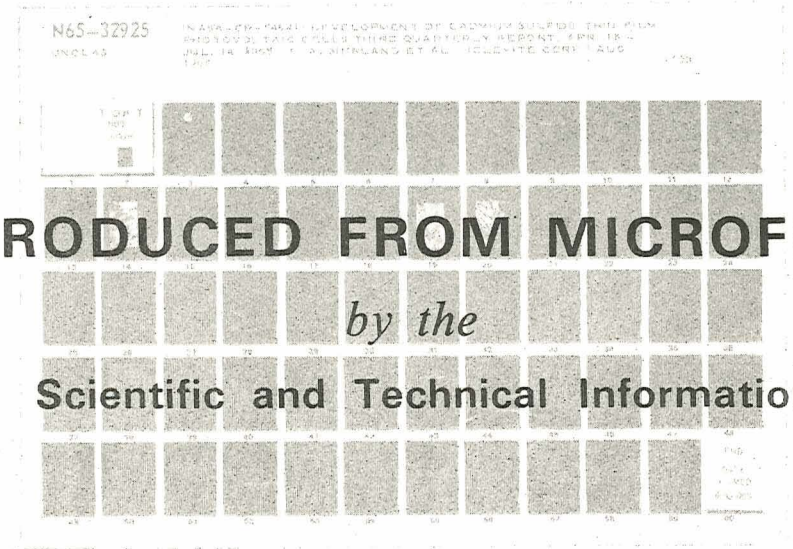
a NASA facsimile reproduction

OF



REPRODUCED FROM MICROFICHE

by the

NASA Scientific and Technical Information Facility

FACILITY FORM 602

N70-16937 (ACCESSION NUMBER)

(THRU)

10 (PAGES)

1 (CODE)

CR-107799 (NASA CR OR TMX OR AD NUMBER)

(CATEGORY)

AW/69-7

Paper # BNL 13623

CONF-690516--1

MASTER

RECEIVED BY OSTI

RADIOBIOLOGICAL STUDIES OF PLANTS ORBITED IN BIOSATELLITE II

L. A. Schairer, A. H. Sparrow and K. M. Marimuthu

Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Research supported in part by the U. S. Atomic Energy Commission and in part by the National Aeronautics and Space Administration (Purchase Order R104-7).

To be presented at the Space Biology Session of Working Group V of the COSPAR Meeting in Prague, Czechoslovakia, May 11-24, 1969, and to be published in the Proceedings.

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

ABSTRACT

Radiobiological Studies of Plants Orbited in Biosatellite II.

L. A. Schairer, A. H. Sparrow and K. M. Marimuthu. Biology Department Brookhaven National Laboratory, Upton, New York 11973 U.S.A.

The Biosatellite II Tradescantia experiment probed the effects of the space environment on spontaneous and radiation-induced mutation rates and on cytological changes in Tradescantia clone 02. Thirty-two young flowering plants were arranged in a plastic housing with the roots immersed in nutrient solution, were exposed to gamma radiation from an on board 85 Strontium source during the two-day orbital flight. Unirradiated plants were flown in a package in the spacecraft behind a tungsten radiation shield and identical nonflight control packages (with and without irradiation) were maintained at the launch site. After retrieval of the spacecraft near Hawaii, samples of root tip, ovary and stamen tissues were collected. These and the intact plants were flown to Brookhaven for observations on the following endpoints: somatic mutation, cell size, loss of reproductive integrity resulting in stunted stamen hairs, pollen grain mortality, frequency of micronuclei in pollen, disturbed mitotic spindle function and chromosome aberrations. Analysis of data on somatic mutation, cell size and chromosome aberration endpoints showed no significant differences between flight and nonflight samples. However, pollen abortion, frequency of micronuclei in pollen and loss of reproductive integrity (stamen hair stunting) showed increases associated with weightlessness in irradiated material. Root tip and microspore cells showed effects of disturbed mitotic spindle function in orbited plants both with and without irradiation. Clearly differences exist between flight and nonflight material and the significance and possible mechanisms for these effects are being studied in continuing nonflight tests. (Research supported by NASA (Purchase Order R104-7) and by USAEC.)

Spaceflight factors acting alone or with ionizing radiation have been shown to induce both synergistic and antagonistic effects as well as no significant differences, depending on the organism and assay system used. This paper discusses the current status of the experiments with the flowering plant Tradescantia and summarizes briefly the final results of the experiment with the bread mold Neurospora crassa in the Biosatellite II mission.

MATERIALS AND METHODS

The Tradescantia experiment on board Biosatellite II[1] was designed to determine the effects of weightlessness and other spacecraft environmental conditions on spontaneous and radiation-induced mutation rates and on various cytological changes in a special clone of the higher plant, Tradescantia. This hybrid clone of Tradescantia has a chromosome number of 12; it is heterozygous for flower color; and it has a high mutation rate at the locus determining flower color (scorable in both petal and stamen hair cells). During the 45 hr Biosatellite flight, 32 young plants were arranged in a plastic housing so that the flower buds were exposed to about 220 R of gamma rays while the roots, immersed in nutrient, were exposed to known radiation levels from about 125 to 285 R. Thirty-two flight control plants were flown in an identical package in the spacecraft behind a tungsten radiation shield and similar unflown control packages (with and without irradiation) were maintained at the launch site. The internal spacecraft environment was rigorously controlled and conditions monitored throughout the flight. The temperatures were maintained between 65 and 70° F in the Tradescantia packages, the atmospheres held at about 55% relative humidity and the gravitational force at about 10^{-5} g

during the irradiation phase of the flight. Telemetered and on-board records were made of vibration, shock, acceleration, etc. for use in subsequent tests. Immediately after retrieval of the spacecraft near Hawaii, root-tip, ovary and stamen tissues were collected. These and the intact plants were flown to Brookhaven for observations on the following end points: somatic mutation, cell size, loss of reproductive integrity (cell death and stunting in stamen hair growth), pollen grain mortality, spindle malfunction and chromosome aberrations.

Following preliminary analysis of the engineering and biological data, several post-flight Earth-based experiments were performed under conditions as close as possible to those of the flight. These tests, run in the same flight hardware, were designed to establish or recheck base-line data on the effects of irradiation, vibration, biocompatibility of the spacecraft, etc.

RESULTS:

The somatic mutation rates were determined for pigment changes from the dominant blue to pink in both petals and stamen hairs and to colorless in only the stamen hairs (see Table 1). The mutation rates in general showed the expected increase due to the gamma exposure but the flight and nonflight samples showed no differences attributable to flight factors. Although the peak mutation rate for the pink stamen hair cells was lower than expected in the flight sample, little significance was attached to this reduced rate because a similar result was observed in a postflight test. All unirradiated plants had consistently low mutation rates and showed no differences between flight and nonflight samples.

The frequency of dwarf and giant cells increased following gamma exposure, but there were no significant differences in the peak values of flight and nonflight irradiated samples.

The loss or partial loss of reproductive integrity of terminal or subterminal stamen hair cells results in a short or stunted hair. Hair lengths normally vary somewhat, but the incidence of stunted hairs increases with increasing exposure and postirradiation time. The percentage of stunted hairs generally was higher in the flight-irradiated than in nonflight-irradiated samples and average values for the period between 9 and 17 days after retrieval are given in Table 1. Many more very short hairs (5 cells or less) were observed in the flight sample than in the comparable non-flight sample, indicating that the same radiation exposure produced a greater deleterious effect on cell division in the biosatellite than in the Earth-based plants.

Mature pollen was collected daily, and, after staining with cotton blue, the percentages of aborted pollen were determined for all four treatments (see Table 1). It should be emphasized that this clone of Tradescantia is characterized by an abnormally high rate of spontaneous pollen abortion (average control levels were 37 and 39% for flight and ground controls, respectively). The data, however, indicate that there was generally a higher abortion rate in the flight-radiation sample than in the nonflight-radiation sample with some points being significantly different at about the one percent level. These results were further supported by the data on the frequency of micronuclei observed after staining with propiono carmine (see Table 1).

The 2-fold increase on certain days in frequency of micronuclei in

the irradiated flight sample reflects an enhanced effect of flight factors and irradiation on cells treated during the sensitive stages of meiosis (14-18 days before anthesis).

Collections of young microspores made after flight showed an unexpectedly high death rate in both flight samples. All of the microspores in buds treated during microspore mitosis had an abortion rate of more than 95%. Subsequent Earth-based tests in the flight vehicle (301) produced similar microspore death to that of the orbited material. This effect may therefore be due to some environmental variable in the vehicle rather than to flight conditions. Preliminary experiments indicate that ethylene may be one of the factors contributing to this cell death.

In the surviving fraction (<5%) of the microspores, abnormalities reflecting disturbances in the spindle mechanism were observed at a much higher frequency in the flight control (27.54%) than in the nonflight control (0.18%).

Evidence of a disturbance in the spindle mechanism also was seen in root tips of flight material in the form of multinucleate cells and in peculiarly-shaped nuclei. Table 1 gives the percentage of such cells in nonflight and flight materials. The percentages are small when expressed as a per cent of all cells scored but would be much larger if only cells at metaphase to late telophase stages were scored. However, it is apparent that not all cells in division showed evidence of a spindle inhibition or malfunction.

Chromosome aberrations were scored at metaphase in root-tip cells in material from all treatments. No significant differences were found between

data from the respective control and irradiated flight and nonflight samples.

Other post-flight tests included studies of the effects of vibration and the clinostat on the various end points used. Work with the clinostat (2 revolutions per minute) with and without irradiation can be quickly summarized as having had no effect on any of the end points scored. Flight-simulated vibration tests on both the entire capsule and individual packages also failed to show any significant effect on the Tradescantia plants.

The results of the Biosatellite II and Gemini XI Neurospora experiments of de Serres, et al. of the Oak Ridge National Laboratory [2,3] will be summarized only briefly here since this work is essentially complete and has been reported at the last COSPAR Meeting (May, 1968).

Dose response curves were made from data on cellular inactivation and the induction of mutations (recessive lethal mutations occurring over the entire genome as well as at two specific loci). In both the Biosatellite II and Gemini XI experiments, using samples collected on millipore filters, there were no effects of spaceflight alone or with ionizing radiation on inactivation or mutation-induction. However, when the Neurospora samples were flown in suspension in the Gemini XI experiment the irradiated samples showed a higher survival rate and significantly lower rate of specific locus mutations than the nonflight samples.

CONCLUSIONS

Thus there are some effects on spontaneous and radiobiological events in Tradescantia plants which may be attributed to orbital and simulated flight environments. These effects are rather small but significant and appear to be

related to effects on normal cell division and cell survival. Bud blasting and microspore death are probably due to some vehicle environmental effects and not flight factors. High rates of pollen abortion, stamen hair stunting and formation of micronuclei are unique to the irradiated, orbited sample and reflect an enhanced interaction between radiation and weightlessness during the more sensitive stages (meiosis and mitosis). Nuclear misorientation associated with mitotic spindle malfunction in pollen and root tip cells were increased significantly in both irradiated and unirradiated flight samples as compared with simulated flight samples. These latter abnormalities were similar to those observed by the Soviet scientists [4,5] and are probably attributable to weightlessness alone but may conceivably be due to weightlessness preceded and/or followed by vibration or other dynamic factors associated with orbital flight. Clearly differences exist between flight and nonflight samples both with and without irradiation. The significance and possible mechanisms for these effects as well as developmental studies involving orbited megaspore tissues are being investigated in continuing nonflight tests.

In the Neurospora experiment the genetic effect of radiation on nondividing and metabolically inactive cells was identical for flight and nonflight samples (the samples collected on millipore filters). Differences were found between flight and nonflight samples when metabolically active cells (the samples in suspension) were used.

REFERENCES

1. Sparrow, A. H., L. A. Schairer and K. M. Marimuthu. 1968. Genetic and cytologic studies of Tradescantia irradiated during orbital flight. BioScience 18: 582-590.
2. deSerres, F. J. and B. B. Webber. 1968. Inactivation and mutation-induction in Neurospora resulting from combined radiation and weightlessness on the Biosatellite II mission. Bioscience 18: 590-595.
3. deSerres, F. J., I. R. Miller, D. B. Smith, S. Kondo, and M. A. Bender. 1969. The Gemini-XI S-4 spaceflight radiation interaction experiment II. Analysis of survival levels and forward-mutation frequencies in Neurospora crassa. Radiation Res. (in press).
4. Delone, N. L., V. F. Bykovskiy, V. V. Antipov, G. P. Parfenov, V. G. Vysotskiy and N. A. Rudneva. 1964. Effect of space factors on the microspores of Tradescantia paludosa in the Vostok 5 and Vostok 6 spacecraft. NASA TT F-8896. Natl. Aeronautics and Space Administration, Washington, D. C. (Transl. from Russian). 17 p. Original publ. in Kosmicheskiye Issled. 2: 320-329 (1964).
5. Delone, N. L., A. S. Trusova, E. M. Morozova, V. V. Anmunov and G. P. Parfenov. 1968. Effects of space flight of the sputnik Cosmos-110 on the microspores of Tradescantia paludosa. Kosmicheskiye Issled. 6: 299-303.

Table 1. Summary of Data for Various Tradescantia Endpoints from Biosatellite

Endpoints	Flight (301) Irrad.	Flight (301) Unirrad.	Nonflight (201) Irrad.	Nonflight (201) Unirrad.
NO SPACE FLIGHT EFFECTS				
Mutations (somatic)				
Pink/petal	20.6 ± 2.9	1.4 ± 0.2	15.4 ± 2.9	1.1 ± 0.3
Pink/100 stamen hairs	4.2 ± 0.8	0.3 ± 0.2	8.2 ± 1.3	0.2 ± 0.1
Colorless/100 stamen hairs	7.6 ± 1.6	0.1 ± 0.1	7.3 ± 1.1	0.3 ± 0.1
Dwarf cells/100 hairs	12.3 ± 1.4	3.2 ± 0.6	15.4 ± 1.7	3.0 ± 0.7
Giant cells/100 hairs	5.18 ± 0.67		4.51 ± 0.70	
Chromosome aber./cell (Roots)	0.53 ± 0.091	0.04 ± 0.021	0.48 ± 0.096	0.02 ± 0.01
ENHANCED EFFECTS				
Loss of reprod. integrity (stunting)/100 hairs	27.0 ± 2.6	10.3 ± 1.5	12.9 ± 1.7	8.2 ± 1.2
Pollen abortion (%)	69.8 ± 5.7	37.4 ± 4.5	49.6 ± 5.8	39.4 ± 5.8
Micronuclei/100 cells Pollen	21.1	4.0	10.5	2.9
Microspore death* % buds	100%	100%	16.7%	33.3%
Disturbed spindles (%cells) Roots	0.55 ± 0.08	0.25 ± 0.05	0.06 ± 0.03	0
Microspores	All aborted	27.54 ± 0.92	0.3 ± 0.09	0.18 ± 0.07

*>95% abortion