed more dramatic differences. Soil aged for 0, 4, 7, and 14 days yielded seedlings which averaged 187, 216, 284, and 256 mm long, respectively, with each mean significantly different from every other at $P < 0.05$.

Aging in columns -

In another study of fuel aging, 50-cm long soil columns were contaminated with 0 or 90 ml JP4-P or JP4-S (0 or 2 ml fuel per cm^2 cross-section). Columns were packed and treated with fuel 16, 8, 4, 2, and 1 week before bioassays were performed. Soil columns were cut into sections and each section was potted and sown with sorghum seeds. One week after sowing, seedling lengths were measured.

The factors in the experiment were two fuel types (JP4-P and JP4-S), two doses (0 or 90 ml fuel/column), five dates when columns were prepared and fuel applied to soil surface (1, 2, 4, 8, and 16 weeks prior to cutting columns and planting seeds), two column replicates per treatment, 10 pots per column (one for each section), and 10 sorghum seeds per pot. Germination and emergence rates and shoot and root lengths were measured.

The hypotheses were: (1) that fuel becomes less phytotoxic the longer it remains in the soil, (2) that petroleum is no more toxic than shale-derived jet fuel, (3) that fuel in soil decreases seed germination, (4) that column replicates will be no different and will not affect seedling growth, (5) that with a short aging period, phytotoxicity will only be associated with the upper quarter to half of the soil column, and (6) that with longer aging periods, phytotoxicity will be reduced but remaining toxicity will be associated with greater column depths.

Summing seedling lengths provided a single number per pot which represented total growth and took non-germinated seeds into account. Analyses of variance, calculated initially using all data, revealed that depth, age, and dose were significant but that replicates and fuel type were not (Table 63). When no-fuel controls (0 ml/column) were removed from the data base, analyses revealed significance at $P < 0.05$ with depth, age, and fuel type; replicates were still not significant (Table 64). Shale-derived fuel had more effect on seedling growth than petroleum fuel at $P < 0.05$ but not at $P < 0.01$. Concerning depth, the soil from the first three or four sections (15 to 20 cm from column top) supported significantly less growth than the bottom two sections (30 to 50 cm from column top). Fuel did not seem to affect growth at the 30 cm level and beyond. Results of the analysis concerning the length of time soil was aged was more difficult to interpret. Some differences existed between these means at $P < 0.05$, but not at $P < 0.01$. Ranking the means indicated that a clear interpretation was not possible. Aging contaminated soil for 16 weeks or less did not statistically affect seedling growth. Reviewing the depth-age interaction revealed

TABLE 63
ANALYSIS OF ALL SEEDLING LENGTH DATA FOR SORGHUM GROWING IN CLEAN AND CONTAMINATED SOIL AGED IN COLUMNS

Depths (cm)	Lengths (mm/pot)	Age (weeks)	Lengths (mm/pot)	Dose	Lengths (mm/pot)
5	773[1] z[2]	1	896[3] z[2]	Fuel	722[4] a[2]
10	803 z	2	1016 xy	No Fuel	1271 b
15	762 z	4	930 yz		
20	812 z	8	1053 x		
25	879 z	16	1088 x		
30	1047 y				
35	1147 xy				
40	1264 x				
45	1257 x				
50	1218 x				

Replicates	Lengths (mm/pot)	Fuel	Lengths (mm/pot)
1	1004[4] NS[2]	Petrol	1022[4] NS[2]
2	984 NS	Shale	971 NS

[1]Total length of all sorghum seedlings in each pot at a given depth, mean of 40 pots

[2]Means followed by the same letter(s) in each column are not significantly different at $P < 0.05$ by DNMR test. NS = means not significantly different

[3]Total length as in (1) but mean of 80 pots

[4]Total length as in (1) but mean of 200 pots

reasonably clear separation among soil depth means, but not among ages (Table 65).

Several conclusions can be made based on these analyses: (1) jet fuel left in soil up to 16 weeks decreased seed germination and subsequent growth, (2) in the present experiment, no differences between replicates were found, (3) shale fuel was slightly more phytotoxic than petroleum-derived fuel, (4) decreased growth was associated with the upper 15 to 20 cm of 50-cm columns, the approximate distance 2 ml/cm^2 fuel penetrates into a column of sandy soil; however, seedlings grown in soil from the bottom 20 cm of the column were not significantly reduced, (5) aging soil for up to 16

TABLE 64
ANALYSIS OF SEEDLING LENGTH DATA FOR SORGHUM GROWING IN CONTAMINATED SOIL AGED IN COLUMNS

Depths (cm)	Lengths (mm/pot)	Age (weeks)	Lengths (mm/pot)
5	221[1] yz[2]	1	646[3] z[2]
10	184 z	2	771 y
15	197 yz	4	616 z
20	377 xy	8	794 y
25	506 x	16	785 y
30	835 w		
35	1172 v		
40	1224 v		
45	1263 v		
50	1245 v		

Replicates	Lengths (mm/pot)	Fuel	Lengths (mm/pot)
1	752[4] NS[2]	Petrol	765[4] Y[2]
2	692 NS	Shale	680 Z

[1]Total length as in Table 63, note 1 but mean of 20 pots

[2]Same as Table 63, note 2

[3]Total length as in Table 63, note 3 but mean of 40 pots

[4]Total length as in Table 63, note 4 but mean of 100 pots

weeks before sowing seeds did not significantly affect inhibition of seedling lengths, and (6) the phytotoxic components did not migrate downwards in the columns during the 16-week period.

Effect of Aging Fuel-Contaminated Soil on Tomato Yield -

Another experiment checked the effect that aging fuel-contaminated soil had on the yield of tomato seedlings transplanted into the soil.

Gallon pots were filled with ca. 2450 g glasshouse soil mix at 16, 8, 4, 2, or 1 week before the tomato-transplant date. Zero or 28 ml (ca. 1% v/w) of JP4-P or JP4-S was applied to the soil surface at the time pots were filled. The gallon pots were insulated from excessive heat by placing each within a two-gallon pot and packing the excess space with perlite. Filled

TABLE 65
INTERACTION OF DEPTH AND AGE FOR LENGTH OF SORGHUM SEEDLINGS GROWING IN SOIL FROM COLUMNS TREATED WITH FUEL

Depth (cm)	Soil aging period (weeks) 1	2	4	8	16
5	257[1] yz[2]	50 z	207 yz	269 yz	322 xyz
10	173 yz	182 yz	157 yz	196 yz	213 yz
15	179 yz	243 yz	60 z	260 yz	238 yz
20	282 yz	761 tuvwx	165 yz	340 xyz	334 xyz
25	160 yz	1087 qrstuv	249 yz	644 uvwxy	391 xyz
30	600 vwxy	1199 qrst	455 wxyz	944 rstuv	975 rstuv
35	1029 qrstuv	1114 qrst	1099 qrst	1225 qrst	1393 qr
40	1212 qrst	1043 qrstuv	1051 qrstuv	1498 q	1316 qrs
45	1271 qrs	1165 qrs	1241 qrst	1164 qrst	1475 q
50	1298 qrs	862 stuvw	1479 q	1396 qr	1191 qrst

[1]Sum of all seedling lengths per pot, mean of four pots

[2]Means across the whole table followed by the same letter(s) are not significantly different at $P < 0.05$ by DNMR test

pots were watered three times per week. Five pots per treatment were planted on the same date with 28-day-old Tiny Tim tomato seedlings which were harvested 28, 56, or 84 days later. Harvest data consisted of fruit count and fresh weight, root and stem fresh and dry weight, number of primary nodes, and number, area, and fresh and dry weight of leaves.

In order to successfully analyze and present the great amount of data collected, various manipulations were made. Means of fuel-contaminated soil treatments for a given variable were compared with corresponding no-fuel treatments to obtain a ratio. These fuel/no-fuel ratios could then be plotted for each of the five fuel aging periods and lines could be drawn connecting ratio points of the same harvest. If fuel had the expected inhibitory effect, then the fuel/no-fuel ratio would be less than 1.0. A ratio value greater than 1.0 was generally interpreted to mean no effect from fuel, since little or no evidence supports any beneficial fuel effect. Each point on the graphs usually represented five plants which, in turn, could be composed of a number of leaves or fruits. A t-test was used to compare the fuel data of a particular point to no-fuel data to determine if the differences were significant.

Leaf area of the tomato plants at harvest was typical of data analysis. For both JP4-P and JP4-S (Figure 12), the first two harvests appeared more influenced by soil contamination than the third. Significance was

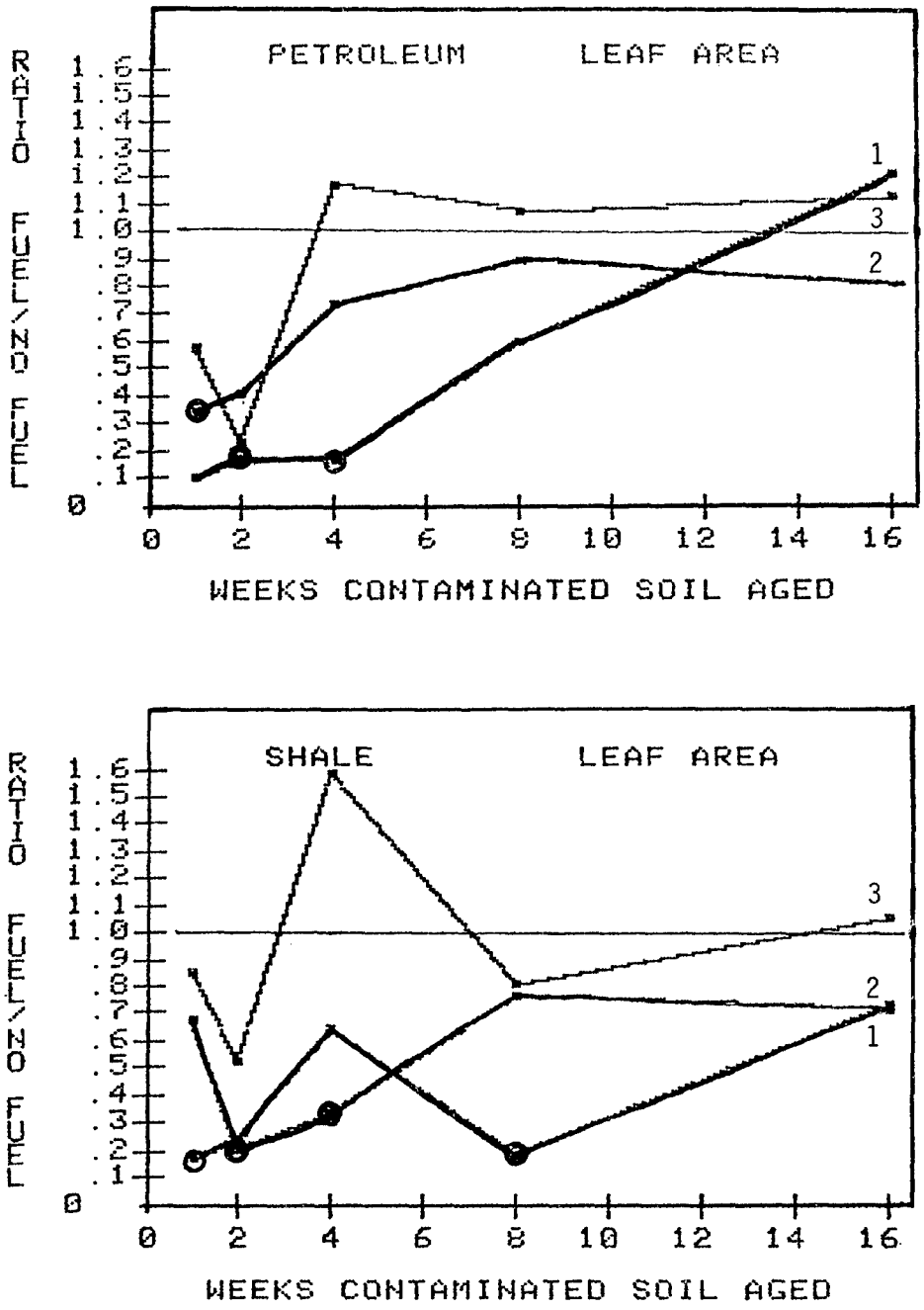


Figure 12. Leaf area of tomato plants after growing 28 (line 1), 56 (line 2), or 84 (line 3) days in soil aged 1-16 weeks after being mixed with petroleum (top) or shale (bottom) forms to JP4 fuel.

limited to soil aged eight weeks or less. The information can also be presented in tabular form (Table 66).

Leaf weight and leaf count were similar to leaf area; the same harvest dates and ages were sensitive.

Root weight and stem weight were analyzed (Tables 67 and 68). In both cases the influence of JP4-P was less than JP4-S. The shale fuel apparently reduced growth of plants even on the third harvest. As in earlier comparisons, only the first two soil ages showed the effect, i.e., toxicity remained only in soil aged one or two weeks before receiving transplanted tomatoes.

TABLE 66
MEAN LEAF AREA OF TOMATO PLANTS GROWN IN SOIL CONTAMINATED WITH JET FUEL

Age[1] (weeks)	JP4-P			JP4-S		
	Fuel	No fuel	t[4]	Fuel	No fuel	t
Harvest[2] #1						
1	66[3]	110	0.73	16	92	3.2 *
2	15	95	4.33 *	17	70	3.49 *
4	18	265	4.92 *	78	122	0.59
8	86	144	2.00	239	45	-4.20 *
16	532	440	-1.07	436	589	2.19
Harvest #2						
1	112	327	3.52 *	177	265	0.90
2	71	176	2.28 *	29	140	2.54 *
4	243	330	.68	179	542	5.11 *
8	350	389	.48	284	368	0.94
16	741	931	1.19	597	847	1.58
Harvest #3						
1	196	336	1.15	311	365	0.38
2	41	176	1.17	68	131	1.83
4	714	613	-0.59	459	291	-0.83
8	713	662	-1.25	453	557	0.87
16	1003	885	-1.04	823	785	-0.24

[1]Weeks that soil was aged (left fallow) between fuel contamination (when treated) and transplanting of tomato seedlings

[2]Harvest #1, #2, and #3 were 28, 56, and 84 days after transplant, respectively; plant was sacrificed at harvest

[3]Leaf areas per plant in cm^2, mean of five plants

[4]Student t-test values, t is starred (*) if fuel no-fuel comparison is significant at $P < 0.05$

TABLE 67
MEAN ROOT WEIGHT OF TOMATO PLANTS GROWN IN SOIL CONTAMINATED WITH JET FUEL AND AGED

Age[1]	JP4-P			JP4-S		
	Fuel	No fuel	t[4]	Fuel	No fuel	t
Harvest #1						
1	2[3]	7	5.15 *	1	7	4.08 *
2	2	9	3.39 *	1	5	3.73 *
4	3	14	3.58 *	5	9	0.77
8	5	9	2.05	12	3	-3.00 *
16	26	19	-1.84	20	26	2.42 *
Harvest #2						
1	7	16	8.81 *	9	16	2.01
2	6	9	1.23	2	8	6.61 *
4	10	15	1.12	10	23	4.54 *
8	14	17	1.65	11	18	2.08
16	28	31	0.45	22	28	1.17
Harvest #3						
1	8	14	1.35	7	12	2.49 *
2	4	8	1.37	5	9	2.82 *
4	20	20	-0.02	15	12	-0.61
8	21	20	-0.21	17	18	0.29
16	35	34	-0.02	32	33	0.23

[1-4]Same as notes for Table 66 except root weight per plant in mg

One of the most important measures of yield was the number and fresh weight of the fruit. No fruit had developed on any plants by the first harvest, but was present for the second harvest, 56 days after transplanting. Both fruit count (Figure 13 and Table 69) and fruit weight (Figure 14 and Table 70) had log-shaped growth curves. Ratios were involved here as in leaf area, and this shape indicated a major influence of the fuel on growing plants. Soil recovery appeared nearly complete after being aged four weeks since plants grown in that soil had fruit counts or weights approaching or exceeding the 1.0 ratio or no-effect point. Almost all significantly low fuel/no-fuel ratios (at $P < 0.05$) occurred with plants in soil aged one, two, or four weeks. One significantly low ratio occurred among fruit weight means of plants in soil aged 16 weeks. Only in this case did fruit on plants growing in fuel-contaminated soil weigh more than fruit on plants in control soil. A beneficial or nutritional effect of the aged fuel may be postulated, or the fuel may release soil nutrients that previously had become bound.

TABLE 68
MEAN STEM WEIGHT OF TOMATO PLANTS GROWN IN SOIL CONTAMINATED WITH JET FUEL

Age[1]	JP4-P			JP4-S		
	Fuel	No fuel	t[4]	Fuel	No fuel	t
Harvest[2] #1						
1	0[3]	2	4.47 *	0	2	3.70 *
2	0	2	4.10 *	0	1	8.18 *
4	1	7	6.04 *	2	2	0.64
8	2	3	1.72	6	1	-3.26 *
16	13	10	-1.51	11	14	1.68
Harvest #2						
1	14	14	0.05	6	14	2.03
2	4	8	3.32 *	2	7	3.69 *
4	9	14	1.10	9	25	5.45 *
8	15	17	0.49	12	16	0.86
16	34	39	1.30	30	38	1.17
Harvest #3						
1	8	13	1.03	8	17	2.50 *
2	2	8	1.76	3	9	3.16 *
4	24	24	0.05	16	15	-0.20
8	27	26	-0.36	20	22	0.38
16	42	37	-1.27	39	36	-0.37

[1]Same as notes for Table 67 except stem weight/plant in g

In summary, this experiment showed that soil aging influenced the toxicity of the fuel remaining in the soil. Yield of tomatoes under our conditions increased significantly ($P < 0.05$) as the soil aged. After four weeks of aging, no differences could be observed for most parameters between plants growing in fuel-contaminated or uncontaminated soil. After 16 weeks, a possibly beneficial effect may have been detected.

Evaporation of Jet Fuel

Petroleum fuels such as JP4 are made up of many components. Workers generally agree that the aromatic, more volatile fractions of oil have greater phytotoxicity (Baker, 1970). Toxicity of fractions was studied by allowing jet fuel to evaporate at room temperature for different lengths of time.

In a preliminary test, 100 ml of JP4-P fuel was placed in each of two 150-ml beakers and 25 ml was placed in a 25-ml cylinder. The fuel in the

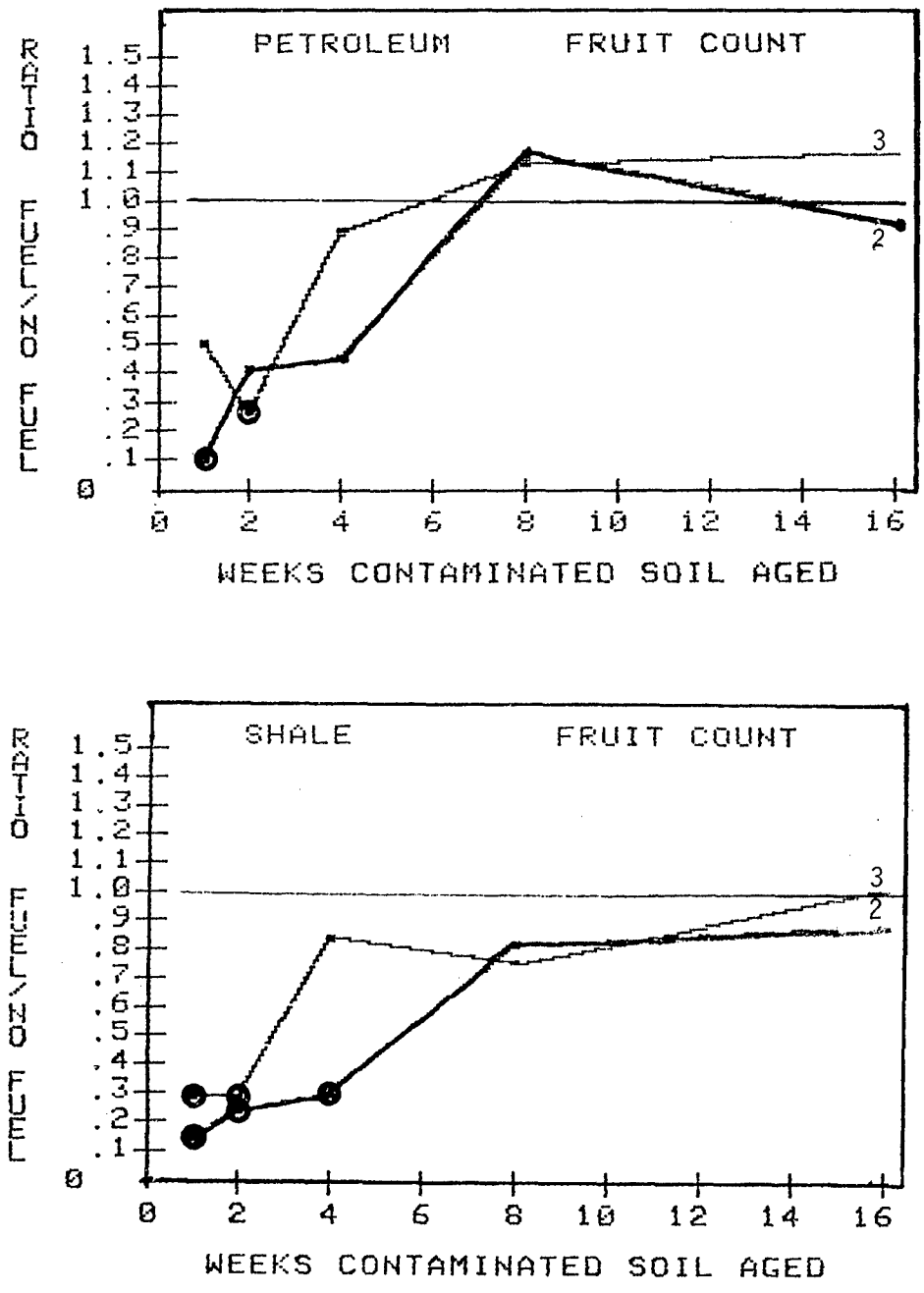


Figure 13: Fruit count of tomato plants after growing 56 days (line 2) or 84 days (line 3) in soil aged 1-16 weeks after being mixed with petroleum (top) or shale (bottom) forms of JP4 jet fuel.

TABLE 69
MEAN FRUIT COUNT OF TOMATOES FROM PLANTS GROWN IN SOIL CONTAMINATED WITH JET FUEL AND AGED

Age[1]	JP4-P Fuel	JP4-P No fuel	JP4-P t[4]	JP4-S Fuel	JP4-S No fuel	JP4-S t
Harvest[2] #2						
1	4[3]	38	8.93 *	5	31	4.11 *
2	9	22	2.15	6	26	2.91 *
4	18	40	1.58	19	65	4.26 *
8	34	29	-0.49	26	32	0.56
16	78	84	0.61	70	78	0.46
Harvest #3						
1	20	41	1.86	16	55	3.70 *
2	6	24	2.65 *	9	33	2.92 *
4	66	74	0.59	41	49	0.47
8	88	78	-1.02	57	76	1.12
16	113	97	-1.27	103	101	-0.11

[1-4]Same notes as Table 66 but fruit counts/plant

TABLE 70
MEAN FRUIT WEIGHT OF TOMATOES FROM PLANTS GROWN IN SOIL CONTAMINATED WITH JET FUEL AND AGED

Age[1]	JP4-P Fuel	JP4-P No fuel	JP4-P t[4]	JP4-S Fuel	JP4-S No fuel	JP4-S t
Harvest[2] #2						
1	4[3]	58	6.68 *	5	40	2.74 *
2	8	28	2.45 *	4	22	3.16 *
4	22	55	1.50	24	99	4.40 *
8	44	35	-0.77	29	33	0.43
16	121	118	-0.25	109	133	0.83
Harvest #3						
1	60	156	1.98	43	195	4.28 *
2	15	72	1.42	27	87	2.77 *
4	223	226	0.06	167	148	-0.25
8	293	248	-2.21	200	239	0.92
16	382	320	-2.48 *	321	309	-0.26

[1-4]Same notes as for Table 66 but for fruit weight in mg

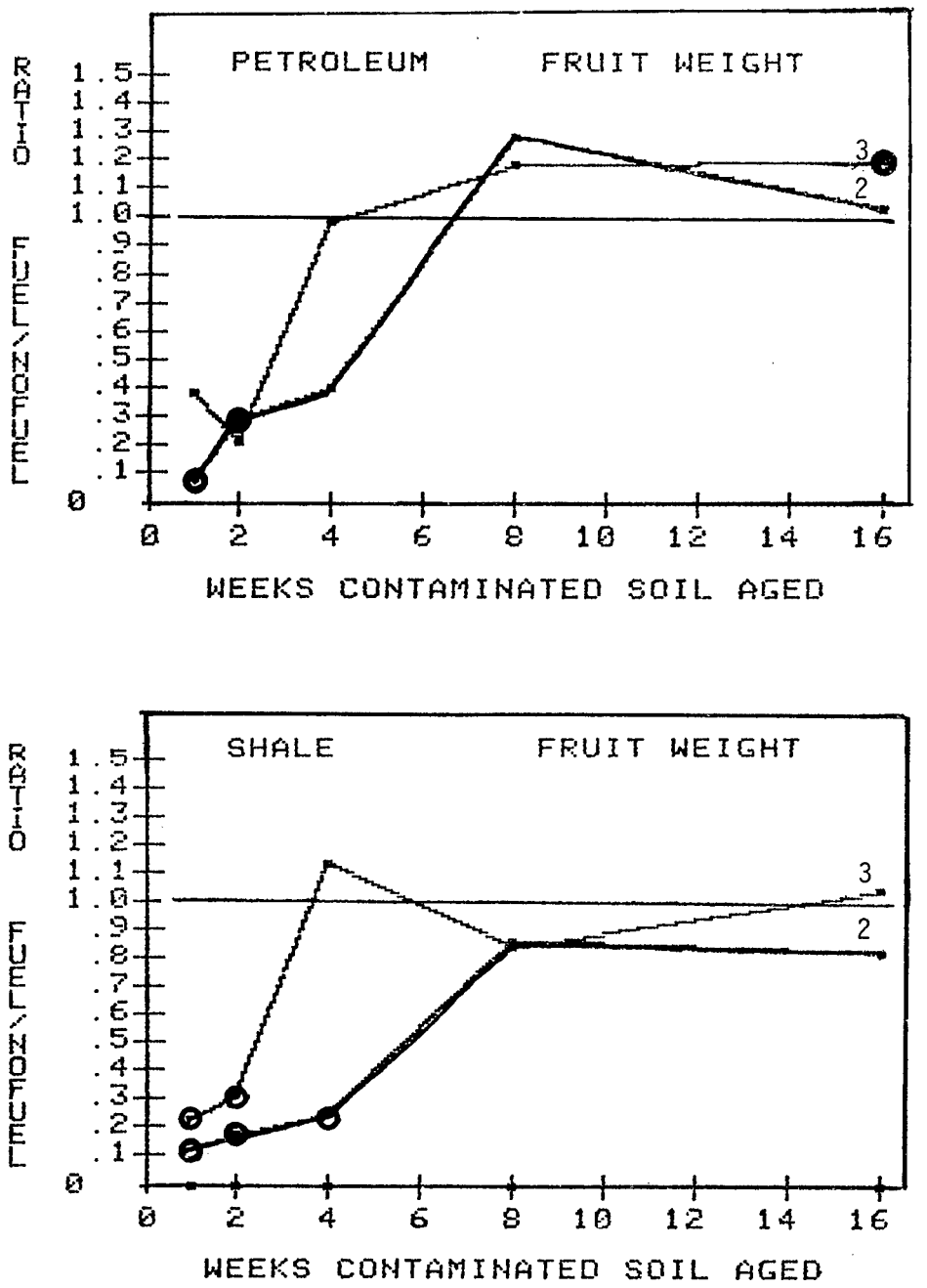


Figure 14: Fruit weight of tomatoes from tomato plants grown 56 days (line 2) or 84 days (line 3) in soil aged 1-16 weeks after being mixed with petroleum (top) or shale (bottom) forms of JP4 jet fuel.

three vessels was allowed to evaporate in a laboratory hood at 28°C (room temperature). Fuel in one beaker was stirred with an air-driven magnetic device at moderate speed. The volume of fuel in each beaker was measured periodically using a 100-ml graduated cylinder (Table 71). The rate of evaporation was calculated taking into account the surface area exposed. Fuel evaporated most rapidly from the stirred beaker. Evaporation rate, greatest during the first hours, progressively decreased thereafter. Amount of fuel remaining, as a function of time, best fits a hyperbolic function.

Fuel in a control beaker was allowed to evaporate and was measured once after 672 hours (28 days). Nine ml remained in this beaker compared to 4 ml in the frequently measured beaker, probably due to many small losses during transfers to and from the measuring cylinder.

Toxicity of Fuel Residue

The lowest boiling-point fraction of fuel oil, the aromatics, are known to be more phytotoxic than higher boiling fractions (Baker, 1970). Our hypothesis was that aromatics would be the first material to evaporate from jet fuel and higher boiling-point fluids left behind would be less toxic

TABLE 71
EVAPORATION OF JP4 FUEL AT ROOM TEMPERATURE

Hours	150-ml beakers[1]				25-ml Graduated cylinder[1]	
	Stirred		Unstirred			
	Remaining (ml)	Rate (ml/cm^2/hr)	Remaining (ml)	Rate (ml/cm^2/hr)	Remaining (ml)	Rate (ml/cm^2/hr)
0	100		100		25.0	
1	87	0.051	88	0.471	25.0	0.000
2	80	0.027	80	0.314	24.8	0.080
3	75	0.196	75	0.196	24.6	0.080
4	70	0.196	70	0.196	24.6	0.000
5	67	0.118	68	0.078	24.4	0.080
6	63	0.157	65	0.118	24.4	0.000
12	51	0.078	52	0.085	24.0	0.027
24	43	0.026	44	0.026	23.4	0.020
60	29	0.015	31	0.014	22.8	0.007
120	20	0.006	23	0.005	21.6	0.008
217	10	0.004	16	0.003	20.4	0.005
420	6	0.001	8	0.002	18.8	0.003
606	2	0.001	5	0.001	17.6	0.003
680	1.5	0.000	4	0.001	17.2	0.002

[1]Cross-section areas: Beaker = 25.5 cm^2, Cylinder = 2.5 cm^2

unevaporated fuel. JP4-P fuel was allowed to evaporate while being stirred at room temperature; 40.5% of the original fuel volume remained after 24 hours. Evaporation residue or unevaporated fuel was mixed at the rate of 4 ml per pot of 420 g glasshouse soil (ca. 1% v/w). The soil was potted and sown with sorghum seeds. Harvest took place two weeks (Group 1) and three weeks (Group 2) after sowing, at which time control shoots were found to average 55 and 80 mm, respectively. Soil was washed away at harvest to measure root and shoot length and fresh weight (Table 72).

Differences of percent germination among treatments were not significant ($P < 0.05$), but germination included all seeds which had begun to grow, even those whose shoots had not yet emerged through the soil surface. Increased incubation would probably not have increased emergence rates in fuel treatments.

Fuel, whether part of the evaporation residue or not, created an inhibitory environment reflected in the lengths and weights of the seedlings. Separate statistical tests on the individual groups revealed separation of the two fuel treatments at $P < 0.05$, but the extra week of growth for Group 2 eliminated differences.

Residues of fuel remaining after evaporation were more, not less, toxic than unevaporated fuel. Evaporating fuel at room temperature apparently concentrated the toxic, presumably aromatic, compounds along with all other fractions.

Toxicity of Different Fractions

To verify and expand the above results that evaporation was apparently concentrating toxicity, four residues or "fractions" were prepared by allowing approximately 0, 25, 50, or 75% of the original JP4-P to evaporate (Table 73). Each fraction was mixed with preweighed soils at the rate of 0, 1, 2, or 4 ml per pot, or ca. 0, 0.25, 0.5, or 1% (v/w) fuel. Ten sorghum seeds were sown on the treated soil of each pot. After two weeks, seedlings were harvested, fresh weights and lengths were measured, and germination and emergence rates were calculated (Table 74). Germinated seeds had visible root growth of at least 1 mm, whereas shoots of emergent seedlings had lengths longer than 10 mm. With either measure, fuel doses (mls applied) were statistically more important than levels of evaporated residues. The experiment verified that seed germination was reduced by 1% (v/w) fuel. Partial evaporation contributed to reduced germination; 75% evaporated residue appeared more toxic or inhibitory than whole, unevaporated fuel.

Similar reductions were observed when seedling weight and length were analyzed (Tables 75-78). In most cases, measurements for the four doses were statistically different when compared at any particular residue level, but measurements did not always differ when residue levels were compared at certain doses.

Evaporating part of the fuel increased the toxicity of the fuel remaining. However, seedling development was more affected by the amount of fuel applied to the soil than by which fuel residue was applied.

TABLE 72
GERMINATION AND GROWTH OF SORGHUM IN SOIL CONTAMINATED WITH FUEL OR EVAPORATED FUEL RESIDUE

Treatment[1]		Germination[2]	Emergence[3]	Lengths (mm)[4]		Weight[5]
Fuel	Harvest group	(%)	(%)	Shoot	Root	(mg)
F	1	57 a[6]	40 (C)	12 C	44 C	57 C
ER	1	55 a	2 (C)	6 C	21 D	39 D
C	1	65 a	76 (B)	55 B	118 B	94 B
F	2	46 a	14 (C)	11 C	34 CD	54 C
ER	2	53 a	14 (C)	10 C	31 CD	46 CD
C	2	64 a	80 (A)	79 A	147 A	164 A

[1]F = JP4-P fuel on soil, ER = Evaporated residue of JP4-P after 24 hr at room temperature, C = untreated control

[2]Percent seeds germinating, means for 50 seeds; transformed for analysis by standard arc sin

[3]Percent seeds with shoots > 10 mm when harvested, mean of 50 seeds

[4]Length of seedlings in mm, mean of up to 40 seedlings

[5]Fresh weight of seedlings in mg, mean of up to 40 seedlings

[6]Same letter(s) following means in columns indicate no difference by SNK test for mean separation at $P < 0.01$ (capital letters) or $P < 0.05$ (lower case)

TABLE 73
EVAPORATION RESIDUES FOR JP4 JET FUEL

Time to evaporate (hr)	Residue remaining (% of original volume)	Residue evaporated (%)
0.0	100.0	0.0
5.0	70.5	29.5
17.5	50.0	50.0
91.5	24.6	75.4

TABLE 74
PHYTOTOXICITY OF EVAPORATED FUEL RESIDUES ON SORGHUM:
GERMINATION AND EMERGENCE RATES

Dose[1] (ml)	Percent whole fuel lost by evaporation			
	0	25	50	75
0	71[2] (71)[3]	79 (71)	85 (74)	79 (55)
1	71 (78)	80 (75)	74 (62)	71 (29)
2	78 (81)	73 (70)	75 (62)	71 (26)
4	66 (78)	64 (66)	65 (42)	64 (19)

[1]Amount of fuel residue in ml applied to 420 g soil

[2]Rate of germination in percent of total of 80 sorghum seeds planted; calculated on presence of root growth

[3]Rate of emergence in percent of total of 80 sorghum seeds planted; count of seedlings with shoot lengths 10 mm or greater

This entire study was repeated with small changes. The greatest evaporation residue was 70% rather than 75%, and the two replicates, of four pots each, consisted of fuel from a previously unopened can. Sorghum seedlings were harvested two weeks after sowing, and emergence, lengths, and weights were measured.

No differences were seen between the replicates. The measured data confirmed the original work (Table 77 and 78): fuel dose was of greater importance than evaporation in reducing seedling emergence, rate, length, or weight, but evaporating the fuel also seemed to increase toxicity, particularly when 70% of the fuel was evaporated. No major differences were seen (at $P < 0.05$) when findings were compared between experimental trials.

Temperature Effect on Evaporation and Toxicity

Evaporation of fuel at room temperature apparently does not differentially drive off the more toxic, lower boiling point, aromatic compounds. The effect of temperature during evaporation was investigated by evaporating JP4-P fuel to 50% of its original volume at four different temperatures: 4, 25, 50, and 70°C. Each prepared residue and adequate controls were mixed into soil at the 4 ml per pot or 1% (v/w) rate. Germination, emergence, and lengths were measured after two weeks (Table 78). Highest emergence rate and longest seedling length were associated with the no-fuel control plants where these factors were significantly greater ($P < 0.05$) than for corresponding plants growing in fuel-contaminated soil. The seedlings in soil contaminated with unevaporated (0%) fuel were significantly longer (at $P < 0.05$) than the evaporative residues. No differences in length were noted

TABLE 75
PHYTOTOXICITY OF EVAPORATED FUEL RESIDUES ON SORGHUM SEEDLINGS: LENGTHS AND WEIGHTS

Dose[1] (ml)	Percent whole fuel lost by evaporation			
	0	25	50	75
		Root length (mm)		
0	126[2] ab[3]	112 b	118 ab	131 a
1	112 b	93 c	91 c	79 cd
2	68 de	74 d	64 de	53 ef
4	54 ef	47 f	42 f	37 f
		Shoot length (mm)		
0	64[2] a[3]	51 abc	66 a	61 ab
1	54 bcd	39 e	37 e	31 f
2	42 e	23 g	22 g	14 h
4	22 g	12 h	11 h	10 h
		Fresh weight (mg)		
0	124[2] a[3]	111 b	120 ab	100 cd
1	109 bc	82 efg	91 de	79 fg
2	90 def	72 g	79 fg	56 h
4	72 g	52 h	58 h	52 h

[1]Amount of fuel residue in ml applied to 420 g soil

[2]Length or weight of sorghum seedlings grown in presence of JP4 fuel residue, mean of eight pots of 10 seeds sown per pot

[3]Means within each measurement variable followed by the same letter(s) are not significantly different at $P < 0.05$ by SNK test

TABLE 76
EMERGENCE OF SORGHUM SEEDLINGS IN SOIL WITH EVAPORATED RESIDUES OF JET FUEL

Dose[1] (ml)	Percent whole fuel lost by evaporation			
	0	25	50	75
0[1]	81 ± 10[2] a	76 ± 9 ab	81 ± 8 a	68 ± 13 ab
1	79 ± 19 ab	69 ± 22 ab	60 ± 16 abc	70 ± 14 ab
2	49 ± 24 abcd	41 ± 10 bcde	26 ± 16 def	20 ± 9 f
4	31 ± 22 ef	36 ± 18 cdef	28 ± 12 ef	22 ± 15 f

[1-3]See notes for Table 75

TABLE 77
DEVELOPMENT OF SORGHUM SEEDLINGS GROWN IN SOIL CONTAMINATED WITH FUEL RESIDUES

Fuel dose[1] (ml)	Percent whole fuel lost by evaporation			
	0	25	50	75
		Root length (mm)		
0	198[2] a[3]	198 a	211 a	201 a
1	169 b	157 b	139 c	126 c
2	91 d	92 d	70 e	64 e
4	68 e	51 e	46 e	50 e
		Shoot length (mm)		
0	96[2] b[3]	126 a	95 bc	96 b
1	69 c	72 c	64 c	67 c
2	52 d	43 de	40 de	40 de
4	49 de	45 de	36 de	32 de
		Fresh weight (mg)		
0	160[2] cd[3]	280 a	196 b	284 a
1	144 d	173 bcd	170 bcd	179 bc
2	97 e	105 e	92 e	83 e
4	72 e	86 e	76 e	71 e

[1-3]See notes for Table 75

among seedlings exposed to residues evaporated at different temperatures. No differences in germination rate were seen with any of the fuel treatments, whereas emergence was lower with the 70°C fuel treatment than with the other treatments.

Fuel toxicity increased with partial evaporation. In addition, a trend of increasing toxicity with increasing evaporation was noted. This evidence implies concentration of the fuel by evaporation. Small amounts (0.1 ml) of JP4-P fuel were injected into a Hewlett Packard 5710A gas chromatograph with an SE54 capillary column, flame ionization detector, and programmed temperature control. As column temperature increased from 50 to 300°C, fuel fractions volatilized and were detected (Figure 15). Three JP4-P samples were tested: non-evaporated fuel and 50% residues after room temperature (22°C) and 50°C evaporations. Certain peaks could be recognized in all three samples and were measured (Table 79). Of nine measured peaks, the first three were larger in the unevaporated fuel than in the evaporated material. Other measured peaks were larger for the residues from evaporation and indicated that the original fuel was being concentrated by the process. In all cases, the material prepared by evaporating at an elevated temperature had greater peak values than the material prepared at room temperature

TABLE 78
GROWTH OF SORGHUM IN SOIL CONTAMINATED WITH JP4-P FUEL PARTIALLY EVAPORATED AT DIFFERENT TEMPERATURES

Treatment[1]	Germination[2] (%)	Emergence[3] (%)	Length[4] Shoot (mm)	Length[4] Total (mm)
No fuel control	75 a[5]	72 a	89 ± 23 a	304 ± 90 a
100% fuel	42 bc	16 b	26 ± 22 b	97 ± 42 b
50% fuel 4°C	56 b	4 b	9 ± 10 c	50 ± 28 c
50% fuel 25°C	43 bc	8 b	14 ± 15 c	61 ± 32 c
50% fuel 50°C	42 bc	4 b	8 ± 10 c	47 ± 25 c
50% fuel 70°C	31 c	0 c	5 ± 2	43 ± 15 c

[1]100% fuel = no evaporation, 50% fuel = evaporated to 50% original volume at stated temperature

[2]Germination is percent number of seeds with any growth, mean of eight pots with 80 seeds total

[3]Emergence is percent number of seedlings with shoot emergence. Analyses done on arc sin transformations

[4]Length of seedlings, mean and standard deviation of 25 to 60 seeds

[5]Means in columns followed by same letter(s) are not significantly different at $P < 0.05$ by SNK test

(Table 79). Visually observing the charts and counting numbers of peaks within 24 column temperature ranges showed that about half the peaks were absent below 74°C and peaks still remaining below 100°C were reduced (Table 80 and 81). The gas chromatographic data indicated that low-boiling point components of the fuel were lost during evaporation and that some concentration of the remaining components takes place.

Petroleum and Shale JP4 Fuel

Much of the fuel work reported herein was conducted with JP4-P, the petroleum-derived form of jet fuel. Shale-derived JP4-S was sometimes more toxic than JP4-P, but often not significantly so. Several experiments explored the two forms of fuels, particularly in conjunction with evaporation trials.

Dose response - JP4-P and JP4-S were compared directly using a seed bioassay. One-half, 1, 2, 4, or 8 ml of JP4-P, JP4-S, or water were mixed into pots of soil for final liquid concentrations in soil of 0.1, 0.25, 0.5, 1, and 2% v/w. Ten sorghum seeds were planted in each pot and there were three replicates of two pots each. Seedlings were harvested two weeks after sowing.

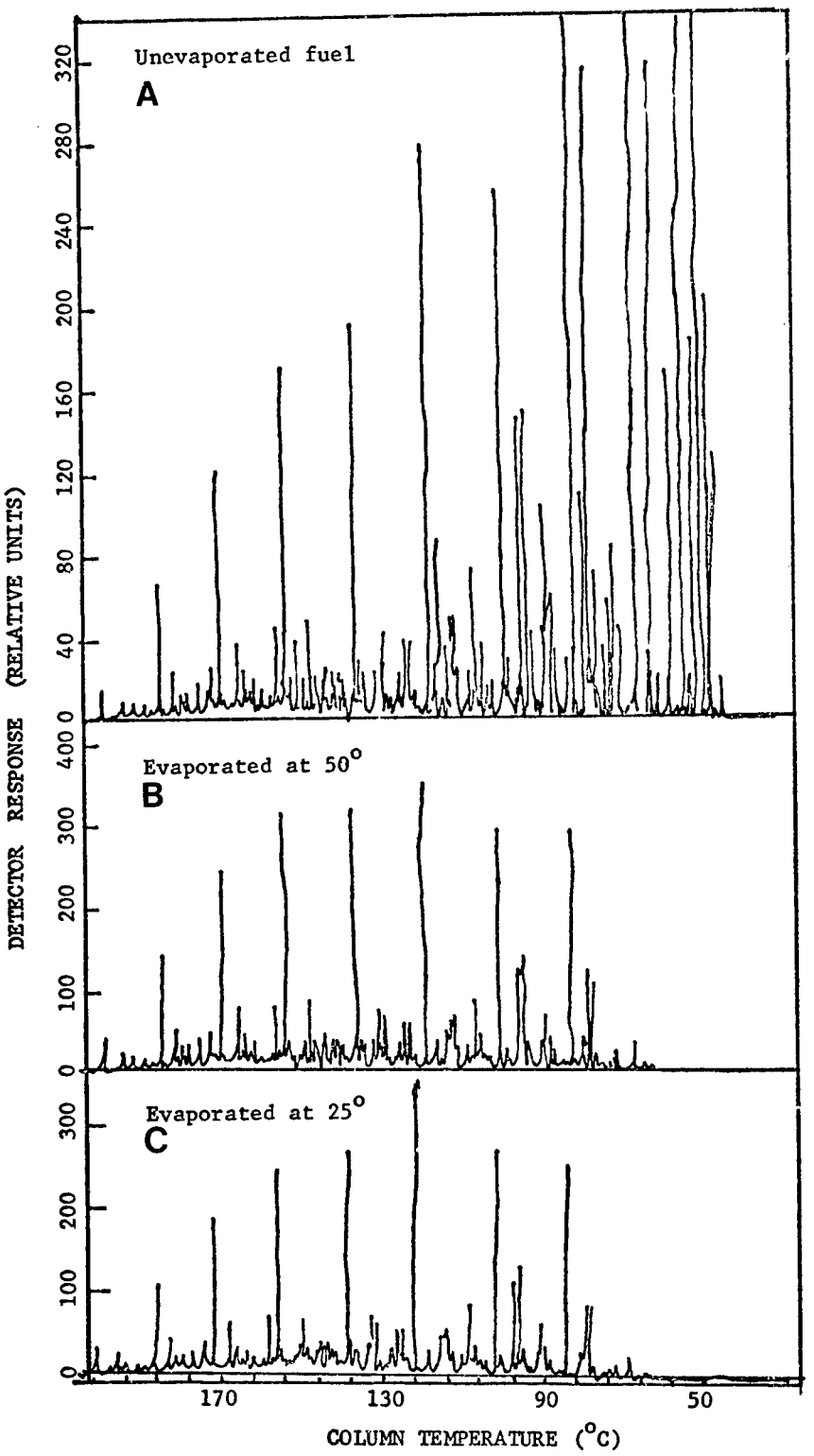


Figure 15. Gas chromatograph tracings of JP4-P (A) unmodified, (B) evaporated to 50% volume at 50°C and (C) at 25°C.

TABLE 79
GAS CHROMATOGRAPHIC DETECTION OF JP4-P FUEL

Temperature of GC peak (°C)	Unevaporated fuel (number)	Residues evaporated to 50% volume at 25°C	Residues evaporated to 50% volume at 50°C
85	> 400[1]	205	232
96	150	108	112
97	147	92	98
102	176	220	240
121	279	286	320
139	191	218	257
156	172	200	255
172	124	152	199
187	68	88	117

[1]Calculated height of detector response on gas chromatograph tracings, attenuation taken into account

TABLE 80
NUMBER OF PEAKS DETECTED BY GAS CHROMATOGRAPHY IN JP4-P FUEL AND FUEL RESIDUES

GC temperature ranges (°C)	Unevaporated fuel (number)	Fuel evaporated to 50% volume 25°C	Fuel evaporated to 50% volume 50°C
50- 74	36[1] (15)	18 (0)	12 (0)
75- 99	31 (15)	29 (6)	28 (8)
100-124	33 (8)	35 (7)	30 (8)
125-149	41 (5)	38 (5)	36 (7)
150-174	33 (5)	39 (4)	32 (6)
175-199	26 (2)	28 (2)	25 (4)
> 200	9 (0)	16 (0)	7 (0)

[1]Number of peaks counted on tracing of gas chromatograph and (number of peaks greater than 40 detector units)

Harvest results (Table 81) showed general trends which could be represented graphically (Figures 16, 17, 18). Emergence rates for all treatments and controls were not significantly different ($P < 0.05$) for all doses and fuels except at 4 and 8 ml per pot. Only 43% of the seedlings emerged in soil to which 4 ml JP4-S per pot had been added. This was less at $P < 0.05$ than the 85% emergence for the water control, but not significantly less at $P < 0.05$ than the 60% emergence after JP4-P treatment. At 8 ml per pot both petroleum and shale forms inhibited emergence.

Total length (Table 81), shoot length (Figure 17), and fresh weight (Table 81 and Figure 18) of seedlings in treated soil showed inhibition by fuel at all dose levels. At the smallest doses, 0.5 ml per pot, seedlings grown in the presence of JP4-P were inhibited significantly more than those grown in JP4-S. This was unexpected since most of the data for larger doses showed larger values for JP4-P. Very low doses of JP4-S apparently were less inhibitory than JP4-P but the evidence does not suggest beneficial effects.

Evaporation and Toxicity Test - Shale and petroleum forms were stirred at 25°C (room temperature) and 70°C until 5% or 50% of the original volumes had evaporated. Glasshouse soil was treated with the prepared materials applied at the rates of 1 or 4 ml fuel per pot (ca. 0.5 and 1% v/w) and each pot was sown with 10 sorghum seeds. Unevaporated fuel and no-fuel controls

TABLE 81
RESPONSE OF SORGHUM SEEDLINGS TO FUEL DOSE

Dose[1] (ml)	Emergence[2] (%)			Length[3] (mm)			Weight[4] (mg)		
	JP4-P	JP4-S	H_2O	JP4-P	JP4-S	H_2O	JP4-P	JP4-S	H_2O
0.5	73 a	78 a	87 a	338 e	390 d	475 ab	565 c	853 b	1229 a
1	78 a	77 a	70 a	334 e	304 e	407 abcd	554 cd	526 cde	1257 a
2	67 ab	58 ab	77 a	310 e	290 e	475 a	475 cde	428 cde	1365 a
4	60 ab	43 bc	85 a	218 f	183 f	450 abc	264 cdef	221 cdef	1261 a
8	33 cd	22 d	70 a	124 g	142 g	425 abcd	97 f	132 f	1174 a

[1]ml of fuel or water mixed into 420 g soil

[2]Emergence rate in percent of sorghum seedlings over 10 mm length, mean of 60 seedlings. Emergence means followed by same letter(s) are not significantly different at $P < 0.05$ by SNK test

[3]Total length of sorghum seedlings in mm, mean of up to 52 seedlings. Mean separation of lengths same as for emergence

[4]Fresh weight of sorghum seedlings in mg, mean of up to 52 seedlings. Same mean separation as above

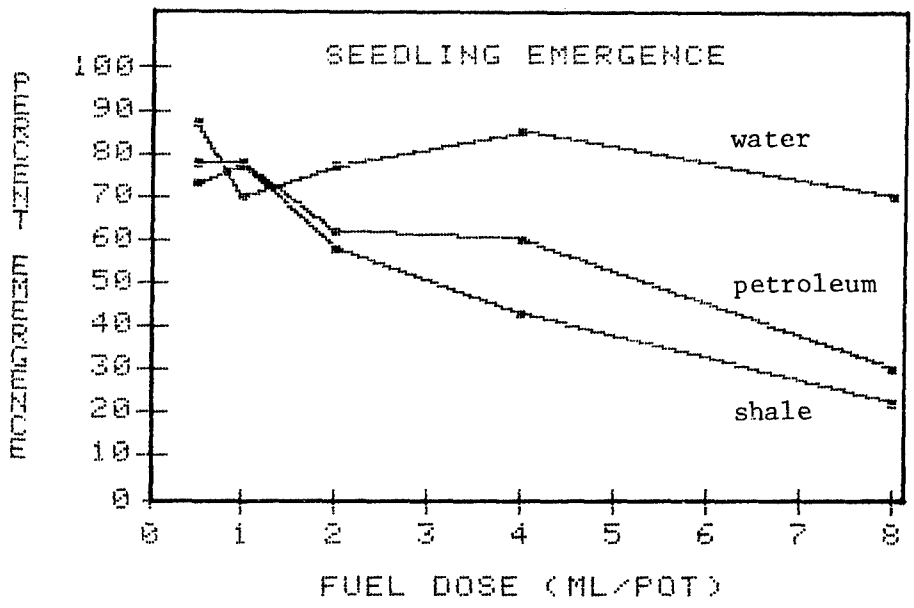


Figure 16. Emergence rate of sorghum seedlings in soil contaminated with 0.5 to 8 ml JP4-P (petroleum), JP4-S (shale), or water per pot.

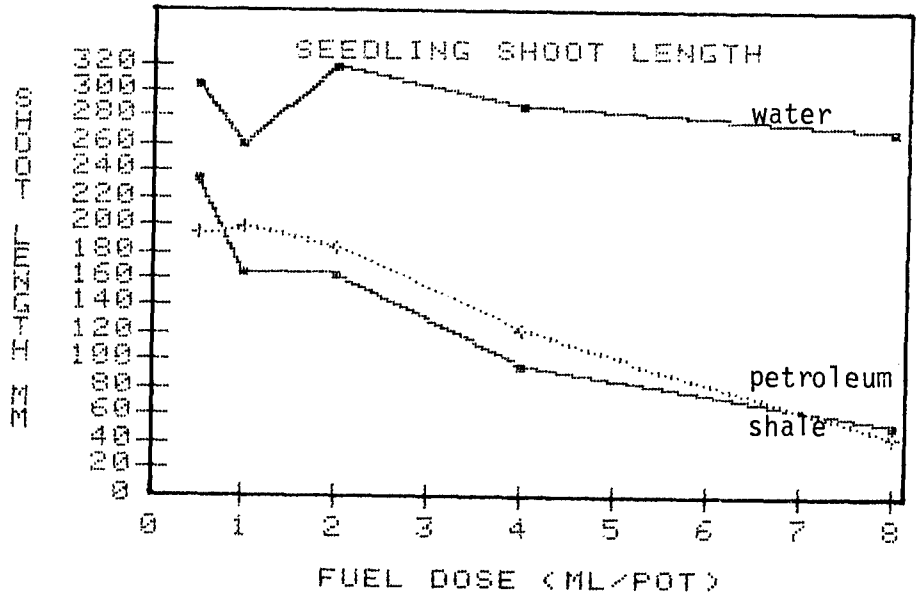


Figure 17. Shoot length of sorghum seedlings grown in soil contaminated with 0.5 to 8 ml JP4-P (petroleum), JP4-S (shale), or water per pot.

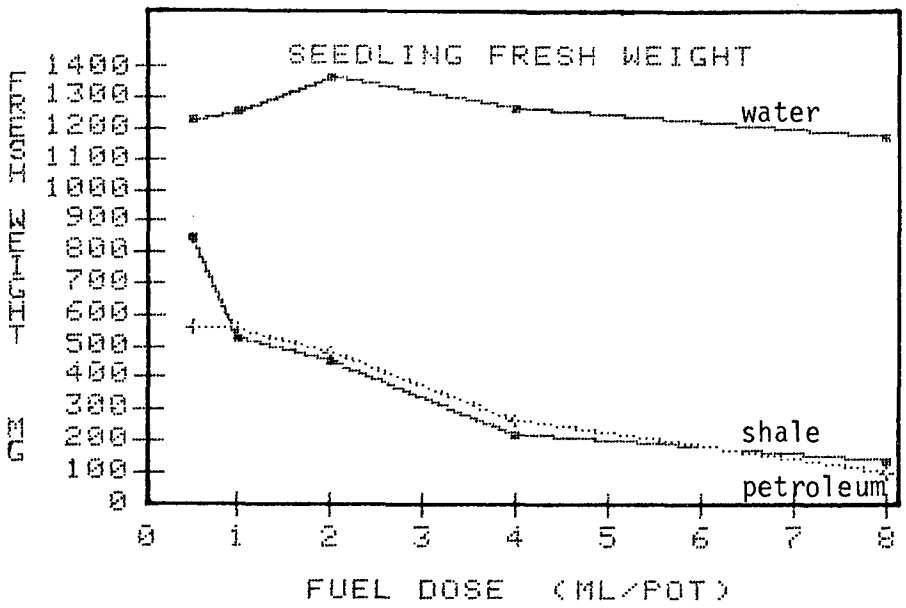


Figure 18. Fresh weight in mg of sorghum seedlings grown in soil contaminated with 0.5 to 8 ml, JP4-P (petroleum), JP4-S (shale), or water per pot.

were also prepared. Shale fuel (JP4-S) did not evaporate as rapidly as JP4-P (Table 82). In addition, JP4-S was significantly denser than JP4-P. The fuel gained density as it evaporated.

Sorghum seedlings were harvested two weeks after seeds were planted. Emergence rate was calculated on the number of seeds which germinated and emerged in each pot (Table 83). The data were analyzed using a one-way ANOVA, and mean differences were separated using multiple range tests. Three separate ANOVA were performed. The first analysis included all 21 treatments of both JP4-P and JP4-S; in the other two ANOVAs, the data for the two fuels were separated. The results of all analyses were similar. No differences were seen among the emergence means for any of the JP4-P treatments, but for JP4-S treatments, emergence rate was larger with 1 ml fuel applications than with 4 ml. Smallest emergence rate (12%) occurred when 5% of the fuel had been evaporated at 70°C. This rate was significantly smaller at $P < 0.05$ than for residues prepared at 25°C or when 0 or 50% evaporation residues were used.

Seedling length (Table 84) and weight (Table 85) were measured. Of the four factors tested, only amount of fuel applied (dose) was consistent in its effect on seedling growth. Four ml was always more inhibitory than 1 ml. Fuel type, evaporation residue or evaporation temperature had no consistent effect on seedling length or weight.

TABLE 82
EVAPORATION TIME AND SPECIFIC GRAVITY OF JET FUEL RESIDUES

Evaporation conditions (°C)	JP4-P Time (hr)	JP4-P Specific gravity (g/ml)	JP4-S Time (hr)	JP4-S Specific gravity (g/ml)
5% evaporated				
25°C	0.18	0.749 ± 0.005[1] d[2]	0.43	0.774 ± 0.002 bc
70°C	0.15	0.749 ± 0.003 d	0.23	0.770 ± 0.001 c
50% evaporated				
25°C	9.58	0.776 ± 0.004 b	39.50	0.799 ± 0.004 a
70°C	1.92	0.778 ± 0.007 c	5.08	0.779 ± 0.005 b

[1]Specific gravity of fuel, mean and standard deviation of four weighings of same residue

[2]Means followed by the same letter(s) are not significantly different at $P < 0.05$ by DNMR test

In summary, this experiment showed that a 4-ml fuel dose reduced sorghum seedling emergence and subsequent growth more than a 1-ml dose. One ml was not significantly ($P < 0.05$) more inhibitory than no fuel at all. Fuel type, evaporation residue, and evaporation temperature did not consistently effect emergence or seedling growth.

In another test comparing toxicities of JP4-P and JP4-S, JP4-P was evaporated to 50% original volume at four temperatures, 4°, 25°, 50°, and 70°C and JP4-S was evaporated at 25° and 50°C. At the same temperature, JP4-S evaporated three to four times more slowly than the JP4-P (Table 86). While JP4-S specific gravity was less than JP4-P before evaporation, no significant differences at $P < 0.05$ were noted after evaporation to 50% original volume (Table 86).

For the bioassay part of this test, 2 ml of each fuel fraction was mixed per pot of soil (ca. 0.5% fuel volume per soil weight) and 10 sorghum seeds were sown in each pot. Plants were harvested and measured after two weeks (Table 87). Emergence rates were significantly less for evaporated fuel compared to fuel that had not been evaporated to 50% of original volume or to no-fuel controls. In this study, JP4-S did not have as great an inhibitory effect on seedling emergence rates as JP4-P.

Considering seedling length (Table 87), either fuel formulation inhibited growth and the fuel residues were more inhibitory than unevaporated fuel. No significant differences existed at $P < 0.05$ among length of

TABLE 83
EMERGENCE OF SORGHUM SEEDLINGS IN SOIL CONTAMINATED WITH JET FUEL AND FUEL RESIDUES APPLIED AT TWO DOSE RATES

Evaporation residue	Fuel type			
	JP4-P dose		JP4-S dose	
	1 ml	4 ml	1 ml	4 ml
0% evaporated	70[1] ab[2] (ab)[3]	49 abcd (ab)	72 a [a][3]	21 ef [bc]
5% evaporated at				
25°C	64 abc (ab)	45 bcd (b)	72 ab [a]	39 de [b]
50°C	68 ab (ab)	46 abcd (ab)	69 ab [a]	12 f [c]
50% evaporated at				
25°C	72 a (a)	48 abcd (ab)	60 abcd [a]	36 de [b]
70°C	66 ab (ab)	48 abcd (ab)	68 ab [a]	41 cde [b]
No fuel control	66 ab (ab)		66 ab [a]	

[1]Emergence rate in percent of sorghum seedlings, mean and standard deviation of eight pots of 10 seeds each

[2]Means followed by same letter or letters in ( )'s or [ ]'s are not significantly different at $P < 0.05$ by SNK test

[3]( ) is for JP4-P analysis, [ ] is for JP4-S analysis

seedlings grown in any of the evaporated treatments or between the two kinds of fuel.

This study indicated that when 50% of the original fuel volume had evaporated, the residue was more toxic to developing seeds than the original fuel, that at 50% volume no difference in toxicity could be detected between different preparation temperatures, and that JP4-S and JP4-P were equally toxic.

In a final residue test, we explored the possibility that inconsistent results between JP4-P and JP4-S toxicity may have been caused by the fuel aging (and changing) during temporary storage in the laboratory. The main bulk of the fuel was stored in a shed in unopened one- or five-gallon shipping drums. One-gallon gasoline cans and glass-stoppered 1-liter flasks were used for storage of the fuel in the laboratory. The fuel may have aged, evaporated, or become otherwise altered before experimental use.

TABLE 84
TOTAL LENGTH OF SORGHUM SEEDLINGS GROWN IN SOIL CONTAMINATED WITH JET FUEL OR FUEL RESIDUES AT DIFFERENT DOSES

Evaporation residue	Fuel type: JP4-P dose (ml) 1 ml	JP4-P dose (ml) 4 ml	JP4-S dose (ml) 1 ml	JP4-S dose (ml) 4 ml
0% evaporated	389[1] bcd[2] (b)[3]	200 h (d)	352 defg [bcd][3]	116 ij [f]
5% evaporated at				
25°C	491 a (a)	208 h (d)	372 cd [b]	154 i [f]
70°C	430 b (b)	167 h (de)	360 cdef [bcd]	79 j [g]
% evaporated at				
25°C	290 bcd (b)	142 i (e)	314 eg [cde]	132 i [f]
70°C	315 efg (c)	127 ij (e)	363 cde [bc]	143 i [f]
No fuel control	412 bc (b)		412 bc [a]	

[1]Seedling total length in mm, mean and standard deviation of up to 58 seedlings per treatment

[2-3]Same as notes in Table 83

Samples of JP4-P and JP4-S were taken from previously unopened storage containers and from the laboratory cans or flasks. Shale fuel (JP4-S) appeared paler than the JP4-P. One set of the fuel samples was allowed to evaporate for 24 hours while another set was stirred until a measured 50% of the original volume evaporated. Amount of fuel evaporated after 24 hours or length of time to complete 50% evaporation was recorded and specific gravities of all residues were measured (Table 88). Before any evaporation, JP4-S had greater specific gravities than JP4-P. After evaporation, comparisons were difficult because amount evaporated and times to evaporate varied with material and storage source. JP4-S of either source took longer to evaporate to 50% original volume and evaporated less fuel in 24 hours than JP4-P under similar conditions.

All fuel treatments were mixed at the rate of 4 ml per pot of glasshouse soil, and each pot was sown with 10 sorghum seeds. After two weeks, the seedlings were harvested and measured (Tables 89, 90, 91). In all measurements made, the no-fuel controls were significantly greater in germination, length, and weight from any of the plants growing in fuel-treated soil. One analysis tested treatment means with the no-fuel control data excluded. The results (part of Tables 90 and 91) indicated little difference from the original conclusion: emergence for all treatment means were statistically identical at $P < 0.05$. Total length and fresh weight

TABLE 85
FRESH WEIGHT OF SORGHUM SEEDLINGS GROWN IN SOIL CONTAMINATED WITH JET FUEL OR FUEL RESIDUE AT DIFFERENT DOSES

Evaporative residue	Fuel type: JP4-P dose 1 ml			JP4-P dose 4 ml			JP4-S dose 1 ml			JP4-S dose 4 ml		
0% evaporation	1156[1]	b[2]	(b)[3]	395	fg	(d)	732	e	[c][3]	145	h	[d]
5% evaporation at												
25°C	1382	a	(a)	410	f	(d)	859	de	[bc]	214	fgh	[d]
70°C	1000	bcd	(bc)	293	fgh	(de)	859	de	[bc]	89	h	[d]
50% evaporation at												
25°C	865	de	(c)	171	gh	(e)	913	cde	[abc]	200	fgh	[d]
70°C	813	de	(c)	176	gh	(e)	987	bcde	[ab]	206	fgh	[d]
No fuel control	1093 bc (b)						1093 bc [a]					

[1]Fresh weight of sorghum seedlings in mg, mean and standard deviation of up to 58 seedlings per treatment

[2-3]Same as notes in Table 83

TABLE 86
TIME TO EVAPORATE JP4 FUEL AND SPECIFIC GRAVITY OF RESULTING SOLUTION

Evaporation temperature[1] (°C)	JP4-P Time[2] (hours)	JP4-P Specific gravity[3] (mg/ml)	JP4-S Time (hours)	JP4-S Specific gravity (mg/ml)
No evaporation	0	750	0	763
4	21.92	776	--[4]	--
25	8.21	789	23.08	784
50	1.63	789	4.33	779
70	1.13	793	--	--

[1]Temperature maintained during evaporation

[2]Time to evaporate fuel to 50% original volume

[3]Specific gravity of fuel in mg/ml, mean of two weight measurements

[4]-- Indicates no preparation made

TABLE 87
GROWTH OF SORGHUM SEEDS IN SOIL CONTAMINATED WITH 2 ml OF EVAPORATED RESIDUES OF JP4-P OR JP4-S FUEL

Evaporation temperature[1] (°C)	Emergence (%)		Length (mm) Total		Length (mm) Shoot	
	JP4-P	JP4-S	JP4-P	JP4-S	JP4-P	JP4-S
No evaporation	35	44[3]	130	141	47	52
4	20	--[4]	95	--	27	--
25	15	33	75	106	18	26
50	20	30	94	112	22	36
70	16	--	104	--	36	--
No Fuel[2]	49		315		162	

[1]Fuel was evaporated to 50% original volume at different temperatures

[2]One no-fuel control group was prepared

[3]Mean percent-emergence of seedlings, where shoot growth exceeded 10 mm

[4]-- indicates no treatment prepared

TABLE 88
EVAPORATION AND SPECIFIC GRAVITY OF JP4-P AND JP4-S FUELS FROM DIFFERENT SOURCES

Source	JP4-P Evaporation % lost[1]	JP4-P Evaporation Time[2] (hours)	JP4-P Specific gravity (mg/ml)	JP4-S Evaporation % lost	JP4-S Evaporation Time (hours)	JP4-S Specific gravity (mg/ml)
Storage shed	0	0	744 ± 11[3] f[4]	0	0	779 ± 7 cde
	50	14.42	784 ± 4 bc	50	40.08	783 ± 3 bc
	60	24	794 ± 6 a	37	24	788 ± 1 ab
Laboratory	0	0	749 ± 4 f	0	0	774 ± 4 e
	50	14.75	779 ± 6 cde	50	33.92	782 ± 6 bcd
	48	24	775 ± 4 de	36	24	783 ± 5 bc

[1]Percent volume of fuel evaporated

[2]Length of evaporation time in hours

[3]Specific gravity of fuel in mg/ml, mean and standard deviation for four measurements

[4]Means followed by the same letter(s) are not significantly different at $P < 0.05$ by DNMR test

TABLE 89
EMERGENCE RATE OF SORGHUM SEEDLINGS GROWN IN SOIL CONTAMINATED WITH JET FUEL STORED IN DIFFERENT PLACES AND EVAPORATED

Storage source	Evaporation residue	JP4-P	JP4-S
Storage shed	50%[1]	30 ± 8[3] b[4]	20 ± 8 b
	24 hr[2]	12 ± 10 b	38 ± 10 b
Laboratory	50%	20 ± 16 b	20 ± 18 b
	24 hr	38 ± 15 b	22 ± 10 b
No fuel control		68 ± 15 a	

[1]Fuel evaporated to 50% original volume by stirring at room temperature

[2]Fuel evaporated for 24 hours by stirring; see Table 88 for concentrations

[3]Emergence rate in percent, mean and standard deviation of four pots of 10 seeds each

[4]Means followed by same letter are not significantly different at $P < 0.05$ by SNK test

indicated that the largest seedlings were those grown in soil contaminated with shed storage can fuel evaporated to 50% volume.

This study showed that source and storage of jet fuel did not seem to affect the fuel's phytotoxic properties. This was true even though the fuel fractions had different specific gravities and evaporation rates.

Aeration of Soil

Background rationale-

Farmers whose land has been impacted with jet fuel spills are recommended to disc or plow their fields (Allen, 1981). These experiments investigated the concepts underlying such suggestions by using small samples of contaminated soil.

Drying and aeration of contaminated soil -

A study was conducted to determine whether drying or aerating fuel-contaminated soil affected seedling growth in the treated soil. JP4-P fuel was mixed into 420 g soil at 0 or 4 ml per pot (0 or 1% v/w). The soil mixture was spread about 7 mm deep on fiberglass trays and aerated for 48 hours in one of several ways (Table 92). Treated soil was potted and sown with 10 sorghum seeds.

TABLE 90
TOTAL LENGTH OF SORGHUM SEEDLINGS GROWN IN SOIL CONTAMINATED WITH JET FUEL STORED IN DIFFERENT PLACES AND EVAPORATED

Storage source	Evaporation residue	JP4-P	JP4-S
Storage shed	50%[1]	256 ± 113[3] b[4] (b)[5]	191 ± 79 bc (ab)
	24 hr[2]	139 ± 75 c (b)	242 ± 81 bc (ab)
Laboratory	50%	225 ± 82 bc (ab)	146 ± 81 c (b)
	24 hr	176 ± 87 bc (ab)	243 ± 85 bc (ab)
No fuel control		554 ± 108 a	

[1,2]Same as notes for Table 89

[3]Total length of sorghum seedlings, mean and standard deviation of up to 27 seedlings

[4]Means followed by the same letter(s) are not significantly different at $P < 0.05$ by SNK test; all means included

[5]Letters in ( ) indicate range test analysis as above but no-fuel control not included

TABLE 91
FRESH WEIGHT OF SORGHUM SEEDLINGS GROWN IN SOIL CONTAMINATED WITH JET FUEL STORED IN DIFFERENT PLACES AND EVAPORATED

Storage source	Evaporation residue	JP4-P	JP4-S
Storage shed	50%[1]	504 ± 390[3] b[4] (a)[5]	247 ± 141 b (b)
	24 hr[2]	140 ± 85 b (b)	296 ± 249 b (b)
Laboratory	50%	327 ± 204 b (ab)	143 ± 137 b (b)
	24 hr	253 ± 171 b (b)	290 ± 145 b (b)
No fuel control		2019 ± 911 a	

[1-5]Same notes as Table 90 except means are seedling fresh weight in mg

TABLE 92
CONDITIONS FOR AERATING AND DRYING FUEL-CONTAMINATED SOIL

Treatment	Conditions
Steam sterilizer	Treated with live steam at low pressure, 87°C
Drying oven	Laboratory drying oven set at 70 ± 5°C
Cold room	10°C for 30 hours (then compressor broke and temperature rose to 25°C)
Shaded glasshouse	In shade of cardboard box in glasshouse
Sunny glasshouse	Unshaded in glasshouse
Outside sun	Outside in open sun, but protected from wind
Control	Soil not dried prior to sowing seeds

Emergence, length, and fresh weight of seedlings were measured two weeks after sowing (Tables 93, 94, and 95). Only the control treatment, in which soil was mixed with fuel but not dried or heated, did differences in seedling emergence rates arise (Table 93). This suggested that soil aeration treatments negated any effect fuel had on emergence.

Seedlings growing in fuel-contaminated control soil also had shortest shoot lengths (Table 94). Seedlings from fuel-contaminated soil which was steam- or shade-treated had significantly shorter (at $P < 0.05$) shoot lengths than seedlings grown in soil with no fuel. Other treatments seemed to diminish the inhibitory effect of the fuel, and seedlings in those soils had the same shoot length as no-fuel controls. With total length, only seedlings growing in fuel-treated control soil had significantly shorter seedlings (at $P < 0.05$) than the seedlings grown in other soils.

Fresh weight means appeared to verify the conclusions reached with shoot lengths (Table 95). Lightest fresh weights were for control seedlings grown in untreated fuel-contaminated soil. The oven and shade treatments of fuel-contaminated soil yielded significantly lighter seedlings (at $P < 0.05$) than the no-fuel controls. Excess water collecting in some of the steam-sterilized soil probably contributed to generally poor growth and lighter seedlings with this treatment, and weights appeared further reduced when fuel was added.

TABLE 93
EMERGENCE OF SORGHUM SEEDLINGS AFTER 14 DAYS GROWTH IN VARIOUSLY-TREATED FUEL-CONTAMINATED SOILS

Treatment[1]	Fuel	No fuel
Steam	65 ± 17[2] a[3]	65 ± 21 a
Oven	75 ± 19 a	80 ± 8 a
Cold	75 ± 6 a	72 ± 10 a
Shade	75 ± 6 a	75 ± 13 a
Sun	68 ± 5 a	78 ± 15 a
Outside	72 ± 12 a	80 ± 14 a
Control	18 ± 10 b	62 ± 5 a

[1]Forty-eight-hour treatment of soil occurred after contamination with fuel but before sowing with seeds

[2]Percent emergence of sorghum seedlings with shoot length > 10 mm, mean and standard deviation of 40 seeds

[3]Means (considering all 14) followed by the same letter(s) are not significantly different at $p < 0.05$ by SNK test

Aeration of contaminated soil over time -

Aeration of fuel-contaminated soil was further studied by spreading soil contaminated with ca. 1% JP4-P fuel on trays. The trays were left in the glasshouse for 0, 1, 2, or 3 weeks so soil would dry. Trays were prepared on successive weeks to complete drying treatments on the same day. Pots were then filled with treated soil and sown with sorghum seeds. Harvest took place two weeks later.

Seedlings were measured and results (Table 96) indicated satisfactory recovery of soil treated in this manner. Only soil which was sown immediately after fuel treatment grew seedlings which significantly differed (at $P < 0.05$) from controls in emergence, length, and weight. Some evidence suggested partial enhancement of growth after three weeks aeration of the fuel-containing soil. If real, this may be due to breakdown of the fuel to components used by the growing plant.

The success of drying and aerating contaminated soil to reduce fuel toxicity lends credence to the recommendation to disc or plow fields to hasten recovery from spills.

TABLE 94
LENGTH OF SORGHUM SEEDLINGS AFTER 14 DAYS GROWTH IN VARIOUSLY-TREATED FUEL-CONTAMINATED SOILS

Treatment	Shoot length (mm)		Total length (mm)	
	Fuel	No fuel	Fuel	No fuel
Steam	102 ± 28[1] d[2] *[3]	174 ± 59 b	255 ± 62 d	301 ± 106 b
Oven	185 ± 43 c	220 ± 56 ab	326 ± 73 c	385 ± 93 a
Cold	200 ± 47 ac	220 ± 66 ab	341 ± 69 ac	367 ± 108 a
Shade	180 ± 39 c *	248 ± 63 a	335 ± 72 ac	403 ± 102 a
Sun	226 ± 40 ab	225 ± 73 a	385 ± 72 a	387 ± 117 a
Outside	227 ± 56 a	220 ± 57 ab	380 ± 89 ab	377 ± 98 a
Control	73 ± 44 d *	215 ± 64 ab	151 ± 78 e *	355 ± 101 ab

[1]Shoot or total length of seedlings, mean and standard deviation of up to 40 seedlings

[2]Means in each column followed by the same letter(s) are not significantly different at $P < 0.05$ by SNK test

[3]Fuel and no-fuel means with a '*' between are significantly different at $P < 0.05$ by SNK test

TABLE 95
WEIGHT OF SORGHUM SEEDLINGS AFTER 14 DAYS GROWTH IN VARIOUSLY-TREATED FUEL-CONTAMINATED SOILS

Treatment	Fuel		No fuel
Steam	211 ± 84[1] c[2]	*[3]	421 ± 207 b
Oven	428 ± 226 b	*	644 ± 300 a
Cold	467 ± 200 b		654 ± 356 a
Shade	435 ± 134 b	*	708 ± 286 a
Sun	583 ± 164 a		634 ± 298 a
Outside	676 ± 301 a		780 ± 339 a
Control	156 ± 145 c	*	778 ± 391 a

[1-3]Same notes as for Table 94 but for fresh weight in mg

TABLE 96
GROWTH OF SORGHUM SEEDLINGS IN FUEL-CONTAMINATED SOIL
DRIED AND AERATED FOR ONE TO THREE WEEKS

Aeration[1] (weeks)	Emergence (%)		Lengths (mm)		Weights (mg)	
	Fuel	No fuel	Fuel	No fuel	Fuel	No fuel
0	30[1] b[3]	65 a	150[4] b[3]	499 a	80[4] c[3]	960 ab
1	78 a	80 a	440 a	483 a	776 b	865 ab
2	58 a	78 a	479 a	464 a	1041 ab	852 ab
3	75 a	77 a	526 a	463 a	1145 b	958 ab

[1]For aeration treatment, soil was spread on trays and left exposed on glasshouse benches for up to three weeks

[2]Percent emergence for seedlings which were longer than 10 mm, mean of 60 seeds

[3]Means in each variable group followed by the same letter(s) were not significantly different at $P < 0.05$ by SNK test

[4]Total length or fresh weight of seedlings (root plus shoot), mean of up to 48 seedlings

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