STUDIES WITH GROWTH REGULATING SUBSTANCES ON THE VEGETATIVE BUD INHIBITION OF ROSE PLANTS DURING COMMON STORAGE.

By

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INTRODUCTION

Rose bushes constitute the greatest percentage of all nursery stock that is stored in common storage. They present a real problem to the nurseryman, since their dormancy apparently is more readily broken by brief exposures to high temperature than other nursery material. In some years, when it is difficult to lower the temperature within common storage houses early in the winter, the bushes start shoot growth in December, making them difficult to handle properly throughout the storage period. Spring temperatures are also of considerable influence. If high outside air temperatures occur in late winter, the temperature within the common storage house also rises and thereby shortens the period that rose bushes can be held safely without excessive shoot production taking place.

The etiolated shoots that grow on rose bushes in common storage are objectionable from two standpoints. First, they must be removed from the plants soon after they develop, since they are very susceptible to destruction by molds and other organisms, some of which, beginning on the new shoots, will grow back into the older canes and may ultimately destroy most of the plant tops. Second, much of the stored food reserves contained in the plants are used up in producing the etiolated shoots and consequently are lost from the plants upon their removal. Many home gardeners have been discouraged
from growing rose bushes due to the poor success they have had with apparently excellent bushes, which in reality were plants of low vigor, because shoot production in storage had depleted the stored food reserves of the plants.

A solution of the dormancy problem of stored rose bushes would appear to come from three sources: first, education of the buying public to make their rose plantings as early as possible and thus avoid a long storage period; second, the use of artificial cold storage facilities which would undoubtedly solve much of the difficulty since the plants could be held at a sufficiently low and uniform temperature that shoot growth would be inhibited; third, improvement in the construction and operation of the common storage house, as well as improvement in methods of handling the plants. The discovery in recent years of the inhibiting effect of certain chemical compounds on bud growth when applied to plants suggested the possibility that these materials might be used to prevent shoot growth on rose bushes while in common storage. The work reported herein deals with experiments that were undertaken to determine if these chemical compounds, commonly referred to as growth regulating substances, could be used to inhibit shoot production on rose bushes when they are held in common storage.
LITERATURE REVIEW

The literature concerned with growth substances and their effects on plant growth and development has expanded rapidly in recent years. Although most of the available references on growth substances have been consulted, only those that seem to have direct bearing on the present problem under consideration have been selected for this review. Several publications (4, 10, 52, 53, 59, 60) are available for those who wish a more comprehensive review of the literature on growth substances and the various plant responses that have been obtained.

"Apical dominance" or the natural inhibition of lateral buds by the uppermost bud on plants has caused much speculation and experimentation over a long period. Goebel (22) in 1900 concluded after studies on the seedlings of walnut that the depletion of terminal bud food reserves contained in the cotyledons would account for any suppression of the lateral buds. Loeb (34) also held to a somewhat similar view after experiments with Bryophyllum stem cuttings of varying lengths. The amount of shoot production on this plant material appeared to be directly proportional to the length of cutting.

Considerable experimental evidence has accumulated in support of the theory that a bud inhibiting substance is formed in plants. Pfeffer (40) as early as 1903 suggested that since many dormant lateral buds contained adequate food reserves for growth, their suppression was caused by some other factor than
food alone. Appleman (1,2) after extensive studies with the potato concluded that the apical bud must produce a growth substance that inhibits development of other buds. He found that if tubers were left intact their apical bud showed greatest development, whereas, upon division, the eye on each piece grew as though it were an apical bud. Cortical incisions made between the buds caused the same response as with separated pieces. Apical dominance appeared to be overcome when intact tubers were infected with the "spindle sprout" disease. More recently Guthrie (24) and Zimmerman and Hitchcock (66) found that all the buds of potato tubers could be inhibited by solutions and vapors of laboratory synthesized growth substances, and in a manner very similar to inhibition by apical buds.

As indicated by Appleman (2) the growth inhibiting substance appeared to move within the cortical region only. Harvey (28) confirmed this by killing a narrow region on the stems of bean plants with steam, which resulted in development of dormant lateral buds below the killed area, in much the manner that an incision above these buds affects their growth. The growth inhibiting substance apparently could not move through dead tissue. Reed and Halma (41) earlier had shown that an incision made above a dormant lateral bud of citrus trees would cause the bud to grow out, since the incision apparently interfered with the movement of inhibiting substances from the apical bud. Cooper (14,15) working with citrus cuttings, found that ringing the cuttings after treat-
ing the top with synthesized growth substance prevented movement of such substances to the base, as indicated by the influence on rooting.

Went (58) and Thimann (53) both credit Snow (48) for first showing that bud inhibition is caused by a definite substance contained in plants. Snow made longitudinal slits in bean seedlings so that they were halved. After binding such seedlings tightly together it was found that some substances passed through the slit zone and caused bud inhibition on the side opposite that carrying the apical bud. Thimann and Skoog (46, 54, 55) later presented data to show that extracts of growth substances from plants, as well as the synthetic compounds, would inhibit buds of *Vicia faba*.

The similarity in plant response that has been found with extracts of living material and with the pure synthesized growth substances has been reported by a number of workers and with widely different plants and various responses. In the case of gall formation on plants, Brown and Gardner (12) have shown that galls produced on bean plants by extracts of a culture of *Bacterium tumefaciens* and those resulting from treatment with indoleacetic acid appear to be very much alike. Detailed histological studies by Kraus, Brown and Hamner (32) on the development of such galls further provides evidence that living cells can produce substances that resemble in action the pure synthesized growth substances. Later work by Mitchell and Hamner (37) on bean and by Hamner (27) on *Mirabilis* indicated that the formation of galls is primarily
determined by the concentration of the growth substance that is in contact with the plant cells. Additional evidence may be found in the literature on parthenocarpy induced by pollen extracts from a wide variety of plants as compared with that induced by a number of pure growth substances, (19, 25, 26, 62).

On the basis of the work reviewed thus far, it would appear logical to suspect that a synthetic growth substance or substances could be found that would successfully inhibit the vegetative buds on rose bushes in common storage. This would appear to be especially true since Guthrie (24) has already reported complete inhibition of sprout formation on potato tubers by the use of growth substance. However, Guthrie applied his treatments and held the treated tubers at $25^\circ$ to $30^\circ$ Centigrade which is considerably higher than the temperature encountered in common storage houses used for storing rose bushes.

The mechanism by which growth substances operate in inhibiting vegetative growth of plants is still not definitely known. Thimann (53) has presented a critical review of the various physiological theories of bud inhibition by naturally occurring growth substances that have been proposed up to 1938. The fact that growth substances either naturally occurring or synthetic do not always inhibit vegetative buds, but may under some conditions cause a stimulation, as shown by the work of Bennett and Skoog (7) and that by Vegis (57), further complicates any theoretical explanations that may be made.
Disagreement also appears in the rather limited amount of literature in connection with the inhibiting effect of growth substances on the opening of flower buds. The work has been largely done with peach, to determine if growth substances applied to unopened blossoms would delay their opening until danger from frost injury is past.

Winklepleck (61) reported in 1959 that naphthaleneacetic acid applied to the branches of dormant peach trees in aqueous sprays at the rate of 125 milligrams per liter caused a delay of eleven days in blossom opening. The sprays were applied just prior to blossoming time, and the delay was measured by comparing the sprayed trees with comparable unsprayed trees. Winklepleck found that the blossoms that developed on growth substance sprayed trees had smaller petals. The retarding effect on the flower opening also carried over into the developing fruits which matured at a slower rate than fruits on the unsprayed trees. The ultimate size of fruits on sprayed and unsprayed trees, however, was the same.

Mitchell and Cullinan (36) after conducting extensive greenhouse and field experiments over a two year period concluded that no retarding effect on the opening of flowers of either the Elberta or Belle peach varieties resulted from spray application containing any one of four growth substances. They used naphthaleneacetic acid, naphthaleneacetamide, indoleacetic acid and indolebutyric acid at varying rates of 15, 30, 50, 300 and 500 milligrams per liter in water alone, in lanolin-emulsion and in oil-emulsion. Naphthaleneacetic
acid caused an earlier opening of greater numbers of floral buds on detached branches of both peach varieties. Naphthaleneacetamide had a similar effect on the floral buds of Belle but not on Elberta flower buds. Indoleacetic and indolebutyric acid emulsions had no effect when applied once. When the applications were repeated on Belle a greater number of flower buds was induced to open. The vegetative buds of peach, however, were retarded by growth substance applications. Mitchell and Callinan concluded from their work that growth substances are ineffective in retarding the blooming date of peaches and under certain conditions may cause an earlier blossoming. They also included branches of Kieffer pear in tests similar to those conducted with peach. The flower buds of pear were not hastened in opening and when repeated sprays with high concentration were used the growth substance caused injury.

Much evidence has appeared in the literature which indicated that indoleacetic acid as well as other growth substances applied to plants and isolated plant parts causes a mobilization of nitrogen, carbohydrates and other substances to the treated region (18,35,50,51).

Cooper (14) found that cutting off the bases of leafless woody cuttings soon after they were treated with growth substance removed some material that caused roots to form. He called this substance rizocaline. Stuart and Marth (50) found that indolebutyric acid caused an accumulation of more sugars in the stems of Ilex opaca cuttings just prior to root formation.
emergence than with similar untreated cuttings. Schneider (44) has reported that sugars are essential for auxin activity in plants. Stuart (51) later discovered that indoleacetic acid also caused a rapid mobilization of nitrogen as well as carbohydrates from the leaves and cotyledons to the bases of treated bean cuttings. A recent paper by Doak (18) with Forsythia cuttings treated with naphthaleneacetic acid confirms Stuart's (51) finding with bean cuttings.

Prior to the discovery and isolation of pure growth substance, early work by Schrader (46) and by Reid (42) with cuttings of tomato has shown that high carbohydrate content plus a relatively low soluble nitrogen content favored root formation.

The experimental evidence obtained with cuttings appears to indicate that growth substances act on the food reserves of the plant and bring about directional changes in its movement. Cooper's (14) work suggests also that a specific, naturally occurring, substance which regulates root formation may also be affected by growth substance applications. However, the amount of food reserves that had been mobilized and lost when he removed the bases of his cuttings would appear to be a factor in any interpretation of his results.

The mode of action by which growth substances cause changes in the food reserves of plants has been greatly clarified by the recent work of Mitchell, Kraus and Whitehead (38). In experiments with intact kidney bean plants they found that α-naphthaleneacetic acid in lanolin-emulsion sprayed
onto the leaves of plants caused a very marked acceleration in the rate of starch hydrolysis. Apparently the growth substance greatly affected enzyme activity. In later experiments, Mitchell and Whitehead (39) found that naphthaleneacetamide had no effect on starch hydrolysis and that phenylacetic acid had very little, whereas indoleacetic, naphthaleneacetic, indolebutyric, indolepropionic, and naphthoxyacetic acids were very effective in increasing the rate of starch hydrolysis. Temperature was found also of considerable importance in the action of growth substances on starch digestion. Greatest enzyme activity was found between 74° and 76° Fahrenheit. Leaves kept at 62° to 64° Fahrenheit and at 90° to 92° Fahrenheit were not affected by growth substance at these low and high temperatures.

For the most part, application of growth substances to plants has been by aqueous sprays or in lanolin paste. Guthrie (34) and Zimmerman and Hitchcock (66) appear to be the first to report the use of growth substances in the form of vapors applied to plants. These authors found that out of 54 physiologically active substances tested 29 were effective when applied as a vapor. In general the substances that were active as growth substances in the vapor state were found much more effective when applied in this way than when applied in lanolin paste. Several compounds that were not active at all when applied in lanolin, caused considerable epinasty of tomato when applied in vapor form.

In a later paper Zimmerman and Hitchcock (67) have
reported that one of the compounds, \( \beta \)-naphthoxyacetic acid applied to plants in aqueous sprays, caused considerable alteration in the characteristic venation pattern and form of the leaves of tomato, hibiscus, mimosa, artichoke, Paris daisy, tobacco and marigold. Apparently these authors believe that the responses obtained were not due to changes in chromosome number but were due entirely to morphological changes in cellular arrangement within the affected plants. Bausor, Reinhart and Tice (5) have also found that this compound will cause histological changes in the tomato.

Greenleaf (23), Levan (33), and Dermen (17) present evidence which show that growth substances may cause changes in the number of chromosomes normally occurring in certain plants. Greenleaf decapitated young tobacco plants and removed all lateral buds. After applying indoleacetic acid paste to the cut surface, callous tissue developed, and from it, shoots were produced some of which were tetraploid while others were octoploid.

Levan (33) found that \( \beta \)-naphthalenesacetic and indolebutyric acids were effective in inducing polyploidy in the roots of 2 species of onion (\( \textit{Allium cepa} \) and \( \textit{Allium fistulosum} \)) whereas phenylacetic and phenylpropionic acids were not effective. The growth substances that caused polyploidy did not appear to affect the dividing cells in the meristematic region of the growing point, but caused the development of abnormally large cells in slightly older tissues. The chromosomes were doubled in number from 32 to 64 in these large cells. Levan
Levan believed that the action of growth substances on the polyploidy obtained was different from the action of colchicine. According to Levan, colchicine affects only cellular divisions in the active meristematic regions and does not affect the more or less mature cells with resting nuclei outside of the meristematic regions.

Dermen (17) treated the stems of young intact bean seedlings with a 0.25 per cent naphthaleneacetic acid lanolin paste and produced tumerlike callous proliferation from which roots developed. Upon sectioning this material he found that .... "Polyploidy was present in the following parenchymatous regions: cortex, endodermal region, phloem, medullary rays and pith of the stem; cortex and endodermal region of the root primordia; and rarely in the central region back of the growing point. No polyploidy cells were observed in the cambial region of the stem and the procambial region of the root primordia...." Dermen concludes from his observations that growth substance treatment may cause partial polyploidy in some plants, resulting in a mixture of diploid and polyploid tissues.
Common Storage

Throughout the experiments the same common storage house was used in storing the experimental lots. Briefly, this house was of concrete block construction, and the house itself was set in the side of a hill so that the side and back walls of the storage room were below ground level. The gable roof was above ground and lined with "Celotex" fiber board on the inside of the rafters, and the ceiling of the storage room was about at ground level and constructed of a layer of "Celotex" overlaid with tongue and groove sheathing. This created a large and a small "dead" air space between the inside of the storage room ceiling and the outside of the house. The storage room was of 28,000 cubic feet capacity and was provided with six boxed-in ventilating openings. These openings were 18 inches square, two extended from inside the ceiling to 4 feet above the gable of the roof, and four ventilators, one at each corner of the room, extended from two feet above the floor to roof level near the eaves. When open, the four corner ventilators allowed cold air to settle into the room, and the centrally located ceiling ventilators permitted the escape of warm air. The only other opening was a double door in the concrete block front wall of the room through which material could be shifted in and out of
common storage.

The floor of the storage room consisted of packed clay soil covered with two inches of fine sand. The sand was kept moist throughout the storage period. Relative humidity records obtained on the same chart with temperature showed that at no time did the relative humidity drop below 90 per cent while the storage was operating.

A weekly recording thermograph was used in the storage room continuously during the storage season. In order to show the temperatures encountered during the common storage experiments, the weekly mean temperatures were calculated from the thermograph charts.

Cold Storage

In experiments where cold storage was used for comparison with common storage, a small room (1200 cubic feet) artificially controlled at 32 \( \pm 1 \) Fahrenheit was available. The relative humidity within this room was unquestionably much lower than that in the common storage. Moistened peat and sand mixture covered the plant roots so that the plants did not become dry at any time during 32\(^\circ\) cold storage.
Rose Plant Material

1939 - 1940 Plants. Since very little work has been reported previously on the effect of growth substances on bud inhibition with material other than herbaceous cuttings, the work in this season was largely of an exploratory nature. For the purpose of testing a large number of compounds as well as various methods of applying them, 1000, one year old rose bushes of the Ami Quinard variety were used. The plants were #1 grade; Texas grown, budded on Rosa multiflora rooted stem cutting stocks.

The Texas plants were utilized in exploratory experiments, with 3 to 5 plants per treatment. As will be noted later many of these trials yielded negative results, but some showed promise of delaying bud growth. In order to further verify some of these initial trials, two lots of plants were obtained. One consisted of 500 plants of the Guinee rambler variety, the other consisted of 350 plants of the Radiance variety. These plants were Eastern grown and were #1 grade budded onto Rosa multiflora seedling stocks.

The plants were secured on March 26, 1940, and the varieties selected were among the few remaining in common storage that were dormant and available at this late date.

1940 - 1941 Plants. A lot consisting of 3200 bushes of Ami Quinard roses was available for the storage experiments on January 3, 1941. The plants, like those used in the previous year, were Texas grown #1 grade, one year old budded on
Eos multiflora rooted stem cuttings. For the most part the plants had stopped vegetative growth when dug in the nursery and were of a better stage of maturity for storage than those received the previous year from the same nursery. The plants were carefully worked over when received from the nursery and were graded further for uniformity, and all broken and weak canes were removed. At this time also, plants that were immature, as indicated by a sappy appearance of the canes and presence of foliage, were separated. After grading and pruning, the plants were stored in racks in common storage with roots packed in moist peat.

In order to determine how much variability existed among the plants that were to be used in the storage tests, a random sample of 40 plants was taken, potted up in composted soil in 6 inch pots and placed in an enclosed case in the greenhouse. The plants were arranged in 20 pairs and were examined at the same time each successive day for the first visible sign of bud breaking. The first plant of each pair to break bud was placed in group A and the other plant in group B. The complete results are shown in table 1.

It is apparent in table 1 that the plants varied markedly, requiring from 6 to 14 days to show visible bud breaking. Some of this variability may have been due to differences in exposure to drying conditions either in handling the plants prior to shipment or to position within the box during shipment. Yerkes and Gardner (64) have shown that the percentage of moisture contained in the packing material as well as in the
**TABLE 1.**

VARIABILITY OF AMI QUINARD ROSE BUSHES AS INDICATED BY NUMBER OF DAYS REQUIRED TO SHOW VISIBLE BUD BREAKING ON 20 PAIRS OF UNTREATED PLANTS. GREENHOUSE TEST BEFORE STORAGE TREATMENTS WERE STARTED.

<table>
<thead>
<tr>
<th>Z</th>
<th>10 10 8 8 14 9 9 10 11 14 7 10 9 9 10 8</th>
<th>7 7 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9 7 8 8 9 6 8 8 8 7 9 7 8 7 8 7 6 7</td>
<td>6 6</td>
</tr>
<tr>
<td>Diff.</td>
<td>1 3 0 0 5 3 0 1 2 4 5 0 2 2 1 3 2 2 1 3</td>
<td></td>
</tr>
</tbody>
</table>

Mean Diff. = 2.00
Standard Deviation = 1.55

Z (from Z chart) = 8 pairs required for 1 percent level

5 = 5 = 5
plants themselves has an important bearing on the production of shoots on rose bushes in storage. Examination by the author of many thousands of rose bushes which had been held in commercial common storage houses for several months indicated that during the storage period the high moisture content of the packing material and air surrounding the plants may tend to reduce variability in shoot production, due to differences in moisture content of the plants. The ricks of plants of a particular variety appeared to be very uniform in shoot length and number per plant. Unfortunately, however, no data were secured to establish this point, and in order to provide a measure of safety for securing reliable results of this study, when it was possible to do so, a minimum of 20 plants was used in each treatment.

In addition to the above mentioned lot of Texas grown plants, other lots were obtained from Eastern nurseries, consisting of 100 bushes each of the following 15 varieties of rose: Ami Quinard, Chatillon, Duquessa de Penaranda, Editor McFarland, Edith Nellie Perkins, Etoile de Holland, Girona, Golden Dawn, Guinee, Margaret McGredy, Poulsen's Yellow, Radiance, Radio, Ramon Bach, and Topaz. These varieties were selected to include those that tended to grow shoots profusely in storage as well as those that were not so troublesome in this connection. These varieties are included in the variety dormancy ratings shown in table 9.

When the plants were set up in experimental lots for treatment, the roots were packed in moist peat and sand, in
paper lined 1/2 bushel baskets. Particular attention was
given to prevent the plants from losing excess moisture.
Plant Materials Used Other Than Rose

Although the experiments were concerned mostly with the effects of growth substances on shoot production of rose bushes, a number of plants representative of species other than Rosa were included to find out if they would be adversely affected by the treatments with volatilized growth substances applied to rose bushes within the same room. Frequently the common storage rooms are built fairly large and often contain large quantities of a variety of nursery stock. If the treatment seriously affected this other material it would have to be removed before treating the roses with volatilized growth substances.

The plants that were included in the test along with roses were as follows: Acer palmatum, Japanese maple, 30 two-year seedlings; Amygdalus persica, Carolina "Natural" peach, 30 one-year seedlings, and 30 Elberta variety peach, one-year trees; Diospyros virginiana, native persimmon, 30 one-year seedlings; Malus sylvestris, Northern Spy apple variety, 30 one-year trees; also Delicious apple, 150 newly-made piece root grafts; Philadelphus grandiflorus, Mockorange, 30 three-year old transplants; Prunus cerasus, Montmorency cherry variety, 30 one year old trees; Prunus serrulata, Japanese flowering cherry, 30 one-year seedlings. Also included for comparative purposes were 60 one-year bushes of Guinee variety of rose.

All the above plants were grown in the experimental
nursery of the U. or. Horticultural Station at Beltsville, Md., except the Guinee rose bushes, which were procured from an Eastern nurseryman.
Growth Substances and Methods of Applying

The following compounds have been reported to cause growth responses, such as rooting, curvatures, and other effects on bud growth: B-indoleacetic acid, B-indolebutyric acid; B-indolepropionic acid; a-naphthaleneacetic acid; a-naphthylmethylacetic; a-naphthylethylacetate; K-naphthaleneacetate; Na-naphthaleneacetate; NH₄-naphthaleneacetate; a-naphthaleneacetonitrile; a-naphthalenepropionic acid; Na-naphthol(4) sulphonate; a-naphthaleneacetamide; a-naphthaleneethioacetamide; a-naphthaleneisothioacetamide; a-naphthaleneoxycetic acid; B-naphthaleneoxycetic acid; a-naphthylmethylthiocyanate; a-naphthylmethyliothiocyanate; a-methylnaphthalene; tetratin-6-acetamide and acenaphthene.

Many of these growth substances have a very low solubility in water. However, in preliminary trials they were all used at their maximum solubility in aqueous solution sprayed on dormant plants. Although several compounds showed a noticeable effect in delaying bud breaking when applied in this way, adequate control was not obtained with any compound. Other solvents and carriers were then tried, such as varying percentage of from 5 to 20 per cent ethyl alcohol, dioxan, acetone, lanolin-emulsion, 1/4 and 1/8 per cent light oil-emulsion, as well as two proprietary wax-emulsions frequently used in waxing rose plants commercially. Of these carriers the wax-emulsions proved to be most satisfactory, for not only could the growth substances be used in higher concentra-
tion than in water alone, but the emulsion appeared to be less toxic to the plants than with the alcohols and other solvents used. (Jones and Richey (31) have reported that wax-emulsions could be applied with safety to young tomato plants.) Further, they had the effect of holding the growth substance on the canes for a longer period, which resulted in an increased effectiveness of a given concentration of growth substance over the same concentration sprayed in water only. In spraying large lots of plants a small, electrically operated, pint capacity paint sprayer was used. This type of sprayer produced a smooth uniform film with better spray coverage than obtained with several types of hand sprayers.

The esters of a-naphthalenesacetic acid, namely, a-naphthylmethylacetate and a-naphthylethylacetate are liquids which volatilize readily on heating and the fumes of those compounds were used in treating plants by the vapor method. Another compound a-naphthalenesacetonitrile, also liquid, was used in the same manner.

In applying the gases it was essential to have air tight rooms or containers to prevent the gases from escaping, also to provide heating equipment within such rooms or containers to volatilize the liquid growth substances and a fan to develop an equal distribution throughout the chamber. Thus treatments were applied in a common nursery storage room of 28,000 cubic feet, in a small stock room of 1000 cubic feet capacity, with openings sealed with putty; in 20 gallon refuse cans having tight fitting lids sealed with adhesive tape; in
a double thickness paper-lined tongue and grooved wooden chamber of 400 cubic feet capacity, used for cyanide fumigation; in large, ground glass bottom, bell jars with a 2 inch diameter opening in the top; also in the chamber illustrated in figure 1 which was constructed of two 50 gallon drums welded end-to-end and with a water sealed lid. In all cases the exact capacity of the chamber was known so that the concentration of growth substance could be reported for a comparable chamber of 1000 cubic feet capacity. In the very small chambers, where low concentration of gas, requiring only a fraction of a drop of growth substance, a quantity of convenient weight of compound was first dissolved in absolute alcohol and a known quantity of growth substance pipetted out. It was found that, upon heating, the pure liquid growth substance would sometimes char, but if it were mixed with 95 per cent alcohol, then placed in a shallow watch glass, volatilization would be complete without visible residue being left. The alcohol also tremendously lessened the time required for volatilization which was of considerable aid in reducing the amount of heat required in the volatilization process. In treatments where it was desired to have a controlled temperature throughout the treatment period it was necessary to have this reduction in heating, especially at the lower temperature employed.

Attempts were made also to volatilize dry crystals as well as alcoholic solutions of a number of growth substances that occur in crystalline form at room temperature since
Figure 1. Diagram showing the apparatus used in treating rose bushes with volatilized growth substance under controlled conditions. The chamber was constructed by welding two 50 gallon drums end to end with a water seal two inches wide and two inches deep welded at the top to prevent gas from escaping past the lid. Growth substance was volatilized with a hot plate and circulated around the suspended plants with a fan as indicated. For treating plants in a partial vacuum a paraffin seal was substituted for the water seal shown. Outlets for vacuum pump and gauge were made next to the thermograph outlet.
Zimmerman and Hitchcock (66) have reported that many are active when used in this way. These attempts met with little success. Although most of the growth substances tried could be volatilized to a greater or less degree, considerable recrystallization and charring took place.

It was felt that under these circumstances the exact concentration of gaseous growth substance would be difficult to determine and repeat under a given set of conditions. For this reason, with the gaseous applications emphasis was placed on the use of the esters of naphthaleneacetic acid in alcoholic solution. These liquid compounds volatilize with a minimum of heat application, and leave no visible residue upon volatilization. This was also true of the naphthalene-acetonitrile compound. Although upon standing at room temperature this compound would form crystals, they would quickly disappear upon warming.

It will be noted that in this study, more emphasis has been given to experiments dealing with gaseous application of growth substances than application in wax or other carriers that leave a visible residue on the plants. The reason for this is that many rosarians discriminate against plants that are waxed. They claim that the presence of wax is indicative of plants of low vitality that may have been held in warm retail display rooms for an uncertain length of time. They prefer to pay a premium for an unwaxed plant of good appearance, for they feel that in order to prevent drying and shoot growth, the unwaxed plants must have been stored under
favorable conditions prior to purchase.

Method of Measuring the Effectiveness of Growth Regulating Substances on Bud Inhibition.

In most of the preliminary experiments in 1939-1940 the plants were moved into the greenhouse immediately after applying the growth substances. Greenhouse forcing was done in order to speed up the testing of a large number of compounds at varying concentrations as well as the effect of different carriers that may be used in applying growth substance. Any delay in shoot production in plants in a given treatment, as compared with similar control plants, was then used as a measure of the effectiveness of the particular treatment on bud inhibition. Unquestionably, the response obtained with plants forced in a greenhouse would not be exactly equivalent to the behavior of similar plants held in common storage with regard to the effect of a given treatment on bud inhibition. However, it was felt that if a particular treatment had absolutely no effect on inhibition of buds under greenhouse condition, it would be of questionable value under common storage condition. Consequently, treatments that showed no effect or, in some cases, an injurious effect on the canes in the greenhouse were not tested further. Other treatments which showed even a slight retarding effect on bud growth were earmarked for further testing under common storage conditions.

In the 1940-1941 experiments the entire set-up was
designed to simulate commercial common storage conditions. Immediately after treatments were applied to plants they were moved into the common storage house and held until the end of the normal common storage period, which is about May 1. Later than this or in years when unfavorable storage conditions occur from 1 to 2 weeks earlier, it is not considered safe to hold rose bushes in common storage.

For the purpose of comparison of the effectiveness of the various growth substance treatments, data on number and length of shoots were taken on an individual plant basis at weekly intervals during the storage tests. At the same time also, record was made of the length of injured portions of the canes that were present on each plant.

Cytological Methods

The work of Greenleaf (23), Levan (33) and Dermen (17) has indicated that growth substances will effect polyploidy in plants. If this is likely to happen in the case of rose bushes it is possible that the characteristic varietal form of the plant and flower may be altered.

The method of treatment and sampling was similar to that used by others on plant material. Rose bushes which had been moved into the greenhouse were given 1.0 gram, 2.0 grams and 3.0 grams per 1000 cubic feet vapor treatments of a-naphthylmethylacetate just as the buds began to show activity. After the buds had started vegetative growth, samples were taken for
sectioning of the growing points.

The buds from control and growth substance treated plants were prepared for sectioning in the following manner: upon removal from the plant they were dropped into a fresh mixture of Wilson's modification of Buin's fixative to kill and fix the material. This fixative is composed of an equal mixture of two solutions, A and B. Solution A is made up of picric acid (saturated solution) 54 cc.; formalin, 50 cc. and urea, 2 grams. Solution B is composed of picric acid (saturated solution), 94 cc.; acetic acid, 5 cc. and chromic acid, 2 grams.

After killing and fixing, the material was washed free of fixative. It was then dehydrated and embedded in paraffin by the usual tertiary butyl alcohol technique. The paraffin sections were cut 15 microns in thickness and after removal of the paraffin with xylol then alcohol, the sections were stained in crystal violet followed by weak IKI solution (1 per cent XI and 1 per cent I). The stained sections were then mounted in balsam for microscopic examination.
Method of Determining Relative Starch Content of Rose Bushes by the IKI Test.

Previous work by Gardner and Yerkes (21) with a variety of fruit tree nursery stocks and by Yerkes, Scott and Swingle (65) with rose plants has shown that, when dug at an immature stage severe losses are encountered during the storage period and a low percentage survival of plants is obtained when such stock is lined out the following spring. In the case of rose bushes even greater losses are encountered when immature plants are stored, since the rose seems to be very susceptible to mold and other disease infection while in storage. The problem of immaturity is further complicated with rose bushes by their tendency to continue vegetative growth until very late in the fall. In years of high rainfall late in the season which delays maturity, even though digging is deferred until the danger of freezing weather is imminent, many immature plants may be stored. Even under optimum maturing conditions there may be low areas in the field where a few plants continue growth later than others and are stored immature along with the mature plants.

After rose bushes have been dug and pruned for storage it is frequently very difficult to determine casually which plants are mature and which are immature. The latter often have the appearance of being the better plants, since they may be larger and often have a more lively green cane color. For a number of years the iodine test for starch content of
the stock has been used with much success in determining the relative stage of maturity of rose bushes. In applying the test a drop of a weak IKI solution is placed on the cross-sectional cut surface of the stock and after a brief interval of 1 - 2 minutes it is examined with a hand lens for the blue color pattern caused by the presence of starch. Immature plants will have very little or no starch present, whereas mature plants have an abundance stored in their stocks.

The IKI method of determining maturity of rose bushes has been somewhat discredited among commercial interests where in certain instances it was found that starch was not present even in the most mature stocks. This discrepancy in the test has been explained by recent work in England by Brandon (11) who found that some rose species and hybrids do not normally store starch reserves in the fall. With such material the IKI test for maturity would be of no value. However, *Rosa multiflora* which constitutes a very high percentage of the stocks used for hybrid tea varieties in this country is one of the species which normally stores starch when the plant matures, and, as indicated by Tukey (56), Carlson (13), Brandon (11), and further substantiated by the work presented here the IKI test with this stock appears to be a reliable test for maturity.

The iodine-potassium-iodide test for starch has been widely used for many years in determining starch in plant tissues. For the most part the test has been used qualitatively, merely to determine whether starch is present or
absent from the material under examination. However, a number of investigators have reported successful use of the test on a roughly quantitative basis in following the relative amounts of starch present at different times of the year or different stages of plant development. For example, Bigelow, Gore and Howard (8), Davis and Blair (16) and Hitz (29) have followed the course of starch disappearance in developing apple fruits to determine the proper time to pick for best storage quality. Archer (3) has followed the seasonal changes in starch content of the different parts of persimmon trees throughout the year. With the rose, Brandon (11) has followed the seasonal changes in starch content of species, interspecific hybrids, and varieties of rose to determine if starch content of the plant were associated with rooting of stem cuttings taken from such plants, and the time of year to take cuttings to obtain optimum rooting responses.

Each investigator has arbitrarily set up for convenience his own system of scoring for starch content, and several (16), (29), (3) have used a numerical system similar to that employed herein. Statistical treatment of the data was applied in this work so that normal variation of the material could be estimated at any particular time of sampling (Snedecor (47)).

The method used in sampling for relative starch content of rose bushes was by making a number of sections of rose stocks which showed varying amounts of starch by the I3I test. These sections were obtained from the stock portion of a
number of plants exhibiting varying stages of cane maturity. Ten sections were selected to represent a range in relative starch content in 10 arbitrary gradations, placed on a scale from 0 to 100, figure 2. The sections were made free hand with a sharp straight edge razor from material killed by boiling in 85 per cent alcohol. After staining they were mounted on glass slides in 50-50 alcohol-glycerin and with cover glasses sealed by paraffin to make semi-permanent mounts. The original material from which each of the ten sections was made was held in 35 per cent alcohol and used for new standard sections throughout the period of sampling so that any changes in the standard could be detected.

Figure 2 shows camera leucida drawing made of all sections except the 100 gradation, since no section was found scoring that high. Scoring for relative starch content was made separately on bark (figure 2-A), which included all tissues exterior to the cambium (figure 2-B); wood, (figure 2-C) composed of xylem tissues from the cambium to the pith, and the pith (figure 2-D).

A random sample of twenty plants was selected from each lot of plants at each sampling period. Segments about 1 inch long were then taken of each plant from the base of the canes, from the mid-portion of the stock, and from the roots at their juncture with the stock. The segments from the different plant parts were dropped into separate flasks containing 85 per cent alcohol and boiled for 30 minutes in a water bath. After cooling, the segments were ready for estimation of relative starch
Figure 2. Camera lucida drawings of cross sections of rose stocks that were used as standards for comparison in estimating relative starch content by the IXI method. (A), bark and cortex region. (C), wood and (D), pith. (B) merely designates the position of the cambium in respect to the other tissues.
content, which was made on the same day that the samples were taken.

For estimating relative starch content a standard slide was placed side by side with slides containing free hand sections of the material being examined on the stage of a microscope. By this method each section was matched to the nearest corresponding standard using a magnification of 10. In the beginning, 20 sections were made of each plant part, but later there was found so little variability within a particular segment that this number was reduced to 10.
RESULTS

Preliminary Experiments with Sprays Containing Growth Substances

1939-1940. Preliminary trials were conducted in the 1939-1940 storage season using the Texas grown Ami Quinard plants previously described. The plants were divided into lots of three plants each and the growth substances previously listed under Materials and Methods were all (except tetralin-6-acetamide, which was not then available) sprayed onto the dormant plant tops in aqueous solution using the following concentrations of each compound applied to individual lots, .001, .01 and .05 per cent respectively. A number of the compounds, such as \( \text{a-naphthylmethylacetate} \), \( \text{a-naphthylethylacetate} \), \( \text{a-naphthaleneacetonitrile} \), indolepropionic acid, as well as the amides and the iso and thiocyanates, have a maximum solubility in water between .003 and .005 per cent, in which case, this concentration was used.

The aqueous sprays were applied on February 2, 1940 and immediately thereafter the plants were set with roots in sand in a propagation case in the greenhouse. Twelve days later (February 14) the plants in all lots had started active growth without any apparent effect on bud inhibition from any concentration of the growth substances that were used.

The experiment was then repeated using sprays of the same compounds at 0.1 per cent concentration. This concentration was obtained by emulsifying the desired amount of
growth substance with a sufficient quantity of light machine oil to make a final concentration of 1/4 per cent oil in the sprays. The tops of two sets of plants were sprayed with the different growth substances on February 15, 1940. One set of sprayed plants was then moved into the greenhouse, the other into common storage.

After 6 days in the greenhouse, control lots sprayed with oil-emulsion only were showing noticeable injury from the oil. However, by the end of 14 days, in spite of cane injury from oil and from the high concentration of growth substances used, differences in time of bud opening were observed between the controls and certain lots treated with growth substance. This retardation of opening did not appear to be a result of injury alone. Records obtained at the end of 18 days in the greenhouse indicated that the following compounds had apparently inhibited the buds for at least from 4 to 10 days: β-indoleacetic acid, β-indolebutyric acid, β-indolepropionic acid, α-naphthaleneacetic acid and the ethyl and methyl esters as well as the Na and K salts of this acid, α-naphthaleneacetonitrile, β-naphthoxyacetic acid, α-naphthaleneacetamide, α-naphthylmethylthiocyaneate, α-naphthalenemethylisothiocyaneate, phenylacetic acid, α-naphthalenethioacetamide and α-naphthalenepropionic acid.

The set of plants that were moved into common storage after receiving the oil-emulsion growth substance sprays also were injured by the oil contained in the sprays. At the relatively low temperature maintained in common storage
injury was slower in showing up, but by the end of 60 days (April 22) of storage the plants were very severely damaged. Records obtained at the end of 45 days of storage (April 7) substantiated the greenhouse results in that the same compounds that had caused a delay in vegetative bud development under greenhouse conditions also noticeably retarded shoot production in common storage.

It was thought that perhaps the failure of the initial tests with aqueous solution of growth substances on bud inhibition was due to the use of too low a concentration of growth substance. Further, it was felt that perhaps if the growth substances had penetrated into the plants better, bud inhibition may have been effected with these sprays.

In order to apply relatively high concentrations of growth substance as well as to cause better penetration into the plants the following solvents in the concentration indicated were used in making up sprays; ethyl alcohol 5, 10, 16 and 20 per cent; acetone 1, 3, 5 and 7 per cent and dioxan 1/8, 1/4, 1/2 and 1 per cent.

Four growth substances were tested at 0.1 and 0.2 per cent. These compounds were B-indoleacetic acid, a-naphthaleneacetic acid, a-naphthylmethylacetate and a-naphthaleneacetamide. In making up the sprays a weighed amount of the chemical was dissolved in a measured amount of solvent then mixed with sufficient water to bring both chemical and solvent to the desired percentages of each. The dormant plants were sprayed on March 6, 1940, using three plants per lot of
the Ami Quinard variety in each concentration of growth sub-
stance - solvent combination so that a total of 96 lots of
plants were used. The plants were set in sand in the green-
house immediately after the sprays were applied.

As in the previous trials with the aqueous solutions,
results were again negative as far as bud inhibition was
concerned. The lower concentrations of growth substance -
solvent combination caused no apparent inhibition or injury
in comparison with unsprayed control lots while the higher
concentration of both growth substance and solvent caused
considerable injury to both canes and vegetative buds.

Failure of the 1/4 per cent oil - 0.1 per cent growth
substance sprays applied on February 14, 1940 was apparently
due to excessive oil in the sprays which caused considerable
plant injury. The experiment was therefore repeated on
March 27, 1940, using both oil and growth substance in lower
concentration. At the same time also, plants were sprayed
with emulsions of both lanolin and wax containing growth
substance, in an effort to find a carrier for the growth
substance that would be non-toxic to dormant rose plants.

The sprays used in this experiment were made up with
the following carrier emulsions: oil, 1/16 and 1/8 per cent;
lanolin, 1/8 and 1/4 per cent, also two proprietary wax-
emulsions which are recommended for the waxing of nursery
stock. The waxes were applied at 1/4 and 1/8 per cent of the
prepared emulsion. All the emulsions were mixed in water and
further emulsified with 0.5 per cent a-naphthylmethylacetate.
The sprays were applied on the above date using 10 plants of the Guinea rose variety in each treatment. Control plants were sprayed with the carriers without growth substance added while one set of plants was left unsprayed. Immediately after the sprays were applied, 5 plants of each lot were placed in the greenhouse, the other 5 into common storage.

At the end of 3 days in the greenhouse a stimulating effect on bud breaking was noted on the control plants sprayed with $\frac{1}{16}$ per cent oil and $\frac{1}{4}$ per cent lanolin emulsions only. The unsprayed controls started growth on the 6th day and by the 14th day in the greenhouse had made considerable growth, having shoots from 4 to 10 inches in length. A very pronounced inhibiting effect was apparent on all lots sprayed with growth regulating substance. These plants were just starting visible bud growth by April 10, 1940 (14 days after treatment). It was apparent also at this time that the concentration of growth substance (0.5 per cent) was too strong, as the plants in all lots receiving it were showing some injury near the cut end of the canes. None of the carriers appeared to be injurious in the concentrations used except the $\frac{1}{4}$ per cent oil which caused a slight amount of cane injury in both sprayed controls and growth substance treatments.
Preliminary Experiments with Volatilized Growth Substance

1939-1940. Experiments with the vapors of three compounds, a-naphthylmethyleacetae, a-naphthylethyleacetae and a-naphthyleacetonitrile, showed much promise by their effectiveness in inhibiting the vegetative buds on rose plants.

Preliminary experiments with volatilized growth substance were begun on February 26, 1940, using Ami Quinard rose plants similar to those used in the spraying experiments. Two sets of plants were treated with vapors from 1, 3, 5 and 10 drops of each of these three compounds using 10 plants for each rate of application. In treating the plants each lot was placed in a 20 gallon container and the growth substance was volatilized from a small watch glass by placing it on a hot piece of metal at the bottom of the container. The containers (20 gallon refuse cans) were sealed so that the plants were exposed to the growth substance vapors for 4 hours at room temperature. Following treatment one set of plants was placed in the greenhouse with roots in moist sand, the other was returned to common storage.

After 14 days it was apparent from examination of plants in the greenhouse that the vapor from one drop (approximately 35 milligrams) of each of the three growth regulating substances had caused bud inhibition without apparent plant injury. The control plants at the end of the same period had produced an average of 24.5 shoots per plant varying in length from 1/2
to 10 inches whereas the growth substance treated plants that had received but one drop of each compound were just beginning active vegetative growth at this time. Plants that had been treated with 3, 5 and 10 drops of growth substance were also inhibited in bud growth, but associated with inhibition and varying directly with it and the concentration was a progressive increase in cane injury so that by the end of the 14 day period in the greenhouse plants treated with 10 drops of each compound were completely killed.

The control plants that had been placed in common storage directly after treatment did not begin active growth until March 20, 1940, or 22 days after treatment. By April 22 they had produced an average of 35.4 shoots per plant, while plants treated with the vapor from one drop of both the esters and the nitrile compound were completely dormant at this time. Plants that had received 3, 5 and 10 drops of the growth substance and held in common storage had by this date developed severely injured canes but injury not so severe as that on the greenhouse plants.

On the basis of these preliminary experiments it was evident that certain chemicals were very effective in inhibiting shoot growth when applied in the vaporized form and suggested that with careful attention to concentration and to other details of application these compounds might be of use in inhibiting shoot growth of dormant rose bushes held in common storage.

The experiments of Zimmerman and Hitchcock (66) indicated
that under greenhouse temperature conditions sufficient volatilization of a-naphthylmethylacetate will take place to cause growth curvature responses on herbaceous plants without the application of additional heat to cause rapid vaporization of this compound. The rate of volatilization under the relatively low common storage temperatures would be very slow in comparison with normally high greenhouse temperatures.

Forty Guineo rose plants were therefore used to determine if slow continuous volatilization of growth substance would cause bud inhibition with plants in common storage. The experiment was started on March 27, 1940. Two lots of twenty plants each were placed in separate 20 gallon refuse cans having tight fitting lids. One drop of a-naphthylmethylacetate absorbed on a piece of paper toweling was suspended 1 foot above the plants in a can. Plants in another can served as the controls. The cans were then sealed and placed side by side in common storage. When the cans were opened on April 15 (19 days after treatment) the control plants had produced an average of 9.3 shoots per plant varying in length from 1 to 12 inches whereas the plants exposed to the growth substance vapors were completely dormant. Ten plants were removed from each can at this time with the etiolated shoots removed from the controls and placed in the greenhouse for observation while the remaining 10 plants were left in common storage after rescaling the cans but without adding more growth substance to the toweling. After 16 days (May 1, 1940) in the greenhouse both control and treated lots of plants had
made vigorous growth, and were very similar in appearance. No evidence of plant injury that could be attributed to the growth substance treatment was found.

The remaining plants that had been left in common storage were removed from the cans on May 1, 1940. Differences in response of typical control and treated plants are shown in the photographs (figure 3). It is apparent that the plants treated with growth substance were just starting active growth at this time whereas the control plants had produced long etiolated shoots. It is of interest also that mold growth was apparently inhibited by the growth substance vapors. This suppression in mold growth may have been due to a better callousing of the cut surfaces shown in the treated lot or to a direct inhibiting action on mold growth by the vapor treatment.
Figure 3. 17. Control plants. 19. Plants treated with regulating substance. Both lots held in common storage. Treatment begun on March 27, 1940 when both lots were dormant. Photographed on May 1, 1940. Control plants showing much more shoot and mold growth than the treated plants.
Comparison of Sprays and Vapors of Growth Regulating Substances on Inhibiting of Bud Growth of Rose and Miscellaneous Nursery Stock

1939-1940. Nurserymen often place plants of species widely different in plant characters in the same common storage with rose bushes. It seemed desirable to know what effect these growth substances would have on such plants, and if it would be necessary to remove them when treating with gases to prolong the dormant period of roses.

An experiment was set up to obtain information on this point on March 27, 1940. At the same time the plants were so arranged as to give information relative to the effectiveness of treatment at different distances between the plants and the gas source during the treatment period.

The plants used are listed in Table 2. For the most part they were selected as being representative of 1 year fruit trees of apple, peach, pear and cherry that are commonly stored as well as several types of deciduous ornamental plants that may be held for brief periods in common storage houses. All plants were dormant when the experiment was set up on March 27 and for comparative purposes plants of the Guiney rose were included in the experiment. Conditions were representative of those encountered when plants are held by the nurseryman for late shipment, or as in the case with apple root grafts, when seasonal conditions prevent earlier planting in the field.
## Table 2

<table>
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<th>Species</th>
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<th>Common Name</th>
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<th>Spray Lot</th>
<th>Volatilized Gas Lots</th>
<th>Remarks</th>
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<td>Japanese maple</td>
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<td>20.7</td>
<td>5.4</td>
<td>2.4</td>
<td>5.0</td>
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<td>Peach seedlings</td>
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<td>0.8</td>
<td>16.5</td>
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<tr>
<td>Amygdalus persica</td>
<td>Elberta 1-year trees</td>
<td>10</td>
<td>14.4</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>8.8</td>
</tr>
<tr>
<td>Ziziphus virginiana</td>
<td>Native seedlings</td>
<td>10</td>
<td>3.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Philadelphus grandiflorus</td>
<td>Mock-orange</td>
<td>10</td>
<td>10.5</td>
<td>0</td>
<td>0.2</td>
<td>1.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Prunus serotina</td>
<td>Montmorency cherry</td>
<td>10</td>
<td>12.8</td>
<td>2.4</td>
<td>0.5</td>
<td>4.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Prunus serrulata</td>
<td>Japanese flowering cherry</td>
<td>10</td>
<td>12.4</td>
<td>0</td>
<td>0</td>
<td>2.2</td>
<td>12.7</td>
</tr>
<tr>
<td>Pyrus communis</td>
<td>Bartlett 1-year trees</td>
<td>10</td>
<td>5.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.9</td>
</tr>
<tr>
<td>Malus sylvestris</td>
<td>Apple, N. Spy 1-year trees</td>
<td>10</td>
<td>6.7</td>
<td>0</td>
<td>0</td>
<td>1.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Malus sylvestris</td>
<td>Apple, Delicious (grafts)</td>
<td>50</td>
<td>2.1</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Rosa sp.</td>
<td>Guine rose variety</td>
<td>20</td>
<td>10.1</td>
<td>0</td>
<td>0</td>
<td>2.8</td>
<td>9.0</td>
</tr>
</tbody>
</table>

* Growth substance = m-naphthylethylacetate. Spray = 0.01% growth substance in 1/4% oil. Gas = 0.5 gram growth substance volatilized per 1000 cubic feet 16 hrs. at 70°F.

** Distance from the gas source.
In treating with gas, a tight common storage room of 28,000 cubic feet capacity was used. \( \alpha \)-naphthylmethyleacetate at the rate of 0.5 gram per 1000 cubic feet was volatilized for 16 hours. For comparative purposes a set of plants was sprayed with an oil-emulsion spray containing 1/4 per cent light machine oil and 0.01 per cent of the same growth substance. The sprayed lot and a control lot were removed from the storage during the period of gas treatment.

The growth substance was volatilized on a hot plate placed at one end of the room. One set of each of the plants listed in table 2 had been grouped on the floor at a distance of 4, 8, 12 and 16 feet from the gas source. A large ventilating fan 20 inches in diameter provided a brisk circulation of air over the hot plate in the direction of the plants throughout the treatment period.

The extent of vegetative growth found on the plants after common storage for 35 days is shown in table 2. The control plants showed considerable growth of vegetative buds during this period, many having produced shoots 4 to 10 inches in length. It is of interest that both spray and gaseous application of growth substance were about equal in effectiveness in inhibiting vegetative buds on the wide variety of plants used.

The data (table 2) show that the volatilized growth substances had apparently condensed onto surfaces nearest its source, even though it had been volatilized into a rapidly moving stream of air provided by the large fan that was used.
Effective inhibition of buds was obtained with plants placed 4 feet distant from the gas source, and the slightly greater number of shoots produced on the plants at 8 feet would not be significantly more than those set at 4 feet. However, at a distance of 12 feet the effectiveness of the growth substance on bud inhibition was unquestionably reduced, while plants placed at a distance of 16 feet from the gas source during the treatment period were very similar to the untreated controls in the number of shoots that subsequently developed in common storage.

The different plant species did not respond alike in all cases to a particular growth substance treatment. Complete dormancy was maintained in all the species used except Acer palmatum, Philadelphus grandiflorus and Montmorency cherry which showed a few vegetative buds, which were in all instances much fewer in number per plant than on the controls (figures 4 and 5).

Injury to either the oil or growth substance was severe in the sprayed lots of peach (both seedling and Elberta) and Montmorency cherry. Since the gas treated lots did not show the injury, it may be assumed that it was either caused by the oil or oil had contributed in some way to its manifestation. The plants of Prunus serrulata, perhaps as a result of penetration of growth substance in oil, showed swellings and root development throughout the entire stem portion of sprayed plants. This response in root production was also noted in certain lots of treated rose bushes (figure 5).
Figure 4. *Philadelphus grandiflora* bushes held in common storage. (9) Control lot. (10) Treated with growth substance.
Figure 5. One year old Montmorency cherry trees held in common storage from March 27, 1940 until May 1, 1940. (1b) Control. (12) Treated with volatilized growth substance.
Figure 6. A. Control.
B. Treated with 2 drops of α-naphthylmethylacetate soaked into paper toweling and allowed to volatilize, without heating, in a sealed 20 gallon can in common storage from March 25, 1940 to April 30, 1940. Roots were beginning growth at several points on the stems as a result of treatment.
The possibility of using this method of pretreating prior to taking dormant stem cuttings of roses and other plants as an aid in rooting such cuttings is suggested. Subsequent to storage the treated plants were lined out in the field. Seasonal conditions were very favorable for transplanting even at this late date (May 2). All the plants lived with the exception of the spray injured lots of peach and the control lots of Delicious apple root grafts. With all growth regulating substance treatments dormancy was maintained from 1 to 2 weeks longer, after transplanting, than comparable control lots of each species. This initial delay in top growth may be of benefit in a less favorable season for transplanting of nursery stock, since it would allow a period for root development to take place before the top began to draw on soil moisture.

The Delicious apple root grafts, illustrated in figures 7 and 8, were the only material to show a pronounced benefit from growth substance in percentage survival. The percentage of grafts that lived of the fifty planted in each lot was as follows: control, 74 per cent; sprayed, 80 per cent; gased, 100 per cent. At the end of the growing season, no significant difference was found in the amount of top growth that the grafts had made in each lot. Scion rooting had not taken place in the first year, as reported by Jones (30) with growth substance applied in lanolin emulsion to grafts of the Virginia crab variety.

A repetition of this work with root grafts of Starking
Figure 7. Condition of Delicious apple root grafts at end of common storage period from March 1, 1940 to May 1, 1940.
1. Control lots with terminal shoots advanced.
2. Grafts which had been treated with volatile growth substance on March 27, 1940, (0.5 gram a-naphthylmethylacestate per 1000 cubic feet for 17 hours at 70° Fahrenheit) showing terminal buds just beginning to swell. Photograph taken on April 27, 1940.
Figure 8. Delicious grafts similar to those shown in figure 7. Upper row, (A). Control. (B), Treated with vapor of growth substance. Grafts potted up after removal from common storage. Lower row, same grafts after two weeks in the greenhouse. Growth substance treatment did not increase the percentage of graft surviving under greenhouse conditions as was noted in similar field planted grafts. (1940).
and Gallia varieties of apple in 1941 also showed a significant increase in stand of grafts as a result of growth substance applications to the graft union. Indolebutyric and indoleacetic acids and naphthaleneacetamide were about equal in effectiveness in the 1941 experiments.

The increase in stand of grafts appeared to be due to a speeding up of callous proliferation, causing a better union between stock and scion pieces of the treated grafts. Suppression of top growth by growth substance treatment would also reduce transpiration until the newly formed union is better able to permit passage of water to the top.
Comparison of Relative Effectiveness of Several Growth Regulating Substances Applied in Wax-Emulsion on Bud Inhibition of Rose Bushes in Common Storage

1940-1941. A number of workers have called attention to the fact that even though a particular growth regulating substance may be very effective in causing one type of plant response, it may be very ineffective with regard to another response. For example, indolebutyric acid causes root formation on Ilex opaca cuttings in a relatively short time (50); on the other hand, when indolebutyric acid was used in experiments to induce parthenocarpy (19) on the same holly plants, it was found relatively ineffective, and naphthalene-acetic acid proved to be much superior. Other examples of the apparent specificity of certain growth substances in causing a particular type of plant response are on record (20), (5).

Of the 23 chemical compounds applied to dormant rose bushes to test their potential bud inhibiting effects in 1939 - 1940 seventeen were of sufficient interest to warrant further testing as growth regulating substances in 1940 - 1941. These compounds are listed in table 3. It seemed desirable also to investigate further the use of suitable carriers for these substances since in the 1939 experiments a light machine oil alone as well as water only as a carrier proved unsatisfactory. The oil produced injury and aqueous sprays did not permit the use of very high concentration of
the compounds of low solubility in water. Trials with several proprietary wax-emulsions miscible with water which are used in waxing rose bushes commercially, indicated that they could be used safely in low wax concentration.

The concentration of wax-emulsion carrier finally selected was 1/4 per cent of the emulsion in water. The desired, weighed, amount of growth substance was dissolved in the smallest quantity of 95 per cent alcohol necessary for complete solution. A minimum of alcohol was used to avoid precipitating wax of the emulsion. The growth substance dissolved in alcohol was then mixed with a measured quantity of full strength wax-emulsion by forcing the two through an emulsifier several times. Varying concentrations of growth substance sprays could then be obtained by adding the desired amount of this stock solution to water, and where necessary adding additional wax-emulsion. This concentration of emulsion when applied with a small electrically operated paint sprayer, left a thin uniform film of wax on the plants that was not readily noticeable.

Rose bushes of the Ami Quinard variety were again used in comparing the effectiveness of the different growth substances. The 1060 bushes used in this experiment were Texas grown #1 grade, one year old budded plants grown on rooted *Rosa multiflora* stem cuttings which had been separated into 53 uniform lots composed of 20 plants per lot. Application of each compound at three concentrations, 0.005, 0.01 and 0.05 per cent, respectively was used on each lot of 20 plants.
Two additional lots of 20 plants each were used for controls, one of which was sprayed with wax-emulsion only, the other was left unsprayed. Each lot of plants was placed in a wax-paper-lined 1/2 bushel basket, and the plant roots and stocks were completely covered with moist sand-peat mixture prior to spray application. The sprays were applied on February 26, 1941 and the plants were placed in common storage. By April 18 the control lots as well as many lots treated with growth substance had produced considerable shoot growth and this date was selected for recording the number and length of shoots produced per plant. After removing all shoots that had developed in storage the plants were potted in soil in 6 inch pots. The plants were then completely randomized on a bench in a 65° to 70° Fahrenheit greenhouse.

In table 3 the compounds used have been arranged in order of their relative effectiveness in preventing shoot elongation of the rose plants during common air storage. It is of interest that the two esters of naphthaleneacetic acid were more effective than the acid itself. As shown in figure 9, at the .005 per cent concentration of the methyl ester complete dormancy was maintained as was also the case with plants treated at this concentration with the ethyl ester. Both esters were also found to be very effective when applied in the gaseous state, which was also true of naphthaleneacetonitrile compound. Indoleacetic acid, indolebutyric acid and naphthaleneacetamide, compounds widely reported for their root promoting effects on cuttings were about equally effec-
### Table 3.

**Effectiveness of different chemical compounds in inhibiting shoot growth of Ami Quinard rose rushes held in common storage, and subsequent shoot growth on the same plants after 21 days in a 65° - 70°F greenhouse.**

<table>
<thead>
<tr>
<th>Chemical Compound</th>
<th>*Mean number of shoots per plant developed in storage 2/26 to 4/16</th>
<th>Mean number of new shoots produced per plant after 21 days in greenhouse 4/16 to 5/6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage concentration</td>
<td>Percentage concentration</td>
</tr>
<tr>
<td>Naphthalene methyl acetate</td>
<td>0.00 0.00 0.00</td>
<td>27.55 35.60 44.95</td>
</tr>
<tr>
<td>Naphthalene ethylacetate</td>
<td>0.00 0.00 0.00</td>
<td>26.90 34.15 40.10</td>
</tr>
<tr>
<td>Naphthalene acetonitrile</td>
<td>0.00 0.00 11.90</td>
<td>21.45 39.10 47.85</td>
</tr>
<tr>
<td>Naphthalene acetic acid</td>
<td>0.00 0.00 12.95</td>
<td>11.50 48.75 49.80</td>
</tr>
<tr>
<td>Naphthalene acetamide</td>
<td>0.00 4.25 10.40</td>
<td>46.40 49.70 45.85</td>
</tr>
<tr>
<td>Indole acetic acid</td>
<td>0.00 5.65 12.80</td>
<td>45.75 46.50 52.25</td>
</tr>
<tr>
<td>Indole butyric acid</td>
<td>0.00 12.60 40.45</td>
<td>45.40 52.70 50.65</td>
</tr>
<tr>
<td>Potassium naphthalene acetate</td>
<td>0.00 16.65 41.70</td>
<td>48.60 49.70 36.40</td>
</tr>
<tr>
<td>Sodium naphthalene acetate</td>
<td>0.00 19.83 48.35</td>
<td>32.50 38.75 31.55</td>
</tr>
<tr>
<td>Naphthoxyacetic acid</td>
<td>19.90 35.75 48.35</td>
<td>40.65 35.45 30.20</td>
</tr>
<tr>
<td>Indole propionic acid</td>
<td>25.75 56.10 70.65</td>
<td>51.50 56.80 35.45</td>
</tr>
<tr>
<td>Naphthalene methyl thiocyanate</td>
<td>26.40 38.70 45.85</td>
<td>42.50 47.85 40.70</td>
</tr>
<tr>
<td>Naphthalene methyl isothiocyanate</td>
<td>29.75 39.35 41.50</td>
<td>40.60 47.45 39.60</td>
</tr>
<tr>
<td>Tetralin-6-acetamide</td>
<td>35.60 54.15 60.40</td>
<td>42.70 56.85 31.70</td>
</tr>
<tr>
<td>Phenylisocetic acid</td>
<td>41.60 46.90 68.15</td>
<td>37.90 40.05 24.60</td>
</tr>
<tr>
<td>Naphthalene thionocetic acid</td>
<td>44.30 49.95 48.10</td>
<td>32.60 38.75 31.55</td>
</tr>
<tr>
<td>Naphthalene proterionic acid</td>
<td>43.10 49.80 47.65</td>
<td>38.70 33.45 45.50</td>
</tr>
<tr>
<td>Control (emulsion spray only)</td>
<td>48.10</td>
<td>40.60</td>
</tr>
<tr>
<td>Control (not sprayed)</td>
<td>40.55</td>
<td>47.55</td>
</tr>
</tbody>
</table>

*Mean of 20 plants. Differences necessary: 5% level 6.8 for significance; 1% level 11.2 for 21.5 1% level 29.7
Figure 9. A. Control plants sprayed with wax-emulsion only. B. Plants sprayed with growth regulating substance (0.005 per cent a-naphthylmethylacetae added to the emulsion). Sprays applied February 26, 1941, plants placed in common storage and photographed April 18, 1941.
tive in preventing shoot growth on rose plants with the amide proving superior at the 0.01 per cent concentration, but none of these compounds were as effective as the esters of naphthaleneacetic acid.

It should be noted here however, that some injury was apparent on all lots treated with 0.06 per cent concentration of every compound except indolepropionic, phenylacetic acid and tetralin-6-acetamide. No injury was apparent even with the highest concentration of each of these three compounds, neither was there any apparent inhibiting effect on shoot development. On the contrary, there was a very marked and significant increase in the number of vegetative shoots produced by each of these compounds at the lowest (.005 per cent) concentration which was used. Stimulating effects on vegetative shoot productions by phenylacetic acid has also been noted by Zimmerman and Hitchcock (66), on potato tubers.

The injury caused by concentration of 0.06 per cent of growth substances with all but the three exceptions noted above is not surprising in view of evidence that such injury has already been shown by Yerkes (63) with concentrations higher than 0.01 per cent on various kinds of hardwood cuttings. In the treatment the cuttings were allowed to stand with their bases only in the aqueous growth substance solution for several hours, whereas the dormant rose bushes used in this experiment had their tops completely covered with a wax-emulsion containing the various growth substances. Further, the growth substance was in close contact with the
plants for almost a two month period (from 2/26 to 4/18) while they were in storage and for a short time following storage. This long exposure to the growth substance may have resulted in cane injury. The wax-emulsion seemed to disappear from all sprayed lots after 3 to 4 days in the greenhouse, probably because the wax melted at the higher temperature and subsequently was washed off the plants in the course of watering.

After the plants had been in the greenhouse 14 days the lots that had received effective growth substance treatments in common storage continued to show a pronounced slowness of development of vegetative buds. This "hold-over" retardation of bud growth is illustrated by the appearance of the control and growth substance treated lots shown in figure 10.

Data on mean number of shoots produced after 21 days in the greenhouse are shown in table 3. With the large differences that were found necessary for significance (21.5 at the 5 per cent level) none of the growth substance treatments applied in common storage induced a significantly greater or less number of shoots to grow out after the plants had been in the greenhouse for 21 days following common storage. The extreme variability of these data with regard to number of shoots was unquestionably due to a marked reduction in number of shoots on certain plants due to strong apical dominance of the uppermost buds, irrespective of prior treatment. These plants produced several strong growing shoots which appeared to inhibit the remainder of the
Figure 10. A. Control plants. B. Growth substance treated. Ami Quinard variety treated by spraying with wax-emulsion of 0.01 per cent a-naphthylmethylacetate. Sprays applied on 2/26, plants held in common storage until 4/19. Photograph taken after 14 days in the greenhouse showing continued inhibition of buds by the treatment in storage.
vegetative buds on the canes that bore them. This behavior threw plants with many shoots and plants with few shoots into the same or different lots in an unpredictable manner. It seems justifiable to conclude from these data (table 3) that growth substance treatments may completely mask individual plant variation as to shoot production in common storage, but that after the growth substance treatment has been removed or dissipated, individual plant variability will again express itself by shoot production in the greenhouse.

Of interest also is the fact that the plants that suffered cane injury from 0.05 per cent wax-emulsion growth substance sprays in storage which in some cases (naphthalene-acetonitrile 0.05 per cent treatment) had considerable top removed when potted up, apparently had sufficient dormant buds at the base of the canes to produce numerous shoots per plant (table 3). The greenhouse conditions employed in this experiment provided humidity and temperature control of the air as well as control of the soil and its moisture content within relatively narrow limits. Under these conditions the control plants (figure 10), and plants from ineffective storage treatments grew remarkably well. This is in direct contrast with the field planting results obtained with plants from similar treatments of the same variety of rose. Under field conditions temperature and moisture of the soil and air are not readily under control to insure optimum conditions for the growth of roses which are plants that are readily injured by growing conditions that are not optimum. The
control plants with a very low reserve after storage due to
the number of shoots produced and removed before trans-
planting grew very poorly when field planted, whereas the
lots receiving growth substances made excellent growth.
Effectiveness of Growth Substance-Wax-Emulsion Sprays on Bud Inhibition when Applied to the Tops Only, to the Stocks Only, and to the Roots Only of Rose Bushes Held in Common Storage

1940-1941. From a practical standpoint, application of the wax-emulsion-growth sprays to the roots, or to the stock portion of rose plants would have at least two distinct advantages, provided such treatments would effectively inhibit shoot formation. First, the wax-emulsion could be applied to a portion of the plant where it would be less conspicuous and thus would be less likely to discourage potential buyers who object to waxed plants. Second, there would appear to be less likelihood of injury, especially with applications to the stock portion of the plant, since it appears to have a thicker and more suberized bark than either the roots or the canes of rose plants.

Snow (49) in 1931 and Went (58) in 1939 have reported that bud inhibition was increased with increased distance from the source of the inhibiting substance. Snow used the effect of the apical bud itself on inhibition of lateral buds. Went used indoleacetic acid-lanolin paste in his experiments on etiolated pea stems, (Pisium sativum var. Alaska). If the findings of these authors should hold for growth substances applied to rose bushes to inhibit shoot development in storage, the implication is quite obvious that such treatments would be most effective if made either to the roots or to the stock.
portion of the plant rather than to the canes. The work of Stuart (51) and others with cuttings has also demonstrated that the inhibiting effect of growth substance will move upward in plants, since the cuttings were treated at the base and the terminal buds above were inhibited.

In conjunction with the previously described experiment (1940-1941) comparing a number of chemical compounds, the data of which are summarized in table 3, two additional lots of plants were used to compare the effectiveness of growth substance on bud inhibition when the applications were made at different distances from the buds. The plants used for such a test were similar to those used in the previous wax-emulsion spray experiment in which tops only were sprayed, and were handled in a similar manner so that direct comparison could be made.

One of the lots of 20 plants received 0.01 per cent naphthaleneacetic acid in wax-emulsion, sprayed on the stem portion of the stocks only. The roots and tops were protected from the spray by covering them with heavy waxed paper sealed tightly with adhesive tape. The other lot of plants received the same spray applications to the roots only by similarly protecting the stems of the stocks and canes. The third lot of plants received the spray application to the tops only, the roots and stocks having been covered with sand-peat mixture, overlaid with sealed wax paper. These plants were therefore used for comparative purposes along with an unsprayed control lot.
In setting up the experiment, the surface area of top to roots was adjusted by pruning so that the amount of spray which adhered to these two plant parts was fairly comparable. No such adjustment could be made with the surface area of stock portion of the plants except as could be done by selecting plants with relatively long stemmed stocks, 6 to 8 inches in length. The plants receiving spray application to this portion only, necessarily received somewhat less growth substance.

The sprays were applied on February 26, 1941 and the plants were placed in common storage immediately after treatment. The data on mean number of shoots that had grown out on plants in the different treatments by April 18, 1941 are summarized in table 4.

### Table 4.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Number of Shoots Per Plant (20 plants per treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray applied to tops only</td>
<td>0.00</td>
</tr>
<tr>
<td>Spray applied to stocks only</td>
<td>38.65</td>
</tr>
<tr>
<td>Spray applied to roots only</td>
<td>42.63</td>
</tr>
<tr>
<td>Control (not sprayed)</td>
<td>40.55</td>
</tr>
</tbody>
</table>
The data in table 4 show that under the conditions encountered, the growth substance was most effective on bud inhibition when applied directly to the vegetative buds themselves. No significant effect on bud inhibition was obtained from application to the roots or stock of rose bushes of wax-emulsion sprays containing 0.01 per cent naphthaleneacetic acid.

Root growth was apparently inhibited in plants that received spray to the roots only, for at the end of the storage period the roots of these plants were calloused at the cut surfaces, but had produced no new root growth; all other lots had some new roots showing on each plant. Bonner and Koepfli (9) have also noted an inhibition of roots from auxin application. These results are somewhat in disagreement with previous experiments that have been reported as to the effect of growth substance on bud inhibition in plants. Direct comparison with other work however is not justified, for the growth regulating substance and the growth status of the plants at the time the inhibiting effects were measured are quite different. Further, the relatively low temperatures prevailing in the common storage house in which the plants used in this study were stored, after treatment, are in striking contrast to the relatively high laboratory and greenhouse temperatures that have been used previously by other workers with other plant material.
Effect of Concentration of Volatilized Growth Substances on Dormancy, Cane Injury and Growth of Molds

1940-1941. Preliminary treatments with the vapors of several growth substances in 1939 - 1940 indicated that three compounds, α-naphthylmethylacetate, α-naphthylethylacetate and α-naphthylacetonitrile were outstanding and about equal in effectiveness in inhibiting the vegetative buds on rose bushes in common storage. The 1939 - 1940 tests were made with a limited number of plants per treatment and with a relatively narrow range of concentration of growth substance. In order to further test these three compounds for better evaluation, they were again used in 1940 - 1941 in a wider range of concentrations and with 20 plants in each treatment. The plants were of the Ami quinard variety previously described. The vapor of each compound was applied at the rate of 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0 and 3.0 grams per 1000 cubic feet for 16 hours at 70° Fahrenheit. The plants were treated on March 12, 1941 and placed in common storage. The compounds and treatments used are given in table 5 together with data on shoot production and cane loss that was found when the plants were examined on May 1, 1941.

Comparison of shoot production on the plants on this date shows that both esters were more effective than the nitrile, the esters having caused complete bud inhibition when applied at the rate of 0.4 gram per 1000 cubic feet while with the nitrile compound the rate necessary to cause complete
TABLE 5.

COMPARISON OF THREE VOLATILE GROWTH SUBSTANCES BASED ON SHOOT GROWTH AND CANE LOSS
WITH AMI QUINARD ROSE BUSHES HELD IN COMMON AIR STORAGE. TREATED MARCH 12, 1941.
DATA OBTAINED AT THE END OF THE STORAGE PERIOD, MAY 1, 1941

<table>
<thead>
<tr>
<th>Concentration of growth substances</th>
<th>a-naphthyl methyl acetate</th>
<th>a-naphthyl ethyl acetate</th>
<th>a-naphthyl aceto nitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>in cu. ft. (16 hours at 70°F., as gas)</td>
<td>Mean number shoots per plant, S.E.</td>
<td>Mean percentage dead canes per plant, S.E.</td>
<td>Mean number shoots per plant, S.E.</td>
</tr>
<tr>
<td>0.100</td>
<td>90.7 ± 17.67</td>
<td>56.70 ± 11.20</td>
<td>86.4 ± 18.14</td>
</tr>
<tr>
<td>0.200</td>
<td>25.6 ± 5.66</td>
<td>38.80 ± 8.41</td>
<td>32.5 ± 9.10</td>
</tr>
<tr>
<td>0.300</td>
<td>2.0 ± 0.41</td>
<td>2.75 ± 0.53</td>
<td>10.7 ± 2.55</td>
</tr>
<tr>
<td>0.400</td>
<td>0.0 ± 0.00</td>
<td>11.90 ± 3.02</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>0.500</td>
<td>0.0 ± 0.00</td>
<td>29.15 ± 4.57</td>
<td>12.65 ± 16.41</td>
</tr>
<tr>
<td>1.000</td>
<td>0.0 ± 0.00</td>
<td>48.42 ± 12.81</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>2.000</td>
<td>0.0 ± 0.00</td>
<td>65.75 ± 2.90</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>3.000</td>
<td>0.0 ± 0.00</td>
<td>90.50 ± 0.75</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>Controls</td>
<td>65.3 ± 7.83</td>
<td>45.3 ± 8.02</td>
<td>-</td>
</tr>
</tbody>
</table>

* 20 plants per treatment. Percentage dead canes calculated from length of dead to total cane length per plant.
inhibition was 0.5 gram per 1000 cubic feet. Further, the methyl ester was slightly superior to the ethyl ester (comparing both at the 0.3 gram concentration). At the lowest concentration used (0.1 gram per 1000 cubic feet) however, all three compounds caused an increase in number of shoots produced per plant in comparison with the control plants. From the standpoint of bud inhibition, therefore the results obtained in the present experiments suggest that of these three compounds applied as vapors the two esters would appear to be the most effective. Of some consideration also is the fact that the esters do not release any substance toxic to animals, whereas the nitrile compound contains a cyanide group that may be released in toxic quantities upon volatilization of the compound.

Injury from a relatively high concentration of growth substance was noted early in the experiments and, as shown in table 5 may result in very severe cane loss while the plants are in common storage. Cane injury in the 1940 - 1941 season was much less severe than in 1939 - 1940. To a lesser degree this seasonal effect held for plants in comparable treatments with growth substance. Although, the nature of the growing season prior to storage as influencing plant reserves may have influenced the degree of injury, and the much higher temperatures encountered during February and March of the 1940 storage period may have also been a factor.

The injury was noticeably different in the control lots than in the lots treated with growth substance. In the
former, the 45.8 per cent injury (table 5) was largely due to cane destruction by disease organisms, starting in the tender etiolated shoots and working back into the canes (figure 11). Material injured by growth regulating substances applied in strong gaseous concentrations was first evidenced by the appearance of small black sunken areas in the internodal regions at and near the ends of the canes. With the three compounds applied as gases at high concentrations of 1, 2 and 3 grams per 1000 cubic feet, injury extended over the entire length of the canes, usually resulting in death. Figure 12 B illustrates plants severely injured by a 2 grams per 1000 cubic feet concentration of a-naphthylmethylacetate. Molds commonly found on the control lots were not usually found on the treated plants, or if spores were present, they did not germinate (figure 3 A and B).

The reduction in amount of cane loss found on plants treated with 0.3 gram of a-naphthylmethylacetate per 1000 cubic feet would suggest that this compound had some direct fungicidal action. To determine if this were true, rose cane media were prepared by cutting the canes into 1/4 inch lengths, boiling them in 200 cc. flasks in water for 1 hour and autoclaving to produce sterile cultures. These media were then transferred to large 9 inch sterile culture dishes, so that a single layer of the cane pieces covered the bottom of each dish. The dishes were then inoculated with a pure
Figure 11. (a) Destruction of extraterrestrial showers on trees.
Figure 12. Appearance of the injury due to highly concentrated volatilized growth substance. (A), Dormant and uninjured Ami Quinard rose stems with slightly calloused ends after 60 days in common storage resulting from low concentration of 0.3 gram per 1000 cubic feet a-naphthylmethylacetate. (B), Almost complete killing of canes as a result of application of the same compound at the rate of two grams per 1000 cubic feet, showing the characteristic black "pitting" type of injury that was found.
culture of Botrytis sp.* a mold organism obtained from badly
infected rose bushes.

After standing over-night the mold had developed and
covered an area of about one inch in diameter. Ten dishes
for each treatment were then selected, and treated respectively
with a-naphthalenemethylacetate at the rate of 0.3, 0.5 and
1 gram per 1000 cubic feet for 17 hours at 70° Fahrenheit.

Subsequent to treatment the dishes, along with 10 inoculated
controls were moved into the common storage room which could
be maintained at 38° to 42° Fahrenheit during the test.

The time, in days, required for mold to completely cover
the media in the bottom of the dishes was used as an index of
the effectiveness of treatment. The following results were
obtained: the control dishes developed mold growth in 2 to
4 days with a mean of 2.5 days; 0.3 gram concentration of
growth substance ranged from 12 to 15 days, and mean of 12.4
days; 1 gram concentration ranged from 15 to 21 days having
a mean of 17.7 days. These results indicate that the growth
substance treatments had a pronounced delaying action on
mold growth which is further shown in figure 13. However,
complete mold destruction was not accomplished in any of the
treated dishes. It is possible that the delay in mold growth

*Acknowledgement is made to Dr. J. S. Cooley who kindly
prepared the pure culture of this organism and who was freely
consulted on the pathological phases of the problem.
Figure 13. Suppression of mold growth on rose cane media by growth substance. A. Control. B. Treated with 1.0 gram a-naphthylmethylacetate per 1000 cubic feet. Both lots held in common storage after treatment. Photograph taken at the end of 12 days. It is evident that mold growth was checked by the chemical treatment, however, complete mold destruction was not accomplished.
amounting to 12 days in the 0.5 gram treatment and 17.7 days in the 1 gram treatment may prove helpful in suppressing mold growth on plants in common storage. However, the data in table 5 show that application of both of these concentrations of a-naphthalenemethylacetate to intact rose bushes resulted in moderate to severe cane injury.

The prophylactic effect of the 0.3 gram treatment of this compound as indicated in table 5 and illustrated by the appearance of canes in figure 12 would appear to be largely due to a suppression of shoot growth, which provide media for molds, even though this treatment may also be mildly fungicidal. Further, no visible sign of chemical injury was noted at this relatively low concentration (0.3 gram per 1000 cubic feet) as was found at the higher.

Plants treated with the relatively high concentration of 1.0 gram of a-naphthylmethylacetate also showed heavy callous formation in addition to injury, figure 14 A. Some callous formation would appear desirable since it tended to seal the cut ends of the canes, and prevent the entrance of mold organisms. Too much callous production, however, would tend to deplete stored food reserves similar to depletion caused by shoot growth on the untreated controls.

Callous formation appeared to be about optimum with plants treated at 0.3 gram per 1000 cubic feet. Many of these plants also developed roots on the variety canes figure 14 B. Whether or not this effect would be desirable from a cultural standpoint would depend largely on how well
Figure 14. (A), Excessive callous formation and cane injury on rose stems in common storage, following too concentrated an application of volatile growth substance. 1.0 gram of $a$-naphthylmethylacetate per 1000 cubic feet. (B), Root formation on similar plants treated at .5 gram per 1000 cubic feet.
a particular rose variety would grow on its own roots as compared with roots of *Rosa multiflora* or other rose stocks.
Effect of Temperatures, Length of the Treatment Interval and Concentration of Growth Substance on Dormancy

1940-1941. The temperature within common storage houses fluctuates over a wide range during the storage season. As shown in table 6 this temperature fluctuation may extend from around 32° Fahrenheit up to 50° Fahrenheit in the same season.

When it is possible to maintain the common storage at temperatures of from 32° to 38° Fahrenheit the nurseryman usually encounters little difficulty in holding rose bushes in a dormant condition during the normal storage season. However, beginning about April 1 (table 6), and in some years somewhat earlier than this date, the temperature within the common storage house raises to 40° Fahrenheit and above. Rose bushes will show some vegetative activity after prolonged storage even at 32° Fahrenheit, at 40° Fahrenheit and above shoot production is usually quite rapid.

Treatment with chemicals to prolong the dormant period of rose bushes should be applied when the plants are still dormant. Normally at this time the storage house would be operating at a relatively low temperature. Other work has shown that temperature at the time that growth substances are applied to plants may greatly affect the response obtained from a given concentration.

It would seem necessary, therefore, for effectiveness of treatment to prolong dormancy of rose bushes by chemical treatment, either to remove the bushes from storage to a warm
### TABLE 6.

**Weekly Mean Temperature in Common Storage House From January 6 Until May 5, 1941**

<table>
<thead>
<tr>
<th>Date</th>
<th>Mean Temp. F.</th>
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<td>Apr 14</td>
<td>44.6</td>
</tr>
<tr>
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<td>Apr 21</td>
<td>46.1</td>
</tr>
<tr>
<td>Feb 17 to</td>
<td>35.8</td>
<td>Apr 28</td>
<td>50.9</td>
</tr>
<tr>
<td>Feb 24 to</td>
<td>37.7</td>
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<td>52.5</td>
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<td>Mar 3 to</td>
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<tr>
<td>Mar 10</td>
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</table>
room, or to raise the temperature of the common storage itself. The former would involve the use of considerable extra labor, since many of the storage houses normally store from 1/2 to several million bushes while the latter method would not be desirable because of the length of time that would be required to bring the storage house back to a favorable storage temperature. An experiment with gaseous method of application was set up, therefore, to determine to what extent a lengthening of the treatment interval, or an increase in concentration of growth substance might tend to increase the effects under low temperature.

The temperatures selected for treatment were 32° Fahrenheit and 40° Fahrenheit, to represent temperature conditions that may be encountered under common storage. Similar sets of plants were treated at 70° Fahrenheit to represent treatment at normal room temperature. The 32° Fahrenheit temperature was secured by treating in an artificially cooled room at 32° ± 1° Fahrenheit. The common storage room was used for treating at 40° Fahrenheit, since it was found to be operating at that temperature. A small laboratory room was used in treating plants at 70° Fahrenheit.

Treatment was of 1, 4, and 16 hours duration with a-naphthylmethylacetate at four concentrations, 0.10 gram, 0.30 gram, 0.5 gram and 1.00 gram per 1000 cubic feet for each of the temperatures and treatment intervals used.

In the experimental set-up, 36 lots of Ami Quinard bushes composed of 20 plants per lot were used. Each lot
of 20 plants was selected for uniformity and prior to treatment was placed in a wax-paper-lined half bushel basket with moist peat-sand mixture settled about the roots to prevent drying. Since the same room was used throughout in treating at each of the three temperatures, and an overnight (16 hours) treatment interval was included for each concentration of growth substance tested, it was necessary to have a time lag of 4 days from the time that the lowest concentrations were applied on March 12, 1941, until completion of treatment with the highest on March 17, 1941. The plants were removed from and returned to common storage with only a brief time allowed for plants to reach temperature of the treatment room before treatment.

The temperatures, treatment interval and concentrations of growth substances that were used are shown in table 7, as well as the mean number of shoots produced on plants in the different lots by May 5, 1941.

The plants showed much variability as indicated from the fact that at the 5 per cent level, differences of 7.4 shoots were required for significance, while at the 1 per cent level this was increased to 11.9 shoots for highly significant differences between treatments.

The data shown in table 7 indicate that growth substance treatments will effectively control shoot growth on rose bushes in storage, even though such applications are made at a temperature of 32° Fahrenheit, as both 0.50 gram and 1.00 gram concentration per 1000 cubic feet were effective when
### Table 7.

**Effect of Concentration of Growth Substance, Temperature and Length of the Treatment Interval upon the Number of Shoots Developing on Ami Quinard Rose Bushes During Common Storage. Treated March 12 to March 17, 1941.**

**Mean** Number of Shoots Grown Out by May 5, 1941.

<table>
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<th>Temperature during treatment</th>
<th>Length of treatment in hours</th>
<th>Mean number of shoots per lot</th>
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<td></td>
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<tr>
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<td>16</td>
<td>75.26</td>
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</table>

* 20 plants per treatment
** Concentration of a-naphthylmethylacetate gas per 1000 cu. ft.
*** Severe plant injury

Difference necessary for significance at 5% level 7.47
at 1% level 11.93
applied over a 16 hour period. It should be emphasized however that the higher, 1.00 gram, concentration would not be considered safe to use, since considerable cane injury took place in several lots receiving this amount of the same growth substance (figure 15 B). Thus, treating at 32° Fahrenheit, the 0.3 to 0.5 gram concentration with a 16 hour treatment interval would appear to be preferable since it was equally effective and is not as likely to induce injury.

Bushes that were treated at 40° Fahrenheit were kept completely dormant by the 0.3 gram concentration applied over a 16 hour period (figure 16). Similar plants treated for 4 hours with 0.5 gram of the same growth substance were not significantly different in shoot growth at the 1 per cent level.

No significant benefit in shoot inhibition was found from extending the treatment interval beyond 1 hour when growth substance was applied at 70° Fahrenheit, except possibly in comparing 1 hour with 16 hours at 0.3 gram concentration, table 7.

Applications of 0.10 gram of a-naphthylmethylacetate at each of the three temperatures and for each of the three intervals of treatment used, not only did not inhibit shoot production but appeared to cause a stimulation (figure 15 A) in their development, as comparable control plants included in another experiment produced a mean of 65.8 shoots per plant. A similar effect has been noted by Bennet and Skoog (7) and by Vegis (57). The most pronounced stimulation occurred in plants
Figure 15. A. Ami Quinard plants stimulated in shoot production by 0.10 gram per 1000 cubic feet for 1 hour at 32° Fahrenheit volatilized growth substance treatment and B. Similar plants severely damaged by 1.0 gram per 1000 cubic feet for 16 hours at 40° Fahrenheit volatilized growth substance treatment. Growth substance used in both cases was a-naphthylmethylacetate.
Figure 16. A. Control plants (Ami Quinard).
B. Similar plants treated with 0.3 gram a-naphthylmethylacetate for 16 hours at 40° Fahrenheit. Treated on March 17, 1941, and both lots held in common storage. Photographs taken on May 1, 1941. The growth substance treatment has inhibited bud growth without apparently injuring the plants.
treated with 0.10 gram of growth substance for 1 hour at 32° Fahrenheit. In this lot of plants, almost every bud appeared to break and produce shoots so that by the end of the storage period a mean of 11.5 shoots per plant had developed (figure 15 A).
Effect of Applying Growth Regulating Substance in Partial Vacuum

1940-1941. The purpose of using a partial vacuum was to determine if, by causing better gaseous penetration of the growth substance between the bud scales of dormant buds, the effectiveness of a given concentration might be increased. This would permit the more effective use of a lower concentration of growth substance, with the added advantage of little or no plant injury.

The effectiveness of fumigants such as hydrocyanic acid gas has been found to be increased by using a reduced pressure in treating. Particular benefit was found in the control of certain tunneling and boring insects which may not be affected by surface applications.

The tank used for treating rose bushes with gaseous growth substances at reduced pressure is illustrated and described in figure 1. A few changes were made that are not shown in the illustration. Instead of using a water seal to prevent gas from escaping at the lid, it was necessary to fill the water seal trough with melted paraffin to seal the lid effectively. To remove the lid, the paraffin was melted by heating the trough with a blow torch. Two additional connections were made in the upper outlet through which the thermograph bulb was introduced. One of these connections was made with a vacuum gauge, the other to a small vacuum pump run by an electric motor. A partial vacuum of 0.7
atmospheres was used during the treatment interval, which appeared to be near the maximum reduction that the apparatus would maintain.

Two lots of 20 plants each of Ami quinard rose bushes were treated with a-naphthylmethylacetate gas at the rates of 0.1 and 0.2 gram per 1000 cubic feet for 17 hours at 70°F Fahrenheit. The lower concentration was applied on March 11, the higher on March 12.

As shown in table 8 treatment in partial vacuum did not increase significantly the effectiveness of low concentration of substance. There was a slight reduction in actual number of shoots in both lots treated in partial vacuum, which may indicate a tendency for slight beneficial effect. As shown elsewhere in this work, growth substance gas appeared to condense on surfaces nearest the gas source. Considering the very minute quantities used, it is possible that this may have occurred and offset any advantage obtained from reducing the atmospheric pressure.
<table>
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<tr>
<th>Concentration of a-naphthylmethylacetate - grams per 1000 cu. ft.</th>
<th>Treated 17 hours at Normal Atmosphere Pressure</th>
<th>Treated 17 hours in Partial Vacuum</th>
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<tr>
<td>.100</td>
<td>Mean 90.7 ± 17.87</td>
<td>Mean 82.4 ± 19.42</td>
</tr>
<tr>
<td>.200</td>
<td>Mean 25.6 ± 5.86</td>
<td>Mean 20.3 ± 6.98</td>
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Differential Behavior of Rose Varieties in Storage and the Effect of Growth Substance on Bud Inhibition of 15 Varieties.

1940-1941. Not unlike other horticultural material, the rose plant exhibits considerable irregularity from one variety to another in the readiness with which the buds break and grow out under the same common air storage condition. A number of varieties of hybrid tea and several of the polyantha types are particularly troublesome each year when held in common storage. In years that fall temperatures are high over extended periods, making it difficult to maintain low temperatures in the storage, buds on these varieties start active vegetation growth in December. The practice among commercial men is to remove the plants from the storage house and prune off the new vegetative shoots and then return the plants to storage. Usually the apical buds on individual canes are first to break and are removed by pruning back the canes 2 to 6 inches. In years of unfavorably high storage temperatures this process may be repeated 2 to 4 times during the storage period, so that by the time spring shipments are to be made in March - April, the plants have had a high percentage of their tops pruned off.

Even greater plant loss would accrue if the storage operator did not adhere to some such procedure. The tender succulent shoots provide an excellent medium for the growth of molds, the spores of which seem to be ever present in
spite of sanitary measures, and fungicidal sprays that can be applied, although such measures are of considerable preventive aid. Once the mold organisms have gotten started, their development may be very rapid and after destruction of the new shoots has taken place, such organisms will work back into the older wood until the greater part or entire top of the plant has been destroyed.

Table 9 shows the relative dormant condition of varieties of roses found in a large commercial common storage house during the 1932*, 1940 and 1941 storage season. Although the data are based on relative scoring which may vary from year to year, depending to a large extent on how near the records were made to the date that the storage operator had last worked over and removed vegetative shoots and mold from the plants examined, as well as climatic seasonal variations, it is felt that they are of sufficient value to present here.

The scoring was done by two workers each year and in setting up the score sheet the procedure was first to examine the plants in the entire storage room as they appeared in the bins. This preliminary examination was continued until the scorers were in close agreement with each other on the scoring of sample lots. The systematic rating of each variety

*Acknowledgement is made to Dr. F. E. Gardner who obtained the records in 1932 and which are presented with his permission.
### Relative Tendency of Rose Varieties to Produce Shoots During Common Storage

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<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2027</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2028</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2029</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2030</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2031</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2032</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2033</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2034</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2035</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2036</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2037</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2038</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2039</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
<tr>
<td>2040</td>
<td>- 2</td>
<td>Gisela</td>
<td>- 2</td>
<td>Charles</td>
<td>- 1</td>
<td>Caprice</td>
<td>- 1</td>
</tr>
</tbody>
</table>

0 = No buds starting growth.
1 = Few buds starting growth.
2 = Moderate number of buds starting.
3 = Very pronounced shoot growth.
In the house was then made. In cases where the two scorers did not agree in their rating of a particular lot, the lot was reexamined. A few smaller lots were made up of approximately 500 plants, but for the most part each variety scored consisted of from 1000 to several thousand plants each.

In order to determine whether or not varieties would differ widely in their response to chemical treatment to prolong the dormant period, fifteen varieties were selected for such a test. Included were those that start vegetative growth quickly as well as those that are more slow in breaking bud when held in common air storage. The varieties selected were given a gas treatment at room temperature (70° to 72°) for 17 hours with a-naphthylmethylacetate in three concentrations viz. 0.1 gram, 0.3 gram and 0.5 gram per 1000 cubic feet. The plants were selected for uniformity in size of canes and pruned to 4 to 6 canes of approximately equal length on each plant. 20 plants were used in each concentration of growth substance tested and 20 plants were included as untreated controls so that a total of 80 plants of each variety were used. The treatments were applied on March 30 and the plants held for 60 days in a common air storage. At the end of this time the control plants in all varieties and certain of the treated lots were showing considerable shoot growth. After recording the number and length of etiolated shoots per plant which had grown out in storage the shoots were removed and the plants transferred to a 65° to 70° Fahrenheit greenhouse for forcing. Although
the plants in some variety lots had started active growth sooner than others at the end of 9 days in the greenhouse. All started and by the 14th day when growth was well advanced, a record was made of the number and length of new shoots that had been produced.

A summary of the results obtained are shown in table 10, although data on the length of shoots are not included in the table in all cases these data were of the same relative magnitude as the bud count data.

It is apparent that the varieties did not respond alike in all cases to a particular treatment given to prolong the dormant period while in storage. The varieties Chatillon, Topaz and Poulsen's yellow were the most difficult one to keep in a dormant condition by treatment. They are also varieties that tend to start growth sooner under commercial common storage conditions.

Treatment with 0.1 gram a-naphthylmethylacetate tended to cause more buds to break than on the untreated control plants in all varieties but one of the fifteen under test. Statistical analysis of the data shows that in the case of 6 varieties this increase in bud break was significant (5 per cent level) and with 4 varieties this effect was highly significant (1 per cent level). It would appear from these results that low concentration of growth regulating substance when applied to rose plants tend to break dormancy or stimulate more shoots to elongate in common air storage than on similar plants not receiving such treatment. This
TABLE 10.

SHOOT DEVELOPMENT IN COMMON STORAGE OF 12 VARIETIES OF ROSE BUSHES 60 DAYS
AFTER TREATMENT WITH GROWTH SUBSTANCE, ALSO SUBSEQUENT SHOOT DEVELOPMENT ON
THE SAME PLANTS AFTER 14 DAYS IN A 60°-65°F. GREENHOUSE.
Treated Jan. 30, 1941. Record April 1, 1941.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mean* number of shoots grown out per plant in common storage</th>
<th>Mean number of shoots per plant on the same plants after 14 days in greenhouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control plants Concentration of a-naphthyl methyl acetate per 1000 cu. ft.</td>
<td>Control plants Concentration of a-naphthyl methyl acetate per 1000 cu. ft.</td>
</tr>
<tr>
<td></td>
<td>.1 gram</td>
<td>.2 gram</td>
</tr>
<tr>
<td>Amed Quinnard</td>
<td>15.65</td>
<td>20.10</td>
</tr>
<tr>
<td>Chatillon</td>
<td>60.45</td>
<td>98.06</td>
</tr>
<tr>
<td>Duquesa de Penaranda</td>
<td>7.20</td>
<td>4.60</td>
</tr>
<tr>
<td>Edith McFarland</td>
<td>19.90</td>
<td>40.95</td>
</tr>
<tr>
<td>Edith Nellie Perkins</td>
<td>5.50</td>
<td>15.15</td>
</tr>
<tr>
<td>Etrole de Holland</td>
<td>12.06</td>
<td>38.90</td>
</tr>
<tr>
<td>Eirota</td>
<td>5.80</td>
<td>11.90</td>
</tr>
<tr>
<td>Golden Dawn</td>
<td>20.05</td>
<td>26.70</td>
</tr>
<tr>
<td>Guinée</td>
<td>1.30</td>
<td>4.05</td>
</tr>
<tr>
<td>Margaret McGredy</td>
<td>14.20</td>
<td>11.60</td>
</tr>
<tr>
<td>Poulsen’s Yellow</td>
<td>26.45</td>
<td>12.05</td>
</tr>
<tr>
<td>Radiance</td>
<td>6.75</td>
<td>12.00</td>
</tr>
<tr>
<td>Radio</td>
<td>9.50</td>
<td>14.85</td>
</tr>
<tr>
<td>Ramon Bach</td>
<td>14.45</td>
<td>30.15</td>
</tr>
<tr>
<td>Tonaz</td>
<td>20.15</td>
<td>25.00</td>
</tr>
</tbody>
</table>

* Twenty plants of each variety per treatment
** Moderate injury from treatment

Difference necessary for significance: 5% level 6.96 1% level 14.01 10% level 10.75
This effect was also obtained with several other chemicals applied to the Ami Quinard variety of rose in low concentration. The responses obtained with four varieties are illustrated in figures 17, 18, 19 and 20.

Comparison of the 0.3 gram and 0.5 gram of a-naphthyl-methylacetate per 1000 cubic feet treatments shows that the former concentration is to be preferred, not only was it effective in prolonging the dormancy but further it did not cause the noticeable cane injury found on 6 varieties treated with the stronger concentration (table 10).

Under greenhouse forcing conditions hybrid tea rose plants are grown largely for cut flower production. Stem length and flower size are of prime importance. For this reason, 2 to 3 vigorous shoots per cane are more desirable than a larger number of weaker growing shoots. It would appear then that since all the hybrid tea roses in this variety response study were pruned to 4 to 6 canes, if the plants produced a total of 8 to 12 shoots each, this number would be considered sufficient for flower production. The variety Chatillon, a polyantha type would more likely be grown as a potted plant and the consideration here would be for the production of plants with many shoots to produce an abundance of flowers for a massed color effect.

The data in table 10 show that upon removal to the greenhouse the control plants were markedly inferior in shoot production to either the 0.3 gram or 0.5 gram of naphthyl-methylacetate treated lots. In all 15 varieties this
Figure 17. Edith Bellie Perkins variety. A. Control plants. B. Plants treated with growth substance. Treated on January 30, 1941. Both lots photographed 90 days after treatment, plants held in common storage. Treatment was with 0.3 gram a-naphthylmethylacetate vapor per 1000 cubic feet for 16 hours at 70°F Fahrenheit.
Figure 18. Red Radiance variety. A. Control plants. B. Growth substance treated January 30, 1941. Photographed, April 1, 1941. taken after both lots were in common storage for 90 days after treatment. Treatment consisted of 0.3 gram α-naphthylmethylacetate vapor per 1000 cubic feet for 16 hours at 70°F Fahrenheit.
Figure 19. Ramon Bach variety. A. Control plants. B. Growth substance treated lot. Treated on January 30, 1941. Both lots photographed on April 1, 1941, 90 days after treatment, plants held in common storage. Treatment was with 0.3 gram of a-naphthylmethylacetate vapor per 1000 cubic feet for 16 hours at 70° Fahrenheit.
Figure 20. Chatillon variety. A. Control plants. B. Growth substance treated January 30, 1941. Photographed, April 1, 1941, taken after both lots were in common storage for 90 days after treatment. Treatment consisted of 0.5 gram a-naphthylmethylacetate vapor per 1000 cubic feet for 16 hours at 70°F Fahrenheit.
The difference between controls and treated plants is highly significant. Treatment with 0.1 gram of this chemical resulted in no significant difference in shoot production.
Comparative Behavior of Mature and Immature Plants Treated With Growth Substance

1940-1941. Even under the most optimum conditions for growing rose bushes in the field, there appear to be always a few at least that are due immature along with the bulk of the crop. These bushes constitute material more readily infected by molds when the plants are stored, and it was felt desirable to know whether or not treatment with growth substances may be of aid in handling such plants, either by conservation of the limited stored materials present in the plants, or by preventing shoot growth that becomes favorable media for mold production in storage.

Two lots of plants were selected, one lot having very mature canes and showing a relative starch rating (figure 2) of 82.3 ± 4.83 in sections taken from the stock portion of the plant. The other lot consisted of plants of about the same size but which were immature in appearance and were found upon sectioning to be 22.6 ± 9.45 in relative starch content. Each lot was divided so that 20 plants received a gaseous treatment of 0.3 gram of a naphthylmethylacetate per 1000 cubic feet for 16 hours at 70°F Fahrenheit, and 20 plants were left as untreated controls. The resulting four groups were then placed in common storage. Records were taken on an individual plant basis of the accumulative number of shoots produced at intervals throughout the storage period.
as well as the percentage cane loss per plant based on the total cane length and the amount destroyed by molds or other causes.

The results are presented in table 11. It is apparent that the growth substance treatment to plants of high starch content (mature plants) resulted in a very marked reduction in shoot growth and in cane loss throughout the storage period in comparison with high starch untreated controls. It should be noted here, that although broken and weak canes were removed from all plants in the test, the remainder of the canes were not cut back. Normally, some topping is given to the bushes before storage. The presence of these slightly immature tops may have tended to accentuate the differences somewhat, since the figures for percentage dead canes at the April 16 and May 2 dates appear somewhat high (35.2 per cent and 39.7 per cent respectively) in the control lot.

Comparison of the growth substance treated and control plants of low starch content shows that in the early stages of the storage period the treatment was effective in controlling shoot growth and reducing cane loss. However, the effective period was at least a month shorter in duration than with plants of high starch content. Not only did the treated low starch plants begin to break buds by March 17, two weeks before shoots appeared on treated high starch plants, but they also appeared to be in a very rapid state of decline as evidenced by a significantly greater cane loss than the comparable control plants after that date. By
TABLE 11.

SHOOT GROWTH AND CANE LOSS ON AMI QUINARD ROSE BUSHES OF HIGH AND LOW STARCH CONTENT (MATURE AND IMMATURE) HELD IN COMMON STORAGE WITH AND WITHOUT TREATMENT TO PROLONG DORMANCY.

Treated on January 6, 1941

<table>
<thead>
<tr>
<th></th>
<th>Plants with high starch content</th>
<th>Plants with low starch content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated*</td>
</tr>
<tr>
<td>Mean no. of shoots</td>
<td>% Dead canes</td>
<td>Mean no. of shoots</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jan. 15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feb. 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feb. 16</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>March 3</td>
<td>2.5</td>
<td>4.5</td>
</tr>
<tr>
<td>March 17</td>
<td>6.4</td>
<td>18.8</td>
</tr>
<tr>
<td>April 1</td>
<td>12.3</td>
<td>28.6</td>
</tr>
<tr>
<td>April 16</td>
<td>18.4</td>
<td>35.2</td>
</tr>
<tr>
<td>May 2</td>
<td>26.5</td>
<td>39.7</td>
</tr>
</tbody>
</table>

*Treatment = 0.3 gm. a-naphthyl methyl acetate per 1000 cubic feet, for 16 hours at 70° Fahrenheit.
May 2 these plants had lost almost 3/4 of their total cane length. It would appear from these results that under common storage conditions it would be hazardous to hold low starch plants over a very long period, even though treatment with growth regulating substance may reduce shoot growth and the plants may appear to be in excellent condition early in the storage period.

The data on "number of shoots" for the two control lots show that the low starch controls produced a greater number of shoots than the high starch controls, especially in the last three record periods. In this connection, it should be mentioned that the two lots were more nearly alike when compared on the basis of "total length of shoots produced." The high starch controls appeared to possess stronger apical dominance. These plants produced a few shoots which were very strong growers, whereas in the low starch controls the plants produced a large number of weak spindly shoots distributed farther downward from the apex of the canes.

The plants were set in the field on May 2 for further observation. Notes taken on June 7 were as follows for each lot: high starch, control, 17 living, all weak; high starch, treated, 20 living, 15 vigorous, 5 weak; low starch, control, 5 living, all very weak; low starch, treated, 2 living, both very weak.
The Rate of Starch Disappearance in Plants Held in Common and in Cold Storage, 32° Fahrenheit as Affected by Treatment with Growth Substance

1940-1941. The data shown in table 12 are concerned with the relative starch content of the stock portion of growth substance treated and untreated (control) Ami Quinard rose bushes held in common and in cold storage. Samplings for the IKI test were made at the beginning of the test and periodically at 2 week intervals during storage until the plants were field planted on May 5, 1941. Growth substance treatment consisted of a gaseous application of a-naphthylmethylacetate at the rate of 0.5 gram per 1000 cubic feet for 16 hours at 70° Fahrenheit.

The treatments were applied on March 10, 1941 and the first sampling for starch was made on that date. This sample of 20 plants was selected at random from the entire lot of plants used, and would be considered as representative of their relative starch content at that time. Comparison of the relative starch present in the wood portion of the stock at this date, March 10, with the readings obtained when the plants were received on January 6, showed that little change in relative starch had taken place during this pretreatment interval, the former being 82.3, the latter 80.5.

As already indicated by Yerkes, Scott and Swingle (65) the starch content of rose canes is so variable between canes
### Table 12

**Effect of Growth Substance on the Relative Starch Content of Stocks of Ami Quinard Rose Bushes Held in Common and in Cold (32°F) Storage, as Shown by the I-K-I Test**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Relative starch content** of rose stocks at 2-week intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3/10/41</td>
</tr>
<tr>
<td></td>
<td>&lt;br&gt;Bark</td>
</tr>
<tr>
<td>Control plants. Common storage</td>
<td>25.0</td>
</tr>
<tr>
<td>Treated with growth substance. Common storage</td>
<td>&lt;br&gt;- &lt;br&gt;- &lt;br&gt;-</td>
</tr>
<tr>
<td>Control plants held in 32°F. storage</td>
<td>&lt;br&gt;- &lt;br&gt;- &lt;br&gt;-</td>
</tr>
<tr>
<td>Treated with growth substance. 32°F. storage</td>
<td>&lt;br&gt;- &lt;br&gt;- &lt;br&gt;-</td>
</tr>
</tbody>
</table>

* Growth substance = 0.5 gram α-naphthyl methyl acetate per 1000 cu. ft. for 16 hours at 70°F.

** Mean rating of 20 plants sampled.

<table>
<thead>
<tr>
<th>Difference necessary for significance:</th>
<th>5% level</th>
<th>1% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark</td>
<td>5.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Wood</td>
<td>10.1</td>
<td>14.8</td>
</tr>
<tr>
<td>Pith</td>
<td>6.9</td>
<td>9.3</td>
</tr>
</tbody>
</table>
on the same plant that they should not be used for sampling to estimate the starch content of rose plants to determine if the plants are mature enough to dig. The data obtained on starch content of the cane portion of the plants used in this study merely confirms the findings of the earlier workers. The data are so extremely variable that differences in plant behavior between treated and untreated lots could not be estimated and for this reason they are not presented. However, these data did show that growth substance treatment apparently did not prevent starch loss in the canes of rose bushes since by the end of the 14th day following treatment starch could not be found in the bark in any sections of any of the 20 plants included in either control or treated lots held in common or in cold storage. By the end of the 28th day following treatment starch was entirely absent from bark, wood and pith portions of the canes in all lots.

Starch present in the roots also did not appear to be affected by growth substance treatment. There did not appear to be large quantities stored at any time in the roots of the plants that were used and as with the canes the data on starch content of the roots were extremely variable. Root growth which occurred in all lots probably would account for the complete disappearance of starch found in the roots by the end of the 28th day following treatment. Although the data on starch content of the roots are variable, growth substance treatment did not appear to accelerate or retard the disappearance of this starch. The time between sampling
dates, however probably would be too great to measure rapid changes that may have taken place which may have shown an effect of growth substance on starch content of the roots.

As shown in table 12, as well as in figure 21 very rapid exit of starch had taken place from the stocks of the common storage control plants 14 days after the treatments were applied. Apparently, the experiment was started very near the critical period for starch disappearance. Delay in growth substance application at this time would unquestionably fail to show the pronounced effect on starch that was obtained.

A proportionally greater amount of starch was found stored in the wood of the stock throughout the entire storage period, than in either the bark or pith tissues. The small amount present in the bark was first to disappear, and growth substance apparently had slight or no effect on its rate of loss, comparing control and treated lots in common storage, table 12.

The rate of disappearance of starch from wood and pith with plants held in common storage was very markedly reduced by growth substance treatment, which was similar to the reduction in starch loss resulting from storage at 32° Fahrenheit. No visible sign of bud breaking or root growth were evident on even the common storage control plants until after the 14th day of treatment. It would appear likely, therefore, that growth substance had affected those processes which bring about starch hydrolysis or which utilize starch in respiration. The large quantity of shoots that formed on
Figure 21. Rate of starch disappearance in "wood" portion of the stock of Ami Quinard rose bushes held in common and in cold storage following growth substance treatment.
the common storage control plants later would account for the ultimate loss of a large quantity of the stored reserves, whether they had originally been present as starch or in some other form.

Cold storage at 32°Fahrenheit is considered near the ideal temperature for holding rose bushes. Treatment with growth substances showed no significant effect on the starch present in plants held at this temperature, until near the end of the storage period. At the last two sampling dates (table 12) starch was found to be significantly higher in the wood and pith of treated plants than of comparable controls held at 32°Fahrenheit. However, at the last sampling date the cold storage controls were showing visible vegetative signs of growth, even at this low temperature. The growth substance treated lots were completely dormant in both common and cold storage until after field planting.

Judging from these results, if the plants are going to be held in 32°Fahrenheit storage, application of growth substances to rose bushes in order to conserve their starch content, would appear to be of questionable benefit. Subsequent behavior of similar plants that were field planted, however, would seem to justify such treatment, since the treated plants became established in the soil quicker and made better growth. Data to support this conclusion are presented elsewhere with the field planting responses that were obtained.

Probably the most significant effect shown in table 12
and figure 21, at least from a practical standpoint, is the close similarity of the starch content of plants treated with growth substance and held in common storage with control plants held in 32°F Fahrenheit storage. Although this effect was true only for starch stored in the wood portion of the stock, and did not hold to the same degree for starch stored in bark or pith, by far the greatest amount of starch was found in the wood tissues by the methods of starch estimation employed in this study.
Growth Responses Obtained on Field Planted Rose Bushes Following Growth Substance Application and Subsequent Storage in Common and Cold Storage

1940-1941. Included in each of the lots of plants that were used for the relative starch tests previously described, was an additional lot that was treated and stored in identically the same way. After storage these plants were used in a field planting to determine if any effects of the different storage treatments would be reflected in shoot and root growth.

These plants at the start of the storage tests had been somewhat more critically selected. They had about the same volume of top and roots as those used in the starch tests but the roots were more uniformly distributed and each plant was root-pruned to six primary roots per plant. This selection was not difficult since the plants had all been propagated on rooted *multiflora* stem cuttings, which have a characteristic form of root system. With such cuttings, almost the entire root system originates from a narrow region near the base of the original cutting and is fairly well distributed around the base. Further, the root system is composed almost entirely of large coarse roots with very few fibrous roots developing the first year in the nursery. This is in contrast to rose bushes propagated on *multiflora* seedlings which develop a relatively fine, fibrous and much branched root system.
The plants were planted in the field on May 5. The 20 bushes of each of the 4 treatments shown in table 13 were set at a uniform depth with the six primary roots of each plant oriented in the same relative positions. This systematic spacing of the roots greatly aided in relocating them to obtain counts on new root production. The plants were set in a loose sandy loam and kept mulched with strawy manure.

**Root Growth.** The first root counts to determine the number of new roots regenerated were made one week after planting on three of the six primary roots per plant. The next counts, a week later, were made on the remaining three primary roots not disturbed at the first sampling. The plants were then given a two week period for recovery after which (28 days after planted), the primary roots sampled originally were again examined. Two weeks later (42 days after planting), the new roots developed on the primary roots examined at the second sampling period were again counted. Root counts were discontinued at this time since the plants had made considerable top growth in some lots. It was felt that further root counts may cause a differential disturbance that would interfere with data on other plant responses.

It is believed that the system employed in obtaining root counts did not materially interfere with normal plant growth, and to insure quick recovery, the plants were watered immediately after each root sampling and were shaded with a 5/8 bushel basket during the two days that followed. The excellent growth made by plants in certain lots by the end of
MEAN NUMBER OF NEW ROOTS PRODUCED ON FIELD-PLANTED AMI QUIKARD ROSE BUSHES AFTER TREATMENT IN COMMON AND IN COLD STORAGE WITH GROWTH SUBSTANCE

<table>
<thead>
<tr>
<th>Treatment**</th>
<th>No. plants*</th>
<th>Number of days following field planting May 5, 1941</th>
<th>7</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean S.E.M.***</td>
<td>Mean S.E.M.</td>
<td>Mean S.E.M.</td>
<td>Mean S.E.M.</td>
<td>Mean S.E.M.</td>
</tr>
<tr>
<td>Control plants.</td>
<td>13</td>
<td>0.0 ± 0.00</td>
<td>1.7 ± 0.15</td>
<td>58.7 ± 6.98</td>
<td>110.7 ± 16.71</td>
<td></td>
</tr>
<tr>
<td>Common storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth substance treated.</td>
<td>20</td>
<td>68.1 ± 6.76</td>
<td>141.5 ± 7.45</td>
<td>184.6 ± 9.70</td>
<td>405.8 ± 24.27</td>
<td></td>
</tr>
<tr>
<td>Common storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control plants 32°F cold storage</td>
<td>20</td>
<td>6.0 ± 1.35</td>
<td>85.8 ± 8.60</td>
<td>230.5 ± 12.24</td>
<td>484.6 ± 19.40</td>
<td></td>
</tr>
<tr>
<td>Growth substance treated 32°F cold storage</td>
<td>20</td>
<td>110.0 ± 8.52</td>
<td>211.5 ± 14.12</td>
<td>246.5 ± 19.75</td>
<td>516.7 ± 15.70</td>
<td></td>
</tr>
</tbody>
</table>

* Number plants surviving out of 20 planted

** Growth substance = 0.5 gram a-naphthyl acetate per 1000 cu. ft. for 16 hrs.

*** When compared with common storage controls all treatments have highly significant t values.
the growing season is shown in figure 22.

The data on new root development of Ami Quinard rose bushes that had been held in common and in cold storage at 32° Fahrenheit are summarized in table 13. As may be expected the individual roots of a plant, as well as the different plants in a particular lot, showed considerable variability in new root production.

Since but half of the primary roots were examined on each plant at a particular sampling date, the values for root counts obtained were doubled in all cases so as to be more nearly representative of the entire root system.

The very poor production of new roots early in the growing season on the control plants that had been held in common storage (table 13 and figure 23) may possibly account for the loss of 7 of the 20 plants included in this lot. The plants that lived were very much weaker throughout the growing season than those in any other lot. Evidently the stored food reserves had been depleted to such a low level, as indicated by the starch test made just prior to field planting, that insufficient quantity was available to supply demands for both new root and shoot growth. On these plants shoots developed before new roots were initiated as shown by comparing common storage controls of table 13 with table 14. It is possible that roots were delayed in formation until the newly formed leaves could supply the necessary materials for root production.

Growth substance treatment to plants in both common and
Figure 22. Showing relative size of plants and differential rate of defoliation on October 20, 1941, of Ami Quintard rose bushes field planted on May 5, 1941, which had received growth substance treatment. (A), foreground, common storage plus growth substance; (A), background, cold storage plus growth substance; (B), cold storage controls; (C), common storage controls. The two rows of plants to the right of C are composed of plants treated with strong concentrations of 1, 2 and 3 grams of growth substance per 1000 cubic feet and were lined out to determine if any polyploidal effects would show up. (Photograph taken through a red filter to show the red flowers.)
Figure 23. Diagram showing relative rate of new root production on field planted Ami quinard rose bushes held in common and in cold storage with and without treatment with growth substance. Field planted May 5, 1941, 56 days after storage treatments were started.
### Table 14.

**Mean Shoot Growth per Plant in Millimeters of Field-Planted Rose Bushes Held in Common and in Cold Storage**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Number of days following field planting May 5, 1941</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of plants</td>
<td>Mean S.E.M.</td>
<td>Mean S.E.M.</td>
<td>Mean S.E.M.</td>
<td>Mean S.E.M.</td>
<td>Mean S.E.M.</td>
<td>Mean S.E.M.</td>
</tr>
<tr>
<td>Control plants, Common storage</td>
<td>13</td>
<td>8.00 ± 1.86</td>
<td>16.10 ± 7.34</td>
<td>47.00 ± 10.21</td>
<td>140.75 ± 5.54</td>
<td>203.75 ± 11.25</td>
<td>247.00 ± 9.60</td>
</tr>
<tr>
<td>Treated with growth substance, Common storage</td>
<td>20</td>
<td>9.20 ± 1.41</td>
<td>69.50 ± 3.54</td>
<td>244.50 ± 12.21</td>
<td>673.00 ± 9.45</td>
<td>1002.00 ± 16.50</td>
<td>1181.75 ± 15.56</td>
</tr>
<tr>
<td>Control plants Cold storage</td>
<td>20</td>
<td>48.50 ± 2.85</td>
<td>85.75 ± 6.40</td>
<td>261.25 ± 13.56</td>
<td>518.00 ± 9.64</td>
<td>578.40 ± 11.45</td>
<td>600.25 ± 12.21</td>
</tr>
<tr>
<td>Treated with growth substance, Cold storage</td>
<td>20</td>
<td>6.00 ± 1.73</td>
<td>58.75 ± 5.42</td>
<td>285.50 ± 9.47</td>
<td>785.50 ± 8.74</td>
<td>1169.00 ± 9.95</td>
<td>1195.10 ± 14.12</td>
</tr>
</tbody>
</table>

* Growth substance = 0.5 gram a-naphthyl methyl acetate per 1000 cu. ft. for 16 hours at 70° F.
cold storage was followed by significantly more root develop­
ment during the first week in the field than on similar un­
treated plants, table 13. This effect was greatest with
plants held in cold storage. Cold storage treated plants had
developed 110 roots per plant 7 days after planting as com­
pared with 68 per plant following treatment in common storage.

As shown previously (table 12), the control plants held
at 32° Fahrenheit had approximately the same relative starch
content as the treated common storage plants (39.5 and 43.7
respectively), at the time of field planting. Theoretically
they should then be able to produce about the same amount of
growth from their stored reserves. At the end of the first
week in the field the cold storage controls had developed an
average of 6 roots per plant, whereas the treated lots from
common storage averaged 68 roots per plant, table 13. On the
other hand, the cold storage controls broke buds very rapidly
after field planting and at the end of the first week had
produced an average of 48.5 millimeters of new shoots as
compared with only 9.2 millimeters of shoots in the treated
lots from common storage, table 14. Apparently, in the one
case, stored reserves were being used up in top growth while
in the other, new roots were becoming established.

One explanation of the stimulative action of growth sub­
stances on root production would appear to be a direct effect
on the roots themselves as has been reported for young pecan
trees by Romberg and Smith (43). Data obtained in these
experiments would indicate that the effect may be an indirect
one. It would seem justifiable to conclude that the main effect of growth substance treatment under the condition of this experiment resulted from inhibition of shoot growth for a short interval after field planting rather than to a direct stimulating action on the roots themselves.

Shoot Growth. A summary of the data obtained on shoot growth is shown in table 14. Records were taken at weekly intervals beginning May 12, 7 days after planting, until June 16, 42 days after the plants were set in the field. Blossom production had taken place on many shoots by this date, causing a temporary stoppage of growth in them.

The common storage control plants made much poorer top growth than any other treatment throughout the growing season. Controls from cold storage made an initial flush of growth that was greater than in any other treatment. However, growth substance treated lots from both common and cold storage caught up with them by the end of 21 days and at the end of 42 days had produced almost twice as much top growth (table 14 and figure 24). The common and cold storage treated lots were very similar in amount of growth at this time, with 1181 millimeters per plant in the former and 1195 millimeters in the latter lots. Controls held in cold storage had grown but 600 millimeters by this date.

From these results it would appear that application of growth substance to dormant rose bushes may be followed by better growth of such plants after planting in the field. The results obtained indicate that the response is not caused
Figure 24. Diagram showing relative rate of new top growth on field planted Ami Quinard rose bushes held in common and in cold storage with and without treatment with growth substance. Field planted May 5, 1941. 56 days after storage treatments were started.
by a direct beneficial action on growing shoots.

**Blossom Production.** The ultimate aim in growing rose bushes is for flower production. In table 15 weekly records of blossom production taken from June 10 until October 24 are summarized by months on the same plants used in the root and shoot growth studies.

As a result of poor root and shoot growth, flower production on the common storage control plants was low, and the flowers had very short stems. Since the cold storage control plants started growth earlier than other treatments it would seem likely that these plants would blossom sooner. Such was not the case. Records during the month of June show that growth substance treated lots, both in common and cold storage had produced more blossoms early in the season. The flowers also had longer stems particularly those from plants treated with growth substance and held in cold storage.

The bushes appeared to have two fairly definite peaks of flower production. The first occurred in June and early July, the second in September and early October. Very few flowers were produced during the month of August, table 15. Seasonal conditions may account to some extent, at least, for this periodic plant response. The plants appeared to be semi-dormant during this mid-summer rest interval, after which they made a second flush of growth on which the second fall flower crop was produced.

Both control lots, common and cold storage, ceased flower production earlier and went into their second (fall) dormancy
<table>
<thead>
<tr>
<th>Treatment**</th>
<th>No. plants</th>
<th>June 18 to 70</th>
<th>July 1 to 71</th>
<th>August 1 to 71</th>
<th>September 1 to 70</th>
<th>October 1 to 24</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control plants, Common storage</td>
<td>17</td>
<td>0.21 ± 0.12</td>
<td>2.00 ± 1.01</td>
<td>2.00 ± 0.00</td>
<td>4.30 ± 0.51</td>
<td>.77 ± 0.15</td>
<td>9.30 ± 2.71</td>
</tr>
<tr>
<td>Growth substance treated, Common storage</td>
<td>20</td>
<td>1.90 ± 1.01</td>
<td>4.70 ± 1.42</td>
<td>1.10 ± 0.41</td>
<td>8.30 ± 2.90</td>
<td>1.25 ± 1.75</td>
<td>21.75 ± 6.45</td>
</tr>
<tr>
<td>Control plants, 70°F, cold storage</td>
<td>20</td>
<td>1.75 ± 0.16</td>
<td>5.50 ± 1.78</td>
<td>6.95 ± 1.25</td>
<td>1.50 ± 0.52</td>
<td>15.40 ± 4.40</td>
<td>15.40 ± 4.40</td>
</tr>
<tr>
<td>Growth substance treated, 70°F, cold storage</td>
<td>20</td>
<td>4.25 ± 1.98</td>
<td>8.30 ± 2.41</td>
<td>1.50 ± 0.51</td>
<td>13.40 ± 6.25</td>
<td>7.50 ± 1.21</td>
<td>21.00 ± 9.45</td>
</tr>
</tbody>
</table>

* Number plants surviving out of 20 planted

** Growth substance = 0.5 gram a-naphthyl methyl acetate per 1000 cu. ft. for 16 hours at 10°F.
sooner than either of the growth substance treated lots. This effect is shown to some extent by the lessened flower production of the controls during October, table 15, as well as the striking differences in amount of foliage shown in figure 22. It is felt that this plant response was due to a difference in vigor of plants at this time, comparing control and treated lots, rather than to any direct effect of growth substance in this connection.

The mean number of flowers produced during the season by plants in the different lots are also given in table 15. It is obvious that cold storage alone was not sufficient to obtain optimum flower production. Plants that had received a combination of cold storage with growth substance treatment produced twice as many flowers per plant during the growing season as those receiving cold storage alone; the former produced 31 flowers per plant, the latter 15. Intermediate between these two treatments were the plants held in common storage with growth substance treatment, these averaged 21 flowers per plant. The common storage control plants that survived produced but 9 flowers per plant, many of which were small and with short stems.

Under the conditions of this experiment, growth substance treatment at the beginning of the common and cold storage period has improved the flower producing capacity of rose bushes. Although treatment was very beneficial to plants when held in cold storage, even greater benefit was found from treatment with plants held in common storage, compared with the respective control plants.
Possible Effects of Growth Substance on Polyploidy in Rose

1941-1942. It has been reported that growth substances have caused the induction of polyploidy in a number of plants somewhat similar to treatment with colchicine.

A very small quantity of volatilized growth substances is apparently necessary to induce bud inhibition of dormant rose bushes, as shown in the experiments that were previously mentioned. The chance of applying over-dosages of growth substances would seem very likely in using such compounds on a commercial scale. The very severe injury to plants that receive over-dosages has also been described. However, as shown in table 5 complete killing was not found in plants treated with as much as 3 grams per 1000 cubic feet for 16 hours at 70°F Fahrenheit with a-naphthalenemethylacetate. Although these plants had 90.6 per cent of their tops killed back, there were a few apparently uninjured buds at the base of the canes at the end of the storage period. If polyploidy would be a factor in treating rose bushes to keep them dormant, since it may alter the varietal characteristics of the plant if it occurred, then it would appear likely to show up to a greater extent in such over-dosed material. It would appear also that plants which were beginning active growth when the growth substance treatment was applied would also most likely be affected, since it has been reported that colchicine solutions are most effective when plants are in
early stages of growth. An experiment was undertaken therefore to determine if polyploidy may not be effected by strong growth substance treatments on rose bushes.

Twenty plants of each of three varieties of rose bushes, Editor McFarland, Ramon Bach and Radio which were beginning shoot growth from apical buds in common storage were potted up in soil and moved into the greenhouse on April 28, 1941. The canes were then pruned back removing all shoot growth that had developed in storage. After 6 days in the greenhouse, active bud growth was visible in a number of buds on each plant. The 20 plants of each variety were then separated into four lots of 5 plants each, and a-naphthylmethylacetate at the rate of 1 gram, 2 grams and 3 grams per 1000 cubic feet was applied for 16 hours at 70° Fahrenheit to three separate lots of each variety, leaving one variety lot untreated for controls. After treatment with the gaseous growth substance the plants were returned to the greenhouse.

One week later the control plants had started active vegetative growth and apparently the plants in the treated lots were also about to do likewise. At this time approximately 10 buds were removed from the base of each plant of the different treatments as well as the controls. Upon removal from the plants the buds were immediately killed and fixed, then sectioned in paraffin by the t-butyl alcohol method. After staining with crystal violet and mounting in balsam the sections were examined under the oil emersion lens of a binocular microscope.
Detailed examination of 10 to 15 slides containing a number of successive sections through the growing points of approximately 50 buds from each of the control plants and 50 each from the growth substance treated lots yielded the following information: all of the control buds showed numerous active and apparently normal nuclear divisions; buds having received the 1 gram treatment contained noticeably fewer divisions than the controls, but the divisions appeared to differ in no way from those in the control buds, either in the apparent number or behavior of chromosomes that were present. The buds from plants treated with 2 grams of growth substance per 1000 cubic feet did not show any active nuclear division. However, in 5 of the 50 buds of the Ramon Bach variety that were sectioned in this treatment a number of abnormally large nucleated cells were found. Apparently the samples were taken either too early or too late to determine the chromosomal compliment of these abnormal cells.

Two weeks after treatment all of the plants in the 3 grams treatment were completely dead. This may account for the fact that no cellular divisions or abnormal nuclei were found in buds of this material that were taken for sectioning.

At the suggestion of Dr. Haig Derment, all of the rose bushes that had received strong (1 gram or more per 1000 cubic feet of volatilized compound) dosages of growth substances in any of the storage experiments previously described were field planted. These plants were lined out in two rows adjacent to the rose planting used in obtaining the growth
response data already mentioned. As shown in figure 22 many of these plants had died by October 20, 1941. However, those that lived throughout the growing season were carefully observed for any abnormalities in plant character or flower structure. As a result of these observations it was found that the few flowers that were produced were normal and the plants themselves did not appear to be at variance with comparable weak growing control plants that had been used in the common storage experiments.

When the plants were dug in late November, 1941, it was noted that many large buds had apparently remained dormant at the base of the larger canes throughout the growing season. These buds were present on the plants when they had been treated with growth substance during their previous storage period. Therefore, it seemed advisable to attempt to force them to grow out. After potting the plants in soil on November they were moved into a 32° to 45° Fahrenheit greenhouse to complete their dormancy. Following this period, they were forced in a warm greenhouse, along with similar control plants.

Figure 25 shows one of the flowers that was produced that was considerably different from the varietal form. The larger flower that is shown as well as another not quite so large were found on the same plant. Both of these large flowers had 150 petals each, whereas petal counts on 25 flowers from control plants ranged in number from 65 to 80 petals each. Another plant of the same variety also produced
Figure 25. Left, flower of Ramon Bach variety of rose, apparently "doubled" in number of petals (count of 150) following treatment with 1 gram of a-naphthylmethylacetate as gas per 1000 cubic feet. Right, flower from control plant of the same variety with 70 petals.
a flower with apparently many more petals than those on the control plants or other growth substance treated plants.

The appearance of these "doubled" flowers strongly suggests that the relatively high concentrations of growth substance that were used caused polyploidy in the buds from which these flowers developed. It is recognized however, that 'doubling' in the case of the rose flower obtained here is no sure indication of polyploidy but may be merely due to the presence of normal petal primordia which require some extra stimulus (such as treatment with growth substance) to cause these petal primordia to develop. The individual petals on all the flowers produced on plants treated with growth substance while dormant in common storage were normal in appearance. Apparently no formative effects had taken place, as noted by Zimmerman and Hitchcock (67) when they applied 3-naphthoxyacetic acid to a number of plants other than rose.

Two of the flowering shoots that produced "doubled" flowers have a number of vegetative buds. These shoots are to be forced out, if possible, to furnish material for propagation as well as for cytological studies.

The results obtained in the present experiments show that changes in the varietal characteristics of rose plants following treatment with growth substance to inhibit shoot formation in storage are not likely to occur unless very strong concentrations of the active compounds are used. From a practical standpoint such strong treatment should be avoided because of the severe plant injury that accompanies
their use, rendering the plants non-saleable. However, the possibility of creating new plant forms from existing varietal forms is suggested.
The evidence obtained in these experiments on roses strongly support Went’s (58) finding that bud inhibition is greatest when a continuous supply of growth substance is available. The increased effectiveness of growth substances when applied in wax-emulsion as compared with comparable concentration applied in aqueous solutions was apparently because the growth substance was condensed and held on the plant surface over a period of time. If penetration by strong concentration of growth substance in a short time were a primary factor in bud inhibition, it would seem that the aqueous solutions as well as sprays containing a penetrating agent, such as ethyl alcohol, would have had an advantage since the plants would be able to take up growth substance from such sprays quicker than from the wax-emulsion sprays. This was not the case, nor did increasing the penetration of the volatile growth substance, a-naphthalenemethylacetate, by applying vapor treatment to plants in a partial vacuum show any increased effectiveness on bud inhibition over treatment under atmospheric pressure with the same rate of application.

Zimmerman and Hitchcock (66) have suggested that the increased effectiveness of vapor treatments over solution treatments with the same growth substance, as also has been indicated in the present experiments, was due primarily to
increased penetration by the vapors. They believed vapor penetration took place in a manner similar to that of ethylene gas which they had used in some of their earlier experiments. It appears logical to arrive at this conclusion, especially since they obtained relatively faster and more pronounced effects from the vapors under the greenhouse conditions and with the vegetative plants that they used. However, in the case of dormant rose plants used in the present experiments, held in common storage and at temperatures of from 32° to 42° Fahrenheit, the increased effectiveness of vapor treatments appeared to arise largely from the fact that the vapor condensed in tiny droplets of the chemical onto the plant surfaces. Slow volatilization of growth substance from these tiny droplets under the low temperature conditions employed appeared to be a primary factor in the bud inhibiting effects obtained. It is recognized that penetration would necessarily be a factor in bud inhibition, but in the present experiments it would appear that a slow and continuous penetration was most effective. Strong evidence in support of this was found with rose plants that were kept in common storage in sealed 20 gallon containers, together with one drop of a-naphthyl-methylacetate (approximately 35 milligrams) soaked into paper toweling and hung one foot above the plants so that it did not come into direct contact with them. Sufficient growth substance emanated from the paper toweling to cause complete bud inhibition over a period of 60 days. Figure 3 shows a
control lot and a lot treated with growth substance in this manner. Apparently, the inhibiting effect could be removed at will by removing the plants from containers into the greenhouse where shoot development took place within a 14 day period.

Injury usually followed when strong concentrations of growth substances were applied to dormant rose plants. The injury did not appear to be manifest primarily in injury to the buds, but showed up as a result of damage to the bark and cambium of the internodal regions on the canes. Evidence of this was found upon removal of the injured plants to the greenhouse, where many of the buds on such plants started growth, but subsequently died because of a girdling effect of the injured tissues above and below the vegetative buds. This is in contrast with the direct bud injury obtained with the peach and cherry material included in this study, as well as that obtained by Mitchell and Cullinan (36) with peach and pear. The bud scales on most rose varieties seem to fit tightly together and are quite waxy when the plants are dormant. Entrance of growth substance as vapors into the plant would therefore seem most likely to occur through the lenticels of the bark rather than directly into the buds. The first noticeable sign of cane injury appeared as small sunken black "pits" in the bark. These "pits" seemed to be centered around the lenticel openings. As injury progressed the "pits" expanded in circumference until they merged, forming a continuous sunken black area on the canes.
Indoleacetic acid, reported by Went (58) and Skoog and Thimann (46) as effective in bud inhibition on herbaceous plants was relatively ineffective with the woody rose plants used in the present experiments. Went however, has shown that with this compound the concentration necessary to cause bud inhibition was very close to the concentration which induced injury. It is possible that inhibition, without injury, may have been obtained on rose plants employed in the present experiments if a concentration of indoleacetic acid had been used that was intermediate between .05 per cent and .01 per cent.

A-naphthylmethylacetate when applied as a vapor was found to have an inhibiting effect on the development of mold (Botrytis sp.) on rose bushes held in common storage. The experiments with the pure cultures of the mold organism suggested that the inhibiting effect may be the result of a direct fungicidal action by the growth regulating substance. However, much of the inhibition of mold on intact plants appeared to be from an indirect effect, since the treated plants produced fewer etiolated shoots which are excellent media for molds. In addition, treatment resulted in better callousing of the cut surfaces of the canes made in pruning before storage, thereby making it apparently more difficult for the mold to become established on the canes.

Mitchell, Kraus and Whitehead (38) and Mitchell and Whitehead (39) have found that under some conditions application of growth regulating substances to bean leaves may have
a stimulating effect on the rate of starch hydrolysis while under others the applications may have no effect. Evidence was obtained in the present experiments indicating that with dormant rose plants stored at relatively low temperatures, application of growth regulating substances may have a retarding effect on the rate of starch hydrolysis. The greatest retardation in starch loss was obtained with treated (0.5 gram methyl ester per 1000 cubic feet for 16 hours at 70° Fahrenheit), plants held in cold storage at 32° Fahrenheit. With plants held in common storage, treatment retarded the loss of starch so that at the end of the storage period there was no significant difference in relative starch content, as indicated by the IKI test, between these plants and similar untreated plants held continuously in cold storage (32° Fahrenheit). However, the untreated plants during common storage produced a larger number of etiolated shoots per plant than the treated plants so that it would be difficult to estimate how much of the reserve starch supply was utilized in producing shoots and how much was conserved by direct action of the growth substance on starch hydrolysis.

Due to the greater amount of stored reserves in the plant and a short delay in shoot growth after field planting the treated plants from both common and cold storage became established in the soil quicker and ultimately produced more roots, shoot growth and flowers than comparable plants that were not treated with growth regulating substances.
SUMMARY
and
CONCLUSIONS

Under the conditions employed in these experiments vegetative buds on rose bushes held in common storage have been effectively inhibited by synthetic growth regulating substances so that the plants remained dormant throughout the normal storage season. The several growth regulating substances when applied to dormant rose bushes in concentrations that were not injurious increased the length of time that the plants can be safely held in common storage without excessive shoot production or mold growth. Applications that were too strong caused severe plant injury, while concentrations that were very low caused a stimulation in the number of shoots that were produced.

Two compounds, α-naphthylimethylacetate and α-naphthylethylacetate, both esters of naphthaleneacetic acid, were very effective when applied either in dilute (1/4 per cent) wax-emulsion sprays or volatilized into the air surrounding the plant tops. When applied in dilute wax-emulsion sprays at a concentration of .005 per cent or as vapors at the rate of 0.3 gram per 1000 cubic feet to the plant tops, both of the above mentioned esters were equal in effectiveness, causing complete bud inhibition on plants held for nearly two months in common storage. Similar results were obtained with α-naphthaleneacetic acid and α-naphthaleneacetonitrile when the concentration of these two compounds in wax-emulsion
sprays was increased up to .01 per cent. The latter compound was also effective when applied in the vapor form at the rate of 0.3 gram per 1000 cubic feet.

Treatments with growth regulating compounds were effectively applied as a vapor at temperatures varying from 32° Fahrenheit to 70° Fahrenheit. Application of growth regulating substances should be made directly to the vegetative buds that are to be inhibited, especially when the compounds are applied in spray form. In vapor treatments the plants should be placed fairly close to the vapor source as it apparently condenses rather quickly. It is believed that the effectiveness of both the vapor and the wax-emulsion treatments was due to a slow continuous penetration of the growth regulating substances.

Treatment with volatilized a-naphthylmethylacetate were effective on bud inhibition when the applications were made at 32°, 40° and 70° Fahrenheit and the plants were stored in common storage following treatment. At the lower temperatures of treatment (32° and 40° Fahrenheit) a longer treatment interval or a higher concentration of growth regulating substance was needed to cause bud inhibition than at the higher (70° Fahrenheit). Consequently with plants treated with a 0.5 gram per 1000 cubic feet concentration of vapor there was found no significant difference between 1 hour of treatment at 70° Fahrenheit, 4 hours of treatment at 40° Fahrenheit or 16 hours of treatment at 32° Fahrenheit.

The food reserves (starch) of rose plants were conserved
while in common storage, as a result of treatment, either by a direct inhibiting effect on the hydrolysis of starch or by prevention of shoot growth which utilize the available reserves. As a result, treated plants after storage produced a much greater amount of root and top growth and potentially more flowers per plant when field planted.

Plants that were dug immature and have a low starch reserve were not materially benefited by treatment. Likewise the vegetative buds of all varieties of rose may not be inhibited to the same degree by a given treatment, some inhibition however, might be expected with most varieties.

Another effect that had a practical aspect was the inhibiting action of several compounds on mold growth. Evidence was obtained indicating that these growth regulating compounds had a direct fungicidal action. However, of possibly greater consideration also was that with plants treated with effective bud inhibiting substance there were much fewer etiolated shoots produced which establish an easy point for mold infection.

In relation to the nursery practice of placing a number of plant species in the same storage room with roses it was of interest to note that treatments that effectively inhibited the vegetative buds on rose bushes also caused bud inhibition of the following plant materials: apple, pear, peach, cherry (ornamental and commercial variety), mockorange, Japanese maple and persimmon. As was found in comparisons between rose varieties, a differential response was obtained with different
plant species in the degree of bud inhibition and injury that was produced by a particular treatment with growth regulating substance.
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