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THE EFFECTS OF GAMMA RADIATION ON *SALIX NIGRA* MARSH. CUTTINGS

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ABSTRACT

Dormant cuttings of *Salix nigra* Marsh. were exposed to gamma radiation from a cobalt-60 source at a dose rate of 1110 roentgens (r) per minute. Exposure doses ranged from 50 r through 100 Kr with 100% mortality at 10 Kr. Low doses of ionizing radiation apparently stimulated growth, while higher doses progressively retarded growth. The growth of the staminate catkins was inversely proportional to total exposure dose (increased dose resulted in progressively decreased growth).

Determination of growth rates at different intervals post-irradiation indicated recovery of the surviving fraction of the irradiated cuttings. At ninety days, the highest growth rate occurred at the highest surviving exposure dose. It was also noted that total RNA and protein concentrations were sensitive even to the lowest exposure doses.

The effects of gamma radiation on development of viable pollen grains were scored on the basis of the aniline-blue (cotton blue) lactophenol test. Pollen grain viability was found to increase with increasing gamma radiation up to 100 r. Progressively lower pollen viability was recorded from 400 r up to 4 Kr.

INTRODUCTION

Numerous investigations have been published describing the effects of ionizing radiation on both plants and animals. Plants have been shown to respond to ionizing radiation by increased chromosomal aberrations; morphological abnormalities of leaves, stems, roots, and flowers; alterations in growth patterns; variations in pollen grain viability and germination; altered seed germination; and death (Gunckel et al., 1953 a,b; Gunckel, 1957; Sax, 1963; Pfahler, 1971; Crest and Ciampolini, 1982; Cordero and Gunckel, 1982).

We have striven for years to better understand such phenomena as radiation recovery and stimulatory effects of low doses. These and other phenomena have been complicated by variations in radiosensitivity between species, between varities, and between individuals of the same variety. Responses of organisms to ionizing radiation also vary with age, the dose rate, the stage of mitosis or meiosis at the time of irradiation, acute or chronic irradiation, physiological condition of the organism when irradiated, and the chromosomal value (Gunckel, 1957).

An attempt will be made to remove possible genetic variability using a monoclonal population of *Salix nigra* Marsh. (black willow) cuttings. *S. nigra* has not, to my knowledge, been studied previously. Its rooting ability, relatively rapid growth, and type of inflorescence (catkin) were important factors in the selection of *S. nigra* for this study.

Variations in growth patterns, morphological alterations, physiological changes, and pollen viability following acute exposures of dormant *S. nigra* cuttings to gamma radiation will be reported in this paper. Special attention will be given to radiation recovery and possible stimulatory phenomena.

MATERIALS AND METHODS

Eight hundred 15 cm cuttings of dormant *Salix nigra* Marsh. (black willow) were exposed to gamma radiation from a cobalt-60 source at a dose rate of 1110 r/min. Exposure doses ranged from 50 r through 100 Kr. Cuttings were potted in peat pots and grown under greenhouse conditions. Each cutting averaged 6-7 buds above the soil level. Rooting was facilitated with a spraying device programmed for seven seconds of spray mist every seven minutes during the day.

Cuttings were carefully monitored to determine survival rates; criterion for survival was opening of the buds. The number of cuttings with growing buds was recorded for each exposure dose (55 cuttings at each dose).

The effect of ionizing radiation on flower growth was determined by measuring the length of the catkins at anthesis. Following abscission of the catkins, vegetative growth was determined by measuring stem elongation from the terminal bud of each surviving cutting at each exposure dose 45 and 90 days post irradiation.

The effects of ionizing radiation on pollen grain viability were determined by the analine blue (cotton blue) lactophenol test by Maneval (1936) as modified by Hauser and Morrison (1964). Twenty slides were made for each surviving exposure dose. Stamens were randomly excised from different cuttings within each exposure group and shaken onto the slides containing the cotton blue dye. Large numbers of pollen grains were counted at each exposure dose to increase statistical reliability of small differences between doses. The significance of differences in pollen viability at each exposure compared to the control and between treatments was determined by the student's "t" test.

The effects of ionizing radiation on protein and RNA concentrations in the upper (youngest) leaves were ascertained using the respective analytical methods of Itzhaki and Gill (1964) and Key and Shannon (1964).

RESULTS

Survival.

Irradiated willow cuttings were found to exhibit one hundred percent mortality at exposure doses above 9 Kr as determined by opening of buds (Table 1). Data suggests significant decreases in survival at dosages above 1 Kr. The great majority of open buds occurred at the terminal position in all exposure groups. Buds exposed to doses above 9 Kr did not open. Two cuttings exposed to 9 Kr showed bud opening; both died within two weeks following abscission of the catkins.

Although some cuttings exposed to gamma radiation above 4 Kr did open (Table 1), vegetative growth did not occur following abscission of the catkin. Consequently, further analyses used only those cuttings exposed to gamma radiation up to 4 Kr.

Growth and Maturation of the Male Catkins.

Catkins were measured at anthesis for all exposure doses. Mean measurements (Table 1) suggest no meaningful effects of gamma irradiation on catkin growth until 3 Kr. Doses above 3 Kr caused a

Table 1. Data summary: Effects of gamma radiation on *Salix nigra* cuttings.

Dose (r)	Percent Survival	Growth of cutting 45 days post irrd. (ratio of treatment to control)	Growth of cutting 90 days post irrd. (ratio of treatment to control)	$\bar{X}$ mean length of male catkin (mm)	RNA concentration (ratio of treatment to control)	Protein concentration (ratio of treatment to control)
Control	83	1.0	1.0	21.6	1.0	1.0
50	94	1.10	0.98	23.1	1.01	0.95
100	93	1.13	1.03	21.3	0.88	0.96
200	84	1.03	0.95	19.6	0.79	0.70
400	98	0.82	1.12	19.0	0.83	0.74
700	84	0.79	1.26	22.1	0.72	1.06
1000	94	0.58	1.85	19.6	0.73	0.15
2000	72	0.55	1.13	23.1	0.66	0.29
3000	58	----	----	17.8	----	----
4000	42	0.46	1.49	11.7	0.64	0.21
5000	45	----	----	7.8	----	----
6000	30	----	----	5.1	----	----
7000	20	----	----	7.7*	----	----
8000	16	----	----	1.0	----	----
9000	4	----	----	6.0*	----	----
10000	0	----	----	----	----	----

*Sample Number less than 4.

precipitous decrease in mean catkin length down to 5 Kr where mean catkin length reduction seems to level off. Measurements of catkin length at doses above 5 Kr were unreliable due to small sample size.

The rate of flower maturation was determined on the basis of anthesis and found to decrease gradually with increasing dosage. These decreases were obvious even at 50 r.

Shoot Growth.

Shoot growth was found to respond to increasing exposure dose initially by increased growth early at dosages of 50 r and 100 r with maximum stimulation at 100 r (Table 1). Shoot growth then decreased with increasing exposure dosages, relative to the control, up to 4 Kr. Cuttings exposed to gamma radiation above 4 Kr did not exhibit shoot growth. However, measurements at 90 days post irradiation indicated a sharp recovery of growth, especially at the highest exposure doses (2 Kr and 4 Kr).

Leaves of all exposure doses showed radiation induced abnormalities such as puckering at the leaf margins and leaf curvature, agreeing with Gunckel (1957) and Venketeswaran and Partanen (1966).

Pollen Grain Viability.

Pollen grain viability was found to increase with increasing gamma radiation exposure up to 100 r (Table 2). Above 100 r, pollen grain viability decreased with exposure dose with minimum viability recorded at 4 Kr. Comparison of "t" values of each exposure dose to the control group indicated significant differences (99% confidence level) at all exposure doses except 400 r. Comparison between treatments indicated significant differences (99% confidence level) between all treatment doses.

RNA and Protein Concentrations

Analyses of RNA isolated from leaves from each dose suggest a slight but gradual decrease in RNA with increasing exposure dose beginning at 100 r. This was unexpected at such low doses (Table 1).

Protein levels also decreased with increasing exposure dosages as compared to the control. These reductions were apparent at 50 r, dropping precipitously at 1 Kr (Table 1).

DISCUSSION

Even low doses of acute exposure to gamma radiation appear to have significant effects on survival and several growth, developmental, and reproductive processes.

Survival of cuttings based on buds opening and producing catkins was misleading since some cuttings died shortly after buds opened. Cut-

Table 2. The effects of ionizing radiation on the development of pollen by *Salix nigra* cuttings.

Exposure Dose (R)	Number Counted	Viable Fraction	"t" Value for Comparison Between Treatment and Control	"t" Value Comparison Between Treatments: Treatments Compared	"t" Value Comparison Between Treatments: "t" Value
0	99,536	0.9506	----	0: 50	6.29
50	43,494	0.9580	6.29	50: 100	11.29
100	42,996	0.9721	20.462	100: 200	12.94
200	44,470	0.9558	4.195	200: 400	4.95
400	79,385	0.9497	0.894*	400: 700	7.53
700	74,030	0.9409	8.798	700:1000	9.63
1000	58,187	0.9276	18.024	1000:2000	14.79
2000	41,540	0.9007	30.784	2000:4000	12.35
4000	37,200	0.8727	41.870	----	---

*Accept H_0:control=treatment
H_0 rejected on 1% level of significance
All others showed highly significant differences ($<$1%)
Accept alternate hypothesis H_A:A≠B

tings exposed to acute dosages of 5 Kr to 9 Kr demonstrated that, although floral development occurred, vegetative growth did not. This explains survival and catkin growth data at doses from 4 Kr to 9 Kr while other determinations did not exceed the 4 Kr exposure dose. Stairs (1964) reported that gamma irradiated male flower buds of two species of *Quercus* both showed complete inhibition of male buds at an exposure does of 4 Kr. However, he noted that the lethal dosage varied with the stage of meiosis at the time of irradiation. When the buds were irradiated from anaphase II on, the lethal point for vegetative growth was extended to 6 Kr.

Although most effects of ionizing radiation are injurious (Schull and Mitchell, 1933), there are numerous claims in the literature that small doses are stimulative (Skor et al., 1965; Moore and Hough, 1962; Gunckel, 1957; Gunckel et al., 1953 a,b,; Kuzin et al., 1964; Iqbal and Zahur, 1975). Skok et al. (1965) suggested that exposure to low doses of radiation may suppress dividing cells but stimulate cell expansion Shull and Mitchell (1933) suggested that the maximum dosage for stimulation would not far exceed 100 r. This agreed with data presented here on pollen grain viability but did not agree with Iqbal and Zahur (1975) who reported stimulation of growth and yield in rice at doses of 1 Kr to 2.5 Kr. However, responses to acute radiation vary with dose rate.

The causes of radiation induced growth stimulation/suppression have been attributed to increased/decreased levels of auxin (Skoog, 1935; Gordon, 1957; Gunckel and Thimann, 1949), cytokinins (Jordan and Haber, 1974), ABA (Degani and Itai, 1978), and translocatable inhibitors (Kuzin et al., 1964). Gordon (1957) suggested that radiation induced auxin reduction is temporary. Recovery of auxin biosynthesis and buildup of metabolites could account for the higher growth rates at 90 days in the 2 Kr and 4 Kr plants. A comparison of the growth of the male catkin with shoot growth 90 days post irradiation (Table 1) suggests that the flower is more sensitive to acute exposures to gamma radiation than the vegetative portion. This agrees with conclusions reported by Cordero and Gunckel (1982).

Venketeswaran and Partanen (1966) reported a precipitous drop in growth above 1,300 r which was not observed here. It is however noteworthy that they reported complete inhibition of the apical meristem at 5,200 r, almost identical to data reported in this study.

Gunckel (1957) suggested that radiation induced auxin production is related to protein synthesis which is controlled by m-RNA. Unpublished results of Van Huystee and Cherry (1967) suggested that x-irradiation inhibits the production of m-RNA. Van Huystee et al. (1968) reported x-irradiation induced reduction in m-RNA from peanut cotyledons but at much higher doses than reported in this paper.

The reduction of pollen grain viability by ionizing radiation is well known (Crest and Ciampolin, 1982). However, Pfahler (1971) and Stairs (1964) suggested that high doses were necessary for pollen grain germination and tube growth. Results presented here indicate high pollen grain sensitivity to low doses as determined by the cotton blue test. However, pollen viability determinations by this method may not be comparable to pollen germination. Uematsu and Nishiyama (1967) reported 50% reduction in seed formation at 2 Kr and no seed production at 9 Kr. Stairs (1964) reported stimulation of pollen tube growth at 1 Kr and a significant decrease in pollen tube germination at doses of acute gamma irradiation of 4 Kr. Crest and Ciampolini (1982) suggest that the initial plateau observed may be due to an accumulative effect and repair mechanisms.

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