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# Exploratory Monitoring of Air Pollutants for Mutagenicity Activity with the *Tradescantia* Stamen Hair System

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**The *Tradescantia* genetic system developed by the late Dr. Arnold H. Sparrow for the study of effects of ionizing radiation is applicable to chemical mutagen detection. Early radiobiological data demonstrated that the stamen hairs were sensitive to as little as 0.25 rad of x-rays and that the number of cells showing a phenotypic change in pigmentation from blue to pink plateaus after approximately 21 days of chronic, low-level irradiation. Exposures to the air pollutants $SO_2$, $NO_2$, and $O_3$ and to vapors of mutagens such as 1,2-dibromoethane (DBE) and ethyl methanesulfonate (EMS) demonstrated the usefulness of the system as a detector of chemical mutagens. A significant number of phenotypic changes was observed following exposures to as little as 0.14 ppm of DBE. The maximum sensitivity of the system is obtained with long-term or chronic exposures because the response increases linearly in proportion to the duration of exposure up to 21 days.**

**To monitor industrial sites for atmospheric mutagens a mobile laboratory was designed to support plant culture in the field. Environment-controlled growth chambers were installed in a trailer so that both ambient air fumigations and concurrent clean-air control exposures could be made. Sites monitored by the mobile laboratory were: Elizabeth, N. J.; Charleston, W. Va.; Birmingham, Ala.; Baton Rouge, La.; Houston, Tex.; Upland, Calif.; Magna, Utah; and Grand Canyon, Ariz. The latter site at Grand Canyon served as a clean air control study. Atmospheric contaminants from petroleum and chemical processing plants generated a significant number of phenotypic pigment changes that were 17 to 31% above the control levels; contaminants from steel and copper smelters, automotive combustion products and photochemical compounds were negative. Chemical analyses are underway to identify the atmospheric mutagens at the sites that showed a positive response.**

## Introduction

Several species in the family Commelinaceae, of which *Tradescantia* is a member, have features particularly well suited for certain radiation and chemical mutagen studies. The effects of chemicals and/or ionizing radiation that are easily measured include: chromosome aberrations in microspores, root tips, and stamen hairs; somatic mutations in petals and stamen hairs in clones heterozygous for flower color; pollen abortion; and cell mortality in stamen hairs. Of the four features mentioned, somatic mutation in stamen hairs is the most versatile, as it requires the least complicated techniques and is more sensitive than the other endpoints to both physical and chemical mutagens. The pattern and magnitude of response of phenotypic changes in pigmentation in stamen hair cells have been studied after treatment with x-rays (*1*), $\gamma$-rays (*2, 3*), $^3$H-$\beta$ rays (L. A. Schairer, unpublished data), nitrogen ions (*4*), monoenergetic neutrons (*5*), and low gravity of space flight (*6*). The parameters studied most extensively with x-rays and neutrons included dose rate (*7*), total dose (*1*), relative biological effectiveness (*5, 8*), and DNA per chromosome (*2*). The x-ray and neutron dose-response curves as well as those for chronic $\gamma$-

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exposures show straight-line relationships over wide dose ranges with no evidence of a threshold dose even at levels as low as 250 mrad of x-rays, 10 mrad of 0.43 MeV neutrons, and 33 mR/hr of $^{137}C_s$ $\gamma$-radiation (*1, 3*).

The significant mutagenic response to an accidental exposure to a gaseous chemical (*9*) as well as the high radiosensitivity were factors that prompted the use of *Tradescantia* as a test system to assay for the mutagenicity of various chemicals and air pollutants (*10, 11*). Newly developed chemical exposure and dosimetric techniques verified the high sensitivity of the *Tradescantia* stamen hair system to gaseous chemical mutagens and these demonstrated its potential for monitoring ambient air pollution for mutagenicity (*10, 12, 13*). Individual compounds or air pollutants can best be studied in the laboratory, but the mutagenicity of unusual and even unique ambient mixtures in urban or industrial sites must be assayed in the field. Perhaps the greatest advantage the stamen hair system affords over other test organisms is its versatility and adaptability to field studies.

## The *Tradescantia* Stamen Hair System

The stamen hair system has been described in detail elsewhere (*11, 14*) so only certain features will be reviewed here. Two clones of *Tradescantia* plants heterozygous for flower color were used in these experiments. Clone 02 is a putative hybrid from a field collection while clone 4430 is an interspecific hybrid (*T. subacaulis* × *T. hirsutiflora*) produced at Brookhaven (Fig. 1). Both clones are hybrids between pink- and blue-flowering parents, blue being dominant over pink. The genetics of pigmentation of clone 4430 in particular is currently receiving much attention and it is anticipated that within a year or two the transmission and characterization at the pink and blue loci will be described. The visible marker used in this test system is the phenotypic change in pigmentation from blue to pink in either petals or stamen hairs. The pigmentation change (hereafter called mutational or pink events) is induced in young developing floral tissue and is expressed 5 to 18 days later as isolated pink cells or groups of cells in the mature flower (Figs. 2 and 3). The pink events are essentially nonlethal so large mutant sectors indicate genetic injury early in the development of that tissue. Although other phenotypic changes such as colorless, plum-colored, dwarf, giant, and misshapen cells do occur, these were generally ignored for the present and the quantitative data gathered was limited to pink-

FIGURE 1. Normal stock plants of *Tradescantia* clones 4430 and 02 showing several mature inflorescences.

FIGURE 2. Shaded area of petal indicates pink mutant sector of flower. The large size of the sector indicates induction of the mutation early in the development of the bud.

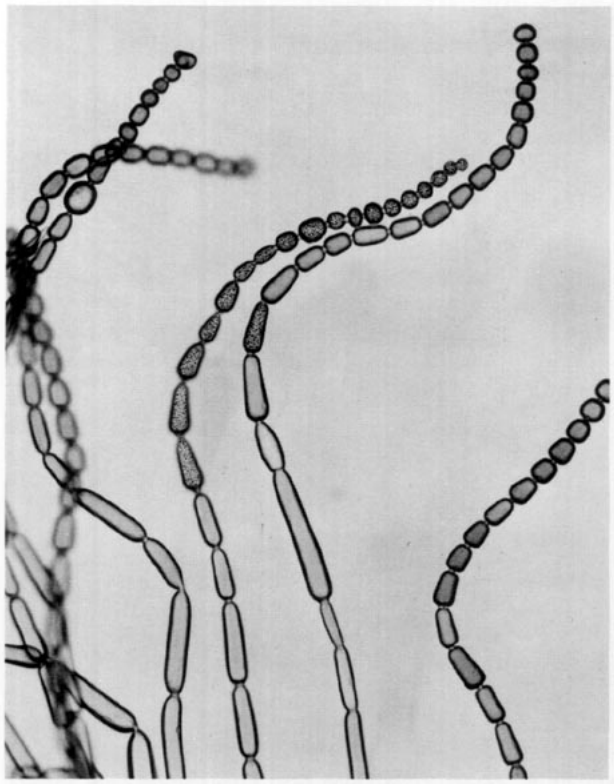

FIGURE 3. Enlargement of stamen hairs with pink mutant events indicated by shading. Mutant events in the flower color locus are not usually lethal; chains of pink cells represent daughter cells of the initial mutated cell.

celled events in stamen hairs. Pink events were considered to be the least ambiguous, allowed the most rapid counting (scoring) and color discrimination in stamen hairs was better than in petals.

The stock plants are easily maintained by vegetative propagation and flower continuously throughout the year in controlled environment growth chambers. The material treated consists of a group of unrooted, fresh cuttings including young inflorescences which contain flower buds in a range of developmental stages as shown in Figure 4. Following exposure to either chemical or physical mutagens, the cuttings are grown in aerated Hoagland's nutrient solution under standard conditions and the flowers are analyzed each day as they bloom for approximately three weeks after treatment. Induced pink event rates are expressed as the mean of the rates for several consecutive peak response days, usually days 11 to 15 for acute x-rays and 7 to 12 for acute chemical exposures (Fig. 5). Detailed descriptions of laboratory techniques for radiation and chemical exposures and calculating mutation rates are given elsewhere (*6, 10, 11*). However, the techniques for field exposures are new and they are described herein.

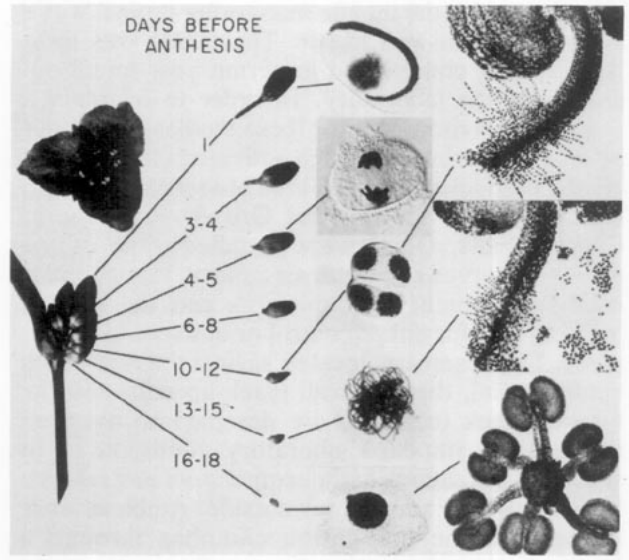


FIGURE 4. Exploded view of *Tradescantia* inflorescence showing representative stages of microspores and stamen hairs in relation to bud location and size.

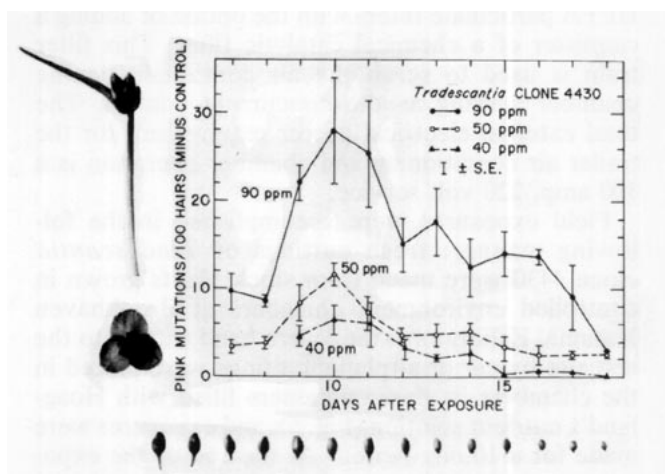


FIGURE 5. Pink mutation rates in *Tradescantia* stamen hairs following 6-hour exposure to gaseous EMS reach peak values 7–12 days after exposure. Pink events are analyzed in mature flowers; the corresponding bud sizes at time of treatment are shown along the abscissa.

## Techniques Employed for Ambient Air Exposures

The criteria for monitoring air pollution for mutagenicity include: a roadworthy vehicle to house the test organism during exposures; exposure of the test organism under suitable culture conditions; a constant flow of untempered ambient air; and a semichronic exposure capability to simulate natural exposures to plants and animals. The vehi-

cle selected for the mobile monitoring project was a 24-ft Clark mini-van trailer. The trailer was insulated and air conditioned to permit year-round operation of the laboratory. In order to maintain a semiclean environment for these studies, the trailer air was recirculated through activated charcoal and HEPA particulate filters. Three Model M-13 growth chambers (Environmental Growth Chambers, Chagrin Falls, Ohio) were installed. One of the chambers serves as a clean air control, the second is used for ambient air exposures and the third a back-up unit for either control or ambient air exposures. The chambers, located against the rear doors (ambient air), the side wall (back-up unit) and the forward deck (control), are designed to maintain any desired standard laboratory condition or to simulate fluctuations in the temperature and relative humidity of the ambient air outside. Ambient air is drawn into the fumigation chamber through a four-inch glass duct at continuous flow rates up to about 18 $ft^3$/min, a maximum of one air change every 2 min. Each chamber is equipped with an air filter train composed of activated charcoal and HEPA particulate filters with the option of adding a cannister of a chemical catalytic filter. This filter train is used to scrub the air continually in the chamber serving as the concurrent control. The total external electrical power requirement for the trailer air conditioning and chamber operation is a 100 amp, 220 volt service.

Field exposures were accomplished in the following manner: fresh cuttings of *Tradescantia* clone 4430 were made from stock plants grown in controlled environment chambers at Brookhaven National Laboratory; they were hand carried to the test site by car or airplane; cuttings were placed in the chambers in glass containers filled with Hoagland's nutrient solution (Fig. 6); and exposures were made for a 10-day period. At the end of the exposure the cuttings were taken back to Brookhaven National Laboratory for posttreatment analysis of the flowers as they bloomed each day. Exposures of a few hours to several weeks could be made, but 10 days was chosen for the *Trandescantia* plants, an interval long enough to maximize the sensitivity of the system and to simulate semichronic natural exposures yet short enough to permit sufficient flower analysis to be done back at Brookhaven. The peak mutation response period following a 10-day exposure is 11 to 17 days after the start of the exposure. The mean of the mutation rates for the 7-day scoring period resulted in an observed rate for a given test site based on an average stamen hair population between 300,000 and 400,000. A population of 300 cuttings in each ambient air and control chamber will yield enough data to resolve as small as a 10% increase in pink events over the background frequency.

FIGURE 6. *Tradescantia* clone 4430 floral cuttings are shown prepared for exposure. Sixty cuttings per dish are grown in aerated Hoagland's nutrient solution during and after treatment.

## Review of Stamen Hair Mutation Response to Acute and Chronic Irradiation

As outlined in the Introduction, a great deal of radiobiological background data exist for the stamen hair system in both clones 02 and 4430. Since many of the principles and techniques of radiobiology are applicable to chemical mutagen studies, certain radiation curves can be used as standards for comparison with those from chemicals. The typical acute x-ray dose-response curves for the two standard clones, shown in Figure 7, demonstrate the good linearity and extreme range of response over at least three log cycles. The high sensitivity of the stamen hair system is indicated by the significant response to as low as 250 mrad and with no evidence for a threshold dose. An even more important response is that for chronic radiation since most exposures to air pollutants are chronic. Data from chronic $\gamma$-ray exposures of clone 02 indi-

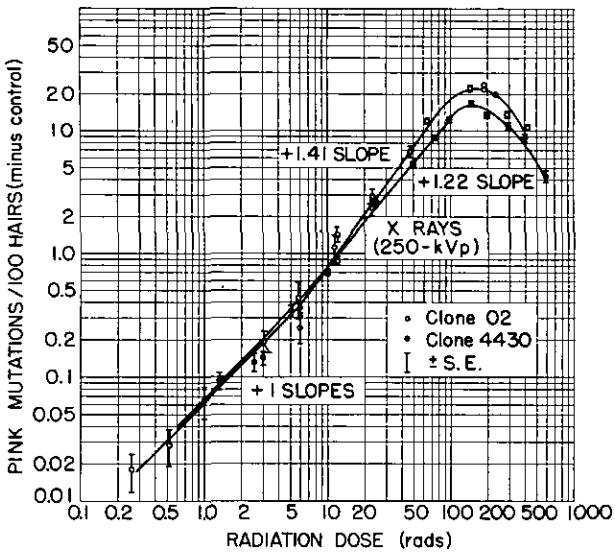


FIGURE 7. Log-log plot shows great similarity in the frequency of pink events in stamen hairs of *Tradescantia* clones 02 and 4430 following acute x-ray exposures.

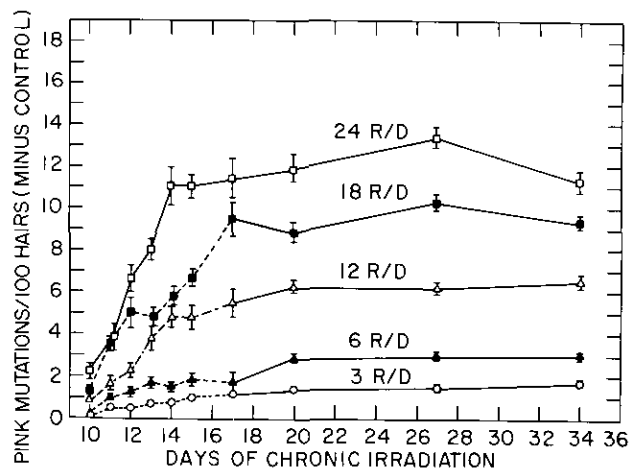


FIGURE 8. Frequency of pink events produced in the hairs of clone 02 during chronic gamma exposure. Frequency of pink events effectively reaches a plateau after about 3 weeks of exposure.

cate an accumulation of pink events for the first three weeks followed by a plateau for as long as the radiation continued (Fig. 8). These data suggest that the maximum sensitivity for the stamen hair system is obtained after about three weeks of exposure and that the plateau response values are proportional to exposure rates and not total dose.

## Chemical Exposures under Laboratory Conditions

Exposures to a standard chemical mutagen, the alkylating agent 1,2-dibromoethane (DBE), in the gaseous state, showed that the number of mutational events increased with exposure time to DBE, at least over the range from 2 to 144 hr, or up to one week (Fig. 9). These data may also be expressed in terms of total dose by plotting induced mutation frequency against the product of concentration and duration of exposure (Fig. 10). For purposes of comparison, a curve for x-ray effect is shown in rads with radiation total dose defined as fluence × time. Slope and shape of the curve for DBE induction of color change resemble those for radiation injury.

Although a large percentage of the effort of this group has been spent on the development of the mobile monitoring vehicle, a number of chemicals have been tested in the laboratory to validate further the system as a monitor for gaseous mutagens. Typical dose-response curves for several chemicals are shown in Figure 11. Chemicals such as the

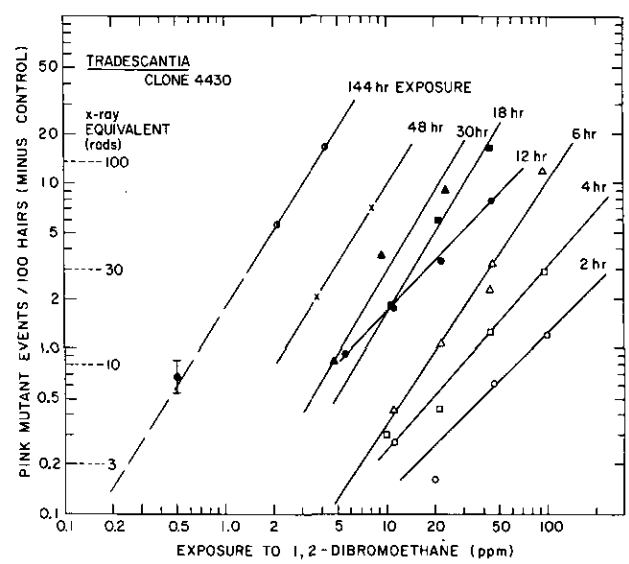


FIGURE 9. Log-log plot of pink events versus gaseous exposure to 1,2-dibromoethane for various exposure periods. X-ray dose-effect scale is given along ordinate for reference.

gasoline additives 1,2-dibromoethane (DBE) and trimethyl phosphate (TMP) were found to be potent mutagens while $SO_2$, $NO_2$, vinyl chloride, and Freon-12 were weak mutagens according to this test system. Other chemicals or air pollutants tested are listed in Table 1. The concentration listed is the lowest value tested which showed a significant mutagenic response.

Mutational events with low-dose experiments were confounded by seasonal variation in background mutation frequency with a consistent cyclic

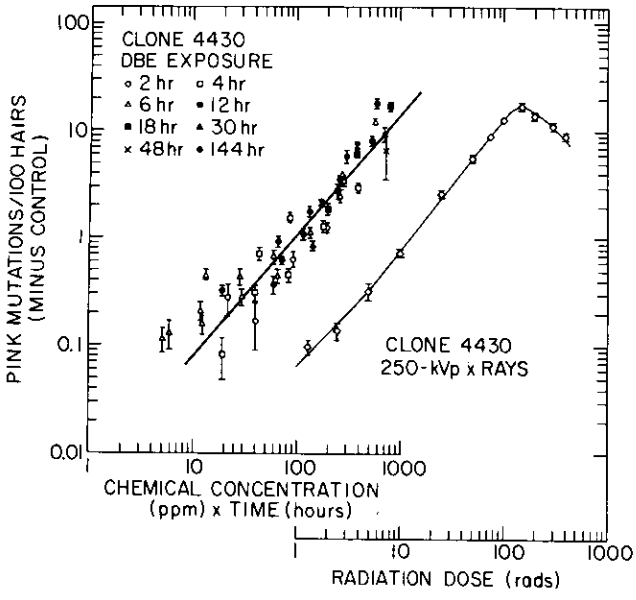


FIGURE 10. Data from Fig. 9 plotted as total dose (ppm × hours of exposure) vs. mutational event frequency. A linear response curve fits all data points from 2- to 144-hr exposures. The standard acute x-ray curve is shown for comparison.

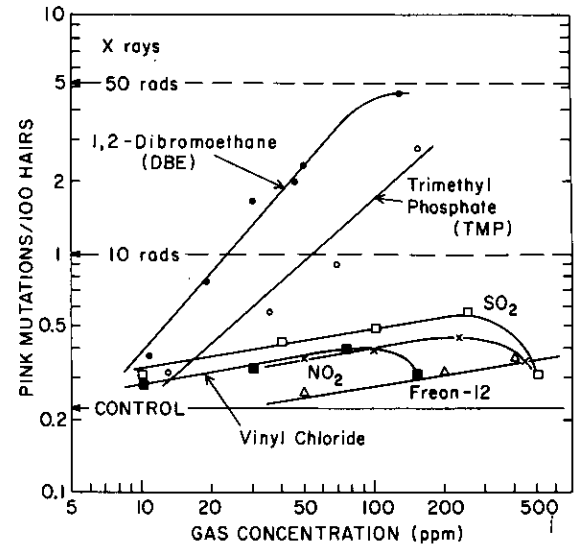


FIGURE 11. Typical dose-response curves for pink events in *Tradescantia* clone 4430 are shown following 6-hr exposures to various gaseous compounds.

**Table 1. Summary of mutation response data for various chemicals used on clone 4430 in terms of lowest concentration giving significant effect.**

| Chemical | Exp. time, hr | Minimum concn, ppm[a] | Hairs scored ×10³ | Total pink events | Pink events per 100 hairs (−Control) | ± SE | Statist. signif., % |
|---|---|---|---|---|---|---|---|
| Air pollutants | | | | | | | |
| Ozone ($O^3$) | 6 | 5.0 | 48 | 153 | 0.098 | 0.040 | 2 |
| Sulfur dioxide ($SO_2$) | 6 | 40 | 41 | 170 | 0.222 | 0.041 | 1 |
| Nitrogen dioxide ($NO_2$) | 6 | 50 | 24 | 87 | 0.112 | 0.056 | 5 |
| Nitrous oxide ($N_2O$) | 6 | 250 | 29 | 115 | 0.117 | 0.055 | 1 |
| Industrial chemicals | | | | | | | |
| Ethyl methanesulfonate (EMS) | 6 | 5 | 20 | 246 | 1.012 | 0.133 | 1 |
| 1,2-Dibromoethane (DBE) | 6 | 1 | 258 | 1088 | 0.118 | 0.027 | 1 |
| | 144 | 0.14 | 148 | 1119 | 0.315 | 0.035 | 1 |
| Trimethylphosphate (TMP) | 6 | 13 | 32 | 115 | 0.125 | 0.051 | 2 |
| Trichloroethylene (TCE) | 6 | 0.5 | 44 | 148 | 0.112 | 0.036 | 1 |
| Vinyl chloride (VC) | 6 | 75 | 34 | 133 | 0.112 | 0.046 | 2 |
| | 24 | 25 | 56 | 281 | 0.151 | 0.041 | 1 |
| Vinylidene chloride (VDC) | 6 | 86 | 30 | 130 | 0.064 | 0.056 | NS |
| | 24 | 22 | 100 | 338 | 0.057 | 0.028 | 5 |
| Vinyl bromide (VB) | 24 | 50 | 49 | 201 | 0.159 | 0.048 | 1 |
| 2-Bromoethanol (2BE) | 6 | 24 | 33 | 131 | 0.107 | 0.046 | 2 |
| Freon-12 (Fr-12) | 6 | 392 | 32 | 103 | 0.095 | 0.059 | NS |
| Freon-22 (Fr-22) | 6 | 194 | 66 | 249 | 0.100 | 0.039 | 2 |
| Hexamethylphosphoramide (HMPA) | 6 | ? | 48 | 314 | 0.277 | 0.051 | 1 |
| Benzene | 6 | 4000 | 43 | 292 | 0.287 | 0.063 | 1 |
| Caffeine | Chronic | $10^{-4}M$ | 39 | 142 | 0.047 | 0.040 | NS |
| Atrazine | Chronic | 0.045 g/pot | 93 | 260 | 0.0 | 0.0 | NS |
| Sodium azide | 3 | $10^{-4}M$ | 19 | 96 | 0.269 | 0.055 | 1 |
| 1,1-Dibromoethane | 6 | 58 | 56 | 219 | 0.073 | 0.039 | NS |
| Dimethylamine hydrochloride | 2 | $10^{-2}M$ | 16 | 83 | 0.151 | 0.080 | NS |
| Vapona | 6 | Sat? | 81 | 278 | 0.0 | 0.0 | NS |

[a] Minimum concentration used which showed a significant increase over background mutation rate.

high rate in the summer months (Fig. 12). The seasonal effect very likely was the result of ambient air pollution since a clean-air chamber (one with a filtered atmosphere) eliminated much of the background variability still seen in plants grown in a standard control chamber (Fig. 13). Henceforth, filters of the type used in the clean-air chamber (activated charcoal and HEPA filters) were employed in stock plant chambers and in the mobile monitoring vehicle to provide more stable baseline control data. The fact that the system responded to differences in filtered versus nonfiltered ambient air was further evidence for the high sensitivity of the color locus and supported its use as a monitor for the mutagenicity of air pollution.

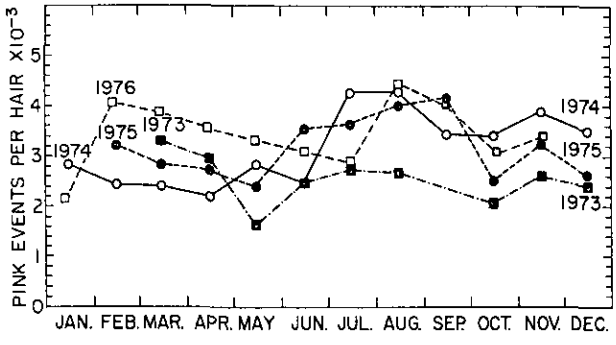


FIGURE 12. Seasonal variation in frequencies of spontaneous pink events of clone 4430 grown in controlled environment chambers. Data points are monthly averages of control values from experiments over four successive years.

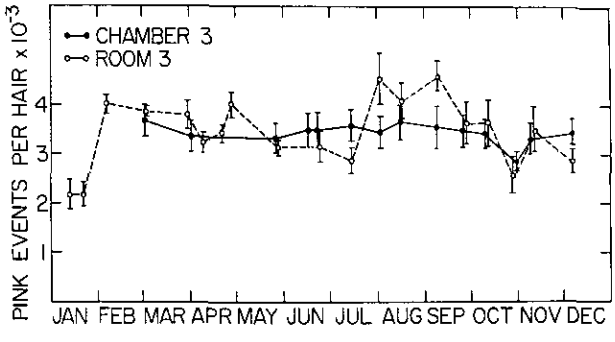


FIGURE 13. Frequencies of spontaneous pink events in stamen hairs of clone 4430 plants grown in either clean air (chamber 3) or standard chamber air (room 3).

## Results of Exposure to Ambient Air Pollution

The first field trials for the mobile monitoring vehicle (MMV) were conducted in the summer of 1976. A location was sought which had high levels of a mixture of pollutants and one which was within about a two-hour drive from Brookhaven National Laboratory.

The first test site selected was in Elizabeth, N. J., beside a New Jersey air pollution monitoring station (Fig. 14). The New Jersey Turnpike, toll plaza, petroleum refineries, Newark Airport, and other industrial pollution sources surround this test site. Two-week exposures were made in July and October 1976 and January 1977 and the data indicate increases in mutational events following exposure to ambient air for all three periods, which were significant at the 1% level (Table 2). The July run yielded a response equivalent to that from a chronic $\gamma$ exposure of about 10 mrad/hr in this *Tradescantia* system. In the third two-week exposure, January 1977, two chambers were exposed to ambient air in order to demonstrate that the induced effects observed in the previous two runs were real and not a unique chamber effect in the third control chamber. Data from the ambient air samples were not different from each other, but both were significantly higher than the concurrent control. Apparently no unique chamber effect exists between chambers, even under field conditions.

Wind direction is an important factor in the location of a mobile monitoring unit. The high induced mutation rate in July occurred with prevailing southwesterly winds, while the October run had prevailing northwesterly winds (Fig. 14). Pollution sources were certainly different in these two exposures, but a much more sophisticated air monitoring facility and a detailed map of the greater Elizabeth area would be required to identify the environmental mutagen and its probable source.

These data were encouraging and supported the use of the *Tradescantia* test system as a field monitor for air pollution. To continue the study, a series of exposures was planned with test sites selected because of high cancer mortality or presumed exposure to high levels of carcinogens (*15*). The MMV experiments were to look for biological activity, while the EPA mobile monitoring van made real-time measurements of the pollution levels. Organic vapors were collected on Tenax absorbers for subsequent identification and quantification. The sites selected for this phase of the study were: Charleston, W. Va., Birmingham, Ala., Baton Rouge, La., Houston, Tex., Upland, Calif.,

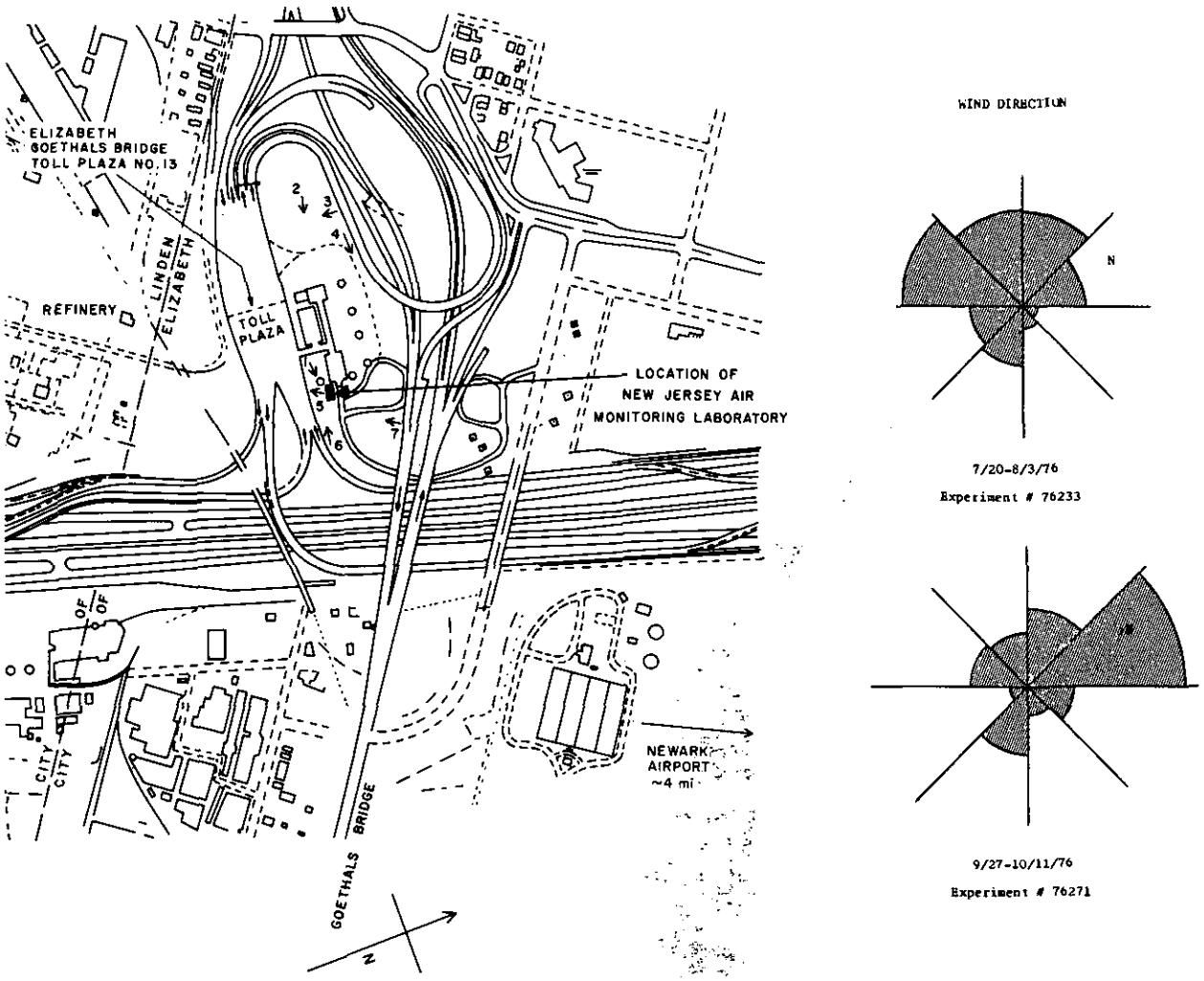


FIGURE 14. Diagrammatic view of New Jersey test site showing some of the sources of high ambient air pollution. MMV was located beside New Jersey Air Monitoring Laboratory (see arrow). Prevailing winds are shown for two exposures.

**Table 2. Mutagenicity of ambient air at Elizabeth, N.J. as measured by *Tradescantia* stamen hairs.**

| Treatment | No. flowers | No. hairs | No. pink events | Events/hair ± S.E. |
|---|---|---|---|---|
| Control | 726 | 299,475 | 1182 | 0.00395 ± 0.00013 |
| Ambient air | 658 | 268,464 | 1386 | 0.00516 ± 0.00016 |
| 7/20-8/3/76 | Ambient air minus control | | | 0.00122 ± 0.00021[a] |
| Control | 892 | 350,824 | 1487 | 0.00424 ± 0.00012 |
| Ambient air | 890 | 358,047 | 1727 | 0.00482 ± 0.00012 |
| 9/27-10/11/76 | Ambient air minus control | | | 0.00058 ± 0.00012[a] |
| Control (1) | 689 | 266,023 | 872 | 0.00328 ± 0.00012 |
| Ambient air (2) | 742 | 291,161 | 1146 | 0.00394 ± 0.00013 |
| | Ambient air (2) minus control | | | 0.00066 ± 0.00017[a] |
| Ambient air (3) | 617 | 231,557 | 873 | 0.00377 ± 0.00014 |
| | Ambient air (3) minus control | | | 0.00049 ± 0.00018[a] |
| Ambient air (2+3) | 1359 | 522,718 | 2019 | 0.00386 ± 0.00009 |
| 1/21/-2/4/77 | Ambient air (2+3) minus control | | | 0.00058 ± 0.00015[a] |

[a] Significant at the 1% level.

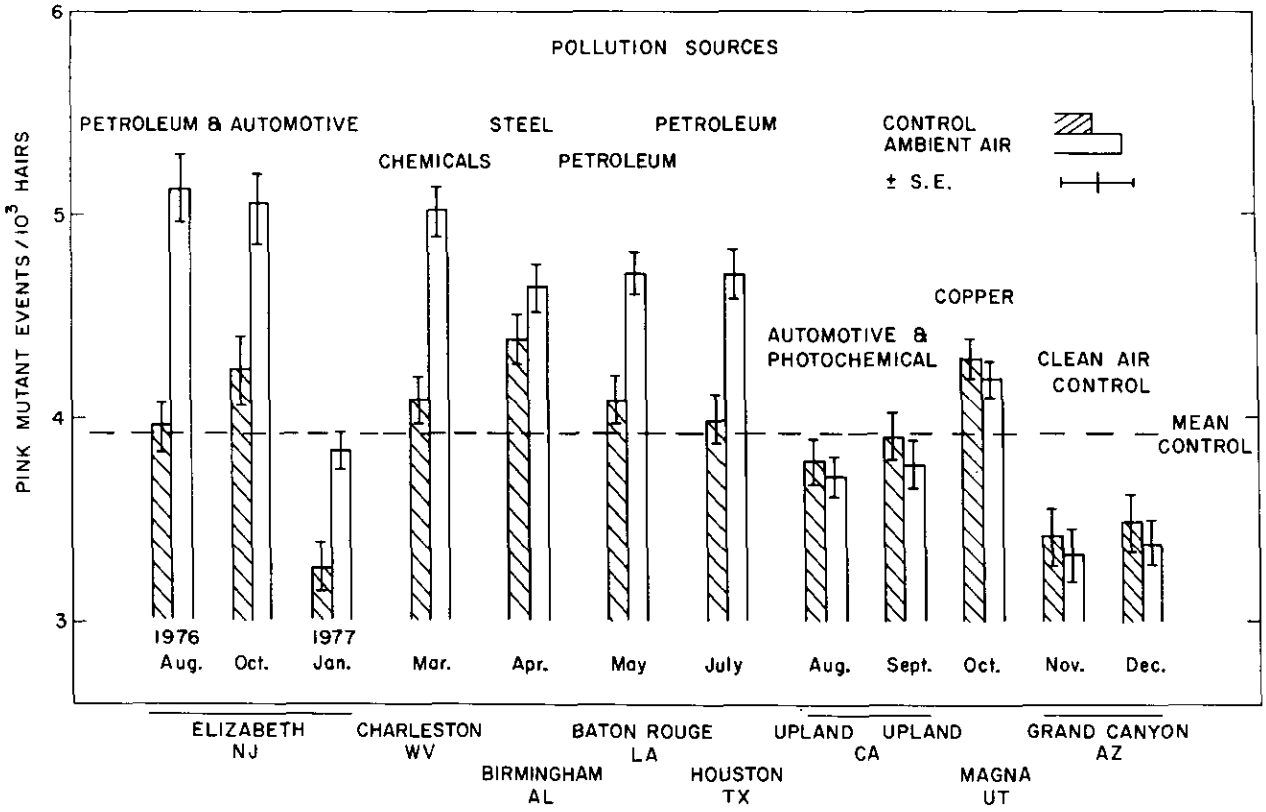


FIGURE 15. Mutagenicity of ambient air as measured by *Tradescantia* in the mobile monitoring vehicle is summarized for the eight test sites visited.

Magna, Utah, and Grand Canyon, Ariz. The latter site at Grand Canyon served as a clean air control study.

The results of these field exposures are summarized graphically in Figure 15. The pollution sources indicated here are only general categories under the heading of the major industries in the area and do not imply a known correlation between mutation response and specific industrial effluent. Statistically significant increases in mutant event frequencies above control levels were observed at Elizabeth, Charleston, Baton Rouge, and Houston. The remaining locations, especially the clean-air site at Grand Canyon, showed no significant response to ambient air. These data are also shown in Table 3 arranged by pollution source and presented as a pollution-induced increase in mutation rate as percent of control. Locations associated with petroleum refining and mixed chemical processing gave increases ranging from 31% down to 17%. The real-time measurements of both organic and inorganic compounds are being analyzed at the present time, and, when completed, these results may provide more specific identification of compounds common to those sites showing induced mutations.

It should be emphasized that a negative response in a single exposure of a test organism may provide inadequate assurance of absence of a health hazard. As pointed out in the Elizabeth experiment, the prevailing wind direction changed from summer to fall and the induced mutation frequency dropped from 31 to 18%. Wind direction, amount of precipitation, industrial complex work schedule, etc., all have a direct bearing on the pollution mixture and level at a fixed monitoring location.

## Conclusion

The body of evidence is growing for a meaningful extrapolation from cytological and genetic effects in microorganisms, cell cultures, plants, insects, and mammals to health hazards in man. The high correlation between mutagenicity and carcinogenicity supports the use of visible genetic markers in test organisms as monitors for carcinogens. The observation of similar chromosome aberrations in both

Table 3. Mutagenicity of ambient air at various industrial sites as measured by *Tradescantia* stamen hairs.

| Site location | Date of exposure | Pollution source | Pollution-induced events/$10^3$ hairs ± S.E. | Increase, % of control |
|---|---|---|---|---|
| Elizabeth, N. J. | July 1976 | Petroleum and automotive | 1.22 ± 0.21 | 30.9 |
| | Oct. 1976 | Petroleum and automotive | 0.80 ± 0.23 | 18.8 |
| | Jan. 1977 | Petroleum and automotive | 0.58 ± 0.15 | 17.7 |
| Baton Rouge, La. | May 1977 | Petroleum | 0.61 ± 0.16 | 15.0 |
| Houston, Texas | July 1977 | Petroleum | 0.72 ± 0.17 | 18.1 |
| Charleston, W. Va. | March 1977 | Chemicals | 0.71 ± 0.16 | 17.1 |
| Birmingham, Ala. | April 1977 | Steel | 0.24 ± 0.17 | 5.5 |
| Magna, Utah | Oct. 1977 | Copper | −0.10 ± 0.14 | −2.3 |
| Upland, Calif. | Aug. 1977 | Automotive and photochemical | −0.08 ± 0.15 | −2.1 |
| | Sept. 1977 | Automotive and photochemical | −0.15 ± 0.17 | −3.8 |
| Grand Canyon, Ariz. | Nov. 1977 | Clean air | −0.10 ± 0.14 | −2.9 |
| | Dec. 1977 | Clean air | −0.10 ± 0.18 | −2.9 |

gametic and somatic tissues gives cytological evidence for the effectiveness of somatic mutation markers as an assay for chemical mutagenicity and hence health hazard potential. The *Tradescantia* stamen hair system encompasses the cytogenetic and somatic potential to make it a useful tool for mutagenicity monitoring of ambient air pollution mixtures. This plant is uniquely adapted to field exposures, hardy enough to tolerate a broad range of environmental conditions, and requires no elaborate sterile culture conditions. The data presented demonstrate the high sensitivity of the system to gaseous compounds and the relatively short time from start of exposure to definition of results (< 3 weeks). In the absence of hard genetic evidence for extrapolation from plants to man, at least this system can become part of a battery of tests which can provide early warning of the potential health hazard of exposure to mixed air pollutants.

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