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BIOMETRIC ANALYSIS OF A GROWTH RESPONSE OF
TWO PLANT SPECIES IN A RADIOACTIVE WASTE AREA

G. L. Plummer

OAK RIDGE NATIONAL LABORATORY

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BIOMETRIC ANALYSIS OF A GROWTH RESPONSE OF TWO PLANT SPECIES IN A RADIOACTIVE WASTE AREA¹

G. L. Plummer²

ABSTRACT

Lengths of pistillate inflorescences of sedges (*Carex* spp.) growing in the contaminated soils of White Oak Lake bed were measured in relation to radiation fields. *Carex Frankii* Kunth and *Carex vulpinoidea* Michx. populations were sampled from areas with air dose rates of 0, 10, 20, and 40 mr/hr. Analyses of variance showed that mean lengths of inflorescences of *C. vulpinoidea* were similar to each other in these areas but that those of *C. Frankii* were significantly different: 0 mr/hr, 93.8 mm; 10 mr/hr, 96.4 mm; 20 mr/hr, 122.0 mm; and 40 mr/hr, 110.7 mm. However, the sites differ in such factors as soil moisture, fertility, and alkalinity, so that these differences in the length of inflorescence may not be accounted for primarily by the exposure dose rate of the radiation field.

INTRODUCTION

Since ionizing radiation in certain environments is increasing both from weapons fallout and from radioactive waste disposal, there is interest in the possible effects of these materials on the native flora and fauna. This investigation employed biometric techniques to analyze a growth habit of two sedges, *Carex Frankii* Kunth and *Carex vulpinoidea* Michx., for evidence of manifestation of radiation effects. The plants inhabit the White Oak Lake bed, a radioactive waste disposal area in which the dose rate of radiation is many times that ever expected to occur in an ordinary environment.

Both morphogenetic and physiological growth responses are known to occur in various plant species exposed to ionizing radiation. Recently, Mewissen *et al.*³ pointed out that chronic irradiation enhanced certain growth characteristics of seed-grown *Andropogon filifolius*, a grass, from uraniferous soils. On the other hand, plants growing in the areas of fallout from the 1954 Bikini hydrogen bomb test exhibit "... some correlation between intensity of observed symptoms

and intensity of recorded fallout."⁴ The responses by plants to ionizing radiation have been widely reported.⁵ Many of the responses are undesirable, but some are quite favorable.

Any effect of ionizing radiation upon plants is of interest to the plant biologist. Gross morpho- and physiogenetic aberrations have been observed at dose rates from 15 r/day upward.⁶ Chromosomal mutations occur with even less radiation. Ionizing radiation is capable of inducing chemical changes both in the soil and within the plant. In general, plants depend upon mineral nutrients that are organized into rather select arrangements. If the suitable arrangement is disturbed by ionizing radiation, a new physiological makeup could produce very subtle responses in growth habits without affecting the obvious morphology of the plant. Therefore, competition, survival, and even evolutionary trends in plant community structure are subject to change.

The objective of this investigation was to determine whether these sedges produced a measurable response which could be attributed to the dose rate from ionizing radiation in their habitat.

¹This work is part of an investigation conducted under the Research Participation Program of the Oak Ridge Institute of Nuclear Studies in the Ecology Section, Health Physics Division.

²Research participant from the University of Georgia Department of Botany.

³D. J. Mewissen, J. Dramblon, and Z. M. Bacq, *Nature* 183, 1449 (1959).

⁴F. R. Fosberg, *Nature* 183, 1448 (1959).

⁵A. H. Sparrow, J. P. Binnington, and V. Pond, *Bibliography on the Effects of Ionizing Radiations on Plants, 1896-1955*, BNL-504 (July 1958).

⁶A. H. Sparrow and W. R. Singleton, *Am. Naturalist* 87, 29-48 (1953).

PLANTS

The sedges in this study are pioneers in the revegetation of lowland habitats. *Carex Frankii* Kunth and *C. vulpinoidea* Michx. are members of a genus consisting of more than 1100 species, some of which are abundant in arctic, alpine, and temperate habitats. They are perennials which propagate from seed and from underground stems. Many species of the genus hybridize readily. The flowering parts, namely, inflorescences (Fig. 1),

Fig. 1. Sedges, Dose Rates, and Collecting Areas of White Oak Lake Bed, Summer 1959.

consist of the pollen-producing structures situated above the seed-producing (pistillate) spikes. *Carex vulpinoidea* has a distinct form, *C. vulpinoidea* forma *segregata*, distinguished by an elongated and obviously segregated group of spikes along the lower portion of the flowering stalk. The ratio of occurrence in the field appeared to be about one forma *segregata* to eight of the typical species. Intermediates and gradations occur suggesting hybridization.

Plants respond to a large number of environmental factors, including soil fertility, soil water, etc., and perhaps to ionizing radiation where present. For the purposes of this study it was assumed that the size of the pistillate spikes is related to the quality of site on which the plants grow, since this is common to nearly all plants. In other words, as the combination of environmental factors becomes more favorable, plants grow larger and reproduce more abundantly.

HABITATS

White Oak Lake bed is a unique ecological system of about 48 acres. The radiation field above the soil surface has been described,⁷ and additional observations show a range in dose rate from approximately 10 to 300 mr/hr. Records are kept on the characteristics of the vegetation and soil,^{8,9} and translocation studies of important radionuclides have been made.¹⁰

Communities of *Carex* and *Juncus* (the rushes) occur in several large patches where the over-all dose rate at 1m above the bed surface is relatively uniform with intensities of approximately 10, 20, and 40 mr/hr (Fig. 1). The wettest places occur at the lower end of the lake bed, which is covered with backwater in the early spring; the dose rate is 20 mr/hr, and the soil is very alkaline, pH 8 to 8.4. The driest radiation site, 40 mr/hr, coincides with the highest elevation, where the soil is slightly alkaline, pH 7 to 7.4. The 10-mr/hr site may be considered as intermediate in respect to all the above factors.

The size and luxuriant growth of the plants suggest that effluents from industrial and domestic wastes brought large quantities of nitrates into the old lake. During the past four growing seasons, following drainage of the lake, the vegetation has flourished in obvious response to this oft-times limiting mineral nutrient.

In areas outside the lake bed, where there are no such radiation fields present, the soils are generally acid, pH 5 to 6.5. The small size of the plants indicates that the supply of nitrates is limited. The sedges consort with the rushes in communities that are not dissimilar, floristically, to those on the radiation sites. Five areas were found as suitable "control" sites. Wet, dry, and intermediate soil moisture sites occur, all of which provided adequate plant materials to harvest for comparison.

⁷P. K. Lee and S. I. Auerbach, *Determination and Evaluation of the Radiation Field Above White Oak Lake Bed*, ORNL-2755 (1959).

⁸H. R. DeSelm, *Plant Taxa Occurring on the White Oak Lake Bed Grid - 1956, 1957, 1958*, Mimeographed list, University of Tennessee, Department of Botany (1959).

⁹R. M. Anderson et al., *H-P Ann. Prog. Rep. July 31, 1957*, ORNL-2384, p 10; S. I. Auerbach et al., *H-P Ann. Prog. Rep. July 31, 1958*, ORNL-2590, p 27.

¹⁰E. R. Graham, *Soil Sci.* 86, 91-97 (1958).

METHODS

Several hundred mature culms of both species were collected in mid-June from different stations on four kinds of sites: no radiation, 10, 20, and 40 mr/hr. Each collection site was sampled at not less than two stations. Each station within a site covered an area of about 50 m². Ten stations on the lake bed and ten stations in the control sites were sampled.

An effort was made to avoid duplicate sampling within individual clumps as well as within groups having a common parent (clones). Also, both species were collected within the same square meter as frequently as possible. Twenty-five plants were picked at random from the mass of plants gathered at each radiation site and from the control areas as a whole. These plants were separated for measurement as representatives of the particular site. An additional group of 25 plants was measured on three of the four kinds of sites.

The length of each flowering head of *C. vulpinoidea*, from the lowest spike to the top-most spike, was measured to the nearest millimeter. On the other hand, each pistillate spike of *C. Frankii* was measured individually and the sum of all spikes per culm was used as an index

of the aggregate length of the flowering part of the plant. As a result of these slightly different measurements on the floral morphology, the two species may not be strictly comparable with each other; nevertheless, the magnitudes of length in both cases are relative.

RESULTS

Table 1 presents the basic information obtained from all measurements. The last two columns are intended to be comparable; that is, the column headed "Mean" represents the averages for all the data obtained from plants growing on the radiation sites, whereas the column headed "Controls" represents the averages of the plants at all the collecting stations without radiation.

The two species appeared to respond differentially to the presence or absence of significant ionizing radiation. The mean length of the spikes of *C. Frankii* was greater for the plants from the lake bed than it was for controls (109.7 vs 93.8), whereas in *C. vulpinoidea* the samples from the control sites had the longer mean head lengths (67.6 vs 63.2).

These relationships suggest that an analysis of variance within each species might distinguish some significant relationships between length and

Table 1. Measurements Taken from Inflorescences of Two Species of Sedges

	Radiation Site			Habitats	
	10 mr/hr	20 mr/hr	40 mr/hr	Mean*	Controls
<i>C. vulpinoidea</i>					
Number of heads measured	50	50	25	50	50
Total length (mm)	3114	2998	1686	3161	3380
Variance (sigma squared) (mm)	187.7	115.7	167.0	167.4	138.5
Mean length of heads (mm)	62.3	60.0	67.4	63.2	67.6
<i>C. Frankii</i>					
Number of spikes measured	212	244	113	112	97
Number of plants measured	50	50	25	25	25
Total length of spikes (mm)	4821	6098	2767	2742	2345
Variance (sigma squared) (mm)	262.0	464.2	290.4	338.8	380.9
Mean length of spikes (mm)	96.4	122.0	110.7	109.7	93.8

*An average derived from measurements on plants at the three radiation sites.

dose rate. The data shown in Table 2 have been adjusted to 25 plants measured from each radiation site. The analyses of variance tables are composited by parts in Table 3. The variance ratio is to be interpreted as the observed F value, and Snedecor's F value, at a given probability level, is shown in the right column.

In tracing the source of the differences in total length shown in Table 1, the data for *C. vulpinoidea* on four radiation sites were treated as shown in Table 3. The observed value of F is 2.46 and the probability that the absolute value of F exceeds 2.70 is 5%. Therefore, the length of inflorescences in this species probably does not differ with dose rate, and those plants growing on the lake bed are quite similar to those on the "control" sites.

Table 2. Table of Sums
C. Frankii and *C. vulpinoidea* - length of
inflorescences on four kinds of sites

Site (mr/hr)	<i>C. vulpinoidea</i>		<i>C. Frankii</i>	
	Σx	Σx^2	Σx	Σx^2
0	1690	117,715	2,345	226,985
10	1557	101,662	2,411	238,970
20	1499	92,773	3,049	383,461
40	1686	117,880	2,767	313,511
Total	6432	430,030	10,572	1,162,927

In another analysis, not shown here, of *C. vulpinoidea* from the control sites and from the 20-mr/hr site (which produced the longest and shortest inflorescences respectively) the inflorescences on the control sites were not significantly longer at a probability of 5% than the ones on the 20-mr/hr sites.

When the data for *C. Frankii* are treated similarly (Table 3), the calculated F value is 12.77 and the probability that the absolute value of F exceeds 3.98 is 1%. The large difference between the values of F indicates that the lengths of the pistillate inflorescences in this species probably differ with site.

The Tukey-Hartley test for comparisons among means is useful to distinguish which sites differ from the others. This test is based upon computing a difference, D , which is significant at the 5% level, then comparing it with the $a(a-1)/2$ sample differences in the investigation;¹¹ D is the product of sample standard error and factor Q , which differs for each range of treatment means. Thus,

$$D = \left(s_{\bar{x}} \right) \left(Q_{f,a} \right),$$

where

f = degrees of freedom,

a = number of treatments compared,

¹¹G. W. Snedecor, *Statistical Methods*, 5th ed., Iowa State College, Ames, 1956.

Table 3. Analyses of Variance

C. vulpinoidea and *C. Frankii* - lengths of inflorescences for 0-, 10-, 20-, and 40-mr/hr sites

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	Variance Ratio	F Value
<i>C. vulpinoidea</i>					
Between sites	1,091	3	363.6	2.46	2.70*
Within sites	14,142	96	147.3		
Total	15,233	99			
<i>C. Frankii</i>					
Between sites	12,913	3	4304	12.77	3.98**
Within sites	32,432	96	337		
Total	45,345	99			

*Probability: 0.05.

**Probability: 0.01.

$$s_{\bar{x}} = \frac{\text{mean square within sites}}{a(a-1)/2} = \frac{336.9}{4(3)/2} = 7.50333$$

When $Q_{96,4} = 3.7$, $D = 27.8$; when $Q_{96,3} = 3.376$, $D = 25.3$; when $Q_{96,2} = 2.812$, $D = 21.1$.

The D values are compared with observed differences in the mean length of inflorescences according to sites in Table 4.

Of the six differences, two exceed their computed difference. These suggest that *C. Frankii* growing in the 20-mr/hr site have inflorescences significantly longer than members of the species growing in both the control and 10-mr/hr sites. The difference between the mean lengths on the 20- and 40-mr/hr sites is not significant at the 5% probability level, but is significant at the 10% level. Similarly, the inflorescences produced on the 40-mr/hr site are probably longer than those produced on the 10-mr/hr as well as the control sites. It is also evident that the inflorescences from the 10-mr/hr area are very nearly the same length as those from the control areas.

The information in Tables 1 and 4 tentatively suggests that the mean length of the inflorescences of *C. Frankii* increases with the dose rate. In addition, the line of regression (Fig. 2) appears to confirm the suggestion that radiation has enhanced the growth of this species. However, certain other environmental factors are present

Fig. 2. Line of Regression Showing Relationship Between Lengths of Inflorescences and Dose Rate of the Radiation Field.

and the influences of these must be considered before this causal relationship can be affirmatively established.

INTERACTION OF ENVIRONMENTAL FACTORS

The size of a plant often varies with available water. The longest inflorescences of *C. Frankii* came from the wettest sites (20 mr/hr); the driest sites (40 mr/hr) produced the next longest; and the shortest grew on several sites (no radiation) where water relations appeared similar to

Table 4. Test for Comparison of Sample Means
C. Frankii - mean length of inflorescence according to radiation site

Radiation Site (mr/hr)	Sample Mean,* $\bar{x}$ (mm)	$\bar{x} - 93.8$	$\bar{x} - 96.4$	$\bar{x} - 110.7$
20	122.0	28.2 (27.8)	26.0 (25.3)	11.3 (21.1)
40	110.7	16.9 (25.3)	14.3 (21.1)	
10	96.4	2.6 (21.1)		
0	93.8			

*Means arranged from high to low; each subtracted from those above it. Observed differences are significant at 0.05 level if larger than the calculated D value shown in parentheses.

both of the above. On the other hand, *C. vulpinoidea* produced the shortest inflorescences on the wettest sites (20 mr/hr) and the longest on the driest sites (40 mr/hr) as well as on sites without radiation.

Obviously, the two species have responded differently to the same kind of environmental factors. In the case of *C. Frankii* it might be assumed from the evidence that radiation had stimulated the production of longer flowering parts; however, more pistillate spikes were produced on the lake bed than on the control sites (Table 1). This kind of plant response is often associated with soil fertility. Apparently radiation has not enhanced the length of the inflorescences in *C. vulpinoidea*. Water relations, mineral nutrition, and soil alkalinity probably have interacted to produce the differences in growth response. The wettest sites are the most alkaline and the driest sites are the least alkaline. A combination of both leaching and runoff could cause these differences between the sites.

The data suggest that *C. Frankii* will tolerate alkali soils (pH 8) and will possibly respond favorably to these soil conditions whereas *C. vulpinoidea* is less tolerant.

The results indicate that these species of sedges respond differentially to the lake bed habitat. *Carex Frankii* develops long pistillate spikes, whereas *C. vulpinoidea* has comparatively short inflorescences. Any effect of dose rate upon this growth response is probably outweighed by the influences of other environmental factors, particularly soil water, nutrients, and alkalinity. Thus, ionizing radiation alone in White Oak Lake bed during the past few years produced no independently measurable effect upon the lengths of inflorescences of either *C. Frankii* or *C. vulpinoidea*.

DISCUSSION

Since ionizing radiation on White Oak Lake bed apparently has not influenced the size of the flower structures of the *Carex* species to an extent that is measurably independent of some other environmental influences, it can be assumed that these plants possess some physiological tolerances to the dose rates involved. The nature of these tolerances may be due to a variety of factors and conditions:

1. The dose rate which will affect various activities in other plants extends over a wide

range. Some species investigated at the Brookhaven radiation field have shown severe morphogenetic effects with dose rates of 30 to 50 r/day. However, *Gladiolus* showed little or no effect from dose rates of 5000 to 6000 r/day.¹² As mentioned earlier, few plants show morphological changes at dose rates less than 15 r/day. Photosynthesis is only partially or temporarily inhibited by 100 to 500 kr.¹³ Chromosome aberrations and mutations have been noted in significant numbers at dose rates below 1 r/day.⁶ Thus, the dose rate anywhere on the lake bed is sufficient to induce genetical disturbances, but even the highest dose rate (300 mr/hr) is rather low compared with the dose rates used to induce physiological disturbances in very sensitive plants. The upper limit of the daily exposure will approximate 7 to 8 r/day only in the most intensely radioactive patches. The typical dose rate over a wide portion of the area ranges between 500 and 1000 mr/day.

2. Polycentric chromosomes, such as those found in *Carex* species, enhance the resistance of the plant to genetic disturbances which frequently accompany fragmentation of the chromosomes. The spindle attachments to the chromosome (or chromatids) are spread along the length, whereas a monocentric chromosome has but one spindle attachment. Fragmentation of a polycentric chromosome would produce less serious disturbances than would a chromosome in which half a chromatid and all the genes for one characteristic move to the wrong end of the dividing cell.

3. The effects of ionizing radiation upon plants can be related also to the number and size of chromosomes present.¹⁴ Plants with rather large chromosomes are somewhat radiosensitive. The size of the chromosomes in these *Carex* has not been compared with other species. On the other hand, polyploids, that is, plants with a multiple of the basic complement of chromosomes, are less sensitive than diploids within a species. The genus *Carex* probably has polyploid species

¹²A. H. Sparrow and J. E. Gunkel, *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1956* 12, 52-59 (1956).

¹³L. P. Zill and N. E. Tolbert, *Arch. Biochem. Biophys.* 76, 196-203 (1958).

¹⁴J. E. Gunkel and A. H. Sparrow, *Brookhaven Symposia in Biol.* No. 6, p 252-79 (1954).

since the somatic numbers range from 12 to 112.¹⁵ The chromosome numbers of these species are not known.

Gray¹⁶ reports that biological disturbances have been observed when the rate of dissipation of energy along an ionizing tract reaches a value corresponding to the production of about 500 ions/ μ . Since the lake bed soils contain 2 to 3 μ c of mixed radionuclides per 100 g of dry weight, it would seem, particularly when the soil is water-saturated, that disturbances could occur in this environment as well as within the plant parts that are underground.

4. Plants tend to show some recovery from radiation damage in ways not wholly dissimilar to the recovery habits of animals. Certain physiological adjustments for recovery and repair of irradiated protoplasm are well known.

It was pointed out earlier that these sedges were temporary residents in the lake bed habitat. If the processes of plant succession proceed normally, the sedges will be replaced by shrubs and eventually trees. At the present time both willows and sycamores are rapidly encroaching upon the communities, and have pioneers already established. Inasmuch as radiation on White Oak Lake bed can, and possibly does, disturb the genetical and physiological systems within these species, there are two levels at which any disturbance may become manifest, namely, within the organism and within the community. Manifestations of any organismal disturbance probably precede any manifestation of disturbance at the community level.

The data suggest that these species show no deleterious effects from the radiation present on

White Oak Lake bed. If, on the other hand, some disturbances have occurred, they have not been observed within these plants. Furthermore, the ecological (sociological) structure within the community is not expected to deviate from a typical pattern since these *Carex* species would normally give way to shrubs and trees.

SUMMARY

This study was conducted on the White Oak Lake bed where radionuclides are incorporated within the top soil in concentrations considerably more than might be expected from fallout. The lengths of pistillate flowering structures of two consorts, *C. Frankii* and *C. vulpinoidea*, were measured as growth response indicators of site quality with particular reference to the field of radiation in which they were growing, namely, 0, 10, 20, and 40 mr/hr.

Lengths varied with dose rate, but are believed to be independent of the ionizing radiation and more closely associated with other soil factors. Each sedge apparently responds differently to the same soil moisture, fertility, and acidity-alkalinity relationships. *Carex Frankii* is probably more tolerant to alkaline soils than is *C. vulpinoidea*.

Ionizing radiation during the four growing seasons has not produced apparent aberrations in these plants. Even though biological disturbances have not been found, they can occur. The sojourn of these pioneering sedges in the lake bed habitat is likely to be so short that gross biological disturbances may not attain obvious magnitudes before these plants are replaced by succeeding forest species.

ACKNOWLEDGMENT

A note of appreciation is extended to S. I. Auerbach for making the facilities in the ecology laboratory available.

¹⁵C. D. Darlington and A. P. Wylie, *Chromosome Atlas of Flowering Plants*, 2d ed., George Allen and Unwin, London, 1955.

¹⁶L. H. Gray, *Radiation Research* 1, 89 (1954).

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