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Growth and bract pigmentation in poinsettia as influenced by fast neutron irradiation

Robert D. Wright

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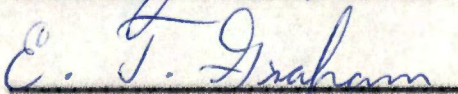
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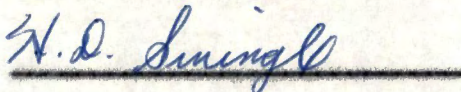
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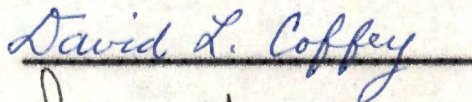

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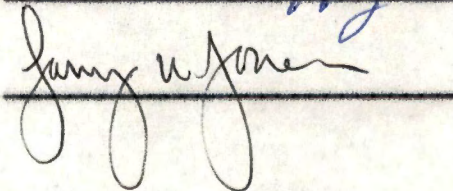
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**GROWTH AND BRACT PIGMENTATION IN POLYMETILLA
AS INFLUENCED BY FAST NEUTRON IRRADIATION**

**A Thesis
Presented to
the Graduate Council of
The University of Tennessee**

**In Partial Fulfillment
of the Requirements for the Degree
Master of Science**

**by
Robert B. Wright**

June 1968



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ABSTRACT

A study was made on growth and bract pigmentation in poinsettia as influenced by fast neutron irradiation. Plants from two varieties of poinsettia, Mikkepink, a periclinal chimera, and Spring Pink, a genetic pink, were irradiated with 300 rads of fast neutrons. Successive generations of cuttings were rooted from these plants and allowed to flower. At this time bract diameter and plant height were measured for growth responses. Color changes on bracts were also recorded according to size, type and frequency.

It was found that plant height and bract diameter were reduced by irradiation only when the shoots from which cuttings were made were present at the time of irradiation. Most of the changes in bract color were found on Mikkepink, the periclinal chimera. Changes from pink to red or white occurred at about the same frequency. These sectors became fewer and larger as successive crops of cuttings were removed from the mother plants.

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CHAPTER I

INTRODUCTION

Physiologists and plant breeders have described bud sports and somatic mutations recovered from many flowering plants (4, 5, 14, 18, 22, 29). Stewart (29) has reviewed the sports which have naturally occurred in certain pink poinsettia varieties. Cusny (5) studied the nature of somatic mutations induced by radiation in several flowering plants. Sagawa and Muhlquist (22) studied some of the mechanisms responsible for X-ray induced changes in flower color of the carnation.

This study will attempt to characterize some of the pigment changes appearing in mutant tissue of poinsettia (Euphorbia pulcherrima, Willd.). A comparison between the morphological and physiological responses of two varieties of poinsettia after acute exposure to fast neutrons will be undertaken. Comparison will be made of radiosensitivity, frequency of visible bract mutations, and characterization of color mutants in bracts considering type and amount of pigment changes. Morphological responses such as plant height, bract diameter, and the number of cuttings taken from irradiated versus control plants will be used to define differences in growth responses to irradiation.

CHAPTER II

REVIEW OF LITERATURE

A number of authors have reported an increase in frequency of somatic mutations or bud sports in horticultural plants after exposure to X-rays, gamma rays or fast-neutrons. For example, following radiation, flower color changes have been observed in Petunia (5), Mimulus cardinalis (20), Chrysanthemum (4), Arthirrhinum majus (5), Dianthus caryophyllus (22) and Tradescantia (18). Chloroplast mutations have also been induced by irradiation in Arabidopsis (21), Euphorbia (28), Dianthus (26) and five other species as reported by Love and Constantin (17). Love (15, 16) also showed both an increase and a decrease in anthocyanin pigments within Coleus leaves following fast neutron irradiation.

The change in flower color and chlorophyll content may be attributed to either a mutation or the exposure of an existing periclinal chimera. For example genetic changes which cause the bract or flower color to vary from red to white or pink have been observed in some varieties of poinsettia (29) and Arthirrhinum majus (17). An accepted explanation of this is that these plants are heterozygous for bract or flower color and the mutation of the Wh allele to wh results in loss of the ability to produce pigment. Change of pink or white to red in William Sin Carnation (22) could be due to a dominant mutation in the outer layer of tissue since flower color in carnation is determined by the epidermis only. However Nagawa and Mählquist (22) attribute this

change to a more plausible theory introduced by Darman (7) which explains this change in color. Darman showed that internal cells may crowd between cells in an external layer and take their place. This he termed "displacement". Hence if the internal tissue were red and the external tissue colorless, as is the case with Minkelpink poinsettia, the resultant sector or flower would be red. Minkelpink is a periclinal chimera; the epidermis is colorless and the underlying red mesophyll gives rise to the pink color. Such species as poinsettia (27, 29), chrysanthemum (4) and carnation (22) seem to demonstrate this theory. A reverse of displacement may occur in which the colorless epidermis may divide periclinally and replace a colored inner layer. This would result in a near white flower or bract sector, a condition seen in Minkeldawn poinsettia, a sport of Minkelpink (29).

The number and size of these sectors, whether they result from a mutation or the exposure of an existing chimera, may vary with time and cultural practice after irradiation. This has been shown in chrysanthemum where Jenk (15) obtained a much higher percentage of sports by pruning his plants seven times between irradiation and flowering. Baur (5) also showed that repeated pruning of irradiated black currant bushes led to the production of mutants several years after irradiation. This may be accounted for since lateral buds with possible mutants were induced to develop because of the severe pruning. Sparrow et al (25) also showed an interrelationship of time after irradiation, sector size and sector number in Anthriscum majus. They showed that spots of color emerging with time were larger but fewer. This is true because

cells which mutate late in the primordial stage of plant parts will not contribute to as much of that part as a mutant cell which starts to divide in early primordial stages of tissue or organ development.

Mutations involving the anthocyanin content of flowering plants have been shown to affect only the amount of anthocyanins present and not the kinds. Stewart (29) reported the same four anthocyanins were found in about the same proportions but in reduced amounts in pink sports and pink seedlings as in red plants of poinsettia. Pollock et al (20) reported the same finding in Mimulus cardinalis which contains different anthocyanins from poinsettia.

The four anthocyanins of poinsettia have been shown by Asen (1) and Stewart (29) to be cyanidin-glucoside, cyanidin-rhamno-glucoside, pelargonidin-glucoside, and pelargonidin-rhamno-glucoside.

Growth reduction and plant death are two visible responses of plants to irradiation. Such visible changes, however, are terminal manifestations of microevents (8). Initially there is a change at the submolecular level of organization. If this is not corrected within a short period of time, there is an alteration of biochemical events controlling developments (8).

Sparrow et al (26) concluded that chromosome structural damage constituted a major factor leading to growth inhibition after exposure to alpha, gamma or X-radiation. Part of this growth inhibition was considered due to the reduced vigor of meristematic cells. Under such conditions it was felt that a plant would not be able to maintain meristematic volume. It was also shown by these (26) and other authors

(6, 9, 23, 24) that nonchromosomal damage may contribute to morphogenic responses. These responses may be secondary effects of induced biochemical and physiological disturbances (9). For instance, localized swellings in stems may be due to local mobilization of nutrients and auxin (10). Auxin level has also been shown to be altered by irradiation (8). For this reason, numerous responses of plants to ionizing radiation have been attributed to destruction of the growth hormones. Auxin, however, is neither radiosensitive nor preferentially inactivated. Thus it was concluded that reduced auxin levels were due to rapid curtailment of auxin biosynthesis rather than an accelerated auxin catabolism (8).

In considering nuclear volume as an influence on radiosensitivity, it must be assumed that a certain number of ionizations are required to produce a chromosome break and that this number does not change significantly as the average chromosome size or nuclear volume varies from species to species (25). Furthermore, the number of ionizations produced within a nucleus is proportional to the nuclear volume (25).

CHAPTER III

MATERIALS AND METHODS

Poinsettia plants of two varieties, Mikkepink and Spring Pink were chosen. Both varieties are diploids but differ in their mechanism for color expression. Stewart (29) reports that Spring Pink is a true genetic pink and progeny grown from its seed will be pink. Mikkepink on the other hand is a periclinal chimera which sported from the Paul Mikkelsen variety. Its pink color is due to a colorless epidermis partially masking an underlying red mesophyll. Genetically this variety is red and progeny from its seed would have red bracts instead of pink (29).

Fifty cuttings of Mikkepink and 27 of Spring Pink were rooted under mist. As soon as plants were growing normally 40 of Mikkepink and 23 of Spring Pink were irradiated with 300 rads of unmoderated fission neutrons at the Health Physics Research Reactor of the Oak Ridge National Laboratory. In order to receive this dose the plants as a group were exposed at a distance of 3 meters from the reactor core for 5 minutes. The actual absorbed dose could not be calculated since this would require a knowledge of the elemental composition of the plants. The remaining plants of each group were retained as non-treated controls. The two varieties were propagated at different times and, therefore, were irradiated at different times. However, both were at about the same stage of growth at dosing and received the same dose.

Since the experiment was initiated under short day conditions, day length was extended by artificial illumination to promote vegetative growth. The first generation of cuttings was taken approximately two weeks after irradiation. Generation is the term used to describe the successive crops of cuttings which were taken from the irradiated plants at approximately four week intervals. These cuttings, ranging from four to six inches in height, were potted in a 1:1 mixture of peat and perlite and placed under a mist system. The rooting bed also was illuminated for long day conditions. Cuttings of both varieties were harvested at one month intervals and after each harvest more shoots were allowed to develop to comprise the next generation or experimental group. Six generations of cuttings were taken from Mikkepink and four from Spring Pink.

Rooted cuttings taken from plants after the termination of short days were placed under shade cloth to simulate short day conditions. Plant height and bract diameter were measured when the bracts were fully expanded. Visible sectors of bracts showing contrasting pigmentation were classified according to color, size and frequency.

In order to evaluate the nature of the color changes in bracts of irradiated plants, it was necessary to classify the color sectors as to type and amount of pigment present. The first step was to determine if the same pigments were present in the mutants as in the normal plants. This was accomplished by using thin-layer chromatography with three different solvent systems. The solvents used

were water-HCl-formic acid (8:4:1,v/v), 1 percent HCl, and acetone-0.5 N HCl (1:3,v/v).

A quantitative comparison of pigments in normal and mutant bracts was attempted by means of colorimetric analysis. A procedure outlined by Asen (1) was used to obtain the four pigments in pure form in order to establish standard curves. Colorimetric analysis could then be made by extracting a known amount of bract tissue with a known amount of solvent. For reasons discussed later this was not a feasible operation. The second alternative was, after acid hydrolysis of the anthocyanins, to establish standard curves using only the anthocyanidins. Again problems were encountered. A further attempt was made by measuring absorption maxima for cyanidin and pelargonidin components of raw pigment extracts. All colorimetric measurements were made on a Beckman DU spectrophotometer.

A technique described by Stewart (29) was employed to demonstrate the morphological distribution of bract pigmentation. The bracts were torn diagonally and observed under a microscope to show the presence or absence of pigment in the upper or lower epidermis and spongy mesophyll tissue.

Radiation damage was more severe in the Spring Pink variety than in Mikhepink, and thus introduced the question of radiosensitivity of the two varieties. For this reason a dose response study was initiated. Twenty-five cuttings from stock plants of each variety were taken and rooted in four inch pots. Five plants of each variety were exposed to 0, 200, 400, 600 or 800 rads of unmoderated fission neutrons

at the Health Physics Research Reactor of the Oak Ridge National Laboratory. Varied doses were administered by varying the distance of the plants from the core of the reactor.

Nuclear volume, a factor related to species radiosensitivity, was measured on apical cells of the two varieties. Apices were excised, fixed in FAA solution,¹ and embedded in paraffin. They were then sectioned at 10 microns and stained with hematoxylin and fast green. Cells in the apical meristem region were examined under oil immersion with an ocular micrometer, and two measurements, "a" and "b", at right angles were made for each nucleus. After seven nuclei in each of the apical cell layers were measured, "a" and "b" values were averaged and average nuclear volume, V, was computed from the formula for an ellipsoid:

$$V = \frac{4}{3} \times \left(\frac{a}{2}\right)\left(\frac{b}{2}\right)\left(\frac{a+b}{2}\right) \quad (19)$$

¹Ethyl alcohol (95%) 50 cc, glacial acetic acid 5 cc, formaldehyde (37-40%) 10 cc, water 35 cc.

CHAPTER IV

RESULTS AND DISCUSSION

Results from the analysis of data for plant height and bract diameter are shown in Tables I, II, III, and IV. Irradiation of mother plants caused a significant decrease in height of plants grown from cuttings taken from these plants. Generation one of the Mikkelpink variety and one and two of Spring Pink were significantly affected by irradiation. The most noticeable effects were observed in the first generations since this tissue was present at the time of irradiation. Irradiation caused no reduction in bract diameter in the Spring Pink variety (Table III) and only in the first generation of Mikkelpink (Table IV). Probably these plants had not yet recovered from the growth inhibiting effects of irradiation at the time of flowering.

There was also an increase in certain morphological responses such as stem fasciation and stem "splitting". The latter term applies to a condition in which the single leader terminates and three to five shoots grow from this point. Both fasciation and "splitting" can possibly be a result of a pathological condition induced by superabundant nutrition (19). The occurrence of a local injury and superabundant nutrition would result in the production of numerous buds in close proximity to each other which could then fuse in the case of fasciation.

A number of different colored sectors were recovered from the irradiated plants. Photographs of the predominant ones in Mikkelpink are

TABLE I
EFFECTS OF FAST NEUTRON IRRADIATION ON PLANT HEIGHT
OF MIMKELPINK POINSETTIA

Group	Mean Height and Standard Deviation in Centimeters		F Test
	Irradiated	Non-irradiated	
1	50.80±10.30	71.78± 9.21	126.22**
2	53.75±11.39	57.45±12.24	N.S.
3 ^a	---	---	---
4	51.33± 9.27	57.04± 7.59	N.S.
5	22.82± 6.31	25.52± 7.77	N.S.
6	38.31± 6.47	40.87± 4.02	N.S.

**Indicates significant differences (.01 level) between means of irradiated and non-irradiated plants.

^aBecause of high temperatures in greenhouse, this generation did not develop and was discarded.

TABLE II
EFFECTS OF FAST NEUTRON IRRADIATION ON PLANT HEIGHT
OF SPRING PINK POLYBETIA

Generation	Mean Height and Standard Deviation in Centimeters		F Test
	Irradiated	Non-Irradiated	
1	30.68±10.71	50.66±10.06	10.0**
2	23.65± 4.82	27.27± 4.60	16.2**
3	18.80± 3.93	18.64± 3.22	N.S.
4	29.94± 5.55	32.57± 5.99	N.S.

**Indicates significant differences (.01 level) between means of irradiated and non-irradiated plants.

TABLE III
EFFECTS OF FAST NEUTRON IRRADIATION ON BRACT DIAMETER
OF SPRING PINK POLYBETIA

Generation	Mean Bract Diameter and Standard Deviation in Centimeters		F Test
	Irradiated	Non-irradiated	
1	12.46± 2.60	10.00± 5.00	N.S.
2	16.83± 2.12	17.72± 2.05	N.S.
3	16.79± 2.19	16.92± 1.97	N.S.
4	19.44± 2.25	19.55± 1.69	N.S.

TABLE IV
EFFECTS OF FAST NEUTRON IRRADIATION ON BRACT DIAMETER
OF MINKELPINK POINSETTIA

Generation	Mean Bract Diameter and Standard Deviation in Centimeters		F Test
	Irradiated	Non-Irradiated	
1	25.90± 4.00	28.25± 2.90	10.90**
2	34.30± 3.56	35.49± 5.04	N.S.
3 ^a	---	---	---
4	23.32± 3.07	23.37± 3.99	N.S.
5	17.44± 6.95	18.83± 5.27	N.S.
6	22.30± 3.46	23.29± 3.26	N.S.

**Indicates significant differences (.01 level) between means of irradiated and non-irradiated plants.

^aBecause of high temperatures in greenhouse this generation was discarded.

shown in Figures 1, 2, 3, and 4. Figure 1 shows the normal condition of Mikkelpink. Figure 2 shows a small red sector and Figure 3 a plant with all bracts changed to red. Figure 4 pictures a change to white similar to the condition found in the Mikheldawn variety. Since Spring Pink is a true genetic pink, few chimeral changes in flower color were to be expected after radiation treatments. The frequency and kinds of changes in Mikkelpink, the periclinal chimera are shown in Table V. Mikkelpink often exhibits changes from pink to red or white under natural conditions. Therefore, an increased frequency of such changes was expected following irradiation. There was a highly significant increase in sectors recovered after irradiation.

The explanation of the recovery of both white and red sectors was discussed earlier as reported by Stewart (29). It is likely that these sectors resulted from the mechanical damage of irradiation to either the outer epidermis or the inner tissue. Damaged tissue would be replaced by adjacent undamaged cells, and the latter would determine expression of bract color. The few color changes found after irradiation in the variety Spring Pink were all a very light pink or almost white. This light color was a result of a colorless epidermis overlying a layer with sparse pigmentation. This same color of internal tissue could also be observed on normal portions of the bracts when the epidermis was peeled off. Probably the colored epidermis was destroyed at the time of irradiation and the almost colorless inner tissue took its place. Furthermore, the chance of a genetic mutation to white in the epidermis is slight since pink bract color is due to a single



Figure 1. Normal bract color of variety Mikkelpink poinsettia.



Figure 2. Small red sector induced by irradiation on variety Mikkelpink.



Figure 3. Irradiation induced bract color change to red on variety Mikkelpink.



Figure 4. Irradiation induced pink and white variegation of bract color of variety Mikkelpink.

factor which is recessive to red pigmentation, $\underline{Wh}/\underline{Wh} \underline{pk}/\underline{pk}$. Thus in order to be genetically changed to white both the dominant genes would have to mutate to a recessive condition or be deleted.

The change to light red shown in Table V cannot be explained at this time.

Number and size of sectors recovered in each generation are shown in Table VI. The sectors became larger in size and fewer in number with time. This result supports the findings of Baur (3), Jank (13), and Sparrow (25). The majority of sectors in generation five and six were red as shown in Table VII. However at the termination of the experiment the mother plants were cut back severely and allowed to produce shoots and flower. New shoots from these old experimental plants produced a population of sectors different from those observed in the latter generations. A number of whites along with a few light reds were present. Also a shoot with leaves variegated was observed. Thus in order to score as many possible mutant cells in irradiated plants, severe pruning is desirable.

Chromatographic analysis of pigment extracts from Mikkepink, Spring Pink and the mutant red, using three different solvent systems, established that the same pigments were present in all three. This conclusion is supported by Rf values in Table VIII. Similar evidence for the color in all the pink and red poinsettias as being due to different amounts of the same four anthocyanins has been reported by Stewart (29).

Colorimetric analysis of the anthocyanins present in the mutant and normal bracts was not successful. Hydrolysis of anthocyanins in the rhamno-glucose form occurred during preparation and purification of the

TABLE VI

EFFECT OF GENERATION (TIME AFTER IRRADIATION) ON SIZE AND
NUMBER OF SECTORS RECOVERED FROM IRRADIATED PLANTS

Generation	Percent in Each Generation Relative to Size ^a					Number of Observations
	1	2	3	4	5	
1	59.5	7.0	25.5	30.2	0.0	43
2	35.0	17.5	32.5	10.0	5.0	40
3 ^b	--	--	--	--	--	--
4	55.5	22.2	0.0	11.1	55.5	9
5	0.0	38.5	3.8	7.7	50.0	26
6	0.0	17.4	0.0	0.0	82.6	25

^aProportion of number of bracts covered by sector.

1 = less than one-quarter

2 = one-quarter to one-half

3 = one-half to whole

4 = one or more

5 = whole plant change

^bBecause of high temperatures in greenhouse this generation was discarded.

TABLE VII
FREQUENCY OF DIFFERENT COLORED SECTORS
FOUND IN EACH GENERATION

Generation	Color			Number of Observations
	Red	White	Light Red	
1	18	26	2	158
2	26	12	3	151
3 ^a	--	--	-	---
4	4	5	0	88
5	15	11	0	108
6	20	3	0	107

^aBecause of high temperatures in greenhouse this generation was discarded.

TABLE VIII
R_F VALUES FOR THE ANTHOCYANINE OF MUTANT
AND NORMAL POINSETTIA BRACTS

Anthocyanins	Solvent System		
	Water:5% Formic Acid (8:1:1, v/v)	1 Percent HCl	Acetone: .5 N HCl (1:3, v/v)
Cyanidin-glucoside			
from-			
Mikelpink	.24	.11	.42
Spring Pink	.23	.10	.20
Mutant Red	.25	.11	.41
Malargenidin-glucoside			
from-			
Mikelpink	.39	.22	.55
Spring Pink	.42	.23	.55
Mutant Red	.38	.20	.54
Cyanidin-rhamno-glucoside			
from-			
Mikelpink	.43	.21	.59
Spring Pink	.46	.22	.58
Mutant Red	.45	.24	.59
Malargenidin-rhamno-glucoside			
from-			
Mikelpink	.60	.31	.68
Spring Pink	.60	.33	.70
Mutant Red	.60	.32	.70

pigment extracts. For this reason the four pigments could not be prepared for calorimetric analysis in the same proportions as they occurred in the bracts. Harborne (12) has reported that anthocyanins in solution are unstable to light and pH change. Also, calorimetric analysis of the anthocyanidin portion of the pigments was not feasible because of the high acid and temperature conditions used in the hydrolysis of the sugar moiety from the basic structure. Harborne (11) concluded that ring opening during hydrolysis would account for loss of color. A further analytical difficulty was encountered because the absorption peak for cyanidin overshadowed the peak for pelargonidin due to the relatively much larger quantity of cyanidin.

Upon histological observation of the bracts for location of pigments the following conclusions were drawn and were in agreement with those of Stewart (29). The normal bracts of Mikkalpink contained no pigment in the upper or lower epidermis, but the inner spongy mesophyll tissue contained red pigment. In the white and pink variegated bracts recovered from irradiated Mikkalpink no pigment was found in the upper or lower epidermis. White bract areas had no pigment in any of the layers and pink areas had a red mesophyll as in normal bracts. The red mutant recovered from Mikkalpink contained red pigment in all histological layers. Spring Pink differed from Mikkalpink in containing some pigment in all tissue layers. The mesophyll of Spring Pink contained very little pigment and had a white appearance when the epidermis was removed.

Results of the radiosensitivity study are shown in Table IX and Figure 5. Table IX, expressing nuclear volume of the two varieties,

TABLE IX
AVERAGE NUCLEAR VOLUME OF TUNICA AND CORPUS LAYERS
IN SPRING PINK AND MIKKELPINK POLYMETTIA^a

	Tunica Layers					Corpus
	1	2	3	4	5	
Mikkelpink	210.18 ±20.76	214.00 ±21.21	171.23 ±19.93	154.89 ±8.85	177.44 ±24.30	148.40 ±13.43
Spring Pink	149.45 ±19.27	158.82 ±13.07	119.97 ±9.35	129.10 ±17.13	120.10 ±9.05	156.05 ±19.60

^aGiven in cubic microns ± standard error and measurements based on seven different cells.

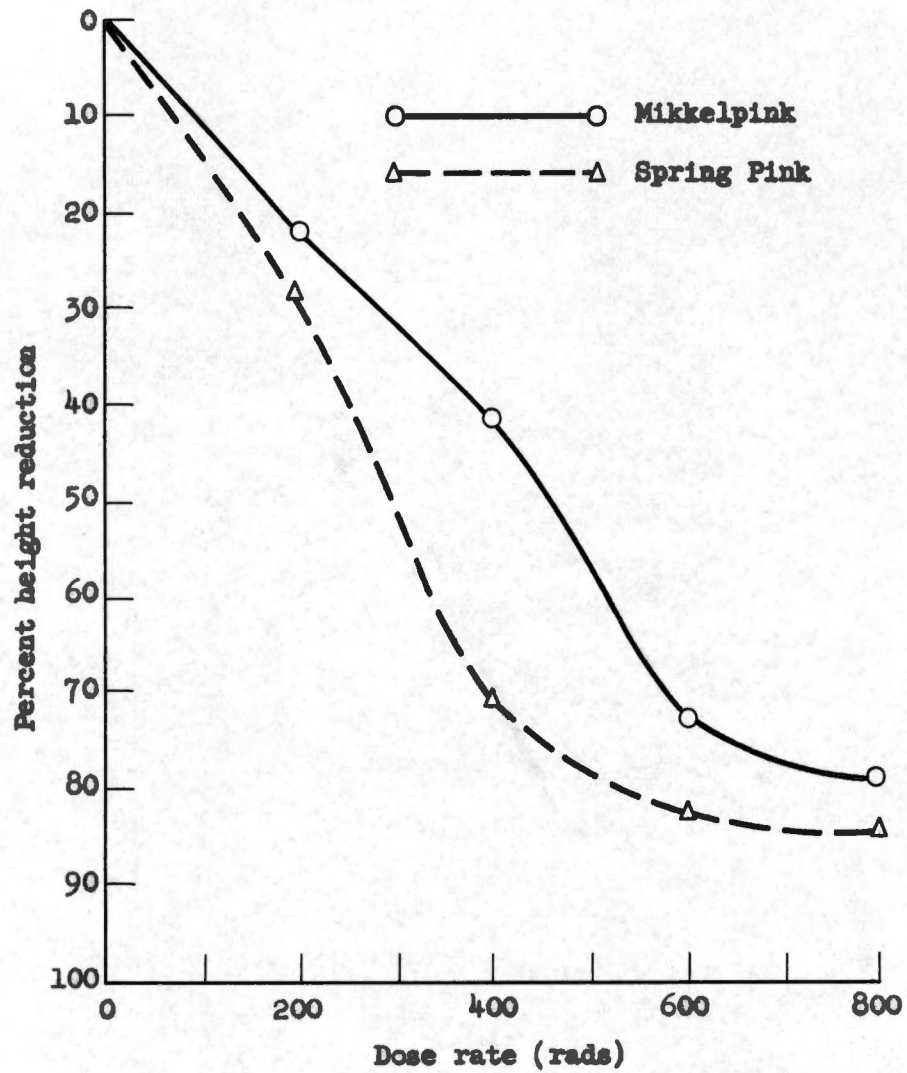


Figure 5. Percent height reduction of Mikkelpink and Spring Pink in response to various doses of fast neutrons.

would indicate that Mikkepink was the more radiosensitive because of the higher nuclear volume of the tunica layers. The relationship of nuclear volume and the sensitivity of growing plants was discussed earlier and in general the larger the nuclear volume the more radiosensitive the plant to gamma irradiation. However, there is very little evidence to support this relationship when using fast neutrons. Furthermore, the dose response studies shown in Figure 5 indicate that Spring Pink is more radiosensitive in that its growth was suppressed much more than the growth of Mikkepink at the same doses.

CHAPTER V

SUMMARY AND CONCLUSIONS

The effect of fast neutron irradiation on bract color and growth was studied in two varieties of poinsettia, Mikkelpink, a periclinal chimera and Spring Pink, a genetic pink. Successive generations of cuttings were rooted from stock plants of the two varieties which had been irradiated with 500 rads of fast neutrons. Chimerical color sectors on bracts were characterized according to size, number, type of pigment present, and histological location of pigment. An attempt to quantitize the pigment content was not successful. A large number of changes from pink to red or white were observed in Mikkelpink the periclinal chimera. Because of its chimerical nature the frequent changes to red or white might not be due to genetic effects, but rather might depend upon alteration of the relative position or rates of growth of genetically different tissue. Spring Pink, the genetic pink, yielded very few color changes. Those observed were to a very light pink or white and were due probably to the destruction of the epidermis by irradiation, thus exposing the sparsely pigmented inner tissue.

Certain growth responses to irradiation such as plant height and bract diameter were measured. In general it was noted that tissue present at the time of irradiation was more severely affected than tissue that developed later.

A measurement of radicosensitivity of the two varieties was accomplished by measuring nuclear volume and plant height reduction to various doses of irradiation. Even though the nuclear volume of Mikispink was significantly larger than the Spring Pink variety, an indication of higher sensitivity to gamma rays, Spring Pink proved to be the more sensitive to variable doses of irradiation.

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