PLANNED INDUCTION OF COMMERCIALLY DESIRABLE VARIATION IN ROSES BY NEUTRON RADIATION

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INTRODUCTION

In March, 1961, the successful induction of a wide range of variations in rose plants grown from high energy neutron radiated Queen Elizabeth rose buds was reported[1]. Among the variants, increase in petal number, elimination of the dominant and undesirable M or magenta factor, and dwarf types, were sufficiently frequent to make this technique seem worthwhile commercially. Frequently as a result of carefully planned cross pollination hybrids are obtained which are desirable in every way except that they have only 15 to 20 petals and accordingly are not useful either as garden or hot house varieties. When breeding for the very desirable currant red color, varieties having all the qualifications for commercial introduction are obtained but, unfortunately, are magenta red. The following experiments were made in hopes of demonstrating that when semi-double hybrids having the right variability potential are radiated, commercially desirable varieties having increased petal number and good form may be obtained. Also, the possibility of eliminating the dominant magenta or M factor from otherwise desirable hybrids was investigated.

MATERIAL AND METHODS

Buds of H55059/16, a rose red hybrid of Queen Elizabeth x Red Delight having 14-18 very large petals, H56024/39, a hybrid of Queen Elizabeth x Red Delight having 15-20 medium large petals, H56022/9, a hybrid of Queen Elizabeth x Cavalier, a large fully double high centered magenta red, heterozygous for the very desirable currant red color, Pink Sensation, a very double salmon pink hot house variety, the well known floribunda Garnette and Queen Elizabeth for a comparison with the results of 1961 were used in the experiments. Fifty buds of each variety were cut from budsticks and placed in petri dishes on moist filter paper. Two petri-dishes or 100 buds of H56024/39 were used in the experiment, placing one on top of the other. This "piggy-back" experiment was included to see if the same unusual decrease in radiation effect occurred in the upper dish furthest from target. The buds in these petri dishes were exposed to 14 MeV neutrons from a Cockcroft-Wahon accelerator, August 9, 1961. These neutrons resulted from deuterons accelerated to 500 kev bombarding a tritium target. The petri dishes were placed 2-4 inches from the target in a basket type of holder. The total dosage in rads of the various petri dishes varied from 1870 rads on one side to 2710 rads on the opposite side of dish No. 1 down to 1020-1460 rads on dish No. 7, the top dish of the "piggy-back" pair. Other dishes ranged from 1440-1890, 1720-1840, 2010-2200, 2080-2440 and 2180-2520 rads. The exposure lasted for about six hours, following which the buds were immediately budded into Rosa *multiflora* understock, August 9, 1961.

ACKNOWLEDGMENT

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RESULTS

With the exception of the buds from the upper dish of the "piggy-back" experiment, all the neutron radiated buds were retarded and slow in starting. Only 8 of the 50 Garnette buds grew into plants. Other buds started growth but were not able to form shoots and soon died. This variety evidently is more sensitive than others to radiation. As the buds of all varieties began growth, they were very deformed in foliage shape and leaf appearance. As in the 1961 experiment, many became more normal by growth of axillary buds from the very deformed primary shoots. However, even these shoots were abnormal, having thick "strap" like sections of leaf tissue and leaves. Segments of heavily pigmented tissue and abnormally light green tissue was characteristic of these first shoots and leaves. By midsummer of 1962, however, most of the plants were superficially fairly normal in appearance. They exhibited as much variability in height and shape of plant, vigor, petal number, and color of flower as most populations of seedlings resulting from cross pollination. The variation was not transgressive, however.

As in 1961, the buds from the petri dish above the one next to the target grew into plants which were typical of the hybrid H56024/39, except for one plant which had only 6-8 petals. Also, the buds were only retarded slightly as compared to those of the petri dish beneath. This differential result in the "piggy-back" experiments continues to be puzzling since the total number of rads recorded for the upper dish were 1020-1460 as compared to 1870-2710 for the lower dish next to the target and 1440-1890 for one of the other dishes.

In the fall of 1962, the most vigorous plants were

harvested, planted in 2 gallon rose-tainers, and placed in the hot house for closer observation and study. The results may best be summarized by variety.

H55059/6. Queen Elizabeth x Red Delight. This population of 20 plants selected from the 47 surviving plants showed more plant and flower variability than any other one. Leaves varied in shape from thin "straplike" ones (Figure 1) to very much thickened rugose ones (Figure 2). As shown in Figure 3, the leaf variability was great, affecting size of leaflets and stipules, thickness of midrib, size of marginal serrations, and color, some leaflets being very dark green and others much lighter green with greater development of anthocyanin pigment.

The variability in plant vigor and habit is shown in Figure 4. Selection 13, having a large dark green leaf and stem much greater in diameter (1½x) was studied with special interest since at first it seemed to be an exception to the general rule that all plants from the neutron radiated buds showing observable variations from the normal were either less vigorous or partially sterile. Careful comparison under hot house conditions indicated that it also was significantly less vigorous. At first, after canning up, it grew fully as rapidly as H55059/6. However, it did not "break" as rapidly following flower production and so in a few months was about 2½ feet lower in height than H55059/6 which was then over 8 feet high. Also, it was almost completely sterile. Examination of the pollen indicated that only about 2% was viable. All of the other selections shown in Figure 4 were definitely weaker than normal, though the slender type 2 and 8 grew as rapidly at first. Several plants in this group had from 25-40 petals but were too weak to be worth testing.

Among the 20 plants, several were indistinguishable from normal and one was particularly intriguing from the commercial viewpoint. It had 29-36 large petals instead of the usual 14-18 of T55059/6. The buds were long pointed and had a lovely regularly imbricate high centered form when ¼-½ open. Comparison of the typical H55059/6 flower and this neutron radiation induced "high double" sport is shown in Figure 5.

Hot house testing shows that it buds true and also breaks fully as rapidly as the normal type. H55059/6 is more vigorous than the average hot house rose, so the slight reduction in vigor of this sport is not a handicap. Whether it will meet all of the other exacting requirements of a good hot house rose remains to be seen.

The significant fact is that we have demonstrated that a semi-double variety can be converted into a commercially desirable one as regards petal number and bud form. Incidentally, the color is very similar to H55059/6.

H56024/39. Queen Elizabeth x Red Delight. The plants from buds in the upper dish of the "piggyback" pair were not transplanted since they showed no variability. 20 of the 44 plants from the lower dish showed great variability in habit of growth, leaf form, vigor and petal number. Several plants had only 8-12 petals. All except one plant had flowers typical in color, and it was a light rose red. One plant had flowers only 2½ inches in diameter with 50-60 small petals instead of the usual 4-4½ inch flower with 15-20 petals. Another had 36 petals and a vivid currant red color. The flower was smaller than H56024/39. As may be seen by reference to Figure 6, the same range of variation in leaf characteristics was found as in H55059/6. Though not clearly evident from the figures one would never mistake a variant plant of H56024/39 for one of H55059/6.

H56022/9. Queen Elizabeth x Cavalier. Tyrian Purple-MMMm. Only a few of the plants observed in the field as having a bright currant red color instead of the usual magenta one were transplanted to rose tainers in the fall of 1962. Considerable variation in color range was observed. Only one selection was sufficiently free of magenta to bud and observe more closely. In the winter of 1963-64 plants of this selection were again dug up and benched in the test hot house. It continued to be much more currant red in color and relatively free of magenta. However, when fully open, especially on the second day and thereafter, it still showed too much magenta to be worthwhile commercially. Though complete success was not attained, enough improvement in color was effected to justify belief that a large scale neutron radiation of varieties or selections having the magenta or M factor would result in complete inactivation of this undesirable dominant factor. The color resulting would, of course, depend on the recessive factors carried by the variety radiated.

Pink Sensation. Sport of Pink Delight having more petals and a somewhat deeper pink color but showing magenta on outer surface of petals when bud opens.

Many of the surviving plants were so much weaker than normal as to be of no commercial value. At least six reversions to a red color identical to Red Delight occurred. Pink Sensation is a sport of Pink Delight, a salmon pink variety which frequently sports to Red Delight. Some of the variations showed less magenta coloration on the outer surface of the petals and so may be an improvement over Pink Sensation which has too much of a magenta tone when in the ¼-½ open bud stage. However, it is questionable if any of these selections are vigorous enough to be worth introducing. Some idea of the range in bud form of the variations obtained may be seen by reference to Figure 7. As with H55059/6 and H56024/39, a great range in leaf form and size occurred.

Garnette. A very popular long lasting floribunda

hot house variety. Though unfortunately only 8 plants survived, each was very distinct from normal and one was a very interesting dwarf having flowers similar to Garnette but only about one inch in diameter. It was hoped that variations to pink scarlet or even white would have resulted since these occur naturally and make up a large "family" of Garnette sports. However, all of the 8 plants were variations of the typical Garnette color, some showing less magenta than others. Five of the variations are shown in Figure 8.

Queen Elizabeth. The same range of variation was found in the 47 surviving plants from neutron radiation as in Experiment I of the 1961 series of experiments. One interesting variation of a scarlet color may be worth introducing. Several almost white variations are being tested, but the leaves are also lighter green in color than Queen Elizabeth. Accordingly, it is doubtful if they would be popular as the public usually associates pale green leaf color with weak growth. Even though these variations are almost as vigorous as Queen Elizabeth, they would suffer from this usually well grounded prejudice.

DISCUSSION

As in 1961, these experiments have again demonstrated that neutron radiation of buds cut from bud sticks and placed in petri dishes produce a remarkable range of variation. In discussing the 1961 results the observation was made that "success of a radiation experiment depends not only on dosage rate, but the variability potential of the hybrid or variety used." This relationship has been clearly demonstrated in the above series of experiments. Thus H55059/6 is a hybrid of Queen Elizabeth with Red Delight and the 14-18 petals are very large ones. As indicated by previous experiments (Lammerts 1945[2]) doubleness is dominant, but also quantitative in its inheritance. Red Delight is a sport of Pink Delight and has 25-30 large petals. Several neutron induced variations had from 25-40 petals. One of these, as reported, was almost identical in every way to H55059/6 except that it had 29-36 petals and was only slightly less vigorous. It had a very lovely long bud, and opened to a high centered regularly imbricate flower. Evidently neutron radiation effected the desired result because H55059/6 was capable of variation to *both* increase in petal number and expression of this in large sized *extra* petals.

By contrast H56024/39 which has a flower only about ½-1 inch smaller in diameter than H55059/6 did not have the variability potential for increased number of large sized petals. Though also a hybrid of Queen Elizabeth x Red Delight, it evidently did not carry factors for large petal size. Accordingly, the vivid currant red mutation with 36 petals was commercially undesirable because most of the extra petals were small.

It would seem then that in order to be successful in converting a semi-double hybrid into a commercially desirable one, at least one of the parents should be a fully double variety in which the extra petals, those from 10 on to the total of 35, are *large* size. Also, the semi-double hybrid selected for radiation should have large petals and relatively few small petaloids which usually indicate that the extra petals which may result from mutation will be small also.

Though the experiment to eliminate the magenta or M factors was only partially successful, the great improvement in color indicates that the dominant M factor is rather easily inactivated. In the case of H56022/9 three M factors were involved since genetically the hybrid was MMMm as regards this locus.[3] Obviously, rose red hybrids carrying only one M factor or solferino purples with 2 M factors would be more easily converted to currant red.

From the viewpoint of origin of varieties in the sense of truly unique and great ones such as Peace, Charlotte Armstrong, Herbert Hoover, Queen Elizabeth and Fashion, mutations have little value. There are, of course, whole "families" of varieties such as the Garnette series of sports. Some of these such as the lovely light pink Carol Amling have sold in fairly large quantities, and the new currant red Mohican sport gives promise of much popularity. Also, as indicated previously, Pink Delight a hybrid of Senator x Florex, sported to Red Delight. Aristocrat and Pink Sensation. Many other examples of such "families" of sports could be given. Better Times, a sport of Briarcliff which sported from Columbia a hybrid of Ophelia x Mrs. George Sawyer is the most successful hot house rose so far discovered. It sells in greater quantity than the combined total of all other varieties. However, the varieties in each of these "families" of sports are in general very similar to one another. Thus any variety which is a sport of Garnette can easily be recognized by anyone familiar with the parent variety.

This is definitely not the case, however, with varieties which are the result of hybridization. Thus, no one looking at Queen Elizabeth could ever guess that it was a hybrid of Charlotte Armstrong x Floradora. This unique genotype is the result of a combination of genetic factors tracing back to at least five and probably six different original species. The "gene pool" of variation resulting from interspecific hybridization of these original species is continually re-shuffled, so to speak, by each generation of rose breeders and occasionally truly distinctive varieties are originated. Once this new varietal pattern of development is set up, mutations can only modify its expression. but can never really change it basically. By this I mean that mutated strains of Garnette or Queen Elizabeth are always distinctly recognizable as such. The mutations are

not transgressive in the sense that they transform Garnette into a variety indistinguishable from for example a sport of Pink Bountiful, another rather successful hot house floribunda. Similarly mutations of Queen Elizabeth do not transform it into a variety similar to Chalotte Armstrong or Floradora.

In terms of the pre-patterning theory, it would seem that mutations can only alter various phases of its expression but the *pattern itself cannot be changed.* In terms of this theory, Queen Elizabeth is the expression of a definite pre-pattern. The expression of this pattern depends on the interaction of the environment and the DNA genetic code. Changes in the environment or mutations in the code can alter the expression of the Queen Elizabeth pattern but cannot basically change it.

It is understood that this concept reduces the role of mutations to a relatively minor one. The variability with which rose breeders, or indeed any plant or animal breeders work is traceable to an original diversity of either species or varieties existing naturally within a species. This diversity is the result of numerous patterns which depend for their physical expression on the environment and the genetic code.

Mutations generally are harmful because the complex genetic code was created to perfectly express the pre-pattern in a hospitable environment. Any derangement in either the environment or the code will result in an imperfectly formed expression of the pattern. As indicated in the discussion of the results of both this and the 1961 experiment by far the greater majority of all mutations resulting from neutron radiation are defective. Most of them are weaker, "break" less frequently or are partially sterile. This is, of course, exactly what one would expect from consideration of the remarkably complex code system created to express the various patterns existing in the mind of God, patterns we recognize as species, varieties, and individuals.

Evolutionists recognize that most mutations are harmful. Thus Fraenkel-Conrat [4] in discussing the genetic code of a virus reports on 200 chemically induced mutants of the tobacco mosaic virus. One of them made the protein coat of the virus much more susceptible to digestion by an enzyme that removes amino acids from the carboxyl (COOH) end of a protein chain. This enzyme was able to digest or chop off the amino acid threonine at the end of a protein chain. The very first mutant studied by Fraenkel-Conrat and his group made the virus protein much more susceptible to digestion by the enzyme. As a result the enzyme was now able to clip three amino acids off the virus, thus rendering it distinctly less viable. Other RNA mutations render the RNA incapable of even forming the protein coat. Fraenkel-Conrat comments, "One can assume that the protein coat of the common strain of the virus as it evolved by natural selection, is highly efficient, and that any mutation is likely to reduce the virus' ability."

This sort of reasoning inverts the logical deduction from the overwhelming burden of evidence that mutations tend to be harmful, hence cannot be useful in explaining the evolution *assumed*. Actually, our evolution minded colleagues are now saying, "Yes, of course, most, or possibly all mutations are harmful since natural selection has eliminated all except those most effectively integrated into the DNA code." Hence, *all* plants and animals *now* have the most perfect combinations of mutations and it would be unreasonable to expect to find mutations increasing the viability or conferring any other advantage to the organism studied. This is actually saying that the course of the evolution they postulate is now completed. One might well ask just when in the past was it incomplete? Presumably, according to orthodox paleobotanical theory, very distinctive flowering plants originated in the Miocene time. At least 11 are described as new by MacGinitie in his very excellent report on the Kilgore Flora.[5] Whether they originated somewhat earlier in the Eocene or late Cretaceus is not the question. Rather one might well ask "if geneticists had been living at that time, would they have concluded that evolution was complete since almost all mutations studied are harmful ?" One could thus go back to the Devonian time and argue that because mutations of the then existing simple plants such as Psilophyton (actually probably Psilotum *nudum)* were harmful, natural selection had eliminated all except those most effectively integrated into the DNA code. In fact, Axelrod does essentially this when he tries to explain the survival of Psilotum as being due to slow evolving rates and calls them bradytelic types.[6]

Actually, this whole argument of our evolution minded colleagues is very amusing. In essence they first say that all existing lines of evidence clearly prove that evolution has occurred and that all existing plant and animal species trace back to one, or at most, a few "primitive" forms. Then, in searching for a possible mechanism by which this presumed evolution has occurred, they find a source of variation in mutations. Natural selection is then assumed (since actually no one has demonstrated even the origin of a variety by it) as the effective mechanism for selecting the best of these mutations and assembling them into a new or distinct plant or animal. Once these assumptions are granted, they then say, "Well, of course we cannot demonstrate evolution as occurring since it has all been completed."

In essence then evolution is reduced to the same status as creation with DNA, mutation and natural selection taking the place of God in presenting us with a *completed* product!

A little reflection should show that *if* evolution occurred by natural selection of mutations, it

should be a continuing process. Surely this is not the most perfect of worlds and plants and animals should be changing in their basic patterns of expression to meet the challenges of new environments. The fact that they change only within the limits of their variability potential and that most mutations are harmful should make evolution minded scientists reconsider their basic assumptions. Do the various lines of evidence from an impartial study of nature conclusively point to evolution as the mechanism by which the remarkable diversity we see around us originated? As we have endeavored to show in various articles, many of us. competent in various fields of sciences, do not think so. Rather we see clear evidence for creative design. a past environment much better suited for the ideal expression of the various patterns of life. and a series of catastrophes[7] which have marred a world originally created perfect in every way.

SUMMARY

1. Data are given which indicate that semi-double rose hybrids having only 14-18 petals may be converted into fully double ones having commercially desirable high centered regularly imbricate flowers by neutron radiation of the buds cut from bud sticks and placed on wet filter paper in petri-dishes.

2. Elimination of the dominant M or magenta factor may easily be accomplished by neutron radiation thus allowing the desirable crimson or currant red factor to be expressed.

3. The importance of selecting hybrids having the right variability potential for effecting these changes is discussed.

4. Biologically, all of the mutations were defective variations from the pattern of development characteristic of the variety radiated.

5. The interrelation of environment, pre-pattern and mutation is discussed and the observation made that mutations obtained are not transgressive.

6. Mutations can only alter various phases of the basic varietal pattern expression, but the pattern itself is not changed. Truly unique and outstanding varieties such as Peace, Charlotte Armstrong or Queen Elizabeth would never result from the accumulation of mutations.

7. The evolutionary concept that mutations are harmful because natural selection has accumulated the most efficient combination of mutations is examined in terms of its implication that the assumed process is complete. The conclusion is reached that environments are not always ideal for plants now. Therefore, if the present variation we see is due to natural selection adapting species and varieties to their environment, the process should be continuing and experimentally demonstrable.

8. The overwhelming lines of evidence indicating that under the usual normal environmental conditions, mutations are for the most part harmful, indicates a need to re-examine the presumed evidence for evolution.

9. It is our belief that the evidence clearly indicates creative design, a Past environment much better suited for the ideal expression of the manifold patterns of life, and a series of catastrophes which have marred a world originally created perfect in every way.

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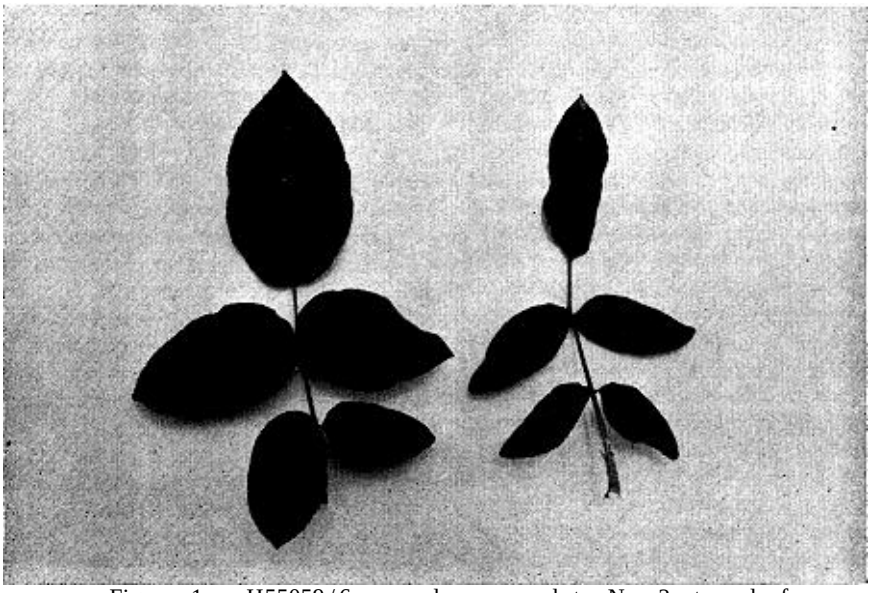

Figure 1 -- H55059/6 normal compared to No. 2 strap leaf.

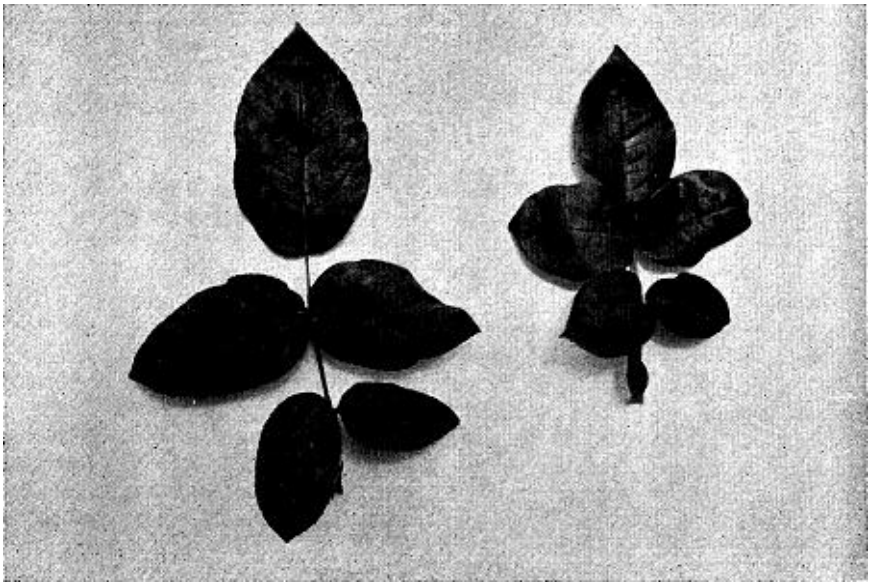

Figure 2 — H55059/6 normal compared to No. 13 rugose thick leaf.

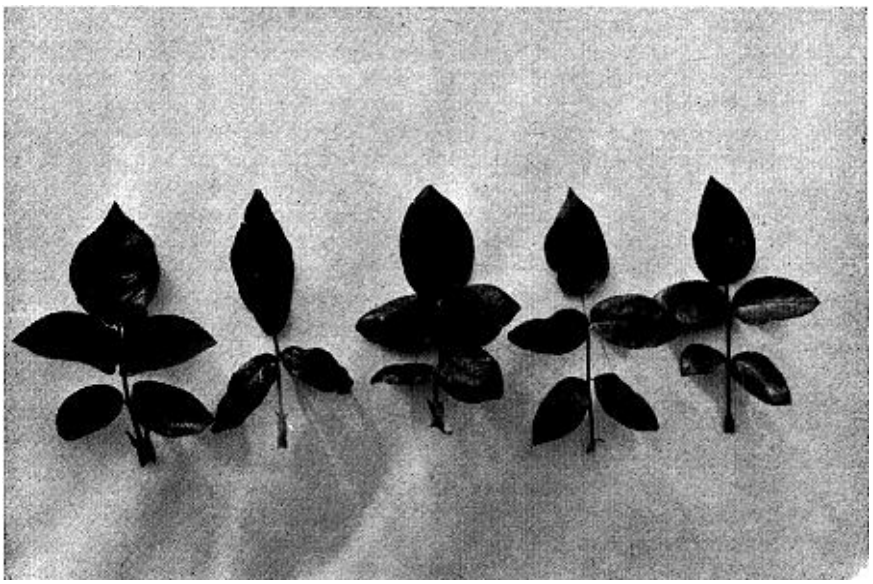

Figure 3 — H55059/6 normal leaf on left compared to four leaf variations on right.

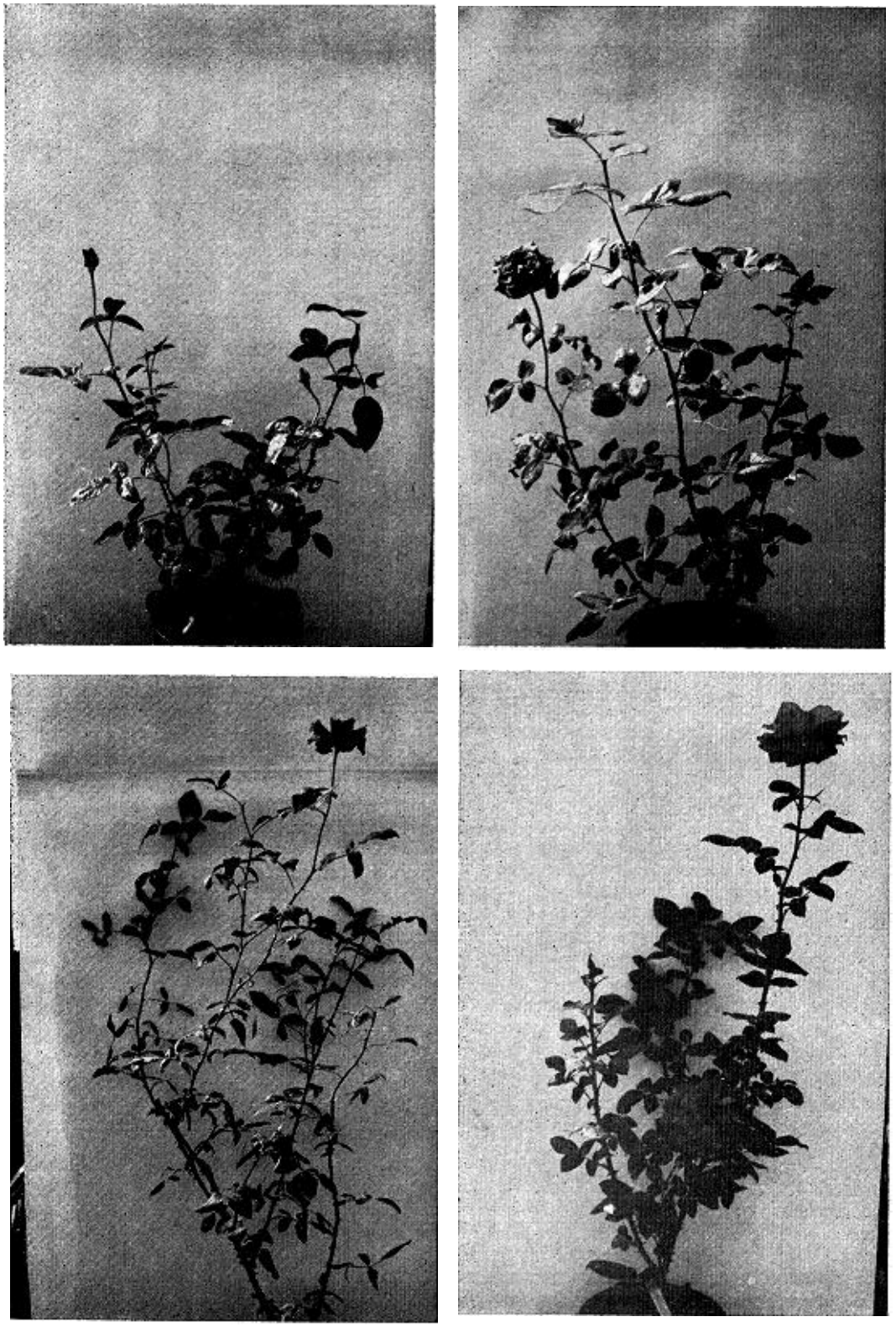

Figure 4 -- H55059/6 (Top left) No. 20—Dwarf. (Top right) No. 9—Dark red large double flower. (Bottom left) No. 2—Slender elongate, and (Bottom right) No. 13—Thick dark green leaf and stem.

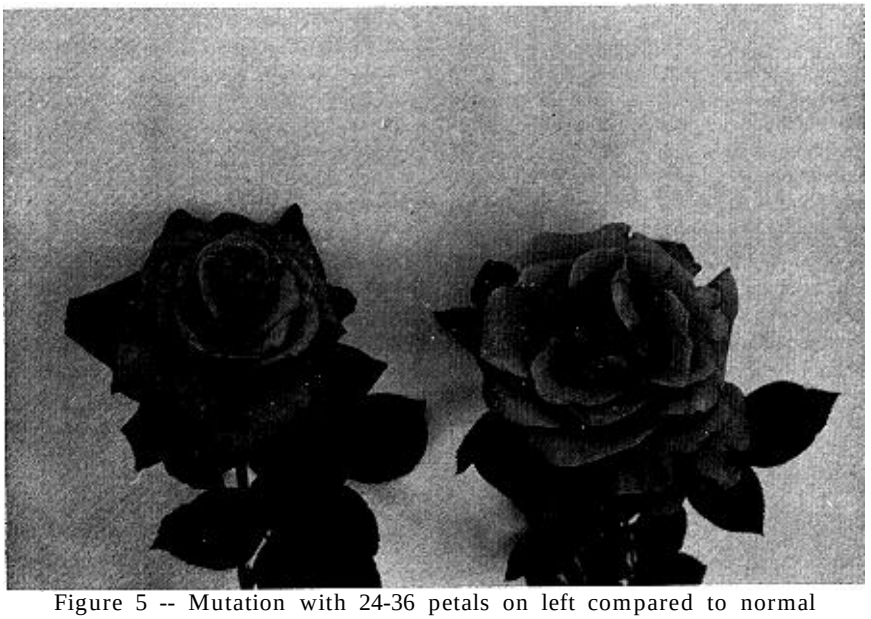

Figure 5 -- Mutation with 24-36 petals on left compared to normal H55059/6 having 14-18 petals on right.

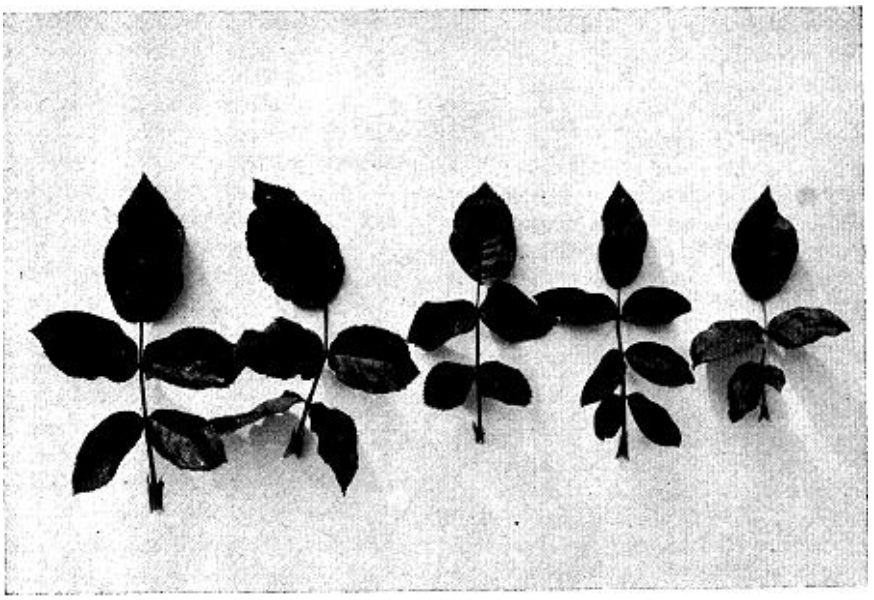

Figure 6 — H56024/39 normal leaf at left compared to four leaf variations on right.

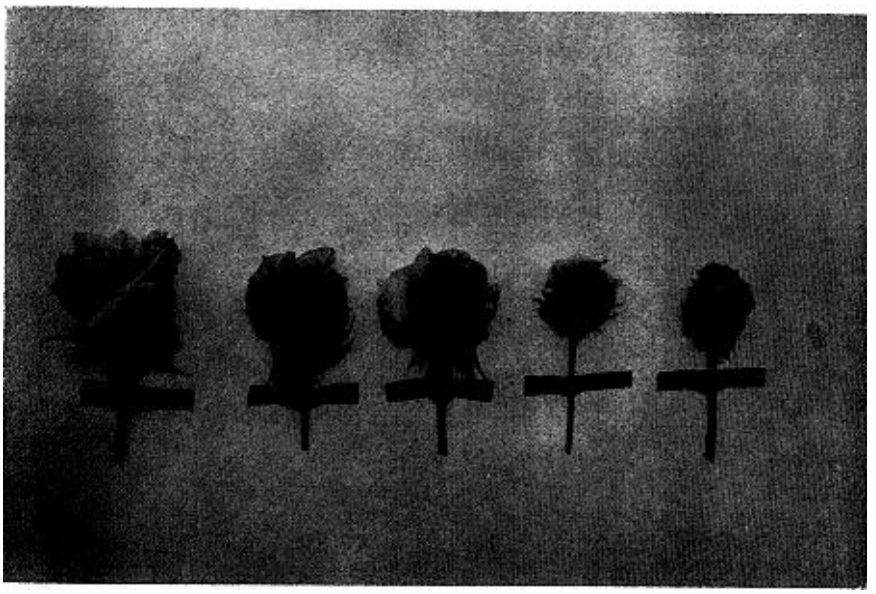

Figure 7—Flower bud of Pink Sensation at lefnt compared to induced mutations on the right.

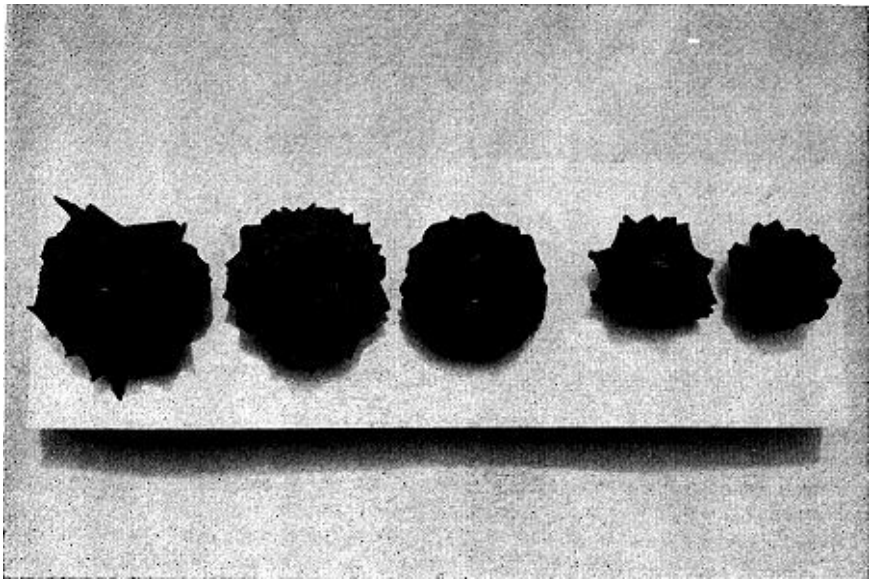

Figure 8 —Variations in flower form induced by neutron radiation of Garnette. Typical Garnette at left.