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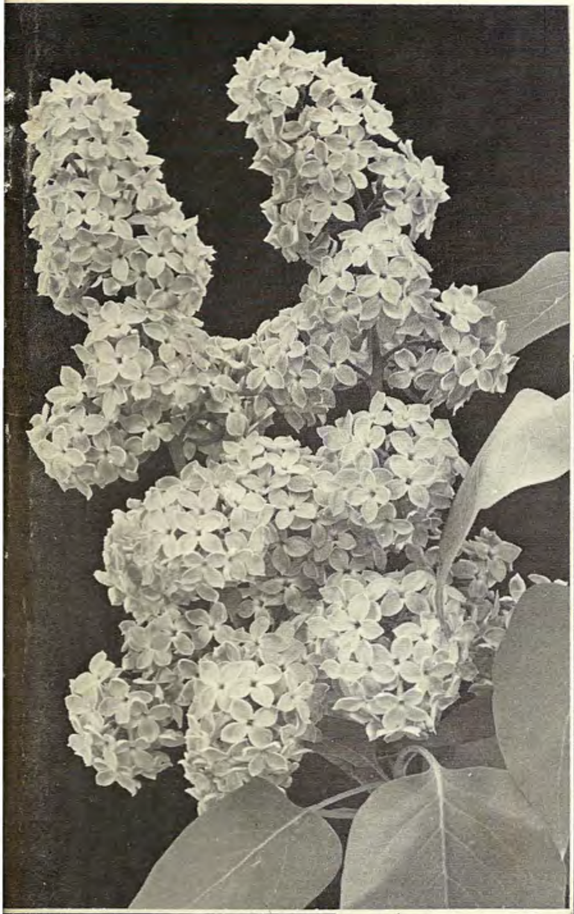

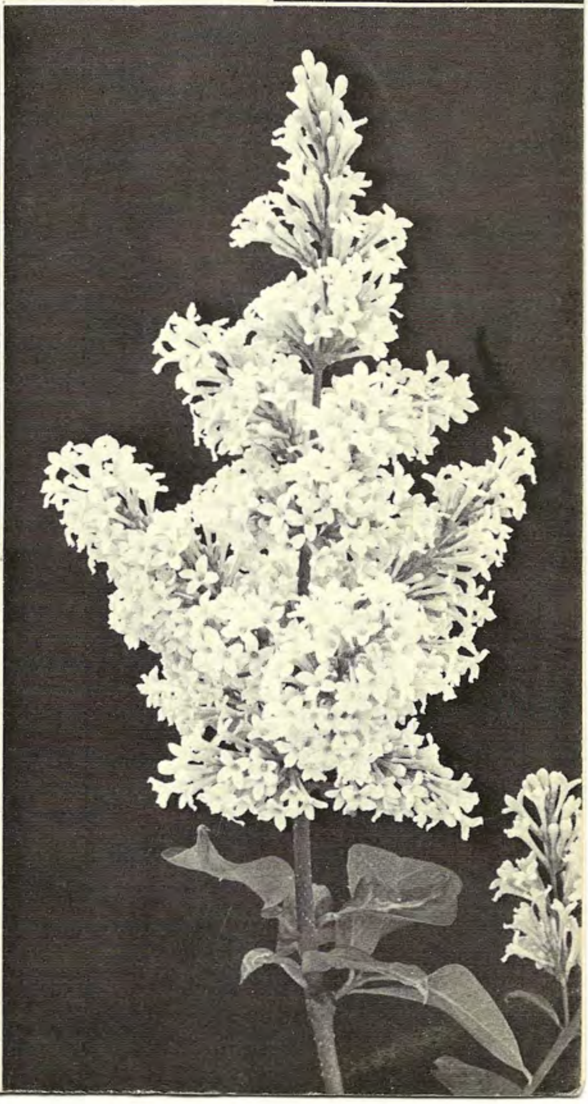

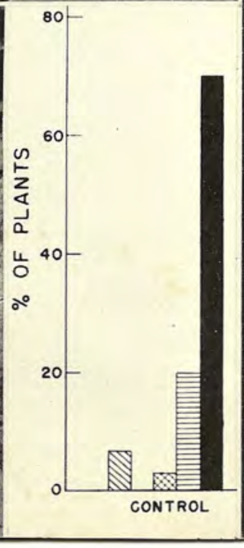

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SOME EFFECTS OF THERMAL-NEUTRON RADIATION ON SEEDS OF SYRINGA[1]

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Introduction

A project in the use of ionizing radiation to induce mutations in Syringa was initiated in 1958 by the late Dr. Albert F. Yeager of the University of New Hampshire and Agricultural Experiment Station, Durham. The present author's studies, reported here, were concerned with those effects of the radiation treatments which were evident in the germination of seeds and in the early development of seedlings.

The study of the effects of radiation on plants began shortly after the discoveries of various types of natural radiation late in the Nineteenth Century. The earliest studies were concerned with effects on the growth of plants subjected to irradiation, rather than with genetic effects. The first experiments demonstrating that mutations could be induced in plants through exposure to ionizing radiation were apparently those of Gager & Blakeslee, reported in 1927. They exposed seeds of a highly uniform, inbred line of Datura stramonium L. to gamma radiation, and obtained numerous mutations among the plants raised from these seeds. They also discovered a number of abnormalities in chromosomal structure associated with some of these phenotypic variations, and determined the chromosomal locations of certain mutant genes. Following these and other early experiments, considerable interest arose in using ionizing radiation as a mutagenic agent in practical plant breeding. The use of radiation for such purposes increased greatly during the late 1940's and 1950's, as nuclear reactors became available for research purposes and more kinds of radiation could be used. Use of various types of radiation, as well as other mutagenic agents, has now become a standard procedure in applied plant breeding (see Singleton, 1955, and Gregory, 1966, for reviews and bibliographies). Although the advantageous results may not have fully matched some early expectations, a number of cultivars, especially of grain crops, have been derived from material exposed to mutagenic treatments (Anonymous, 1972).

The literature on the biological effects of thermal-neutron radiation is now extensive and will not be reviewed at length here. The effects of the neutrons are believed to result from nuclear-capture reactions with elements of biological importance, notably hydrogen, nitrogen, and boron. With nuclear capture, these elements are converted to unstable isotopes, which emit high-energy secondary radiation. Upon capture of a neutron, H becomes H^2 and produces gamma rays; B^{10} is converted to B^{11}, which emits an alpha particle and thus becomes Li^7; and N^{14} temporarily becomes N^{15}, which emits a high-energy proton and electron and becomes C^{14}, which decays slowly (half-life 6000 years) to form N^{14}, emitting beta particles. Thus both electromagnetic and particulate radiation originate within and

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[1]Contribution No. 18 from the Royal Botanical Gardens, Hamilton, Ontario, Canada. This paper is adapted in large part from a thesis (Pringle, 1960) presented to the Graduate School of the University of New Hampshire in partial fulfillment of the requirements for the degree of Master of Science. The author is grateful to the late Dr. Albert F. Yeager and to Dr. Owen M. Rogers, who successively served as his major professor, and to Prof. Russell M. Eggert and the late Dr. John T. Kitchin, who were members of his advisory committee. Drs. Bertram Husch, William R. Lee, Douglas G. Routley, and Shih-an Yu, Profs. Elwyn M. Meader and J. Lincoln Pearson, and the late Dr. William W. Smith provided valued advice and information. Unpublished data were graciously supplied by Mr. Bernard Harkness, Dr. Owen M. Rogers, Dr. Seymour Shapiro, and the late Mr. Glenn Viehmeyer.

irradiate the contents of the cells; also, an atom emitting an alpha particle recoils in the opposite direction, breaking bonds. The secondary, internal radiations cause the atoms through which they pass to eject electrons, thus becoming ionized. Thermal neutrons, therefore, are classed with fast neutrons, X-rays, and alpha, beta, and gamma rays as ionizing radiation. Ionization of an atom is almost certain to effect changes in the molecule of which it is a part, which may be a nucleic acid or nuclear protein (Conger & Giles, 1950; Lea, 1955; Bender, 1970).

Thermal neutrons have been found to produce both "chromosomal" mutations, i.e., visible changes in chromosome structure, and "genic" mutations, apparently affecting a single gene, although the former type appears to predominate (Bender, 1970). In this respect, the effects of thermal-neutron radiation are similar to those of X-rays and gamma rays. With regard to efficiency, however, thermal neutrons have found favor with plant breeders in that they appear to induce a higher proportion of recoverable mutations in relation to lethal damage to the embryos or other plant parts irradiated (Conger & Giles, 1950; Brock, 1970; Gaul et al., 1972). Conger & Giles (1950) have suggested that radiation originating internally may be more effective in producing chromosomal changes because boron, the most important atom emitting particulate radiation and recoiling, is apparently most concentrated in the nucleus, and because injury to the ends of chromosome fragments, preventing rejoining in the original order, may be caused by recoiling atoms or alpha particles. Recent studies with chemical mutagenic agents, however, have indicated that some of these, especially ethylmethanesulfonate (EMS), may be more efficient than any type of ionizing radiation (Bender, 1970; Gaul et al., 1972).

In addition to mutations, the effects of ionizing radiation on plant tissues include numerous modifications of growth and development. Gunckel & Sparrow (1950) have reviewed many studies of these effects. Growth is commonly retarded or inhibited by high doses of ionizing radiation. Bud development is often abnormal, and numerous adventitious buds may develop, producing witches'-brooms. Stems and peduncles may be fasciated, and leafy growths and other abnormalities sometimes appear in inflorescences. Foliar abnormalities are especially common, including reduction in size, roughening or puckering of the blades, asymmetry, distorted venation, forking, fusion, cup-shaped or tubular blades, and early abscission. Probably the most common abnormality is a mosaic pattern or other variegation in leaf color, due to uneven distribution of chlorophyll.

Some of these abnormalities are believed to be related to chromosomal damage. Retardation of growth appears to be due in part to suppression of mitosis resulting from the depolymerization of nucleic acids and from the destruction of sulfhydryl groups in enzymes involved in nucleic-acid synthesis, by the products of the ionization of water (Gunckel & Sparrow, 1950; Hevesy, 1952; Barron, 1952). Retardation of growth appears also to be due in part to inhibition of cell elongation through the destruction of auxins (Skoog, 1935; Smith & Kersten, 1942). According to Barron (1952), numerous other compounds, including various enzymes, ascorbic acid, cytochrome C, and the energy-transfer compound adenosine triphosphate, may also be destroyed through oxidation by the products of water ionization.

Such effects, categorized as "physiological," appear in many cases to be due to extrachromosomal damage to cells already differentiated to some extent at the time of irradiation. As long as nuclei in the meristematic tissues retain the genetic information necessary for the production of a normal complement of enzymes and other cytoplasmic compounds, growth may be expected eventually to become normal, as destroyed compounds are replaced. Plants exhibiting physiological abnormalities in growth produced shortly after irradiation commonly produce normal growth later.

Effects on seed germination have been highly variable. Stimulation of germination has frequently been reported with relatively low doses of ionizing radiation, although heavier doses have caused retarded or reduced germination. Studies by Singh (1941) and Haskins & Chapman (1956) have indicated that low doses of radiation increase catalase activity in seeds, thus paralleling the effects of after-ripening.

Only three other experiments in which lilacs have been subjected to ionizing radiation are known to the author. Viehmeyer (in litt., 1960) reported that seed of Syringa X chinensis Willd. (pro sp.) and cultivars of S. vulgaris L., irradiated with thermal neutrons for five hours and stratified for about 60 days, germinated almost normally, and that seedlings from this seed grew normally. Seed subjected to ten hours' irradiation and stratified for the same period also germinated fairly well, but only three of the seedlings from this seed produced any top growth above the cotyledons. One of the S. vulgaris seedlings was described as being an extreme dwarf, only five inches high after three growing seasons. The other two seedlings grew normally. Harkness (in litt., 1959) subjected cuttings of S. vulgaris cultivars to several doses of thermal neutrons and X-rays. He found that survival might have been somewhat reduced by the greatest exposure to thermal neutrons, although the small numbers of cuttings used, the use of different cultivars with each treatment, and the absence of controls precluded definite conclusions. No variations from expected growth patterns nor any morphological abnormalities were observed. At the Brookhaven National Laboratory, several lilac plants were grown in a field exposed to chronic gamma radiation. According to Shapiro (in litt., 1960), these plants exhibited a number of abnormalities of sorts commonly associated with radiation, but there were no advantageous changes.

In limited experiments with a chemical mutagenic agent at the Royal Botanical Gardens in the autumn of 1966, seeds of S. emodi Royle, open-pollinated, were soaked for six hours at room temperature in water and in 0.04 M, 0.08 M, and 0.12 M aqueous solutions of EMS. This experiment was repeated in 1967 with additional seeds of S. emodi and with seeds of S. tomentella Bur. & Franch., open-pollinated. The seeds were sown outdoors in the autumn. Total germination the following spring was not significantly different among the seeds of each species subjected to the different treatments, although germination was much slower among the seeds of S. emodi subjected to the highest concentration of EMS. Visible physiological effects were few. The only conspicuous abnormality was the production of widely divergent branches in the early growth of a seedling of S. emodi, in contrast to the strongly ascending branches characteristic of this species. No chimeras were detected. Because of the evidently limited effects of EMS in the doses employed, these experiments were not continued into additional generations.

Materials and Methods

Seed sources. Seeds designated "series Villosae mixed" were collected by Dr. Yeager from open-pollinated plants at the Horticulture Farm of the New Hampshire Agricultural Experiment Station. The source plants were first- and second-generation seedlings of the cultivar 'Royalty', and included the cultivars 'Anna Amhoff', 'Maybelle Farnum', and 'Nellie Bean', as well as unnamed sibling seedlings.[2]

The late Dr. William W. Smith collected seeds of S. 'James Macfarlane', another seedling of 'Royalty', at his farm in Gilford, New Hampshire. Most of these seeds were believed to have come from remontant flowering, and therefore to be the result of self-pollination, since no other plants flowered in the autumn of 1958. Some seeds, however, may have developed from open pollination by

[2]Although designated S. X prestoniae McKelvey by Yeager et al. (1959), 'Royalty' is a cultivar of S. josikaea X S. reflexa, according to reports of the Experimental Station at Morden, Manitoba (Leslie, 1938). Since Dr. Yeager's plant of 'Royalty' was grown with 'Coral' (S. villosa X S. reflexa, = S. X. prestoniae) and 'Hedin' (S. villosa X S. sweginzowii), as many as four species have been involved in the ancestry of the plants used in these experiments.

plants of similar parentage during the June flowering season.

Seeds of S. vulgaris, also the products of open pollination, were collected by Dr. Yeager from plants of several cultivars in the Lilac Arboretum of the New Hampshire Agricultural Experiment Station.

Radiation treatments. Seeds collected in 1958 were subjected to thermal-neutron radiation on 10 February 1959 at the Brookhaven National Laboratory, through the courtesy of Dr. Seymour Shapiro. Seeds from all three lots were exposed to the three doses of thermal neutrons shown in Table 1. Because the doses varied almost entirely in the length of exposure rather than in the flux, or density, of the neutrons, the lengths of exposure can conveniently be used to represent the respective treatments. Seeds from each lot which were not irradiated were used as controls.

Table 1. Doses of thermal neutrons, in neutrons/cm^2, to which Syringa seeds were exposed.

Time	Flux	Total dose (Flux x time)
4 hrs.	6.30×10^8	9.07×10^{12}
8 hrs.	6.26×10^8	18.0×10^{12}
12 hrs.	6.34×10^8	27.3×10^{12}

[a]Figures carry a possible error of $\pm$ 15%.

Germination tests. Shortly after their return from Brookhaven National Laboratory, all groups of seeds were tested for germination in the seed-testing laboratory of the New Hampshire Agricultural Experiment Station. Very small, shriveled seeds and broken seeds were avoided, but otherwise no discrimination was exercised in their selection. The seeds were placed atop four layers of moist blotters and then moved into closed, humid germinators. From about 8 a.m. to 4 p.m. for five successive days each week the seeds were kept in a germinator with a glass panel facing a window, at 30° C. At night and on the remaining two days they were kept in a dark germinator at 20° C.

Daily counts were made of germinated seeds. A seed was counted as having germinated if any part of the embryo could be seen, regardless of its apparent quality. The first test was begun 2 March 1959, and continued until four days had passed in which no further germination occurred, a total time of 37 days after the seeds were placed in the germinators. The second test, begun 16 March 1959, was also continued for 37 days.

Seeds were also sown in a greenhouse, in flats containing a mixture of equal parts soil, sand, and peat. In these flats, however, emergence was irregular and often much delayed, so that usable data on total emergence were not obtained.

Subsequent handling of seedlings. Seedlings were moved from the germinators to flats in the greenhouse, containing the soil mixture described above, as their survival required. Both these seedlings and those from seeds planted directly in the flats were left in the flats until early May. All surviving seedlings were then potted in two-inch-diameter rose pots containing a mixture of two parts soil to one part peat and one part sand. Shortly before their removal from the greenhouse in early June, Dr. Yeager examined the seedlings and discarded those which he considered unlikely to produce any appreciable growth beyond the cotyledon stage.

In early June 1959, the remaining seedlings were transplanted to a field at the Horticultural Farm of the New Hampshire Agricultural Experiment Station. The plants were set in rows 3.5 feet apart, with approximately six inches between the plants in the rows. They were cultivated periodically for control of weeds, but no fertilizers, herbicides, fungicides, or insecticides were applied. Observations of the growth of these seedlings and the appearance of their foliage were made at intervals during the remainder of 1959.

Results and Discussion

Germination tests. Statements and calculations in the following paragraphs are based on the combined results of the two tests of seed germination. Results of the germination tests with the series Villosae mixed seed are shown in Table 2. The highest percentage of germination occurred among the seeds irradiated eight hours, those not irradiated, and those irradiated twelve hours were successively lower in that order. However, when analyzed according to the method described by Davies (1950) for determining significances of differences between percentages, the differences among the percentages of seeds which germinated were found not to be statistically significant.

Table 2. Results of germination tests of Syringa x series Villosae mixed seeds subjected to three levels of thermal-neutron radiation.

Length of exposure	Total seeds	Germination No.	Germination %	Mean days to germinate	Median days to germinate	Transplanted No.	Transplanted %[a]
Control							
I	102	74	72.6	9.61	8.11	73	98.6
II	100	77	77.0	8.49	6.78	77	100.0
Total	202	151	74.8	9.04	7.25	150	99.3
4 hrs.							
I	101	78	77.2	10.69	8.38	78	100.0
II	100	77	77.0	10.34	7.69	76	98.7
Total	201	155	77.1	10.52	7.96	154	99.4
8 hrs.							
I	97	75	77.3	10.65	9.21	73	97.3
II	99	75	75.8	9.37	8.41	75	100.0
Total	196	150	76.5	10.01	8.56	148	98.6
12 hrs.							
I	94	68	72.3	12.50	10.30	66	97.1
II	99	71	71.7	10.49	9.30	70	98.6
Total	193	139	72.0	11.47	9.96	136	97.8

Correlation of days required for germination with dose
$r = +.1624$**

[a]of those which germinated.
**Statistically significant at the 1% level.

Correlation of the numbers of days required for germination with the dose of radiation to which the seeds had been exposed was positive and highly significant according to Student's t test. Table 2 includes the mean numbers of days required for germination by the seeds subjected to each dose. The median, i.e., the time required for half of the seeds which eventually germinated to have done so, is also shown in this table and in Figure 1 since in some cases there is a highly significant correlation with the radiation dose received.

Nearly all of the seeds from this lot which germinated survived long enough to be transplanted to the flats. There are no significant differences among the percentages of seedlings which were transplanted.

Table 3 shows the results of the germination tests of the seeds of S. 'James Macfarlane'. The generally low germination of these seeds largely eclipsed any effects of radiation. However, although the differences among the percentages of seeds which germinated were not statistically significant,a similar trend to that found with the other two lots of seed was observed, in that the highest percentage of germination occurred among the seeds irradiated four hours, while among the seeds which had been subjected to higher doses, percentages of germination were successively lower.

Table 3. Results of germination tests of seeds of Syringa cv. 'James Macfarlane' subjected to three levels of thermal neutron radiation.

Length of exposure	Total seeds	Germination No.	Germination %	Mean days to germinate	Median days to germinate	Transplanted No.	Transplanted %a
Control							
I	99	44	44.4	12.32	11.17	43	97.7
II	100	47	47.0	9.91	8.95	45	95.7
Total	199	91	45.7	11.08	9.63	88	96.7
4 hrs.							
I	99	51	51.5	11.45	9.42	51	100.0
II	98	56	57.1	12.64	10.25	50	89.3
Total	197	107	54.3	12.07	9.85	101	94.4
8 hrs.							
I	99	53	53.5	12.75	11.08	50	94.3
II	98	45	45.9	12.88	10.25	41	91.1
Total	197	98	49.7	12.81	10.75	91	92.9
12 hrs.							
I	97	44	45.3	15.70	12.90	42	95.5
II	97	51	52.6	12.61	11.25	42	82.4
Total	194	95	48.9	14.04	12.58	84	88.4

Correlation of days required for germination with dose
$r = +.6997$**

a Of those which germinated.
** Statistically significant at the 1% level.

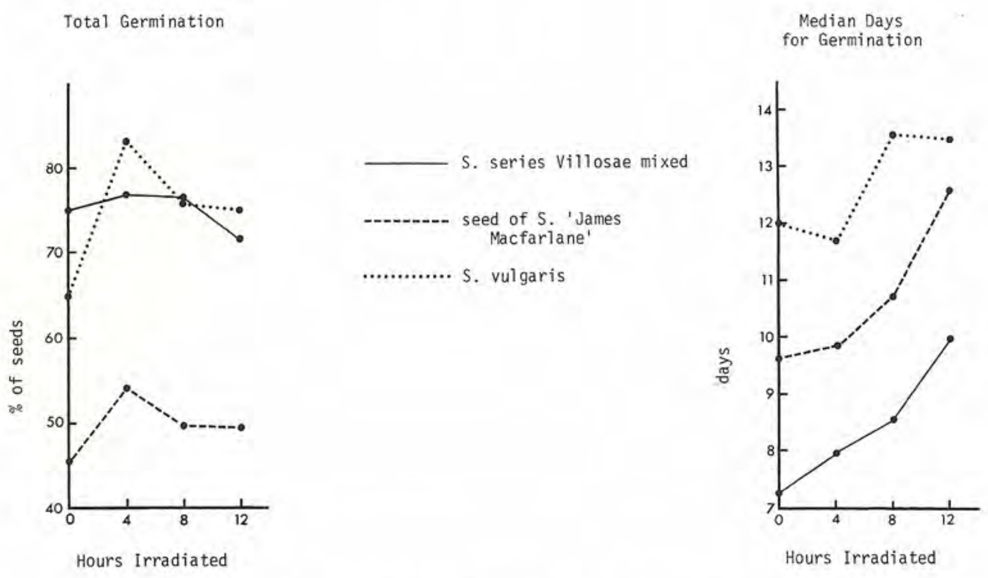

Figure 1. Results of germination tests of seed of Syringa subjected to three levels of thermal-neutron radiation.

With this lot of seeds the rate of germination was again found to have been slower with each increasing dose; the correlation of the rate with the dose is highly significant. Table 3 shows the mean and median numbers of days required for germiantion, and Figure 1 includes a comparison of the median for this lot with those for the other lots.

As with the series Villosae mixed seeds, nearly all of the seedlings survived long enough to be transplanted to the flats, with no significant differences existing among the percentages.

The results of the germination tests of S. vulgaris seed are shown in Table 4. Again, the highest percentage of germination occurred among the seeds which had been irradiated four hours, with germination among the seeds irradiated eight and twelve hours being successively lower. Germination was lowest with the control seeds. The percentages of seeds which germinated among those irradiated four hours was significantly higher than that of either the seeds not irradiated or those exposed twelve hours. The other differences among the percentages of seeds which germinated were not statistically significant.

As with the other two seed lots, there was a significant positive correlation between the doses of radiation to which the seeds had been exposed and the number of days required for germination. The mean and median numbers of days are shown in Table 4, the median also in Figure 1.

With S. vulgaris, there were significant differences among the percentages of seedlings from the different radiation treatments which survived long enough to be transplanted to the flats, as indicated in Part B of Table 4. Most of the seedlings which were not transplanted produced a short root which soon died. In a few cases only a portion of the seed coat protruded from the seed coat while the radicle never appeared, or the hypocotyl appeared with no growing point at its radicle end.

That the seeds used in these experiments had not been stratified is probably relevant to the interpretation of the above data. Hartmann & Kester (1975) have stated that the germination of lilac seeds is significantly improved by after-ripening treatments. In view of such studies as those of Singh (1941) and Haskins & Chapman (1956), it may be hypothesized that the lower radiation doses (all doses with S. vulgaris) produced effects similar to those of after-ripening, thus stimulating germination through an increase in catalase activity. The lower germination of the seeds exposed to higher doses is presumably due to interference with growth processes, which has frequently been reported to have resulted from irradiation.

As Figure 1 shows, with all three lots there was a trend toward slower germination as the length of exposure to radiation increased beyond four hours. Retardation of cell division and enlargement was probably at least partly responsible for this effect.

Selection and survival of seedlings. Tables 5, 6, and 7 show the numbers of seedlings which Dr. Yeager considered to be suitable for field planting and the survival of these seedlings during the summer and autumn of 1959. In all lots, an appreciable decline in the numbers of seedlings deemed to show potential for growth can be noted among those from seeds which received greater doses of radiation.

Table 4. Results of germination tests of Syringa vulgaris seeds subjected to three levels of thermal-neutron radiation.

A. Numbers and percentages of seeds which germinated and seedlings transplanted; time required for germination.

Length of exposure	Total seeds	Germination No.	Germination %	Mean days to germinate	Median days to germinate	Transplanted No.	Transplanted %[a]
Control							
I	100	72	72.0	12.90	12.00	54	75.0
II	99	65	65.7	12.06	11.83	55	84.6
Total	199	137	68.8	12.50	11.97	109	79.6
4 hrs.							
I	98	81	82.7	12.78	11.85	74	91.4
II	99	83	83.8	11.77	11.25	83	100.0
Total	197	164	83.2	12.27	11.75	157	95.7
8 hrs.							
I	99	76	76.8	16.62	14.64	55	72.4
II	98	74	75.5	13.62	12.64	68	91.9
Total	197	150	76.1	15.14	13.53	123	82.0
12 hrs.							
I	100	74	74.0	16.34	14.67	63	85.1
II	100	77	77.0	14.34	12.70	75	97.4
Total	200	151	75.5	15.32	13.47	138	91.4

Correlation of days required for germination and dose r = +.3214.**

[a] Of those which germinated.
** Statistically significant at the 1% level.

B. Significance of differences between percentages of seeds which germinated and seedlings transplanted.

Lengths of exposure compared	Germination	Transplanted
Control - 4 hrs.	**	**
Control - 8 hrs.	n.s.	n.s.
Control - 12 hrs.	n.s.	*
4 hrs. - 8 hrs.	n.s.	**
4 hrs. - 12 hrs.	*	n.s.
8 hrs. - 12 hrs.	n.s.	*

Correlation of days required for germination and dose r = +. 3214.**

* Statistically significant at the 5% level.
** Statistically significant at the 1% level.
n.s. Not significant.

Table 5. Survival of *Syringa* series *Villosae* mixed seedlings from seed subjected to three levels of thermal-neutron radiation.

Length of exposure	Potted	Selected for field planting		Surviving 13 July		Surviving 9 Nov.	
		No.	% of those potted	No.	% of those planted	No.	% of those planted
Control							
From 500 seeds[a]	215	199	92.6	183	92.0	173	86.9
4 hrs.							
From 500 seeds	266	250	94.0	232	92.8	214	85.6
Total	-	-	-	311	-	282	-
8 hrs.							
From 500 seeds	252	217	86.1	190	87.6	164	75.6
Total	-	352	-	299	84.9	263	74.7
12 hrs.							
From 500 seeds	285	165	57.9	140	84.8	126	76.4
Total	-	-	-	195	-	173	-

[a] No additional seedlings grown.

Table 6. Survival of seedlings of Syringa cv. 'James Macfarlane' from seed subjected to three levels of thermal-neutron radiation.

Length of exposure	Potted	Selected for field planting		Surviving 9 Nov.	
		No.	% of those potted	No.	% of those planted
Control					
From 431 seeds[a]	155	123	79.4	103	88.7
4 hrs.					
From 500 seeds	171	107	62.6	88	82.2
Total	-	-	-	96	-
8 hrs.					
From 500 seeds	163	75	46.0	50	66.7
12 hrs.					
From 458 seeds	114	43	37.7	23	53.5

[a] No additional seedlings grown.

Table 7. Survival of *Syringa vulgaris* seedlings from seed subjected to three levels of thermal-neutron radiation.

Length of exposure	Potted	Selected for field planting: No.	Selected for field planting: % of those potted	Surviving 13 July: No.	Surviving 13 July: % of those planted	Surviving 9 Nov.: No.	Surviving 9 Nov.: % of those planted
Control							
From 500 seeds[a]	163	156	95.7	153	98.1	126	80.8
4 hrs.							
From 500 seeds[a]	259	239	92.3	238	99.6	233	97.5
8 hrs.							
From 500 seeds[a]	201	115	57.2	106	92.2	106	92.2
12 hrs.							
From 500 seeds	129	44	34.1	39	88.6	37	84.1
Total	-	-	-	51	-	47	-

[a] No additional seedlings grown.

Table 8. Distribution of shoot length on 13 July 1959 of _Syringa_ series _Villosae_ mixed seedlings from seed subjected to three levels of thermal-neutron radiation.[a]

Length of exposure	Total plants	Up to 2 in.	2.5-4 in.	4.5-8 in.	8.5-16 in.	16.5 in. and over	Mean ht. (in.)
Control							
No.	183	80	23	43	30	7	4.67
%		43.7	12.6	23.5	16.4	3.8	
4 hrs.							
No.	311	194	35	59	19	4	3.02
%		62.4	11.3	19.0	6.1	1.3	
8 hrs.							
No.	299	205	49	37	7	1	2.05
%		68.6	16.4	12.4	2.3	0.3	
12 hrs.							
No.	195	147	29	16	3	0	1.78
%		75.4	14.9	8.2	1.6	0.0	

Correlation of shoot length with dose to which seeds had been exposed r = -.2711**

[a] To the nearest 0.5 inch.
** Statistically significant at the 1% level.

Tables 5, 6, and 7 also show the total selection and survival of the seedlings from the seeds used in the germination tests plus the counted seeds planted directly in the flats, of which there were 300 for each treatment when possible. In cases in which additional seedlings were set out, total survival in the field is also indicated. Because the seedlings planted in the field were selected, differences in survival may to some extent reflect artificially induced factors. A few plants may have died as a result of root destruction by June-beetle larvae or as a result of injury in cultivation. However, with all seed lots, survival in the field followed a similar pattern of decline among the plants from seeds subjected to the higher radiation doses.

Growth of plants in the field. The total growth, including branches, of all surviving seedlings from the series Villosae mixed seeds was measured on 13 July 1959. The data obtained are shown in Table 8 and Figure 2. There was a highly significant negative correlation between the mean shoot lengths of the seedlings from the respective treatments and the doses of radiation to which the seeds had been exposed.

Assignment of the seedlings to arbitrarily defined classes according to the growth they had produced, as shown in Figure 3, indicated that the lower mean shoot lengths associated with higher radiation doses was not due to uniform retardation but to greater proportions of slow-growing seedlings. Had all of the seedlings been transplanted to the field, the proportions of very slow-growing plants would presumably have been greater. A general approximation of the total effect of the irradiation treatments can probably be obtained by adding the numbers in the lowest size category. The presence of slow-growing seedlings among those from the control seeds is probably due to the fact that the seeds had not been stratified.

The numbers of branched seedlings were also recorded at this time (Table 9 and Figure 2). With these plants, branching appeared to be related simply to total growth, rather than having been independently retarded or stimulated by irradiation.

Table 9. Numbers of branched seedlings on 13 July 1959, from Syringa series Villosae mixed seed subjected to three levels of thermal-neutron radiation.

Length of exposure	Branched seedlings	
	No.	%
Control	27	14.8
4 hrs.	35	11.3
8 hrs.	24	8.0
12 hrs.	10	5.1

Growth of the seedlings of S. 'James Macfarlane' and S. vulgaris was not measured on 13 July. These plants were in general much slower growing, and at that time were still too small to exhibit appreciable differences related to irradiation treatments.

On 19 October 1959, when shoot elongation appeared to have ceased, measurements were made of the total shoot lengths of a sample consisting of every fifth plant in all the lots of seedlings. The data obtained with the series Villosae mixed seedlings are presented in Table 10 and Figure 4. Greater proportions of seedlings which had produced very little growth were still found among those plants from seeds exposed to higher doses of radiation. Correlation

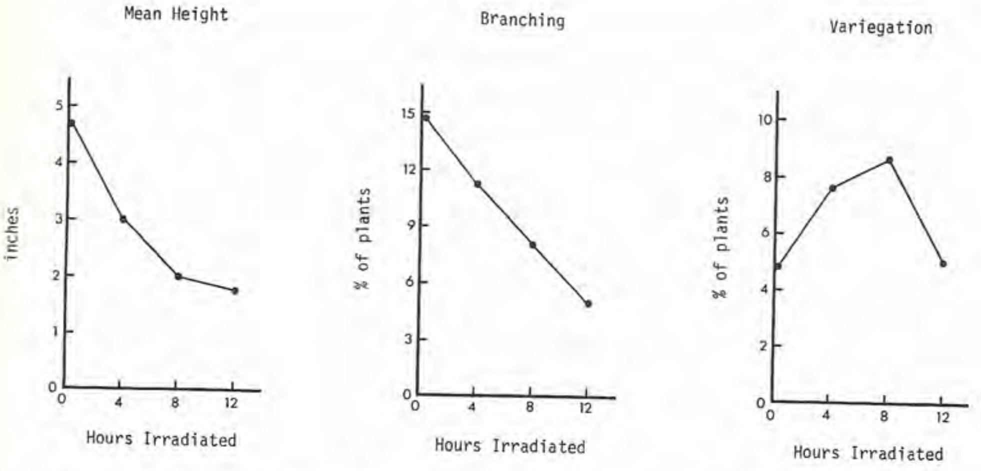

Figure 2. Observations on 13 July 1959 of Syringa series Villosae mixed seedlings from seed subjected to three levels of thermal-neutron radiation.

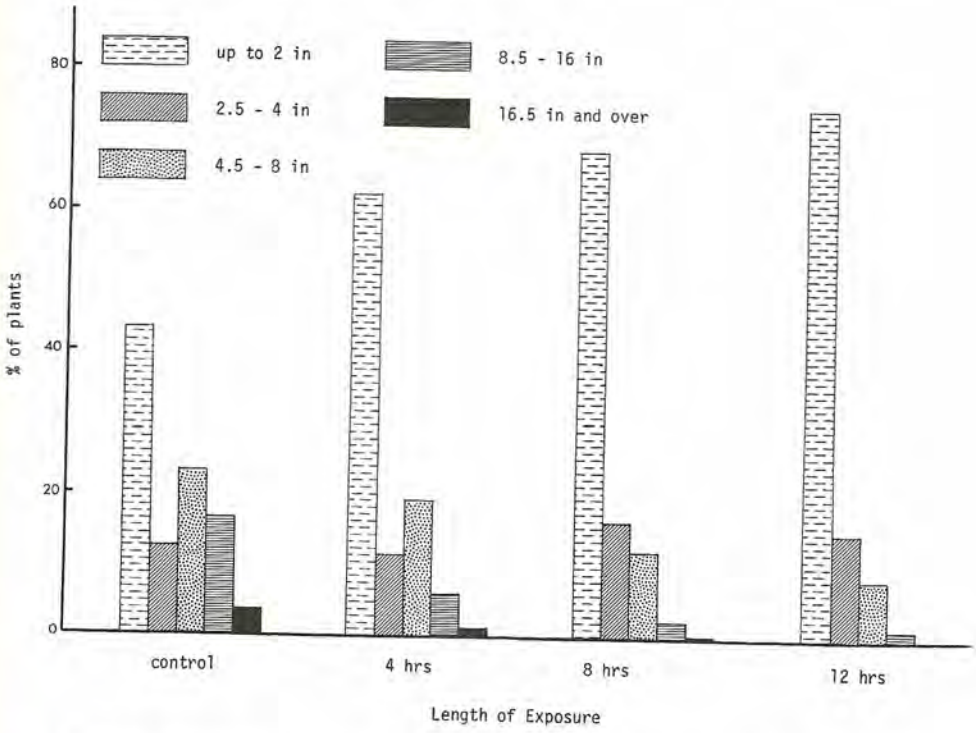

Figure 3. Distribution of height on 13 July 1959 of Syringa series Villosae mixed seedlings from seed subjected to three levels of thermal-neutron radiation.

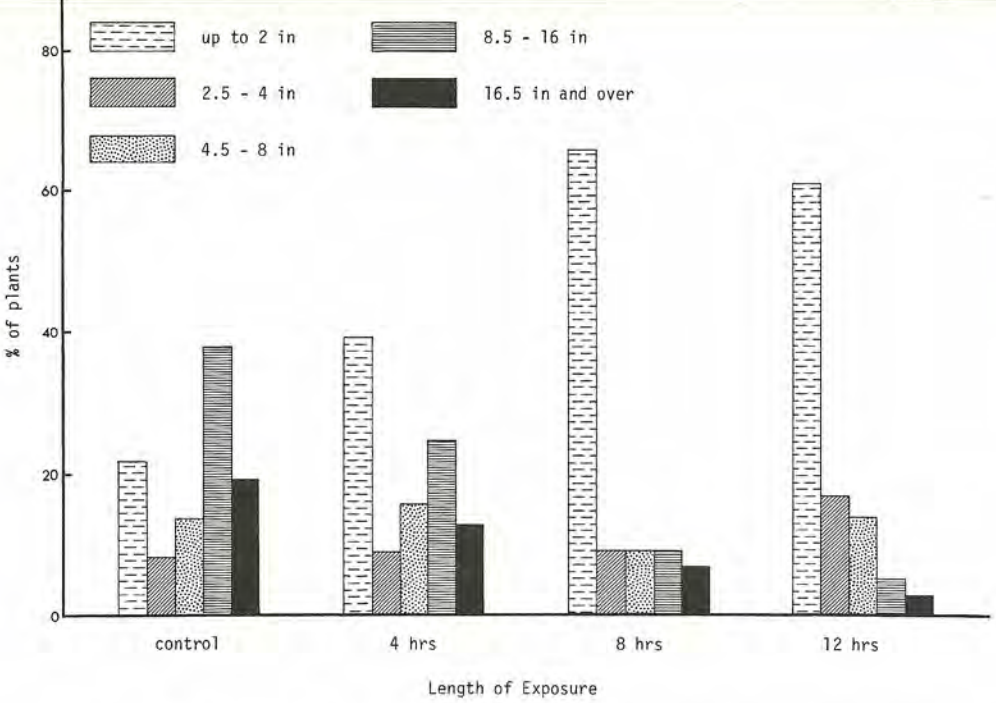

Figure 4. Distribution of height on 19 October 1959 of Syringa series Villosae mixed seedlings from seed subjected to three levels of thermal=neutron radiation.

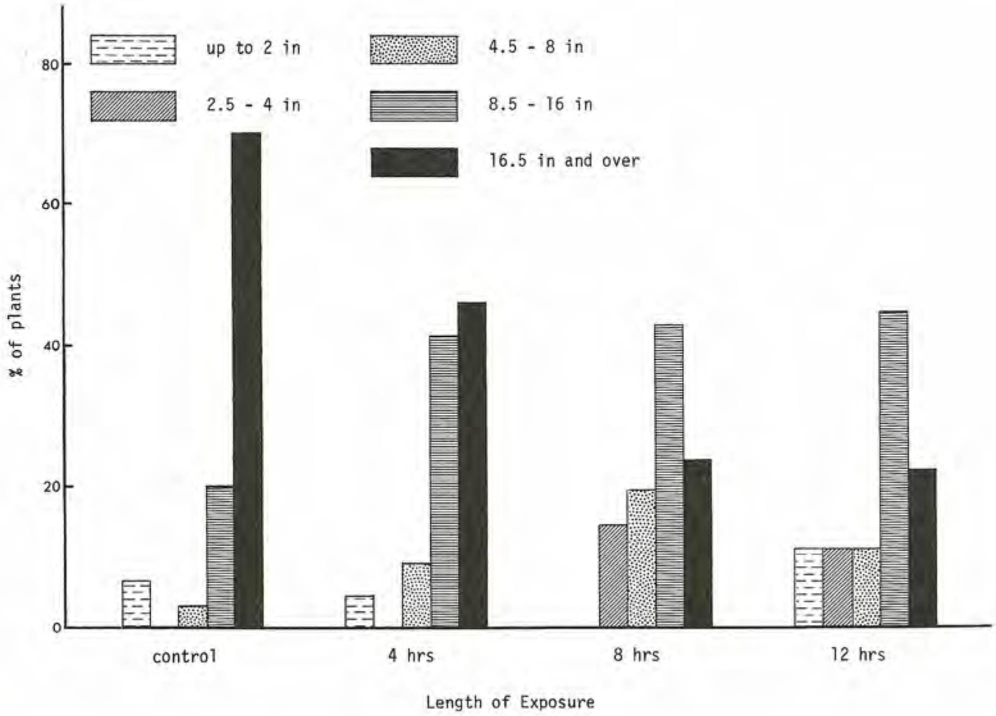

Figure 5. Distribution of height on 19 October 1959 of Syringa vulgaris seedlings from seed subjected to three levels of thermal-neutron radiation.

Table 10. Distribution of shoot length on 19 October 1959 of Syringa series Villosae mixed seedlings from seed subjected to three levels of thermal-neutron radiation.[a]

Length of exposure	Plants in sample	Up to 2 in.	2.5-4 in.	4.5-8 in.	8.5-16 in.	16.5 in. and over	Mean ht. (in.)	Increase in mean ht. since 13 July(in.)
Control								
No.	37	8	3	5	14	7	11.26	6.59
%		21.6	8.1	13.5	37.8	18.9		141.1
4 hrs.								
No.	57	22	5	9	14	7	6.49	3.27
%		38.6	8.8	15.8	24.6	12.3		114.9
8 hrs.								
No.	56	37	5	5	5	4	4.41	2.44
%		66.1	8.9	8.9	8.9	7.1		119.0
12 hrs.								
No.	36	22	5	5	2	1	3.20	1.42
%		61.1	13.9	13.9	5.6	2.8		79.8

Correlation of shoot length with dose to which seeds had been exposed r = -.3322**

[a] To the nearest 0.5 inch; based on a sample consisting of every fifth plant.
** Statistically significant at the 1% level.

Table 11. Distribution of shoot length on 19 October 1959, of seedlings of Syringa 'James Macfarlane' from seed subjected to three levels of thermal-neutron radiation.[a]

Length of exposure	Plants in sample	Up to 2 in.	2.5- 4 in.	4.5- 8 in.	8.5- 16 in.	16.5 in. and over	Mean ht. (in.)
Control							
No.	21	19	0	2	0	0	1.53
%		90.5	0.0	9.5	0.0	0.0	
4 hrs.							
No.	19	11	4	2	2	0	2.61
%		58.9	21.1	10.5	10.5	0.0	
8 hrs.							
No.	11	10	1	0	0	0	1.23
%		90.9	9.1	0.0	0.0	0.0	
12 hrs.							
No.	4	3	1	0	0	0	1.50
%		75.0	25.0	0.0	0.0	0.0	

Correlation of shoot length with dose to which seeds had been exposed r = -.0836 n.s.

[a] To the nearest 0.5 inch; based on a sample consisting of every fifth plant.
n.s. Not significant.

Table 12. Distribution of shoot length on 19 October 1959 of <u>Syringa vulgaris</u> seedlings from seeds subjected to three levels of thermal-neutron radiation.[a]

Length of exposure	Plants in sample	Up to 2 in.	2.5- 4 in.	4.5- 8 in.	8.5- 16 in.	16.5 in. and over	Mean ht. (in.)
Control							
No.	30	2	0	1	6	21	25.16
%		6.7	0.0	3.3	20.0	70.0	
4 hrs.							
No.	46	2	0	4	19	21	16.86
%		4.3	0.0	8.7	41.3	45.7	
8 hrs.							
No.	21	0	3	4	9	5	13.05
%		0.0	14.3	19.0	42.9	23.8	
12 hrs.							
No.	9	1	1	1	4	2	10.06
%		1.1	11.1	11.1	44.4	22.2	

Correlation of shoot length with dose to which seeds had been exposed r = -.3965**

a To the nearest 0.5 inch; based on a sample consisting of every fifth plant.
** Statistically significant at the 1% level.

of the total shoot length of the plants with the dose to which the seeds had been exposed was negative and highly significant.

Table 11 shows the measurements obtained from the seedlings of S. 'James Macfarlane'. In this case, the extremely slow growth of nearly all of the seedlings, and the small numbers of seedlings remaining among those from the more heavily irradiated seeds, precluded the appearance of significant differences in the measurements.

The measurements of the sample of S. vulgaris seedlings are shown in Table 12 and Figure 5. Among these seedlings, too, there was a highly significant negative correlation between shoot length and the dose to which the seeds had been subjected. There were, however, very few extremely stunted seedlings; growth thus appeared relatively uniform within each of the four treatment groups. The paucity of stunted plants is probably due at least in part to the selection of seedlings for field planting; it is likely that, at the time of their selection, most of the stunted seedlings of this lot had produced no appreciable growth and were therefore discarded. Again, an approximation of the total effects of irradiation on growth can probably be obtained by adding the numbers of seedlings discarded to the numbers in the lowest size category.

The evident retardation of growth resulting from the irradiation treatments in both series Villosae and S. vulgaris is believed to be a physiological effect caused by the destruction of growth substances in the embryos.

Morphological abnormalities. None of the young seedlings of any lot, except for a few albinos which quickly perished, showed any abnormalities in the amount or distribution of chlorophyll while in the greenhouse. By 13 July 1959, however, a number of plants of the series Villosae mixed lot had produced leaves with color variegations. Table 13 and Figure 2 show the number and distribution of all plants which exhibited any abnormal leaf coloration on this date. The percentages of plants with variegated leaves are higher among those from the seeds irradiated four and eight hours, but the differences are not statistically significant. Absence of chlorophyll from the tips or margins of leaves was a common form of variegation. Also frequent were plants with stippled or finely mottled leaves, in which the non-green portions were often pinkish. In some cases, such leaves remained almost devoid of chlorophyll until quite well expanded. Leaves which appeared in late summer were usually free of such abnormalities; by autumn, very few plants could be found in which variegation was visible.

Table 13. Numbers of variegated seedlings on 13 July 1959 from Syringa series Villosae mixed seed subjected to three levels of thermal-neutron radiation.

Length of exposure	Variegated seedlings	
	No.	%
Control	9	4.7
4 hrs.	24	7.7
8 hrs.	26	8.7
12 hrs.	14	5.1

Two plants in this lot showed chimeric chlorophyll abnormalities which appeared very probably to have had genetic bases. One plant, from a seed irradiated twelve hours, bore one leaf with a distinct yellow sector, and another leaf of which one half was virescent. The other plant, from a seed irradiated four hours, produced two branches, one with leaves of a distinctly lighter shade of green than those of the other. A third plant, from a seed irradiated four hours, produced leaves which, in addition to being extremely rugose and white-margined, were rather coarsely splotched with areas of dark green and gray-green; this splotching may also have had a genetic basis.

The leaves of the slowest-growing plants generally appeared smoother and more coriaceous than is usual in plants of this ancestry. Almost all leaves, however, were normal in shape, except sometimes for the first two or three leaves on a few seedlings. No other morphological abnormalities were noted during the period of observation.

Because of their slow growth and consequent small size, few seedlings of S. 'James Macfarlane' reached sufficient size during 1959 to exhibit any chlorophyll abnormalities for which tendencies might have existed. On 13 July 1959, mottling could be discerned definitely in only four plants in this lot, derived respectively from seeds subjected to each of the four treatments.

No definite chlorophyll abnormalities of any sort were detected among the S. vulgaris seedlings.

General discussion. The radiation doses employed in these experiments do not appear to have been sufficient to cause many abnormalities other than temporary slowing of growth rates, as indicated by the total absence of morphological abnormalities among the S. vulgaris seedlings. Among seedlings of interspecific parentage in series Villosae, such foliar abnormalities as mottled, chlorotic, or temporarily non-green leaves, which may be puckered or otherwise malformed, are not uncommon (Preston, in McKelvey, 1928; the author, in press). For this reason, and because they appeared among the control seedlings in this experiment with seeds of series Villosae, the appearance of such abnormalities in small numbers cannot definitely be attributed to the effects of thermal-neutron radiation.

The physiological, rather than genetic, basis of most of the leaf variegation was indicated by its tendency to disappear from the later vegetative growth during the first year.

Radiation doses also appear to have been too low to cause many genetic changes, as indicated by the extremely small numbers of chimeras detected. Some additional chimeras might have been detected had the observations been continued through one or more additional growing seasons, because some might not have attained visible size until the first year's axillary buds had given rise to leafy branches. A general examination of the seedlings in the spring of 1960, however, did not indicate any significant increase in the number of chimeras.

Some radiation-induced genetic changes, constituting recessive mutants, often do not appear until later generations. Following Dr. Yeager's retirement and the author's completion of his studies at the University of New Hampshire, the seedlings came under the purview of Dr. Rogers, with further observations being directed toward the selection of superior plants for introduction or breeding. In view of the interspecific hybrid origin of the series Villosae seedlings, and the variability among the source plants of S. vulgaris, it is impossible to be certain that any genetically controlled trait appearing in subsequent generations is attributable to a radiation-induced mutation. It is of interest, however, that "double-flowered" plants appeared among the progeny, or R_2 generation, of plants raised from the irradiated series Villosae seed (Rogers, in litt., 1975). Such plants are not unknown in this series,as indicated by McKelvey's (1928) discussion of S. villosa 'Semiplena', but they do not appear to have been cultivated in recent years, and have not otherwise been reported by persons working with lilac populations descended from those which gave rise to 'Royalty' and the other cultivars named in this paper.

The Potential Role of Mutagenic Agents in Lilac Breeding

Lilacs are hardly suitable plants for use in experiments which have the specific objective of assessing the genetic effects of ionizing radiation or chemical mutagenic agents. Because selections are propagated vegetatively, no attempts have been made to establish inbred lines. Therefore, lilac plants can generally be assumed to be more or less heterozygous. In S. vulgaris and S. X prestoniae, in which cultivars are generally the direct or indirect products of crosses between conspicuously dissimilar plants, heterozygosity related to visible traits is doubtless especially high. Moreover, some cultigens of hybrid origin are sterile, and others, although fertile, often produce some seedlings exhibiting such abnormalities as mottled or chlorotic leaves. Specific genetic phenomena among the derivatives of treated propagules could therefore rarely if ever be attributed with confidence to the treatment.

Mutagenic agents may nevertheless be of value in applied programs having the objective of producing new lilac cultivars. In practical plant breeding, mutagenic agents are generally employed for the purpose of increasing the genetic diversity available to the breeder. In subgenus Syringa series Vulgares, a great deal of diversity is already available, including all that is present in S. vulgaris, S. oblata Lindl., S. laciniata Mill., and their hybrid derivatives, as well as that of S. pinnatifolia Hemsley in series Pinnatifoliae, all of these species being genetically compatible (Sax, 1945). Likewise, the ten species in series Villosae all appear to be interfertile (Pringle, 1974,in press), providing the breeder with highly diverse genetic resources. In these groups, mutagenic agents might most profitably be applied to propagules of generally superior cultivars which exhibit the greatest known expression of some desirable trait. For example, S. vulgaris 'Primrose' is not only a choice cultivar with regard to such traits as abundance, size, and carriage of panicles, but is also the only cultivar in which the corolla color approaches yellow. Mutagenic agents might well be used in attempting to obtain deeper yellow corollas from derivatives of 'Primrose', since no other selection is an obvious source of genetic material to enhance this color.

In series Pubescentes, such species as S. meyeri Schneider, S. microphylla Diels, and S. patula (Palibin) Nakai have attracted considerable attention in recent years because of the value of their relatively compact habits in modern landscaping. Species in this series have proved difficult to hybridize (Sax 1945; O.M. Rogers and the present author, unpublished). Combining the compact habit and the attractive foliage of S. patula with a greater range of corolla colors was a special goal of Dr. Yeager's, which has not yet been achieved through interspecific breeding. Mutagenic agents might profitably be used in attempts to increase the variability of some of these relatively uniform and often incompatible species.

Subgenus Ligustrina exhibits even more uniformity within each of its few species and botanical varieties. Despite the similarity of S. pekinensis Rupr. and S. reticulata (Blume) Hara, both species in this subgenus, the author's attempts to cross these species have been unsuccessful. Mutagenic agents might be especially valuable in enhancing the variability and thus the horticultural potentials of these species.

The data from the experiments discussed in this paper and from the other experiments with lilacs previously cited indicate that mutagenic agents, whether ionizing radiation or chemical mutagens, must be employed in relatively heavy doses to be effective with seeds of Syringa. The seed coats of lilacs are thick and fairly hard, and presumably are quite effective in shielding the embryos from damage. Also, the embryos of lilacs are relatively large when the seed is ripe. Consequently, any one cell in which a mutation has occurred may give rise to a smaller proportion of the seedling than would be the case with plants having smaller embryos, and may therefore be less likely to produce a visible chimera.

Where the objective of a plant-breeding program is an improvement or enhancement of one trait in an otherwise satisfactory, asexually propagated cultivar, Broertjes (1972) has recommended the use of mutagenic agents on vegetative propagules. Thus there may be a better chance of inducing a mutation affecting one trait without disrupting the combination of other desirable traits for which the cultivar was selected. As noted above, such objectives may often prevail in work with Syringa series Vulgares, in which existing cultivars can be expected to be much superior to the great majority of seedlings. With lilacs, however, practical vegetative propagules consist of relatively large cuttings or scions, and new growth develops from multicellular buds. A large proportion of the mutations induced, consequently, may be in cells which do not give rise to a chimera of detectable size. Heavy applications of mutagenic agents would therefore be required with vegetative propagules of Syringa. According to Broertjes, neutron radiation has generally been more effective than chemical mutagens in work with vegetative propagules, although supplementary compounds increasing penetrability offer some promise in enhancing the effectiveness of chemical mutagens with larger propagules.

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