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ACID RAIN RESEARCH PROGRAM
ANNUAL PROGRESS REPORT

SEPTEMBER 1975 THROUGH JUNE 1976

September 1976

ENVIRONMENTAL PROGRAMS
DEPARTMENT OF APPLIED SCIENCE

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SEPTEMBER 1975 THROUGH JUNE 1976

LANCE S. EVANS* AND GILBERT S. RAYNOR

September 1976

*Laboratory of Plant Morphogenesis, Manhattan College, Bronx, N.Y. and Consultant, Department of Applied Science, Brookhaven National Laboratory.

ENVIRONMENTAL PROGRAMS
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Abstract

The aims of the research program are: (a) to observe the minimum threshold dose of simulated acid rain to produce visual and histological effects on plant foliage, (b) approach threshold limits of simulated sulfate acid rain that affect plant growth and reproduction, and (c) to measure chemical and meteorological parameters of incident rain. Acute leaf injury to several plant species resulted from exposure of foliage to simulated sulfate acid rain of pH level 2.3 to 2.9. Only slight injury occurred at 3.1. Scanning electron micrographs showed that injury to upper leaf surfaces occurred mostly at the base of trichomes (leaf hairs) and near stomata. An association of lesion development near vascular tissue was also noted. Histologically, lesions are characterized by an initial collapse of the epidermis with eventual lysis and collapse of more internal leaf tissues on the upper leaf surface of pinto beans which complemented detailed descriptions of visual lesion development after daily exposures to simulated rain.

Initial experiments with gametophytes of *Pteridium aquilinum* show that reproduction of this fern species is very sensitive to solutions of pH 5.2 while vegetative development is not affected at pH levels of 2.2.

Initial rain samples from the sequential sampler have been obtained. Initial portions of rain events exhibit a pH near 3.0 in some cases. More complete chemical analyses are anticipated.

I. Background

The direct effects of air pollutants on plant foliage have been observed for over several decades. Attention has been focused upon (a) the minimum threshold dose to produce acute leaf injury, (b) visual and histological alterations caused by acute foliar injury, and (c) threshold levels necessary to produce reductions in plant growth and productivity. In addition, researchers have attempted to estimate injury in many plant species in order to view relative species sensitivity. Such data may be helpful in predicting sensitivity at other dose rates and may be helpful in predicting changes in species sensitivity under field conditions. In a similar manner, studies that describe visual and histological injuries after exposure to air pollutants may indicate the location of primary effects in leaves. Detailed histological studies of leaf tissue injury coupled with visual and physiological parameters may be helpful in predicting with some reliability (1) the pollutant present, and (2) the possible degree of injury which will eventually develop by long exposure. In research with low levels of ozone, investigators have shown that very low levels given over a long time period (as little as 12 hrs) results in a reduction of the leaf expansion rate and a reduction in crop productivity. In these experiments, the researcher varies (a) acid solution concentration, (b) duration of exposure, and (c) number of exposures.

The recent increased use of fossil fuels since 1950 as sources of energy, coupled with current pollution control techniques (which include increased height of smoke stacks and greater use of particulate precipitators) have given rise to

increased concentrations of SO_2 in the atmosphere in widespread areas of New England and Long Island (Likens and Bormann, 1974). It has been shown that this increased SO_2 in the atmosphere may cause plant injury by direct absorption of the gas by plant leaves as SO_2 on particulate matter or dissolved in rain water (acid rain).

Much research has been conducted into the biological effects of gaseous SO_2 on plants, but much less is known about injury that may be caused by SO_2 deposition on foliage in particulate matter or in precipitation. In addition, the direct effects of lowered pH of precipitation with high SO_4 levels may augment the detrimental effects to plants.

Some investigations of acid precipitation have taken place which point to the various effects possible. Necrosis of leaf tissue on yellow birch exposed to aqueous H_2SO_4 at pH 3.0 was shown to occur by Wood and Bormann (in press), while HSO_3^- and H_2SO_4 may cause toxic effects. In addition, Gordon (1972) has shown that emerging needles of several western pines showed limited elongation when sprayed with solutions of H_2SO_4 at pH levels below 4.0.

Reduced growth rate, partial disruption of the leaf cuticle, unavailability of elements to plant roots as the result of soil pH changes, and nutrient leaching of leaves that may occur when plants are exposed to acid rain can result in increased susceptibility of plant leaves to pathogens, herbivores, plant competition, and other environmental stresses such as air pollutants. The injury of pine trees by oxidants was augmented by the infestation of initially injured trees by bark beetles (Miller, et al., 1963). Such indirect effects on plant growth and survival in an ecosystem must always be considered in any investigation.

II. Specific Aims

The specific aims of the research during the first several years are outlined under the objectives section and are discussed in detail in a following section. They are:

A. Establish the minimum threshold dose necessary to produce acute leaf injury from (1) acid rain alone, and (2) acid aerosol and acid rain in combination.

B. Establish the visual and histological effects of both (1) acid rain alone, and (2) acid aerosol and acid rain in combination.

C. Establish threshold levels of acid rain to produce reductions in leaf growth rate and/or crop productivity.

D. Describe the morphological and anatomical changes that occur in plant leaf tissues brought about by frequent exposure to low dose rates of acid rain.

E. Set threshold limits of sulfate acid rain these plants species can withstand so (1) no visual or anatomical malfunctions occur, and (2) no growth inhibition and/or loss of crop productivity occurs.

F. Follow the rates of accumulation of sulfur and nitrate ions in plant leaf tissue after exposure of acid aerosol and acid rain. Note how the rates of accumulation relate to leaf injury.

G. Monitor the rain events of Long Island and observe changes in rain chemistry among rain events and among various times during single events.

During the first year, our efforts were aimed at constructing necessary equipment and starting exploratory experiments. This report is divided into four parts: (A) Instrumentation, (B) Procedures, (C) Present Results, (D) Anticipated Experiments for Next Year.

III. Instrumentation

A. Greenhouse Facility

The greenhouse facility (625 ft^2) is used to house propagated plant materials prior to their exposure to acidic conditions. The construction is of the normal glass type with summer shade provided by netting. A 15-ton capacity air conditioner is added to the greenhouse to cool the atmosphere during warm summer days. The air conditioner is necessary because the ventilators of the greenhouse will be closed to avoid contamination from ambient atmosphere. In this manner plants are protected continually from ambient levels of gaseous and particulate air pollutants. Filters are present to remove traces of oxidants, particulates, and sulfur dioxide.

B. Controlled Environment Growth Chamber

A large controlled environment chamber is used to house plants between exposures to rain treatments. This facility is approximately 450 ft^2. Such environmental parameters as temperature, light intensity, relative humidity, and air quality are controlled. Air quality (protection against introduction of gases, particulate and rain) are in force continually. Very low levels of ozone (0.03 ppm) and sulfur dioxide (0.01 ppm) were recorded before introduction of filters. Since introduction of filters, no ozone or sulfur dioxide has been detected.

C. Rain Chamber

A facility was constructed to administer rain solutions in a random pattern over all the experimental plants under study. Several nozzles have been tested for this application and several more will be included. The criteria for evaluation are (1) distribution patterns, (2) necessary flow-rates to simulate natural rates, and (3) average droplet diameter to simulate natural rainfall (see below).

A simulated rainfall solution containing most of the known chemical components has been designed (Table 1), with information from several surveys in the Northeastern United States, with particular emphasis on Long Island rainfall data. Supplemental acids are added to the simulated rain solution. Acid solutions with high concentrations of SO_4, NO_3, and Cl will be used to test the effect of these anions on the system.

D. Small Greenhouse and Rain Chamber Facility

On July 16, 1976, another greenhouse (160 ft^2) with a rain chamber facility will be converted for use in experiments to view the botanical effects of acid rain. This facility (although small) represents enough area to conduct one or two experiments at temperature and conditions that more closely resemble the environs compared with conditions in the controlled environment growth chamber. Two factors (high temperatures and high humidity) present in the greenhouse facility, which are not available in the growth chamber, are necessary for growth of plants to measure growth, yield, and reproductive capacity over a summer growing season.

E. Aerosol Administration Facility

An aerosol-administration facility has been constructed that meets many plant physiological and meteorological requirements. This facility will be used to administer aerosols of several different chemical compositions and of various sizes to plants. Experiments are underway with $(NH_4)_2SO_4$ with a mean particle diameter of 0.6 μm. Eventually aerosols of H_2SO_4 and possibly NH_4HSO_4 (ammonium bisulfate) will be administered.

Exposures to aerosols will be augmented by exposure to rain solutions. Experiments by other investigators suggest that (1) rain or (2) very high leaf moisture conditions are necessary for plant response to aerosols.

After an understanding of the administration dynamics has been established, experiments with plants will begin. Presently, modifications are being made to more accurately mesh both plant physiological and meteorological considerations.

Preliminary experiments for administration of $(NH_4)_2SO_4$ have begun and all components of the facility are operating efficiently. With $(NH_4)_2SO_4$ our injection schedule attempts to maintain relatively constant atmospheric concentrations at levels such as 10 and 100 $\mu g/m^3$. Appendix C illustrates a typical curve. A balance between the gradual decline in concentration with time after injection, the time between injections, and the duration of each injection must be established. After such relationships are achieved initial primary interest will be focused on (1) the rate of aerosol deposition on plant leaves versus aerosol concentration, (2) rate of aerosol

accumulation within leaves versus concentration in atmosphere, (3) leaf injuries after prolonged exposure coupled with rain episodes, (4) response of plants to aerosols at various levels of relative humidity.

Presently, modifications have been made to place plants on a large turntable instead of carts. Modifications are planned to administer rain as well as gaseous SO_2 to plants in addition to aerosols of various compositions.

F. Sequential Precipitation Collector

Design and construction of an automatic, sequential precipitation collector was completed during the period. Several basic considerations determined the design chosen and the dimensions necessary. Precipitation rates range from 0.01 inch per hour to an extreme of over 2 inches per hour, but over 90% of all precipitation rates at BNL are under 0.2 inch per hour. The chemists determined that at least 50 ml should be collected for each sample. In order to get 50 ml in an hour from a rain of 0.02 inch per hour, a funnel about 16 inches in diameter is necessary. A 2-quart collection bottle was chosen so that the catch would exceed this amount in only a few percent of all precipitation hours.

About 100 precipitation events and about 1000 hours of precipitation occur on the average each year. The chemists decided that 1000 samples per year could be analyzed for the constituents of interest. A few rains last for several days so for convenience in changing bottles during normal working hours as often as possible, an array of 30 sampling bottles was selected.

The sampler consists of a box containing a turntable holding the 30 sample bottles. The turntable is stepped periodically by a machine tool indexer to bring each bottle under the funnel in turn, starting when precipitation starts. The funnel is covered during dry weather. A precipitation sensor actuates a mechanism to open the cover and start the timing control when precipitation begins. The times of cover opening and closing and sample changing are recorded on an operations recorder in the Meteorology building.

On the normal timing cycle, the turntable is stepped each hour from the time precipitation starts. When a bottle becomes filled during a heavy rain a photo-cell liquid level sensor causes a step to the next bottle. For sampling short, heavy rainfalls such as thundershowers, an alternate five-minute cycle is selected manually. For changing bottles and servicing the unit, a switch is included for stepping the turntable at will.

The rain collection funnel is fabricated of stainless steel with an offset spout requiring minimum space. The funnel is wrapped with thermostatically controlled heating tape actuated at 35°F to melt snow and sleet during cold weather. The rain sensor includes a heating unit to dry the sensor quickly when rain stops.

Three sets of bottles are available so previously used sets may be cleaned conveniently during periods of continuous or frequent rainfall. After each rain or bottle set change, the precipitation collected is stored in smaller containers at 4°C until analysis. The collection funnel is cleaned after each period of precipitation. Samples are analyzed by the Atmospheric

Chemistry Group for pH, acidity, specific conductance, ammonia, nitrate, chloride, sulfate and sodium. Specific conductance and pH are measured as soon as possible after collection. Chemical components are analyzed by a four-channel auto analyzer system.

Use of this sampler does not account for dry fallout between rain events. This system enables a detailed study of changes in chemical constituents during each rainfall in detail proportional to the intensity and duration of the event.

Results of the chemical analyses are used in several ways. They give guidance in the preparation of artificial acid rainfall which will be applied in a range of acid concentrations to selected plant species under controlled conditions over a range of rainfall rates and durations.

Results will also be used to verify a sophisticated meteorological transport model currently in use by the Meteorology Group. The model uses a comprehensive source inventory over much of North America and weather observations over the same region to predict concentrations of selected chemical species during successive time periods. It can also predict both dry fallout and washout.

Results are also expected to provide information on the relative importance of washout and rainout in removing material from the atmosphere. Material removed by washout is expected to predominate during the early stages of a rain while gases and particles incorporated into the drops during the drop formation stage should be more uniform throughout the rain.

G. Plant Species Use--Criteria for Use

The experimental species will be chosen with the following criteria in mind.

1. Uniform genetic background to minimize variation during experimentation. This aspect has been cited by Taylor (1974) as a limiting factor in many air pollution studies.

2. Inclusion of forest species which are indigenous to the forests of northeastern United States.

3. Species used previously in air pollution studies for the purpose of comparison of the injury response with respect to other air pollutants.

4. Ease of propagation; amenability to laboratory investigations.

5. Leaf surface characteristics.

6. Ease of study of sensitive reproductive stages.

The following six plant species have been selected:

1. Pinto bean (*Phaseolus vulgaris*)--Seed is obtained with known genetic background from a commercial seed source. Although it is not a forest species it has been used extensively in air pollution studies and is easy to manipulate in the laboratory.

2. Eastern White Pine (*Pinus strobus*)--Clonal plant material is obtained through the United States Forest Service. Because we can obtain clonal plant material, variation between the response of plants is diminished. Eastern white pine is abundant in the northeast and has been used in numerous studies involving air pollutants.

3. Pin Oak (Quercus palustris)--A clone of sovereign pin oak is commercially available and can be propagated in the greenhouse. Although Pin Oak has not been used in air pollution studies, it represents another forest species to investigate the relative response to acid rain.

4. Cottonwood (Populus deltoides hybrids)--This hybrid of poplar can be propagated vegetatively from stem cuttings. Although it has not been used extensively in air pollution studies (only some work by investigators at Delaware, Ohio), it is a broad leaf forest species which can be used to study leaf growth rates at low doses of acid rain.

5. Tradescantia (Tradescantia paludosa)--Plants were selected because of many advantages. Early results indicated that lesions resultant from acid rain exposure, were preferentially localized near trichomes (leaf hairs) and stomata. We were fortunately able to select clones of individuals from an F2 segregation of glabrous and pubescent parents. The four selected clones were evenly divided: two pubescent and two glabrous clones.

6. Pteridium aquilinum--Experiments are focused at viewing the sexual and vegetative stages of fern reproduction. Details are given below. This system allows the researcher to separate the effects of acid rain on the sexual and vegetative stages of fern development independently.

IV. Procedures

A. Plant Experiments

Plants are propagated in facilities listed above by staff of the Biology Department to fit experimental specifications. A standard potting soil mix is used. Seeds or cloned plant

materials are placed in the soil mix and allowed to progress. Standard overhead mists are provided when necessary. Plants are transported from one facility to another in closed vans to minimize atmospheric exposures to gases, particulates, and rain.

Initial propagation usually occurs in the greenhouse and plants are transported only once to the other facilities for experimental treatments. Transport of plants occurs several days prior to experimental treatments to insure acclimatization of plants to any differences in environmental conditions.

Plants are fertilized through irrigation water as required. Plants that grow rapidly require weekly fertilization while pines generally need fertilization once or twice annually.

Plants selected for experiments are fitted with a plastic hood around the base of each plant stem to cover the soil. In this manner, simulated rain solutions never enter the soil. During experiments, plants are irrigated under the cones so test solutions are not washed from leaves. Irrigation water is never applied to foliage at any age.

Experimental plants are sprayed in a fully enclosed chamber (height = 10 ft., width = 4 ft., and length = 5 ft.) with dim light provided. Plants are placed along the circumference of a three foot diameter turntable which revolves at 2.5 rpm. Plants are exposed to a spray mist from a set of nozzles three feet above the turntable.

B. Histology

Samples were fixed in cold acrolein for 4 hr. After fixation samples were dehydrated through a series of cold alcohol solutions (Feder and O'Brien, 1968). Samples were

These two drop sizes simulate rain and fog conditions simultaneously. In general, plants are exposed to 0.78 mm/hr (0.28 in/hr) for six minutes only daily. Under the conditions of our experiments, drop velocities are about twice that found in nature.

B. Histology

Samples were fixed in cold acrolein for 4 hr. After fixation samples were dehydrated through a series of cold alcohol solutions (Feder and O'Brien, 1968). Samples were placed in each alcohol for at least 4 hrs. After complete tissue dehydration, the last normal butanol solution was brought to room temperature. After several hrs., normal butanol was decanted, molten paraplast was added and the tissues were transferred to 62°C to facilitate infiltration. After several changes of paraplast, tissues were embedded and sectioned at 20-30 μm. Sections were stained according to Evans and Miller (1975).

C. Scanning Electron Microscopy (SEM)

Leaf samples for scanning electron microscopy were fixed in acrolein and dehydrated in ethanol. Samples were placed in a critical point apparatus cooled to 4°C. Subsequent desiccation occurred after exposure to CO_2 liquid at the critical point temperature of 31°C. The temperature was later increased to 40°C at a pressure of 1700 psi. The pressure was released eventually at the rate of 100 psi/min. and the sample was removed. Each dried leaf was mounted on two-sided tape on a SEM plug. A layer of carbon or silver was applied in a vacuum evaporator with a rotating and precessing stage. An operation voltage of 5 kv or 10 kv in the SEM was used to avoid excessive electron penetration of thin edges and hairs. Electron angles of 15 to 45 degrees were used to improve resolution of certain leaf surfaces.

D. Methods with *Pteridium aquilinum*

Under aseptic conditions, spores of *Pteridium aquilinum* were washed with fresh chlorox and aerosol OT and placed on either (1) Moore's medium with agar or (2) sterile vermiculite with Moore's medium added in liquid form (Moore, 1903).

Cultures were placed under lighted conditions at 20°C throughout the entire growth period. Table 1 gives a list of developmental events of organisms under these culture conditions.

E. Sperm Viability Experiments

To view the duration of sperm viability of *Pteridium aquilinum* at various pH levels. Spores were inoculated on Moore's medium with agar and allowed to grow for about 21 days. Sperm were obtained from prothalli in the following manner. Prothalli were removed from culture flasks and were placed in a small petri dish. Specific amounts of water were added to the prothalli so that antheridia would release sperm into the water. The maximum degree of release occurred after 12 minutes.

After 12 minutes, citrate-phosphate stock buffer (Jensen, 1962) solution was added to the specific amounts of water to obtain a specific buffered pH level in the dish. After the buffer solution was mixed, 2 to 3 drops of the sperm suspension were placed in the center of a ring of methyl cellulose on a microscope slide and covered with a cover slip.

Each suspension was viewed under Phase Contrast optics for 2 minute intervals from 2-4, 5-7, 9-11, and 13-15 minutes after addition of the buffer stock solution. All sperm observed in each 2 minute interval were scored as either alive or dead dependent upon flagellar movements. The slide was moved frequently to avoid scoring the same sperm twice in any 2 minute time interval.

F. Sporophyte Counts

To view the degree of fertilization at a single pH level, spores were inoculated into sterile vermiculite with Moore's medium added in liquid form. Petri dishes were reinoculated

6 days later with a second batch of spores. When plants from the first inoculation had been cultured for 28 days, citrate-phosphate buffers at pH levels of 2.2, 3.2, 4.2, 5.2, and 5.8 were added to the vermiculite medium for 3.5 hours only. Control cultures had no buffers added. After 3.5 hours the buffer was removed and a buffer of the original pH of 28 day cultures was added back to the dishes. After several minutes, this second restoration buffer was also removed. Dishes were then returned to the controlled environment room for an additional 14 days. Almost all fertilization was restricted to the 3.5 hour exposure to the buffer solutions. The 14 day period after the buffer treatments was necessary for development of the sporophyte plants that resulted from fertilization.

V. Results

Initial experiments were focused on the response for plants of *Phaseolus vulgaris* and *Helianthus annuus* of different ages to sulfate acid rain. The results indicate that very slight plant age effects were present in *Phaseolus vulgaris* when visual injury effects were viewed (Table 3). Only a slight effect was evident at less acidic solutions. The results illustrate that the lower the pH of the simulated rain solution, the greater the percent leaf area injured at any time period after the start of the experiment. In addition, an increase in the number of rain events usually increases the percent leaf area injured at pH levels below 2.9.

In plants exposed to pH 2.7 an increase of percent leaf area injured was noted after several days of repeated rain exposure. Older plants exhibited a greater degree of injury compared with younger plants. Presently, further experiments are focused at viewing leaf age--lesion sensitivity interactions in more detail in P. vulgaris.

In H. annuus as in P. vulgaris, as the pH decreased, the the area injured increased, and as the number of events increased, the degree of injury also increased. However, unlike plants of P. vulgaris, plants of H. annuus exhibited a strong interaction between plant age and percent leaf area injured. At pH levels of 2.3 to 3.1, plants at the 3-4 leaf stage were injured more compared with plants of leaf stages 5-6 and 7-8. During floral initiation, plants were almost insensitive to the acid rain compositions used.

In older plants of H. annuus, chlorosis occurred. Chlorosis occurred in plants exposed to rain at pH 5.7 as well as plants exposed to simulated rain at lower pH levels. Chlorosis was accelerated by exposure to low pH acid rain. In addition, necrosis of leaf tips of older plants resulted from acid rain exposure. So, although no significant reduction in the leaf surface area affected occurred with plant age after exposure to acid rain, the amount of chlorosis was accelerated and necrosis of leaf margins resulted from exposure to acid rain.

A. Visual leaf injury symptoms

The description that follows presents generalizations derived from 13 experiments of plant injury sustained after exposure to simulated sulfate acid rain pH 2.7.

Injury types were selected to characterize the sequential stages of injury progression through time. The sequence varied slightly between Phaseolus vulgaris and Helianthus annuus but was generally consistent for both plant species. The injury types were labeled as #A, #B, #C, and #D in order of increased degree of injury in which injury #A occurred initially after rain events.

Injury #A was the first observable injury to the first trifoliate leaves of P. vulgaris after treatment. This type of injury was not found on leaves of H. annuus and was found rarely on primary leaves of P. vulgaris because leaf surfaces of young trifoliate leaves of P. vulgaris were smooth compared with the more expanded and rough-textured unifoliate leaves of P. vulgaris and leaf pairs of H. annuus. Injury #A was characterized as a very small, shallow, circular depression in the leaf surface (Fig. 16-a,b,c,d). On many trifoliate leaves, slight traces of chlorosis occurred in later stages of #A injury. This injury stage was found generally near veins but also occurred in intervenial areas.

Injury #B was characterized by a slightly larger, circular depression compared with #A injury (Fig. 17(2) and (8)). The depression was greater than in #A and chlorosis was always present. Often, #B was the first stage detected in older or rough-textured leaves (primary leaves of P. vulgaris and all leaves of H. Annuus).

Injury #C developed when lesions enlarged to at least 1 mm in diameter. The depression of #C was larger than that of #B and was usually enlarged in an irregular pattern, especially adjacent to vascular tissues. The lesion was marked clearly with distinct side walls. In general, necrosis occurred throughout the depression (Fig. 17(3) and (9)).

Injury #D, the most advanced stage characterized, exhibited a large irregularly shaped lesion about 2 mm in diameter (Fig. 17 (4), (5), (6) and (10)). In most cases necrosis on the abaxial leaf surface corresponded to the lesion on the adaxial surface so the lesion penetrated throughout the leaf in cross section. Injury #D was usually found initially at vein junctions, and (1) was due to coalescence of two or three smaller injury areas, or (2) resulted from enlargement of a single #C lesion.

Often the epidermis near vascular tissues was injured initially, possibly due to small areas of leaf curvature. These small areas of curvature may accumulate rain solutions with greater frequency compared with areas away from vascular tissue. It should be noted that such an estimate of injury associated with vascular tissue may be misleading when acid rain results in large necrotic areas. However, as the area of injury increased, the probability of injury more associated with vascular tissues increased. On the other hand, in very small leaves the location of vascular tissues is difficult to define. For this reason, the significance of initial injury adjacent to vascular tissues cannot be defined by leaf surface observations alone.

B. Histology

Histologically, #A injury was characterized by collapse of adaxial epidermal cells. In cross section epidermal cells exhibited compressed, distorted cell walls with little or no cytoplasm, collapsed adjacent to the palisade cell layer. At this stage the closely aligned epidermal cell walls stained darkly. In #A lesions, the upper palisade parenchyma cell

layer frequently exhibited distorted side walls concommitant with an increased stain of both cell walls and cytoplasm. The collapse of these tissues resulted in a depression of the adaxial leaf surface described previously.

Complete distortion of both the adaxial epidermal and palisade layer cells occurred to give #B lesions. In general, injury areas stained greater than adjacent cells as noted above. Frequently, epidermal and palisade parenchyma cells collapsed completely adjacent to spongy mesophyll cells. Lesions developed frequently in concave leaf surface areas near vascular bundles. When lesions were located near vascular tissues with associated supportive tissues, the degree of tissue collapse was not as marked compared with areas removed from vascular tissues. In sections with #A, #B and #C lesions, a high frequency of lesions adjacent to stomata and trichomes was noted. In sequential serial sections through all lesions observed, most injury areas were associated with either stomata or trichomes in accord with previous observations.

In #C lesions, almost all cells were distorted. In general, however, tissues remained distinct except for the adaxial epidermis. The adaxial epidermis collapsed completely in all #C lesions observed. In #D lesions no tissues remained distinct except for the more rigidly walled vascular tissues.

C. <u>Time sequence of events</u>

In experiments to view the time sequence of effects of simulated acid rain, plants were exposed to a six minute rain event daily. Just prior to a rain event an assessment of injury was made. So the time sequence of events was delayed by 24 hours.

D. Phaseolus vulgaris

In Phaseolus vulgaris, after one rain event (24 hr.) most injured areas of primary leaves were categorized as #A with a few #B lesions. Lesions of type #C were rare and no type #D injury was present. In first trifoliate leaves, most lesions were #A level with only a few at #B injury level. One day after the first rain event less than one-half of all lesions were associated with vascular tissues.

After 48 hours, after two rain events, most lesions were #B with some #C and #D injury types on primary leaves. On trifoliate leaves, some #A lesions were present but most were #C and #D. On both leaf types, lesions were located adjacent to vascular tissues in 75% of all cases. After 72 hours, #A lesions were found on both unifoliate and trifoliate leaves. Frequency of #C and #D lesions increased from earlier #A and #B lesions. The frequency of #B lesions remained similar to 48 hour samples.

After 72 hours the frequency of all lesions remained stable. Two processes occurred during this time period. Lesions #A and #B progressed to #C and #D while new lesions of #A and #B originated with continued rain events.

E. Helianthus annuus

After the first rain event (24 hours) the first leaf pair exhibited a great amount of leaf injury. Injury levels of #B, #C, and #D were all present. On the second leaf pair #A, #B and #C were all present but much lower in frequency compared with the first leaf pair. Corresponding to this pattern, the third leaf pair exhibited very little injury. On this leaf pair, #B lesions predominated. As stated previously, #A was very infrequent on leaves of H. annuus.

After 48 hours (two rain events) many large areas of the first leaf pair were almost completely affected by the simulated rain. Most lesions coalesced to give large areas of affected leaf surface. In general, about 10-20% of the entire leaf was injured and about 50% of this injury was related to vascular tissues. In the second leaf pair most lesions were #B with an increase in frequency of #C and #D lesions. About 75% of all lesions were associated with vascular tissues. In the third leaf pair, three simultaneous characteristics were present. Most lesions were #B, occurred on the distal portion of leaves, and almost all were associated with vascular tissues.

After three rain events, 50% of the leaf surface of the first pair was necrotic (D). Most lesions on the second and third leaf pairs were #B and #C with some #D. As after 48 hours, most lesions were associated with vascular tissues. After four rain events, most lesions of leaf pairs 2 and 3 were #C and #D. Eventually, all areas with #C and #D lesions became fully necrotic. At this time period about 75% of all lesions were associated with leaf veins.

F. *Populus* clones

Tables 5-10 summarize the response of various clones of Poplar to simulated acid rain. Each table gives an injury rating for each injury type for each pH per day. Also, the percent of total leaves injured is given.

If consideration is given mostly to injury rating and not to percent of total leaves injured, the following order of greatest to least injury is indicated: clone 8, 207, 44, 211, 327, 43. If the percent of total leaves injured is used as the sole criteria, the order of greatest to least injury was: 8, 211, 207, 43, 44, 327.

In general, the same injury indices of #A, #B, #C, #D were used in clones of Poplar as in *P. vulgaris* and *H. annuus*. Visually the symptoms were the same among the three species except for some minor variations. Presently, samples are under examination for (a) visual progression of injury, (b) initial effects via scanning electron microscopy, and (c) histological progress of injury. These results will augment similar results above for *P. vulgaris* and *H. annuus*.

Table 11 shows the number of stomata per unit area on the adaxial (upper) and abaxial (lower) leaf surface. Preliminary results indicate that the number of stomata on the adaxial leaf surface is positively related to the injury index among the clones studied. These resultant correlations are of interest in light of the above localization of injury near stomata. Almost no trichomes are present on leaves of the poplar clones studied.

G. *Tradescantia* clones

Because trichomes and stomata were the chief points of entry of simulated acid rain into foliage, a search for closely related plants with and without trichomes and varying numbers of adaxial stomata would represent a method to check responses of plants with and without these structures.

Plants with and without trichomes were obtained from the late Dr. Arnold H. Sparrow. He donated representatives of F_2 progeny from a cross of a pubescent clone 2451 with a glaborous clone 02. Four clones, 10, 18, 21, and 56 were initially selected from all the F_2 progeny available. Table 12 gives the frequency of stomata and trichomes on both adaxial and abaxial leaf surfaces. Both leaf surfaces were viewed because both would be subjected to simulated acid rain. Clones 10 and 18 exhibited lower frequencies of trichomes compared with clones 21 and 56. In general, however, the frequency of stomata were not different among the clones. Presently, these four clones as well as the parents are under propagation for future tests. Experiments with simulated rain should begin in September 1976.

H. *Pinus strobus* clones

Eastern White Pine (*Pinus strobus*) represents a forest plant species indigenous to the northeastern United States. Over the last year, I have corresponded with Dr. Bill Sites, Chief Pathologist at Forest Insect and Disease Management Office, Asheville, North Carolina. Dr. Sites has donated 6 clones of Eastern White Pine originally propagated for their diverse responses to ambient ozone and sulfur dioxide levels in forests.

Although the present categorization of their responses is unclear, these clones represent a wide divergence of responses to these air pollutants. Two clones, WVA and W1, are very sensitive to either ozone or SO_2 and because of this sensitivity are almost non-existent in forests. Successful propagation of these clones can only be done in facilities such as ours.

Preliminary analyses suggest that no great differences appear in stomatal frequency or internal needle characteristics. Preliminary Scanning Electron Micrographs suggest that stomata of pines are occluded with gums or resins. These resins may be a chief factor in resistance of gaseous pollutant entrance. Does acid rain help dissolve these resins or waxes? If acid rain helped dissolve these waxes, resistance to gas flow would be reduced and gas pollutants would enter at a more rapid rate.

Experiments are designed to test this hypothesis by (1) acid rain and aerosol treatments in combination with (2) SO_2 fumigations in the aerosol chamber. In the autumn of 1976, preliminary experiments are planned to view sensitivities of the clones to acid rain alone. In the spring of 1977, the combination experiments outlined above will be performed.

I. *Pteridium aquilinum*

The gametophyte stage of development in *P. aquilinum* represents a unique system to study the effects of aqueous pollutants in rainwater or surface waters in general. Table 13 outlines the stages in development of gametophytes of *P. aquilinum*. The specific methods of culture are outlined above. Two types of experiments were conducted: (a) the response of sperm to buffered solutions, (b) the response of vegetative and sexual stages to buffered solutions.

When sperm are exposed to buffered solutions at various pH levels the sperm remain active for various time periods. If sperm are exposed to buffer pH 5.8, 32% remain viable (flagellar movements) for 8-10 minutes (Table 14). However, if the pH is dropped to 5.2 no sperm are viable 5-7 minutes after the beginning of exposure although 27% are viable 2-4 minutes after exposure. If the pH is adjusted to 4.2 or lower very few sperm remain viable.

In experiments to view the sensitivity of vegetative and reproductive stages of P. aquilinum to buffered solutions, the buffered solutions are added for only 3.5 hours at a stage where most of the viable sperm are borne by plants of the second inoculation while only plants of the first inoculation have mature archegonia. For fertilization to occur, over 90% of the sperm must travel from plants of the second inoculation to plants of the first inoculation in the buffered solutions provided.

Twenty-eight days after the first inoculation, 43% of all prothalli in the dish (which includes plants of the second inoculation) are notched (Table 15). The notched condition is prerequisite to the presence of mature archegonia. In fact, 95% of all notched gametophytes had mature archegonia at 28 days. After the buffer is added fertilization can take place, then the buffer is removed. From the lower percentage (9%) of sporophytes produced with no buffer treatment, most plants are fertilized during the 3.5 hr. period. If this background percentage of sporophytes is subtracted (see last column of Table 15), it is evident that the percent of sporophytes per notched gametophytes is dependent on buffer pH.

The third column of Table 15 reflects the ability of notched gametophytes at day 28 to survive the buffer treatment. Since all the values of column three are similar, we concluded that the buffer treatments have little or no effect on gametophyte survival. This Table clearly shows that a decrease in buffer pH lowers the level of sporophytes produced in a similar manner to the sperm counts of Table 14. Presently, experiments are underway to test the effects of additives such as sulfate, nitrate, and chloride to test anion effects in this sensitive assay system.

VI. Anticipated Experiments for Next Year

As outlined in the specific aims section and further reinforced in latter sections, the following areas of experimentation are underway and will be completed by the end of FY 1977:

(A) Description of lesion development on Poplar clones similar to that outlined above for *P. vulgaris* and *H. annuus*. Because Poplar has few if any trichomes, experiments will be focused on injury localization near stomata.

(B) Description of lesion development in clones of *Tradescantia*. Experiments will focus on frequency of lesion development near trichomes as the clones available differ in frequency of trichomes with little variation in frequency of stomata.

(C) Finish experiments that view leaf growth rate reductions in *P. vulgaris* at pH levels near 3.0. Finish experiments that view overall productivity of *P. vulgaris* and *H. annuus* with respect to total biomass, seed yield, resultant seed viability, etc.

(D) Follow rates of accumulation of sulfate and nitrate in plant leaf tissue after exposure to acid rain with respect to leaf injury, no growth reductions, and no yield reductions.

(E) Observe rates of ion permeability to plant leaves exposed to acid rain treatments. Rates of ion permeability should be related to nutrient accumulations and/or leaching from acid rain treatments.

(F) Observe effects of treatments to plants after exposure to acid aerosols in combination with acid rain as compared with acid rain only. Techniques similar to those used previously will be used.

(G) Observe the effects of various anions in the very sensitive *Pteridium aquilinum* system. It is hoped that sporophytes of these plants will be available in the summer of 1977.

(H) Conduct acid rain experiments with cloned material of *Pinus strobus* (Eastern White Pine) and Sovereign Pin Oak. Because of propagation difficulties these two species were unavailable.

(I) Monitor rain events of Long Island and observe the major constituents in rain among rain events. Emphasis will be placed on sequential samples during single events with regard to rain chemistry.

VII. References

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Table 1

Constituents of simulated rain solution

Constituent	Conc. (mg/l)
Ammonium	0.40
Sodium	1.20
Calcium	0.506
Magnesium	0.17
Potassium	0.20
Iron	0.010
Manganese	0.020
Lead	0.050
Zinc	0.10
Nickel	0.10
Copper	0.02
Cadmium	0.02
Hydrogen ions	0.060
Fluoride	0.100
Sulfate	5.0
Chloride	2.7
Nitrate	0.80

Table 2

Characterization of Droplet Sizes
Generated by the SRN[a] and Oil Burner Nozzles[b]

	SRN Nozzle	Oil Burner Nozzle
Mean radius, r_m, sum of all droplet radii divided by total number of droplets	0.0604	0.0012
Mode radius, r_d, radius that corresponds to the maximum number drops	0.300	0.0138
Mean volume radius, r_v, radius of a droplet whose volume is equal to the average droplet volume	0.353	0.0128
Predominant radius, r_p, radius that corresponds to the maximum volume of water	0.300	0.0138
Median volume radius, r_n, radius of the droplet such that half the water is comprised by larger drops	0.410	0.0139

a/ SRN - Polyvinyl chloride nozzle (14 holes; each hole 0.010 inches in diameter; 9 inch diameter spray pattern at height of 3 feet) purchased from Bete Fog Nozzle Company, Greenfield, Mass.

b/ Sid Harvey Oil Burner Nozzle, 80 degree spray pattern. Flow rate = 0.85 gph - 80 p.s.i. Semi-solid spray pattern.

Table 3.

Effect of Sulfate Acid Rain on Visual Leaf Injury of *Phaseolus vulgaris*

	pH of simulated rain					
	2.3	2.5	2.7	2.9	3.1	5.7
	Days after exposure					
	3	3	3	3	3	3
	6	6	6	6	6	6
	9	9	9	9	9	9
	12	12	12	12	12	12
Phaseolus vulgaris Trifoliate leaf stage						
FIRST	2	1	1	1	0	0
	3	2	1	1	0	0
	3	2	1	1	1	0
	3	2	2	1	1	0
SECOND	2	1	1	1	0	0
	2	2	1	1	0	0
	3	2	1	1	1	0
	3	2	2	1	1	0
THIRD	2	1	1	1	0	0
	3	2	1	1	0	0
	3	2	2	1	0	0
	3	2	2	1	0	0
FOURTH	2	1	1	1	0	0
	3	2	2	1	0	0
	3	2	2	1	0	0
	3	2	2	1	0	0

Table 4.

Effect of Sulfate Acid Rain on Visual Leaf Injury of *Helianthus annuus*

	pH of simulated rain					
	2.3	2.5	2.7	2.9	3.1	5.7
	Days after exposure					
	3	3	3	3	3	3
	6	6	6	6	6	6
	9	9	9	9	9	9
	12	12	12	12	12	12
Helianthus annuus						
Leaf pair stage						
3-4	2	1	1	1	0	0
	3	1	1	1	0	0
	3	2	1	1	1	0
	3	2	1	1	0	0
5-6	1	1	0	1	0	0
	1	1	0	0	0	0
	2	1	1	1	0	0
	2	1	1	0	0	0
7-8	0	0	0	0	0	0
	0	0	0	0	0	0
	2	0	0	0	0	0
	1	1	0	0	0	0

Table 5

Response of Poplar Clone 8[a/] After Exposure to Simulated Sulfate Acid Rain

Day[c/]	pH	Injury type[b/] A	B	C	D	X	Percent of Total Leaves Injured
1	2.7	1[d/]	2	1	0	1	54
	2.9	1	1	0	0	1	36
	3.1	1	1	0	0	1	35
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
2	2.7	1	2	1	0	1-2	69
	2.9	1	1	0	0	1	41
	3.1	1	1	0	0	1	38
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
3	2.7	1	2	1	1	1-2	74
	2.9	1	1	0	0	1	39
	3.1	1	1	0	0	1	35
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
6	2.7	2	2	2	1	1-2	91
	2.9	1	1-2	1	1	1	56
	3.1	1	1	0	0	1	40
	3.4	0	0	0	0	1	0
	5.7	0	0	0	0	0	0

a/ Clone 8-hybrid of *P. nigra* L. X *P. laurifolia* Ledeb.

b/ Injury type #A, #B, #C, #D, #X refers to injury types. See text for description of injury symptoms for each type.

c/ Day - refers to number of days after the start of simulated rain events. Ratings are made 24 hours after each rain.

d/ Rating: 0 = no injury
1 = 1 to 10% of leaf surface injured
2 = 20 to 40% " " "
3 = 50 to 100% " " "

Table 6

Response to Poplar Clone 211[a/] After Exposure to Simulated Sulfate Acid Rain

Day[c/]	pH	Injury type[b/] A	B	C	D	X	Percent of Total Leaves Injured
1	2.7	1[d/]	1	0	0	1	65
	2.9	1	1	0	0	1	27
	3.1	1	0	0	0	1	9
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
2	2.7	1	1	1	0	1	72
	2.9	1	1	0	0	1	51
	3.1	1	1	0	0	1	15
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
3	2.7	1	1-2	1	1	1	72
	2.9	1	1	0	0	1	48
	3.1	0	1	0	0	1	13
	3.4	0	1	0	0	1	0
	5.7	0	0	0	0	0	0
6	2.7	1	1-2	1-2	1	1	89
	2.9	1	1-2	1	0	1	57
	3.1	1	1	0	0	1	26
	3.4	0	1	0	0	1	0
	5.7	0	0	0	0	0	0

a/ Clone 211-hybrid of P. deltoides Bartr. X P. trichocarpa Torr. and Gray

b/ Injury type #A, #B, #C, #D, #X refers to injury types. See text for description of injury symptoms for each type.

c/ Day - refers to number of days after the start of simulated rain events. Ratings are made 24 hours after each rain.

d/ Rating: 0 = no injury
1 = 1 to 10% of leaf surface injured
2 = 20 to 40% " " "
3 = 50 to 100% " " "

Table 7

Response to Poplar Clone 207[a] After Exposure to Simulated Sulfate Acid Rain

Day[c]	pH	Injury type[b] A	B	C	D	X	Percent of Total Leaves Injured
1	2.7	1[d]	2	1	0	1	42
	2.9	1	1	0	0	1	32
	3.1	1	0	0	0	1	13
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
2	2.7	1	2	1	0	1-2	70
	2.9	1	1	1	0	1	48
	3.1	1	1	0	0	1	22
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
3	2.7	1-2	2	1	1	1-2	75
	2.9	1	1	1	0	1	48
	3.1	1	1	0	0	1	18
	3.4	1	1	0	0	1	6
	5.7	0	0	0	0	0	0
6	2.7	2	2	1-2	1	1-2	80
	2.9	1	1-2	1	0	1-2	66
	3.1	1	1	0	0	1	22
	3.4	0	1	0	0	1	6
	5.7	0	0	0	0	0	0

a/ Clone 207-hybrid of *P. deltoides* Bartr. X *P. trichocarpa* Torr. and Gray

b/ Injury type #A, #B, #C, #D, #X refers to injury types. See text for description of injury symptoms for each type.

c/ Day - refers to number of days after the start of simulated rain events. Ratings are made 24 hours after each rain.

d/ Rating: 0 = no injury
1 = 1 to 10% of leaf surface injured
2 = 20 to 40% " " "
3 = 50 to 100% " " "

Table 8

Response of Poplar Clone 44[a/] After Exposure to Simulated Sulfate Acid Rain

Day[c/]	pH	Injury type[b/] A	B	C	D	X	Percent of Total Leaves Injured
1	2.7	1[d/]	1	0	0	1	52
	2.9	1	1	0	0	1	27
	3.1	1	1	0	0	1	16
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
2	2.7	1	1	1	1	1	62
	2.9	1	1	0	0	1	38
	3.1	1	1	0	0	1	22
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
3	2.7	1	1	1	1	1	65
	2.9	1	1	0	0	1	43
	3.1	1	1	0	0	1	27
	3.4	0	1	0	0	1	2
	5.7	0	0	0	0	0	0
6	2.7	1-2	2	1-2	1	1-2	71
	2.9	1	1	1	1	1	56
	3.1	1	1	0	0	1	32
	3.4	0	1	0	0	1	5
	5.7	0	0	0	0	0	0

a/ Clone 44-hybrid of P. maximowiczii Henry X P. berolinensis Dipp

b/ Injury type #A, #B, #C, #D, #X refers to injury types. See text for description of injury symptoms for each type.

c/ Day-refers to number of days after the start of simulated rain events. Ratings are made 24 hours after each rain.

d/ Rating: 0 = no injury
1 = 1 to 10% of leaf surface injured
2 = 20 to 40% " " "
3 = 50 to 100% " " "

Table 9

Response of Poplar Clone 43[a] After Exposure to Simulated Sulfate Acid Rain

Day[c]	pH	Injury type[b] A	B	C	D	X	Percent Total Leaves Injured
1	2.7	1[d]	1	0	0	1	33
	2.9	1	1	0	0	1	32
	3.1	1	1	0	0	1	9
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
2	2.7	1	1-2	0	0	1	53
	2.9	1	1	0	0	1	38
	3.1	1	1	0	0	1	23
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
3	2.7	1	1-2	1	0	1	65
	2.9	1	1	1	0	1	54
	3.1	1	1	0	0	1	38
	3.4	1	1	0	0	1	11
	5.7	0	0	0	0	0	0
6	2.7	1	1-2	1	1	1	69
	2.9	1-2	2	1	1	1-2	61
	3.1	1	1	0	0	1	38
	3.4	0	1	0	0	1	8
	5.7	0	0	0	0	0	0

a/ Clone 43-hybrid of P. maximowiczii Henry X P. berolinensis Dipp.

b/ Injury type #A, #B, #C, #D, #X, refers to injury types. See text for description of injury symptoms for each type.

c/ Day - refers to number of days after the start of simulated rain events. Ratings are made 24 hours after each rain.

d/ Rating: 0 = no injury
1 = 1 to 10% of leaf surface injured
2 = 20 to 40% " " "
3 = 50 to 100% " " "

Table 10

Response to Poplar Clone 327[a/] After Exposure to Simulated Sulfate Acid Rain

Day[c/]	pH	Injury type[b/] A	B	C	D	X	Percent of Total Leaves Injured
1	2.7	2[d/]	1-2	0	0	1	48
	2.9	1	1	0	0	1	39
	3.1	1	1	0	0	1	20
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
2	2.7	1	1-2	1	0	1	56
	2.9	1	1	0	0	1	58
	3.1	1	1	1	0	1	24
	3.4	0	0	0	0	0	0
	5.7	0	0	0	0	0	0
3	2.7	1	2	1	1	1	60
	2.9	1	1	1	0	1	52
	3.1	1	1	1	0	1	24
	3.4	1	1	0	0	1	5
	5.7	0	0	0	0	0	0
6	2.7	1	2	1-2	1	1	60
	2.9	1	1	1	0	1	58
	3.1	1	1	1	0	1	30
	3.4	1	1	0	0	1	7
	5.7	0	0	0	0	0	0

a/ Clone-327-hybrid of P. balsamifera L. c/. candicans X P. berolinensis Dipp

b/ Injury type #A, #B, #C, #D, #X refers to injury types. See text for description of injury symptoms for each type.

c/ Day - refers to number of days after the start of simulated rain events. Ratings are made 24 hours after each rain.

d/ Rating: 0 = no injury
1 = 1 to 10% of leaf surface injured
2 = 20 to 40% " " "
3 = 50 to 100% " " "

Table 11

FREQUENCY OF STOMATA ON ADAXIAL AND ABAXIAL LEAF SURFACES OF *POPULUS SP.* HYBIRDS

Hybird Clone #	Adaxial Surface (stomata/mm^2)	Abaxial Surface (stomata/mm^2)
8	1.07 ± 0.19[a]	0.73 ± 0.14
43	0.71 ± 0.04	2.36 ± 0.18
44	0.51 ± 0.04	2.92 ± 0.17
207	0.41 ± 0.05	1.27 ± 0.06
211	0.54 ± 0.03	0.95 ± 0.04
327	0.41 ± 0.02	1.57 ± 0.14

[a]Each figure represents the average and standard error of the mean from 3 impressions on each leaf surface from each clone. Eight areas on each impression were scored.

Table 12

FREQUENCY OF STOMATA AND TRICHOMES
ON ADAXIAL AND ABAXIAL LEAF SURFACES
OF TRADESCANTIA SP. CONES

Clone #	Leaf Surface	Stomata (no./mm^2)	Trichome (no./mm^2)
10	Adaxial	0.45 ± 0.06[a]	0.04 + 0.02
18	"	0.66 ± 0.06	0.01 ± 0.01
21	"	0.61 ± 0.07	0.26 ± 0.02
56	"	0.68 ± 0.27	0.30 ± 0.04
10	Abaxial	1.52 ± 0.06	0.0 ± 0.0
18	"	1.51 ± 0.33	0.08 ± 0.03
21	"	1.27 ± 0.16	0.46 ± 0.05
56	"	1.74 ± 0.09	0.41 ± 0.04

[a]Each figure represents the average and standard error of the mean from three impressions on each leaf surface from each clone. Twelve areas on each impression were scored.

Table 13

Stages in the development of *Pteridium aquilinum* under standard culture conditions

Day After Inoculation	Developmental Stage (Range)
5	Spores ungerminated.
11	Recently germinated to Gametophyte with Rhizoids and Antheridial initials.
12	Antherial initials on most--Notch beginning to form.
14	Mature Antheridia present.
19	Mature Antheridia--some have live sperm.
21	--Some Filamentous Gametophytes with mature Antheridia. --Heart-shaped (Notched) Gametophytes with (a) mature Antheridia and (b) Archegonial initials.
28	Heart-shaped Gametophytes with mature Archegonia.

Table 14

Response of sperm of *Pteridium aquilinum* to buffered solutions

	Percent Sperm Alive		
	Exposure Time (min)		
pH	2 - 4	5 - 7	8 - 10
2.2	0 ± 0	---	---
3.2	0 ± 0	0 ± 0	---
4.2	2.2 ± 0.7	0 ± 0	---
5.2	27.4 ± 3.9	0 ± 0	---
5.8	66.5 ± 4.4	48.7 ± 3.1	31.9 ± 3.8

Table 15

Response of Vegetative and Sexual Stages

of *P. aquilinum* to buffered solutions

Buffer Solution pH	Notched gametophytes - 4 weeks - (percent)	Notched gametophytes - 6 weeks - (percent)	Sporophytes per notched gametophytes (percent)	Sporophytes per notched gametophytes (percent of control)
Control- no buffer added	43.0 ± 1.8	50.7 ± 2.8	8.9 ± 2.9	0
2.2	"	41.8 ± 4.2	13.1 ± 5.9	4.2
3.2	"	48.9 ± 2.4	30.0 ± 7.2	21.1
4.2	"	45.7 ± 4.5	33.0 ± 5.5	24.1
5.2	"	54.5 ± 6.4	47.6 ± 7.2	38.7
5.8	"	46.6 ± 1.5	51.7 ± 3.1	42.8

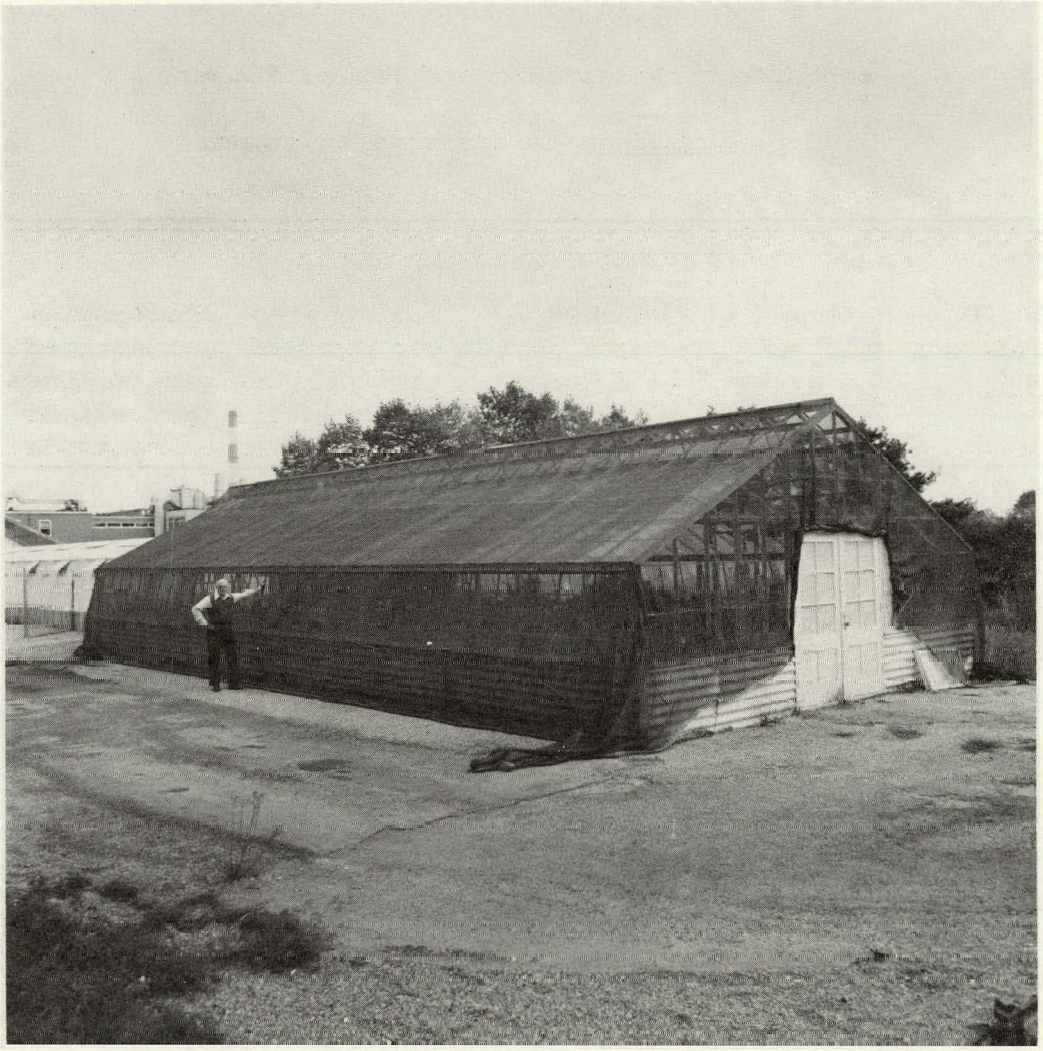

Figure 1. Exterior view of large greenhouse facility with shade provided.

Figure 2. Interior view of large greenhouse facility. Note supplemental lighting provided.

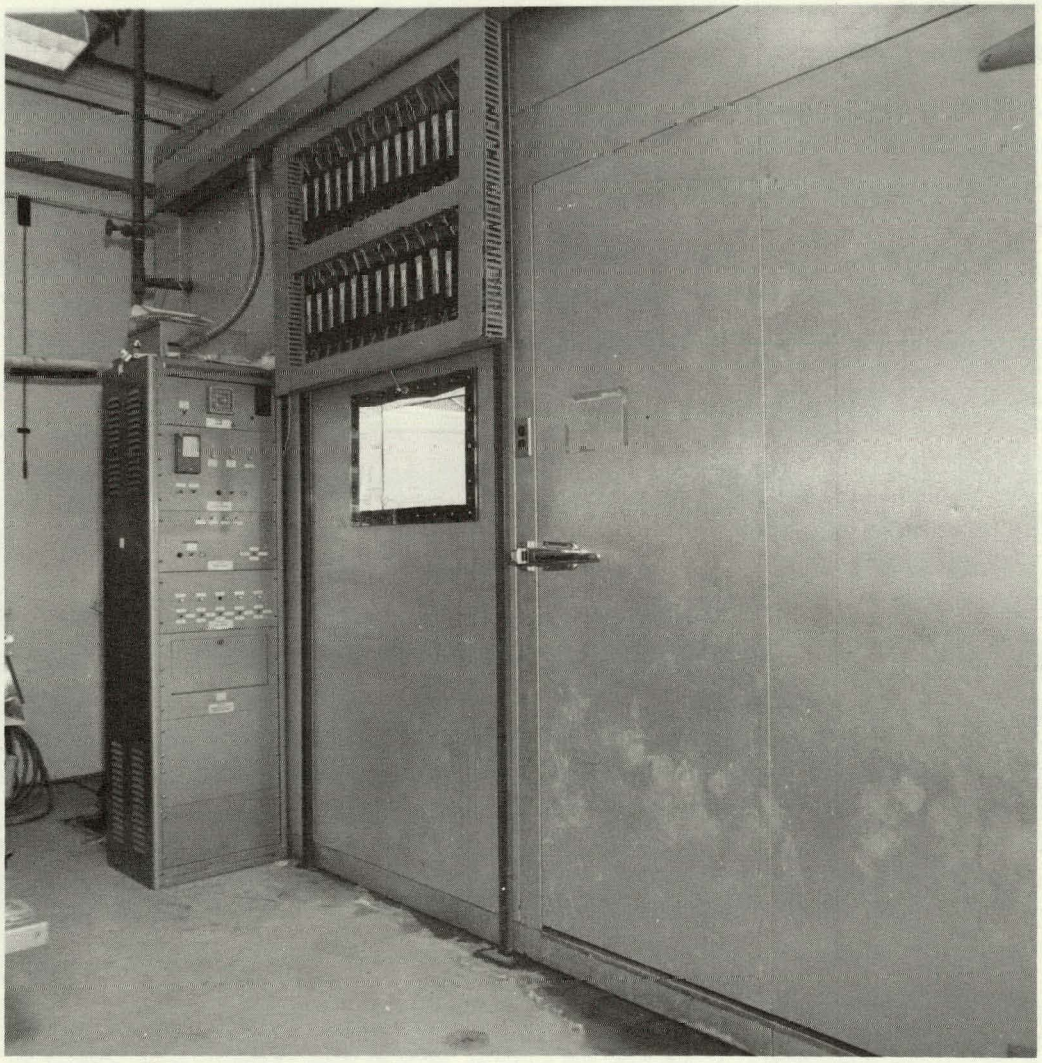

Figure 3. Exterior view of growth chamber.

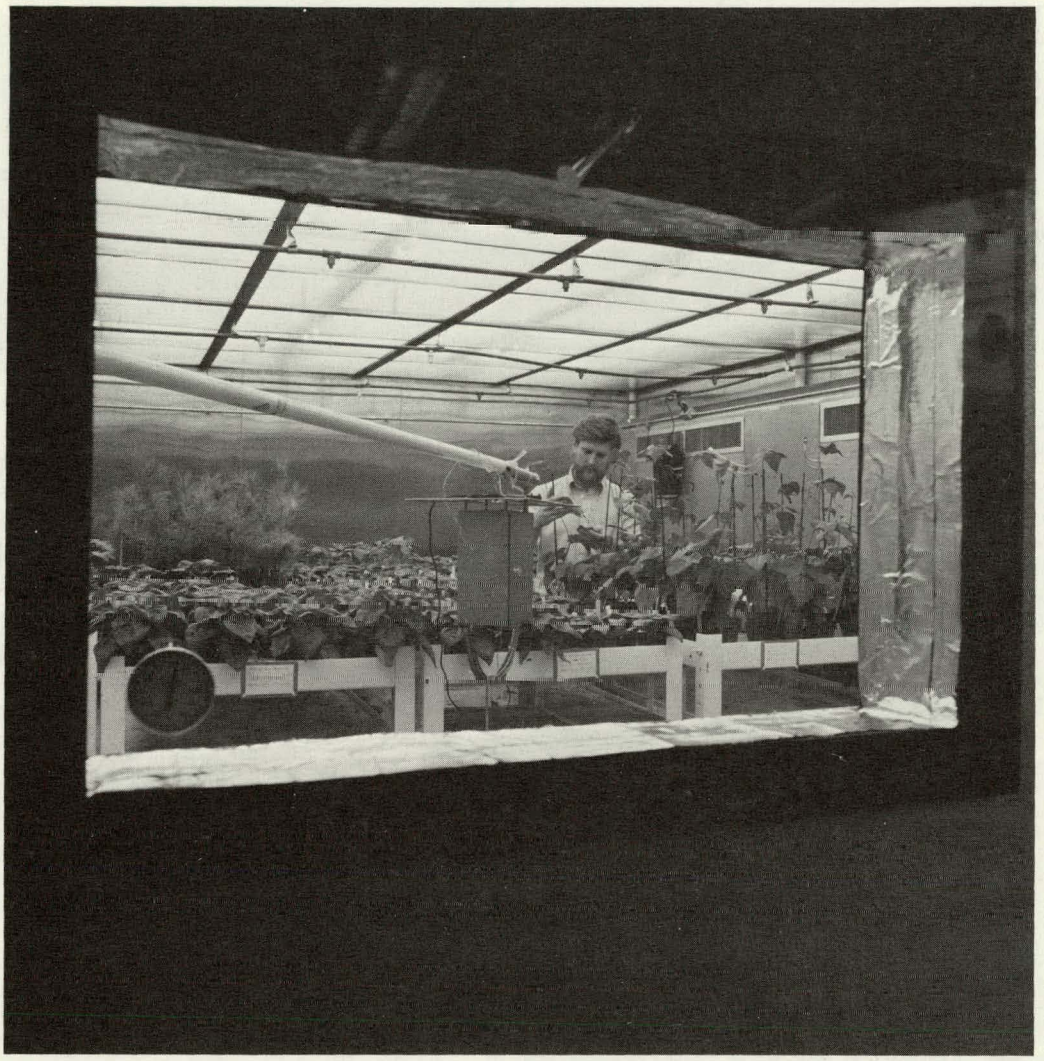

Figure 4. View into growth chamber from exterior.

Figure 5. Interior view of growth chamber.
Samples of plants are in foreground.

Figure 6. Overview of rain facility: left--compressed air for pressure; left front--nalgene bottle that contains rain solutions; front--turntable to support and rotate plants; upper area--nozzle for spraying (not in view); upper area--plastic covering.

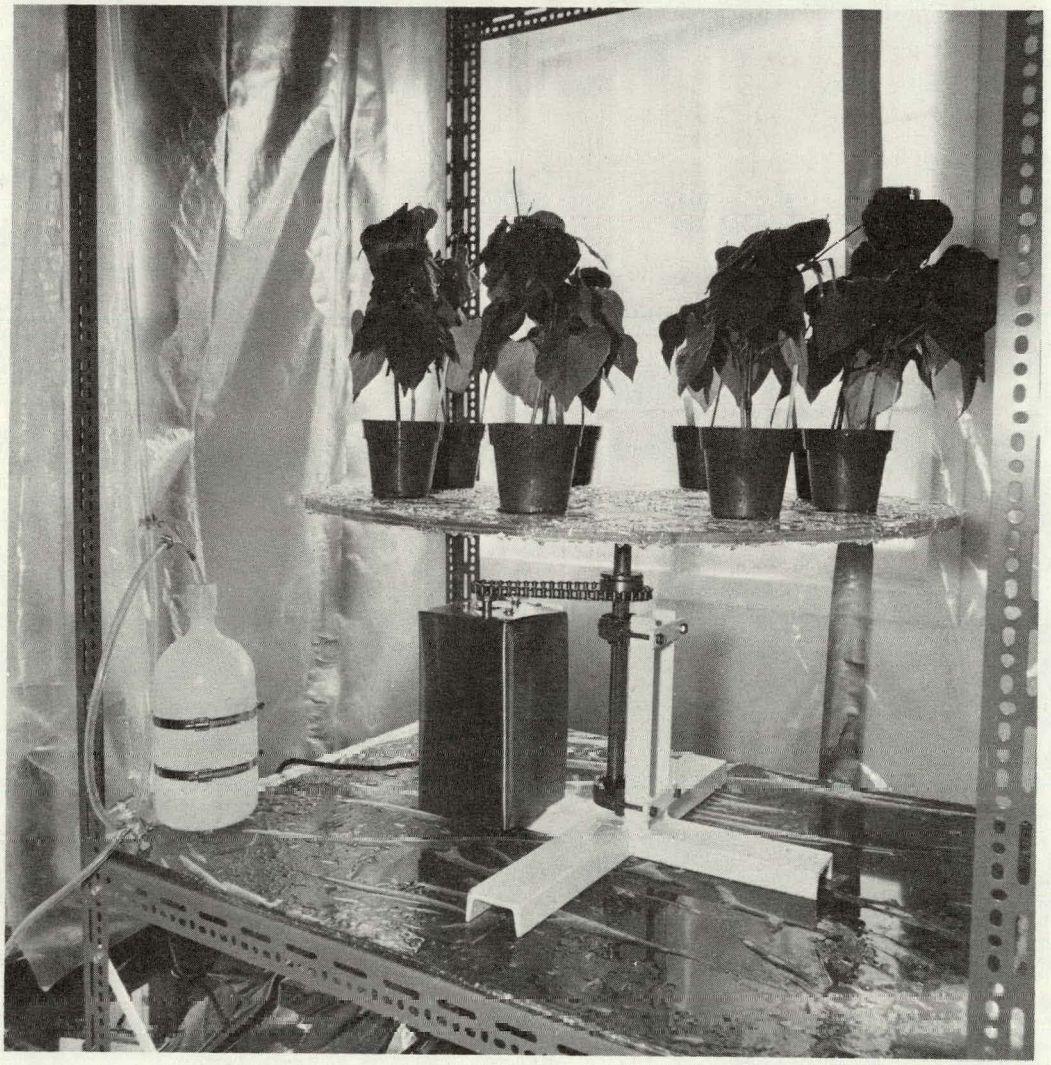

Figure 7. Detail of rain facility with turntable.

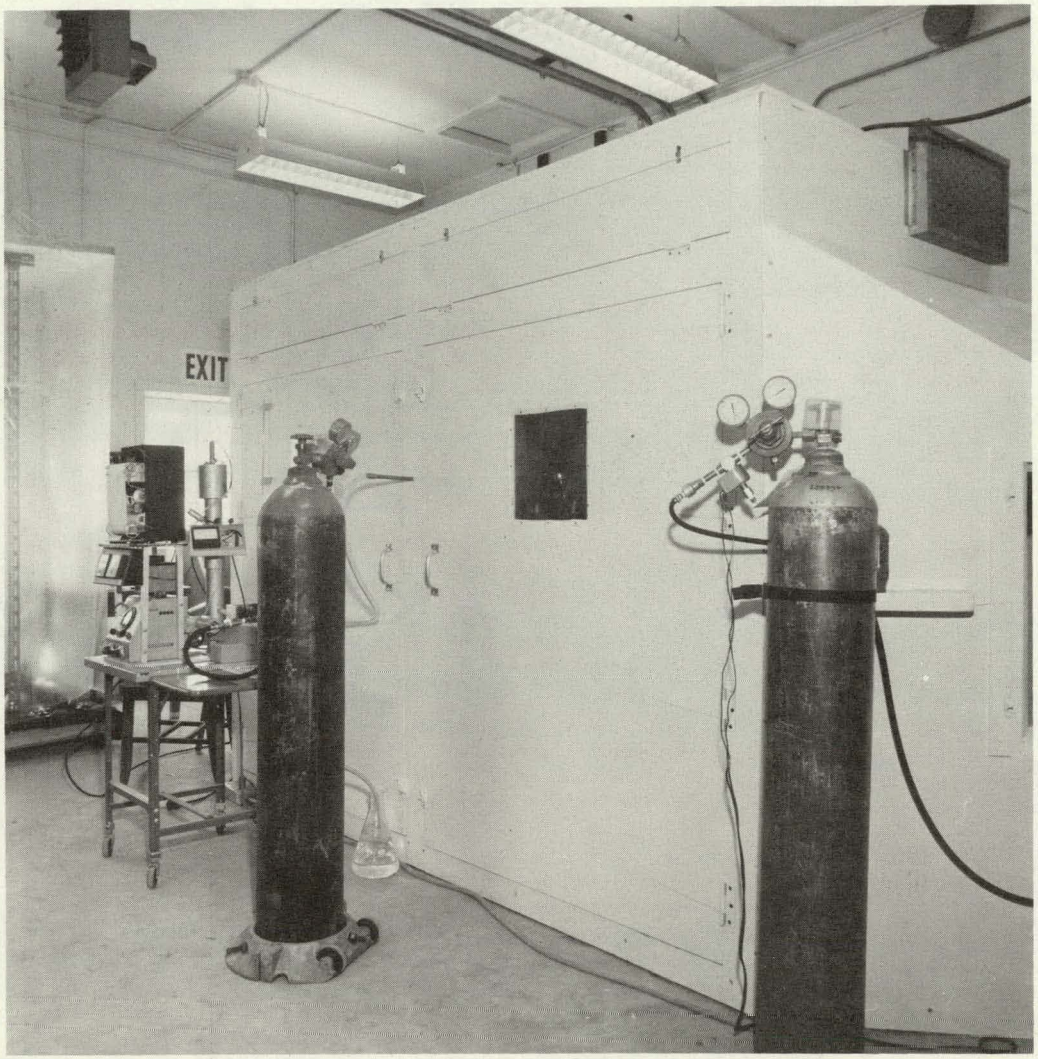

Figure 8. Exterior view of aerosol chamber: left--particle counter; center left--cylinder of helium carrier gas for particle counter; center--aerosol chamber (exterior view); cylinder of compressed air for pressurization of aerosol solution atomization.

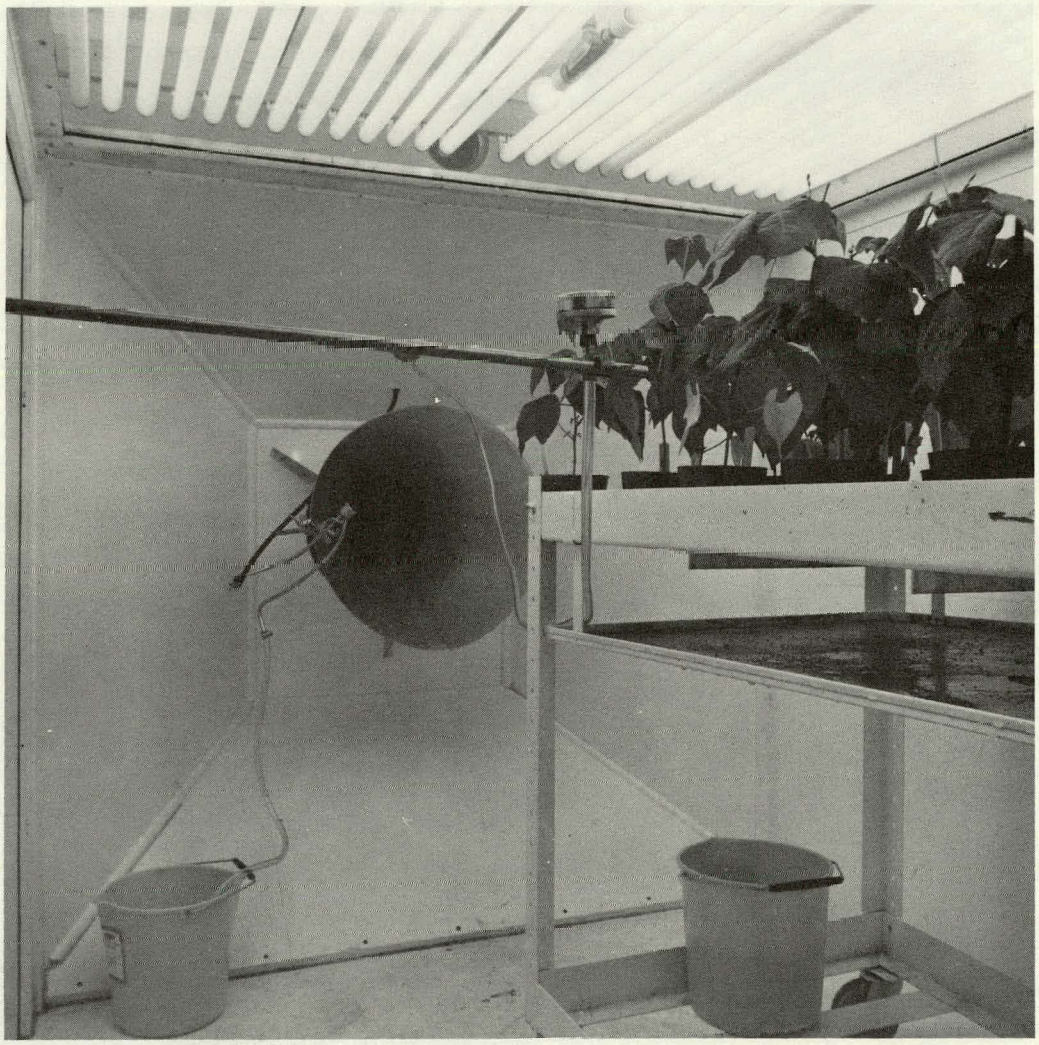

Figure 9. Interior of aerosol chamber. View of opening in which aerosols enter chamber. On left tube for aerosol particle sampler. Above tube is a holder for millipore filters for analytical chemistry.

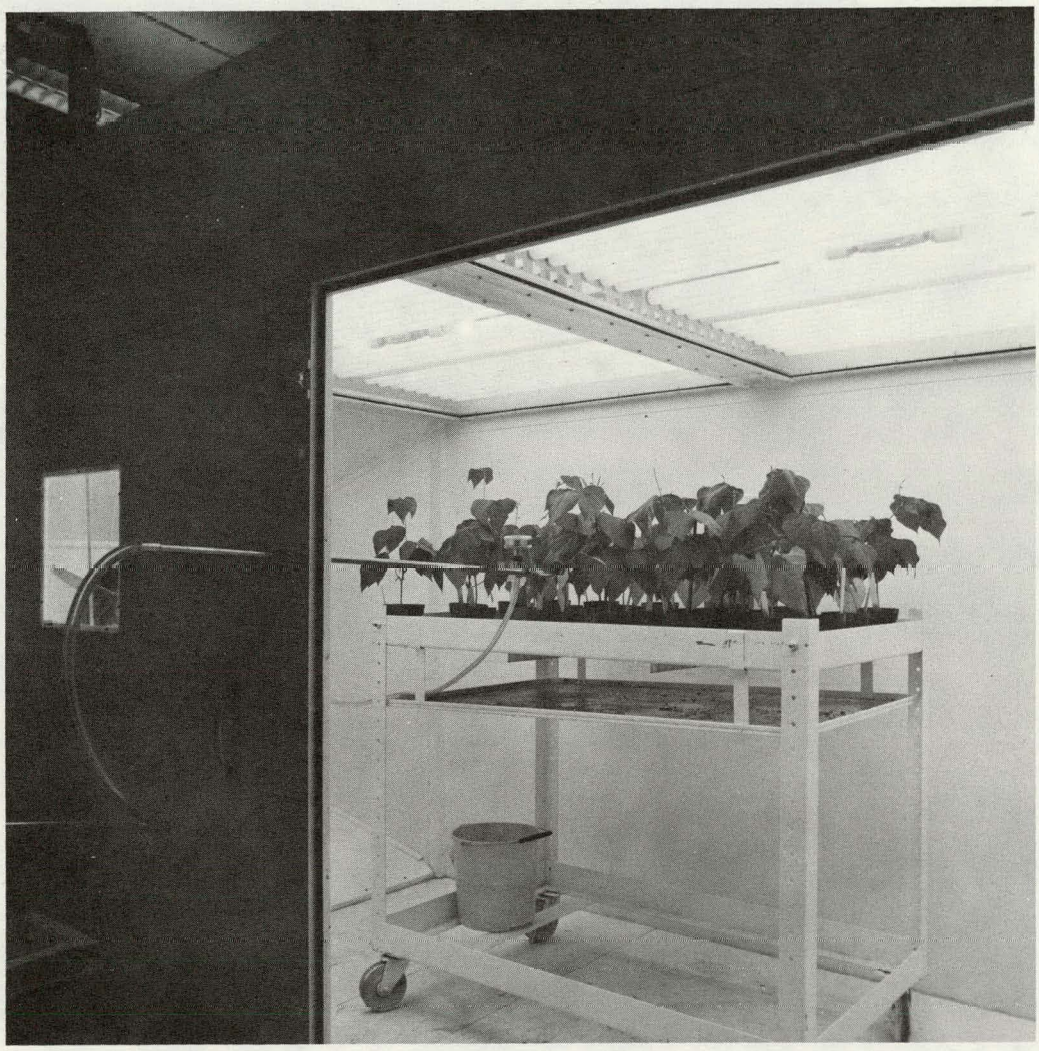

Figure 10. View of plants on cart in aerosol chamber. Carts have been replaced by turntable. Rain facility will be installed shortly.

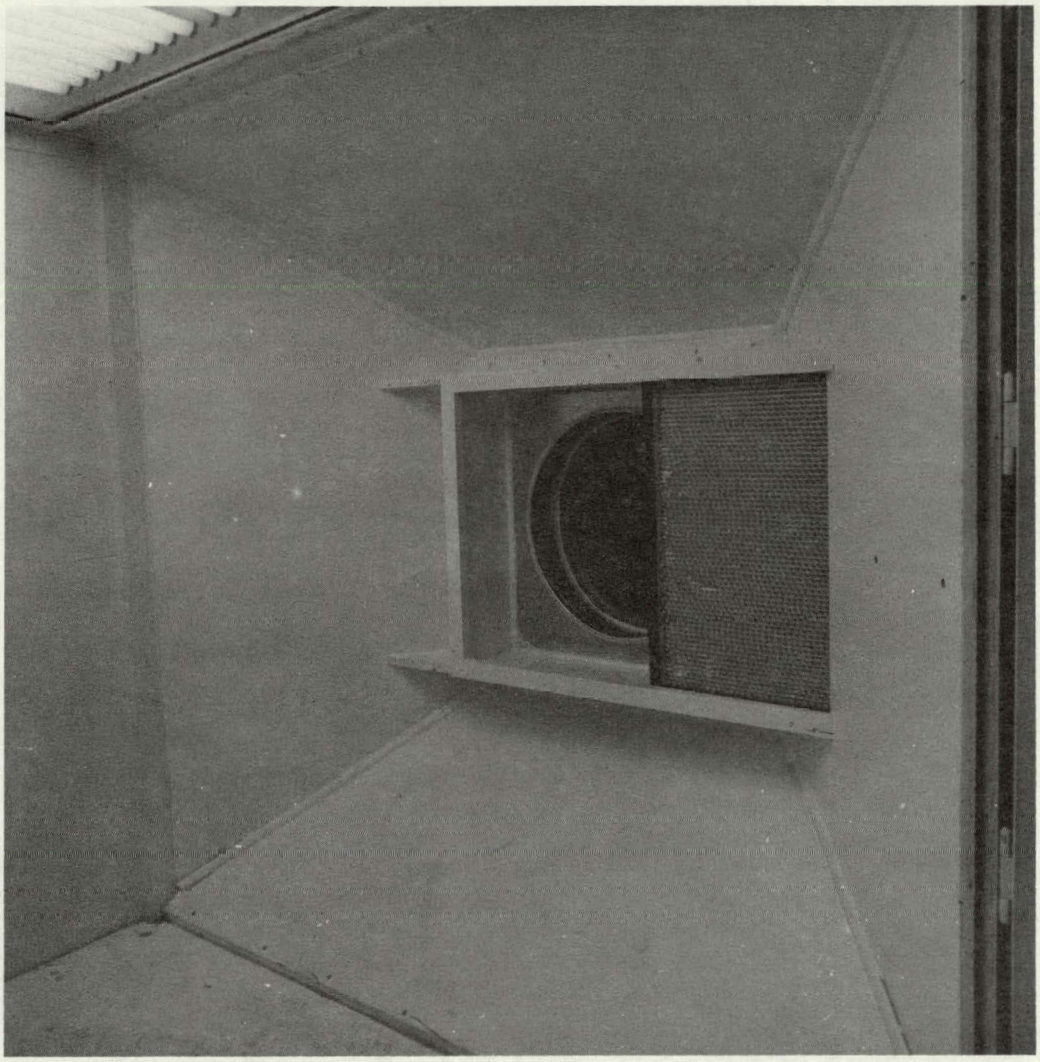

Figure 11. Interior view of aerosol chamber. View of opening in which aerosols leave chamber to begin recirculation through ducting. On right is filter to remove aerosols.

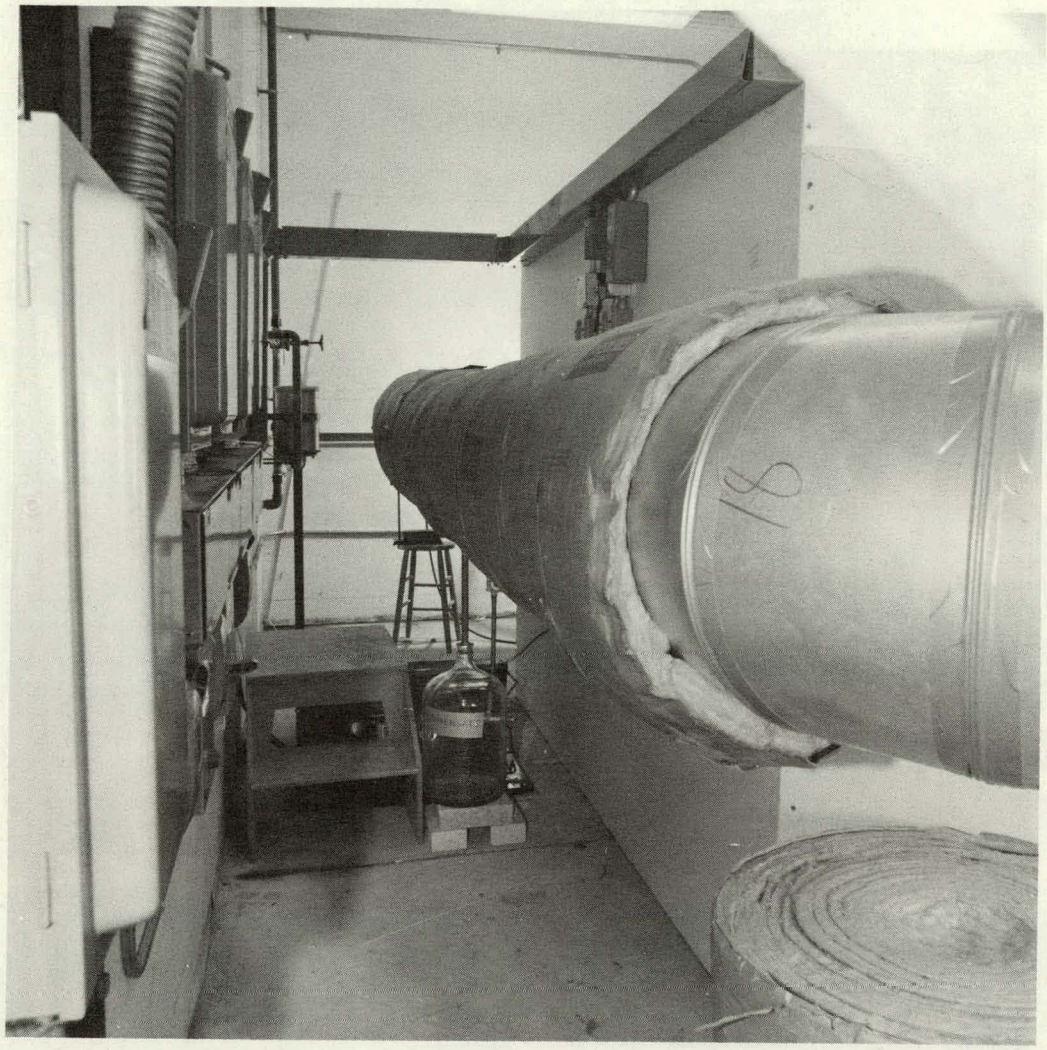

Figure 12. Ducting to recirculate aerosols. Cooling coil wrapped around ducting to lower air temperature. All ducting is insulated during experiments.

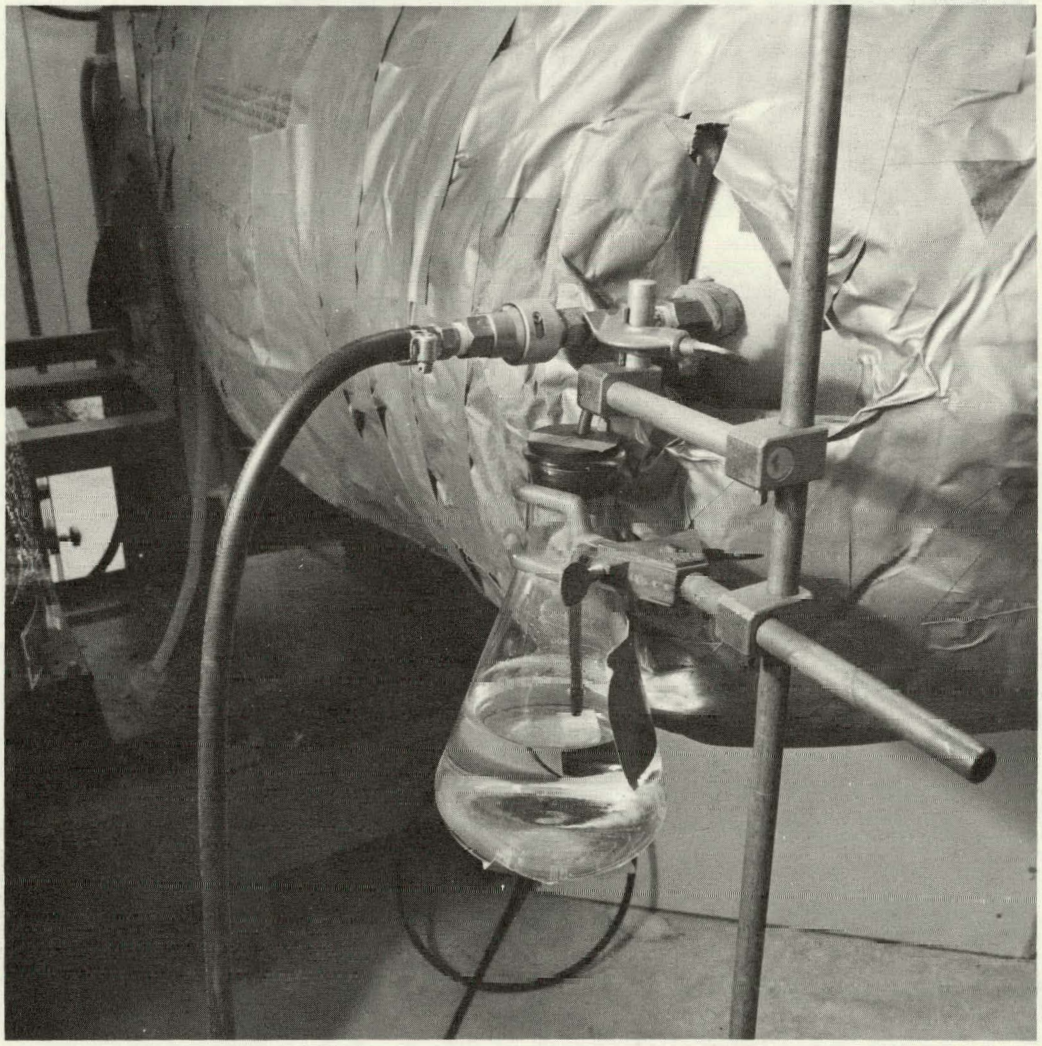

Figure 13. Pressurized aerosol solution and connection with ducting. Aerosol atomizer is placed in ducting.

Figure 14. Comparison photograph of pinto bean plants exposed to 4-6 minute daily rain events of pH 5.7 (control) and pH 2.3.

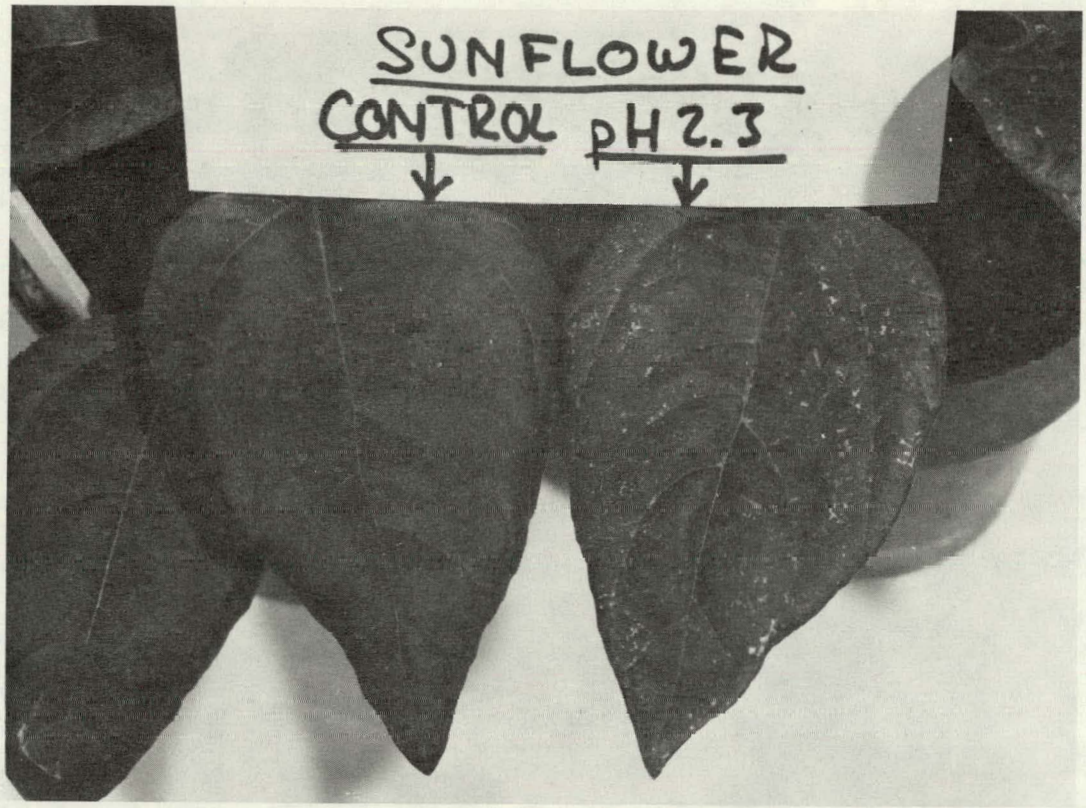

Figure 15. Comparison photograph of sunflower plants exposed to 4-6 minute daily rain events of pH 5.7 (control) and pH 2.3.

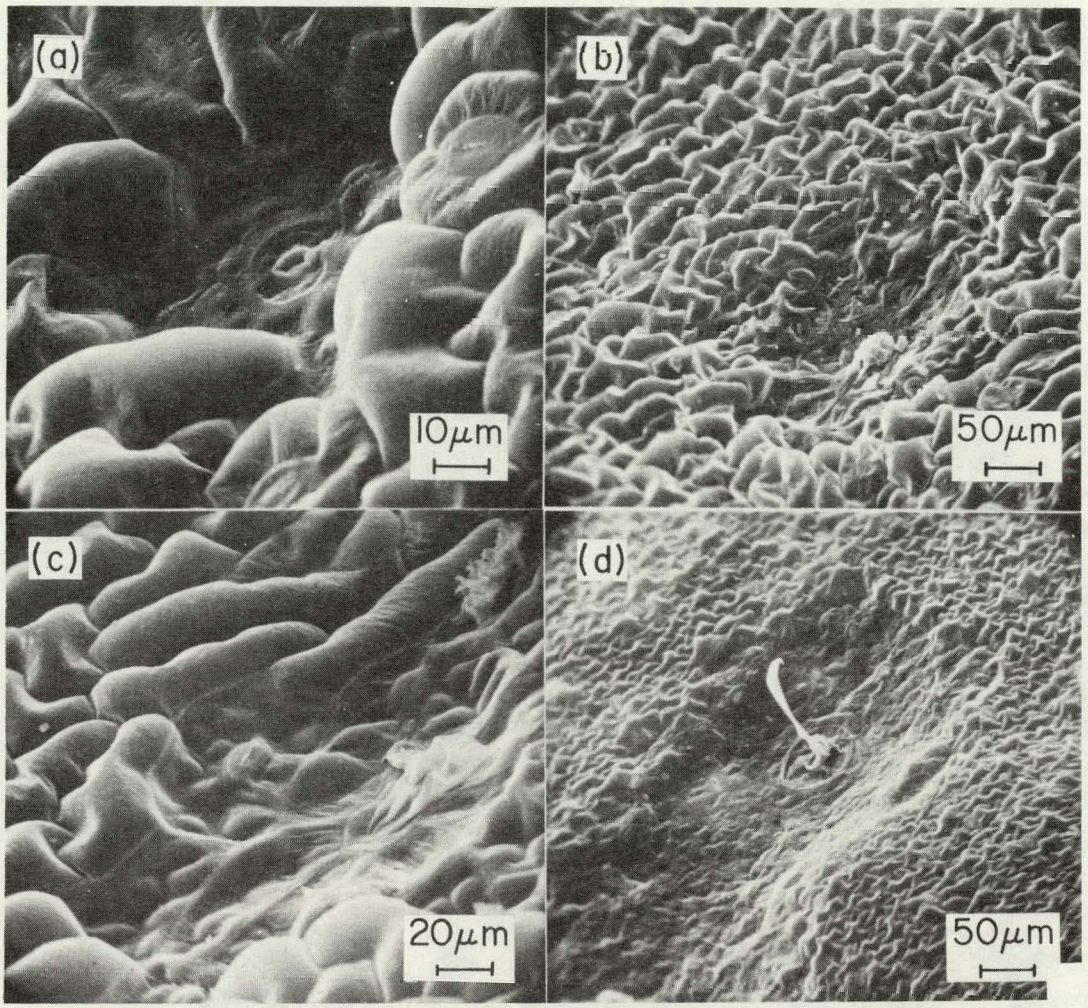

Figure 16. Scanning electron micrographs of adaxial leaf surfaces.

(a) Injury to epidermal cells of Phaseolus vulgaris via scanning electron microscopy 24 hours after a single six minute exposure to simulated acid rain pH 2.7. Healthy turgid cells surround several flaccid epidermal cells and a single stoma.

(b) Epidermal cells of Phaseolus vulgaris 24 hours after a single exposure to simulate acid rain pH 2.7. Injured flaccid cells are adjacent to a hydathode.

(c) Detail of injury by simulated acid rain pH 2.7 in Phaseolus vulgaris leaf epidermis. The injured area contained a hydathode and a stoma.

(d) Leaf surface of Helianthus annuus with injury after several daily exposures to simulated acid rain pH 2.7. The injured area was composed of more internal cell layers. Remnants of a spiked trichome are present within the injury area.

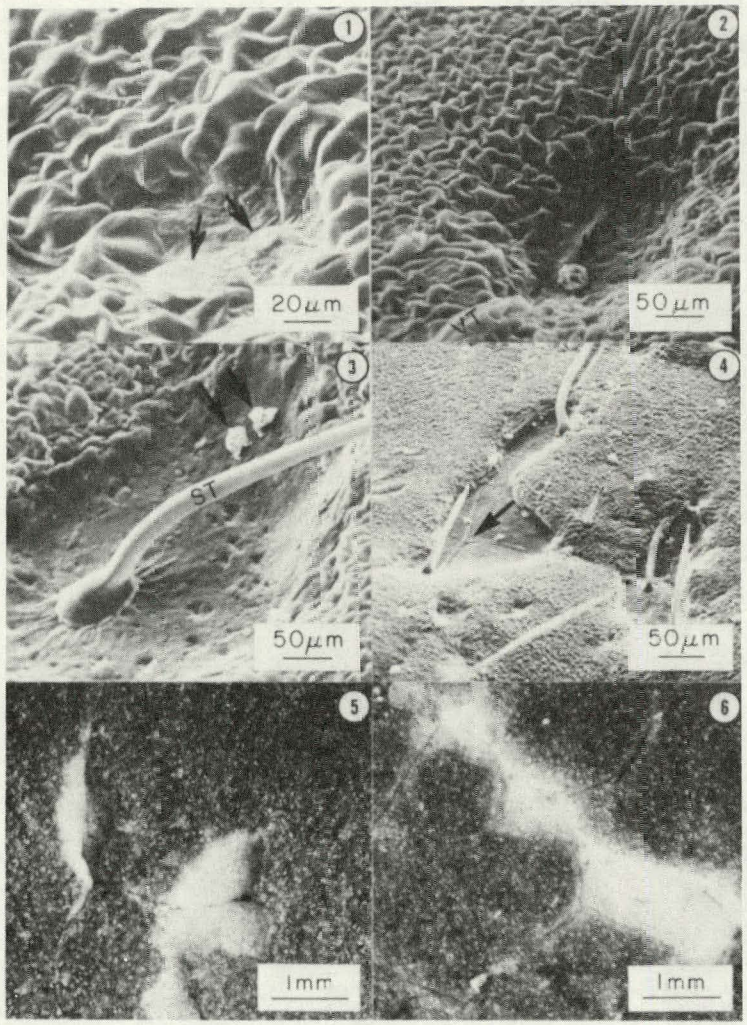

Figure 17(1-4). Scanning electron micrographs of adaxial leaf surfaces of Phaseolus vulgaris and Helianthus annuus that exhibit various stages of lesion development. All leaf surfaces were exposed to simulated sulfate acid rain pH 2.7 or pH 3.0 from one to several rain events.

Figure 17(1). Two stomata present in a lesion of flaccid, injured cells. The lesion is bordered by injured cells. Lesion represents #A injury on H. annuus.

Figure 17(2). Remnants of a trichome hydathode in an injured area near vascular tissue. The lesion is localized and is bordered by uninjured epidermal cells. The lesion shown represents #B injury and may affect cell layers below the epidermis of P. vulgaris.

Figure 17(3). Injured cells surround a spiked trichome and remnants of two trichome hydathodes. The injured cells are localized and are bordered by turgid, uninjured cells. This represents a #C lesion on P. vulgaris.

Figure 17(4). Low magnification of the leaf surface of P. vulgaris that exhibits a #D lesion around several branches of vascular tissue. Many spiked trichomes are evident along with remnants of trichome hydathodes. Apparent uninjured cells border the lesion.

Figure 17(5-10). Photographs of adaxial leaf surfaces of leaves of P. vulgaris and H. annuus that exhibit various stages of lesion development. All surfaces were exposed to simulated sulfate acid rain pH 2.7 from one to several daily rain events.

Figure 17(5). Two lesions adjacent to vascular tissues. Such lesions represent #D injury on primary leaves of P. vulgaris.

Figure 17(6). Coalescence of several lesions along vascular tissue. The resultant large lesion occupies a length of several millimeters on leaves of H. annuus.

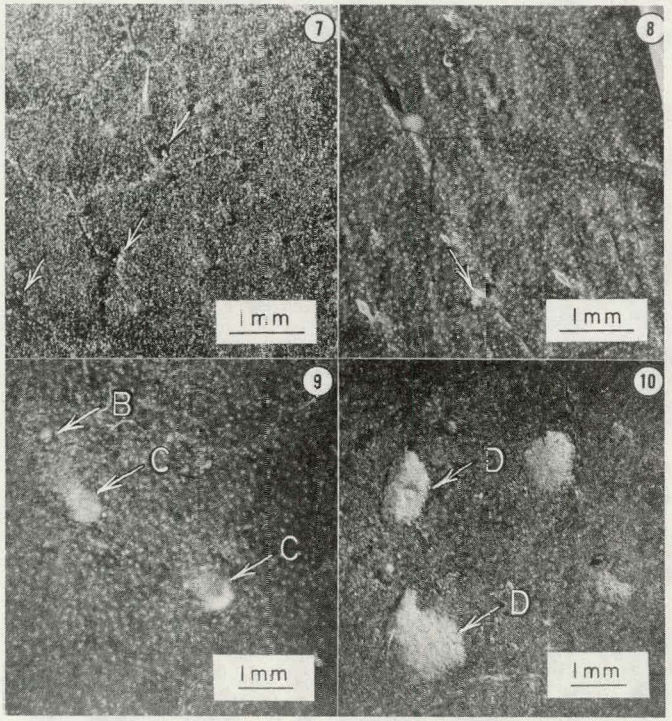

Figure 17(7). Several darkened areas on the leaf surface which represent the first observable evidence of lesions. Such lesions usually encompass only the epidermal cell layer and are termed #A lesions. Frequently lesions are associated with vascular tissues. Areas that border lesions exhibit uninjured cells. Samples were taken from the first trifoliate leaves of P. vulgaris.

Figure 17(8). Three lesions on the leaf surface which represents a later stage of injury compared with the lesions of Figure 17(7). Such lesions usually represent collapse of epidermal cells and are termed #B lesions. Areas adjacent to the lesions exhibit uninjured cells. Samples are first trifoliate leaves of P. vulgaris.

Figure 17(9). Two #C lesions with several #B on primary leaves of P. vulgaris.

Figure 17(10). Three #D lesions with several #C lesions on primary leaves of P. vulgaris.

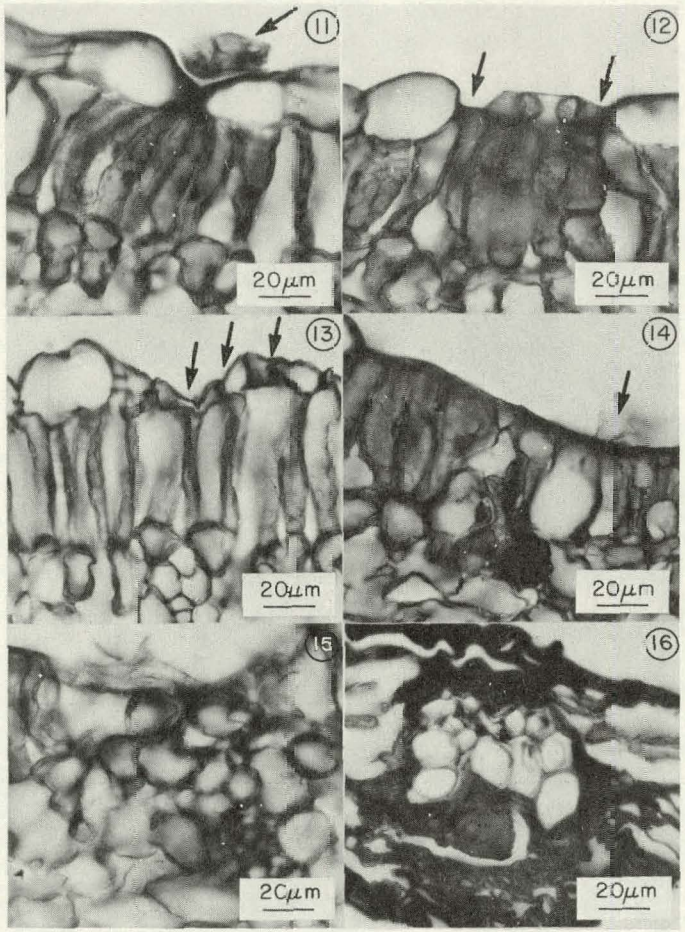

Figure 18(11-16). Cross sections of adaxial primary leaves of Phaseolus vulgaris that exhibit various stages of lesion development from exposure to simulated acid rain. Leaves were exposed to acid rain pH 2.7 or pH 3.0.

Figure 18(11). Initial injury to epidermal cells near a trichome hydathode. Note collapse of epidermal cell walls at the base of the hydathode. This injury represents #A injury.

Figure 18(12). Initial injury of epidermal cells adjacent to stoma guard cells. In one area the epidermal cell(s) collapsed. Many other epidermal cells appear uninjured. Sample represents #A injury.

Figure 18(13). Initial injury of several epidermal cells adjacent on intact palisade parenchyma. This section shows initial collapse of epidermal cells (#A injury) with no apparent injury to palisade parenchyma cells.

Figure 18(14). Micrograph represents #B injury in which epidermal cells are collapsed with some distortion of cells in the palisade parenchyma. Little or no distortion occurred in spongy parenchyma cells.

Figure 18(15). Collapse of epidermal cells and palisade parenchyma cells. Little or no distortion of cells occurred in the spongy mesophyll. Remnants of a trichone remain on the adaxial leaf surface. This micrograph represents a #C lesion.

Figure 18(16). Collapse of many leaf tissues of a #D lesion. Only vascular tissues remain intact.

Figure 19. Whole plant view of two clones of Poplar (cottonwood) fitted with caps at the beginning of an experiment.

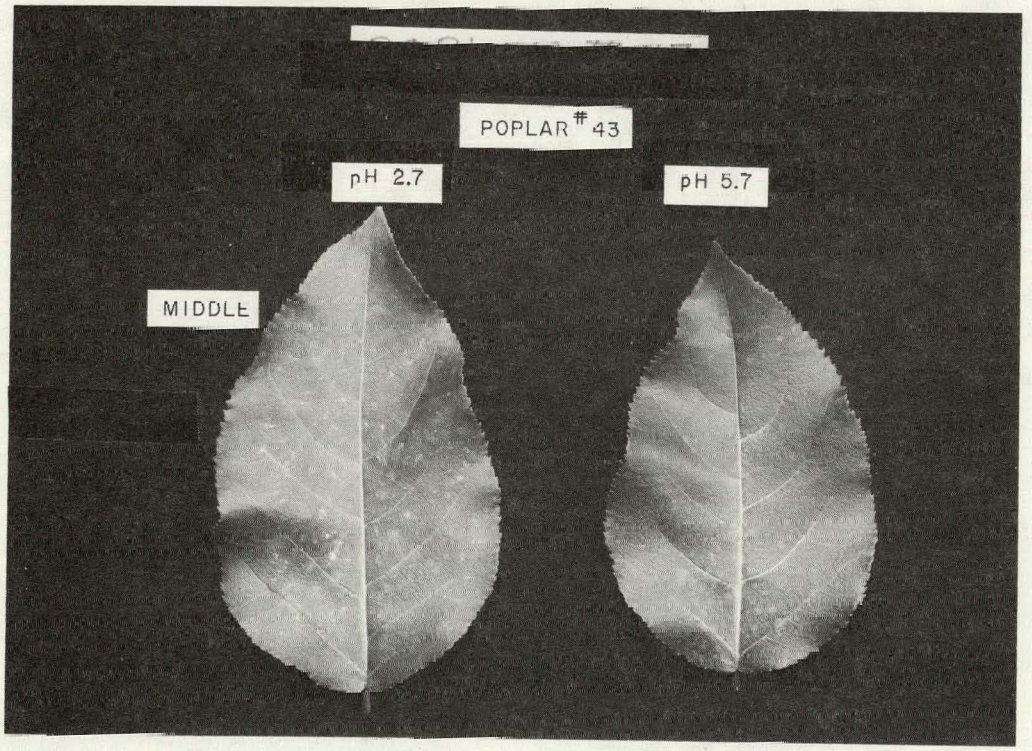

Figure 20. Leaf injury to a middle leaf of <u>Poplar</u> clone 43. Note individual lesions.

Figure 21. Photograph of culms and leaves of Tradescantia clone 02 that exhibits a glaborous surface on leaves.

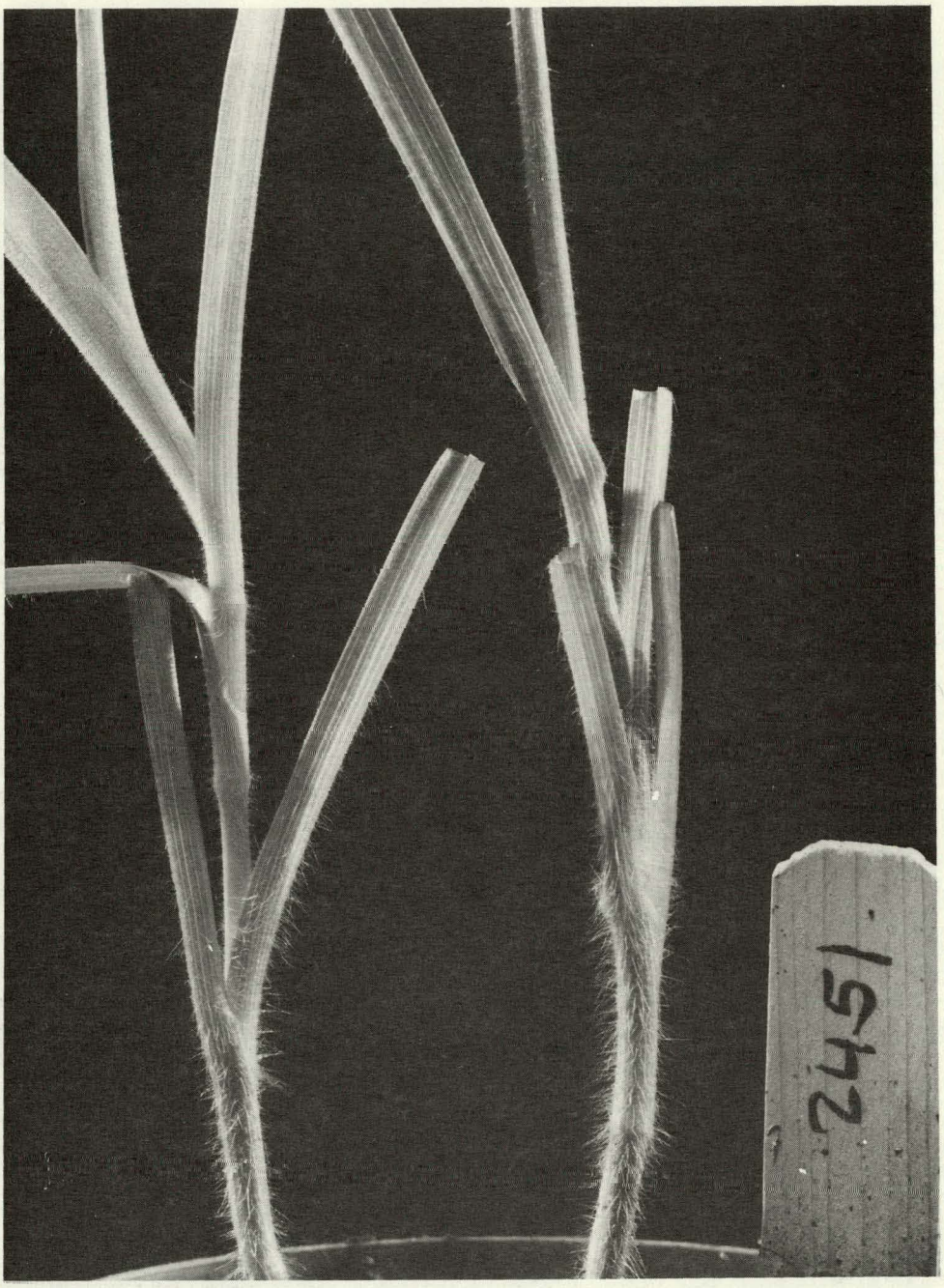

Figure 22. Photograph of pubescent culms and leaves Tradescantia paludosa clone 2451. This clone and Trad clone 02 are the parents of a cross. Four cloned segregates of the F_2 generation of this cross (two relatively glaborous and two pubescent) are to be used in future experiments.

Figure 23. Stamen hairs of flowers of Tradescantia paludosa that has been utilized by the late Arnold Sparrow to assay mutation rates after

(1) gamma radiation
(2) known gaseous and water soluble mutagens
(3) several gaseous air pollutants.

Experiments are planned to view the possible mutagenic effects of

(a) sulfur dioxide
(b) sulfate aerosols
(c) simulated sulfate acid rain.

Combinations of A, B, and C could be tested.

Errata to BNL 50575

Please insert the following paragraph in place of the last four lines on page 12.

Table 1 summarizes the chemical composition of the simulated acid rain solution applied in the treatments. The pH of each solution is adjusted to the desired pH with H_2SO_4. Rain was administered with the apparatus shown in figure 6. The drop sizes of the two nozzles used are outlined in Table 2.