Regeneration in Various Types of Apple Wood

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SUMMARY

Two distinct growth phases were demonstrated to exist in apple trees. These phases were closely related to the ease of regeneration of roots on stems. Stem cuttings of wood in the mature phase were very difficult to root without special treatments, while those made from shoots in the juvenile phase rooted very readily.

The juvenile condition was recognized by the thinness of the leaves and small amount of pubescence. Anthocyanin production was abundant in juvenile shoots. In some species of apple the shape of the leaf changed with maturity from an entire to a lobed form.

Shoots having the juvenile characteristics of young apple seedlings were produced from roots of older trees. Stem cuttings made from these shoots usually formed roots readily. Juvenile shoots were produced from adventitious buds on roots and possibly from adventitious buds on limbs of mature trees.

Watersprouts were found to originate from latent buds rather than from adventitious buds. The only adventitious buds produced on stems were found on sphaeroplasts. Adventitious buds were produced readily on roots, especially if the latter were placed under favorable conditions of temperature and moisture. These buds arose from parenchyma in the secondary cortex and thus had no connection with the cambium of the root.

The change from juvenile to mature form was not related to the beginning of secondary growth in the plant, the loss of primary structures, or to the stage of organization of internal tissues of the stem. The expression of growth phases, together with the accompanying changes in ease of root formation, is believed to be dependent upon certain biochemical factors not clearly understood at present.
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The present interest in the production and use of own-rooted stocks as a means of decreasing variability in the growth and production of apple trees was aroused by two distinct lines of investigation. These were: first, numerous critical statistical studies of the individual behavior of orchard trees suggested that much of the variability which occurred in bearing trees might be due to genetic differences in the seedling understocks; second, certain English investigators, basing their work on experimental observations, reclassified the common European apple stocks and recommended certain types of dwarf and standard own-rooted stocks as a means of producing uniformity in orchard trees.

Although the development of own-rooted or clonal lines of rootstocks may be desirable, the fact still remains that the ordinary vegetative methods for propagating fruit tree stocks are not sufficiently practical to meet the needs of the commercial producers of trees. Certain understocks may be particularly well adapted to special conditions, but their propagation on own roots, except to meet experimental needs, is expensive under present conditions.

The inadequacy of the commonly used seedling rootstocks for some environmental conditions has been recognized by practical growers, who, in an attempt to remedy the situation, have made extensive use of double working (using an intermediate stock between rootstock and desired variety) and top grafting (grafting the desired variety on branches above trunk). The increase in hardiness, vigor and longevity of apple varieties when top-worked on certain intermediate stocks is generally well known. Many varieties form good unions and are very productive on stocks of one variety and are poor growers on others. Stocks may also influence the character of the growth of the top in various ways. Northern Spy has been used as an own rooted stock because of its resistance to infestation by woolly aphis. Grimes Golden and Tompkins King are often double worked or top grafted on another variety in order to escape the destruction of the tree by collar rot. Thus the use of vegetatively propa-

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1Taken from a thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree, doctor of philosophy. Project 467 of the Iowa Agricultural Experiment Station.

2The writer is indebted to Prof. B. S. Pickett and Prof. T. J. Maney of the Department of Horticulture for continued encouragement and assistance with the investigation. The morphological phases of the investigation were conducted in the laboratories of the Department of Botany under the supervision of Dr. J. E. Sas.
gated stocks can be related in many ways to commercial fruit growing.

The present studies were made in an attempt to investigate and describe the capacity for regeneration in various types of wood produced by apple trees. Methods of rooting were sought which would be effective with widely different varieties and species of apple, including those which do not develop burr-knots or other preformed root primordia. A better understanding of the fundamental anatomical and physiological principles of the development of apple trees throughout their complete life history was sought, as it was believed that only by obtaining new information on these fundamental principles could any real advances be made toward solving the problems of vegetative propagation of the apple.

REVIEW OF PERTINENT LITERATURE

There is extensive literature on vegetative propagation of fruit trees and rootstocks. Three authorities, Hatton (16), Auchter (3) and Southwick (37), have reviewed the general field with great thoroughness. Hatton (16) has surveyed it with particular emphasis on the work of European experimenters. Auchter (3) collected and summarized the American publications and uncovered much additional unpublished data by means of questionnaires and correspondence. Southwick (37) listed and summarized a total of 498 publications related to this subject. The existence of these three excellent summaries and bibliographies makes an extensive general review of all the literature unnecessary. Many of the references to literature on the more specialized aspects of the problem have been placed in the appropriate sections. A recent survey by Yerkes (56) has revealed the large amount of attention given to stock problems by experiment stations throughout the country.

The following methods have been used for the vegetative propagation of apple stocks:

1. Layering
2. Nurse grafts
3. Root cuttings
4. Stem cuttings, either hardwood or softwood

All of these methods have been practiced with a good many variations or modifications, and some of the most important of these will be discussed.

Layering has been commonly used by nurserymen for the production of Paradise and Doucin dwarfing stocks. It was the ordinary method used by Hatton (14) for the new rootstocks selected at the East Malling Experiment Station. It has been used to a limited extent by other investigators.
The various types of nurse grafts are designed to encourage the formation of cion roots. Yerkes (54) and Auchter (2) used grafts in which a very long cion was attached to a short root piece. When the graft was planted deeply a considerable amount of cion root formation occurred. Chandler (7) used a method of grafting in which the lower end of the cion projected below the graft union. Maney (26) wrapped the grafts with wire in order to accomplish the same result. Kerr (19) was successful with a novel method of grafting in which the inverted root pieces were grafted to the cions.

When own-rooted trees of the desired variety or species are available, the plants may also be multiplied by root cuttings. Practically all investigators have mentioned an initial period of slow growth in plants propagated from root cuttings. A method of avoiding this disadvantage has been described by the author and associates (40, 41).

A large amount of work has been done on the rooting of hardwood and softwood stem cuttings of apple, but generally with very disappointing results. The problem has received the attention of English workers, including Knight (20), Knight and Witt (21) and Knight, Amos, Hatton and Witt (2) and others. Little success attended their attempts with any varieties except those which are very easily propagated by layering. Auchter (3) summarized the results of American workers and found that some of these experimenters had used as many as 16,000 to 40,000 cuttings with very little success. Vierheller (51) tried many unusual treatments but rooted only a very few cuttings. Shaw (34), Tukey and Brase (45) and numerous others have tried large numbers of stem cuttings with practically no successful rooting.

Only one group of apple varieties may be rooted with some degree of success by using stem cuttings, a group comprising those varieties which form burr-knots on the wood. Burr-knots, so-called, consist of masses of preformed root primordia. The ready adaptability of these varieties to vegetative propagation was described by Loudon (25) in his Encyclopaedia of Gardening over a century ago. Maney (26), Swingle (42, 43, 44), and also Hatton, Wormald and Witt (15), further demonstrated the value of this method of rooting apple cuttings. Van der Lek (49) has summarized some of the literature on burr-knots in relation to propagation.

Recently two treatments have been devised by means of which roots may be formed on stem cuttings of any variety of apple. Gardner (11) has used a method based on etiolation with much success. This is done by protecting the wood of the stem to be used as a cutting from light by means of tape or black paper.
It is necessary to start the treatment very early in the development of the shoot. Etiolation is doubtless a large factor in the success of some methods of layering.

A second type of treatment, which appears to be more promising from a commercial standpoint, is the use of the newly discovered root forming substances. Hitchcock and Zimmerman (17) obtained good rooting in softwood apple stem cuttings with this type of treatment. The most effective compound of this type discovered to the present is indolebutyric acid, according to Zimmerman (57). The newer method of treatment uses the substance dissolved in water rather than in lanolin, and the cuttings are allowed to take up the solution through the stems for 24 hours or more. This work is still in an experimental stage but appears to be very promising.

With the exception of the varieties which produce burr-knots, stem cuttings of apple have been rooted very rarely. The only promising methods of rooting stem cuttings which have been devised are the use of etiolation and the treatments with the newer type of chemical stimulants.

MATERIALS AND METHODS

METHODS AND EQUIPMENT FOR PROPAGATION

The plant materials for this investigation were obtained from the orchards and nurseries of the Pomology Subsection of the Iowa Agricultural Experiment Station. The trials of rooting of various types of apple wood were begun in 1931 and were continued through 1936.

In the early experiments with greenwood cuttings of apple, mixtures of peat and sand were used, as well as sand alone. The use of peat, however, was found to be undesirable as it encouraged decay of the cuttings. Apple greenwood cuttings, especially if very succulent, were found to be rather susceptible to fungous attacks when there was an excess of moisture in the atmosphere or the rooting medium. Sand was later adopted for use with all apple stem cuttings. This made the problem of watering the cuttings much easier.

Manure was used to supply bottom heat in the first 2 years of the investigation, but this was later abandoned in favor of electrical heating, which was more convenient and readily controllable. General Electric thermostats and lead covered soil heating cable were installed in both the indoor and the outdoor frames. The outdoor frames were under a propagating house shaded with muslin cloth. After the cuttings were inserted, the frames were kept closed tightly until rooting began.

In the indoor experiments conducted in the greenhouses during the winter, the equipment consisted of an electrically heated
frame covered with double glazed sash, two steam heated frames and an open propagating bench. The closed frames were used mostly for root cuttings or for starting shoots on root pieces. Stem cuttings were usually rooted on an open propagating bench in sand.

Stem cuttings were made without any particular reference to the position of the basal cut, since the apple stem cuttings rooted abundantly on internodes as well as on nodes. The leaves were removed from the base of the cutting. The length of cuttings was not observed to influence the results. In general, the cuttings made in the latter part of the investigation were shorter than those made earlier and were often only 2 or 3 inches long. The cuttings used in any one particular experiment on rooting were made as uniformly as possible.

Any method requiring a highly refined technique in propagation is not likely to be adopted widely in commercial practice. The methods tried were not considered satisfactory unless the cuttings could be rooted with only such attention as might reasonably be expected in good nursery practice. The percentages of rooting obtained in these studies were believed to be a conservative indication of the possibilities of each method which has been described.

HISTOLOGICAL METHODS

The material for the anatomical studies was usually killed in acetic-formalin-alcohol as this was found to give excellent results with all lignified tissues. With this killing fluid, the materials were left for at least a week before beginning dehydration in order to insure complete fixation. The chrome-acetic-formalin killing fluids were used for very succulent tissues. Early in the course of the investigations the use of an ethyl alcohol or acetone series for dehydration was abandoned in favor of a schedule using butyl alcohol and acetone. The action of the butyl alcohol was found to facilitate the cutting of lignified materials and was especially helpful with paraffin sections on the rotary microtome. Soaking the paraffin blocks in warm water for several days before sectioning improved the cutting quality of the preparations.

Serial sections were used in practically all slides made for the anatomical studies whether cut in paraffin or in celloidin. Some of the thick woody materials imbedded in paraffin were cut on the sliding microtome. Serial order of the sections was obtained by placing the individual sections in order on a slide as they came from the microtome, using a gelatin fixative. These slides were not dried in the oven according to the ordinary procedure but were passed at once through a series of staining solutions in which no water was present. They were stained in alcoholic
safranin 0 and counter-stained with gentian violet which was dissolved in clove oil and added to the carbol-xylol.

Very tough woody materials were imbedded in celloidin. The serial order of the sections was preserved in the following manner: As the sections were removed from the microtome knife, they were placed in serial order on a slide. When completed, the space between the sections was flooded with a thin solution of celloidin. As soon as a film had formed and dried, it was lifted from the glass slide with a safety razor blade. These films were stored in labeled bottles of alcohol and were later stained in small dishes. After staining, the sections were removed from the celloidin matrix by placing the film on a strip of filter paper and submerging it in a dish of methyl alcohol and ether. When the sections were free, they were transferred individually in serial order to a clean slide with a scalpel blade and were mounted in Canada balsam. For the woody material used in this study, Mayer's hemalum stain with a counterstain of safranin 0 was found to be the most generally useful. A more brilliant contrast of blue and red was obtained by adding a solution of gentian violet in clove oil to the final clearing bath of carbol-xylol.

The low power photomicrographs were taken with a Bausch and Lomb photomicrographic camera and a 32mm. Microtessar lens. With a few exceptions, all of the photographs made with this apparatus were taken at a magnification of approximately 18 diameters.

For the higher magnifications, a Leitz microscope fitted with the "Makam" photomicrographic camera was used. A 10x Periplan ocular was used with 16mm. and 8mm. objectives, giving magnifications of approximately 120x and 230x, respectively. A Wratten "B" green filter and an orange "G" filter were most useful in obtaining the proper contrasts with slides stained with the hemalum and safranin combination.

EXPERIMENTAL

THE CAPACITY FOR REGENERATION IN VARIOUS PARTS OF THE APPLE TREE

THE MATURE GROWTH PHASE

STEMS

As was shown by the review of literature, the use of stem cuttings of mature wood of apple has been tried by many experimenters, but with very little success. The burr-knot producing varieties alone have responded to propagation by this method. The preliminary trials of rooting softwood cuttings made in this study likewise yielded negative results. During the summer of 1931, trials of rooting of softwood cuttings of apple taken
from trees in the orchard were made. Two varieties, Sharon and Missouri Flat, were used. The variety Sharon is not readily propagated vegetatively, but the variety Missouri Flat is known to be particularly adaptable to vegetative propagation by layering. These cuttings were about 8 inches long and consisted of wood of the current season.

The cuttings were inserted in various types of sand and peat rooting media during July and August. No rooting was obtained in a total of 660 cuttings. The cuttings remained in excellent condition during the entire period of the test, but when the final observations were made on Oct. 16 no rooting had occurred. Large masses of callus had formed on the cut ends of the cuttings, and in many instances the lenticels on the stems were covered with callus.

During the summer of 1933 trials of rooting of softwood apple cuttings were made from the varieties Virginia Crab, Hibernal, Missouri Flat, Sharon, Hawkeye Greening, Jonathan, Delicious and Whitney Crab. These cuttings were treated with potassium permanganate following the rates recommended by Laurie and Chadwick (24). No rooting occurred either in the treated lots or checks, although 1,700 cuttings were used. Chemical stimulation of this type did not give any advantage in rooting. Other experimenters mentioned in the review of Auchter (3) have reported similar results. Evidently stem cuttings of mature wood of apple trees are particularly difficult to root, and chemical treatments useful with other woody subjects have been unsuccessful. Not until the recent use of specific root forming substances by workers at the Boyce Thompson Institute for Plant Research has chemical stimulation been successful with apple stem cuttings.

PETIOLES

According to Agricola (1), leaves have been used to propagate a number of fruit and forest trees, including apple. He related an experiment in which the leaves were cut off and the ends of the petioles were waxed and inserted into moist soil. The leaf blades decayed but the petioles remained alive and the following year shoots arose from the ends of the petioles. Priestley and Swingle (31) reported that the experiment had been tried at the University of Leeds without success.

Some similar experiments were conducted during the course of this investigation and were successful to a slight degree. Leaves taken from mature trees of several varieties were rooted in sand. It is apparently impossible for an apple leaf to regenerate a new bud at the base of the petiole. If the axillary bud

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5This name has been used locally for a red fruited variety of unknown origin which was found in the State Experimental Orchard at Council Bluffs, Iowa.
was included at the base when making the cutting, however, a new shoot was produced which soon grew into a normal plant. Cuttings of this type have been called "leaf-bud" cuttings by the author and associates (39). Figure 1 shows a young plant started from a cutting of this type taken from a Whitney Crab seedling tree. This cutting was rooted in the spring of 1933. A summary of the trials made in 1934 with rooting of cuttings of this type is presented in table 1. All of these cuttings were taken from trees grown in the greenhouse.

During the same season some trials were made in outdoor propagating frames. The cuttings were taken from large mature trees, except for Missouri Flat cuttings, which were obtained from the tops of 3-year trees growing outdoors in the nursery. The results are shown in table 2.

The data show that the rooting was only slightly better with leaf-bud cuttings than with ordinary stem cuttings. Logically this could be expected, since the petiole is a reduced stem in anatomical structure and has a cambium and other typical tissues of the stem. It should possess approximately the same capacity for rooting as the stem to which it is attached.

**TABLE 1. ROOTING OF LEAF-BUD CUTTINGS OF APPLE IN GREENHOUSE PROPAGATING FRAMES.**

<table>
<thead>
<tr>
<th>Species or variety</th>
<th>No. of cuttings</th>
<th>Number rooted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malus baccata</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Hibernal</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Whitney Crab</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Missouri Flat</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Virginia Crab</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Missouri Flat</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Virginia Crab</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Dudley</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Virginia Crab</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Wolf River</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
The fast succulent growth of young watersprouts was tried as a source of material for softwood cuttings. Watersprouts from trees of several varieties were tried in the summer of 1934, and the results are presented in table 3. These cuttings were inserted in sand in outdoor propagating frames and were given bottom heat. The cuttings remained in excellent condition for a long time and formed abundant callus but did not root. They were removed and counted on Sept. 25.

Although numerous cuttings taken from young watersprouts were tried, in no case was rooting obtained. The watersprouts all exhibited every external character of the mature growth phase. The source of the buds from which the watersprouts had arisen was studied by means of sections of the wood made with saw and plane and finished with a very fine garnet paper. Invariably the buds were found to have originated many years back as the nodal buds of the twig which formed the basis for the mature limb or tree trunk. The points of emergence of watersprouts originating from an older limb often showed plainly the two-fifths phyllotaxy of the original 1-year shoots from which the limb developed. By careful sectioning of a limb or trunk of an apple tree, the latent buds were located together with the gaps left in the xylem by the extension of the leaf trace over a period of years. Thus the possible locations of the watersprouts were determined in the first year of growth of the twig. The location of

Fig. 2. Two latent buds on a limb of a Virginia Crab tree.
two latent buds is shown in fig. 2. The buds are imbedded in the bark at the ends of the traces left in the xylem. Figure 3 shows a watersprout which has grown from a similar latent bud.

Several instances were observed in sections of the wood where the latent bud had received some stimulus and had swollen greatly but did not actually start active growth. When this happened, two new latent buds were often formed. These continued to exist fairly close together, and each was capable of forming a separate watersprout later.

The erroneous idea that watersprouts originate from adventitious buds is rather common. Apparently only Gardner, Bradford and Hooker (12), and Chandler (6), have presented a true view of the situation.

THE JUVENILE GROWTH PHASE

TOPS OF YOUNG SEEDLINGS

Gardner (10) reported that softwood cuttings made from the tops of apple seedlings during the first year of growth could be rooted easily. This was done not only with apple, but also with a wide variety of deciduous and coniferous tree species. Hardwood cuttings made from wood of the same type also root readily. An Iowa nurseryman \(^4\) has used the tops removed from 1-year bench grafted seedling stocks as hardwood cuttings. These were set into the field in the spring and a satisfactory amount of rooting was obtained. The resulting plants were used as grafting stocks. Auchter (3) mentioned that Gardner has used trees

\(^4\)C. C. Smith, Sherman Nurseries, Charles City, Iowa.

<table>
<thead>
<tr>
<th>Date</th>
<th>Variety</th>
<th>Number of cuttings</th>
<th>Number rooted</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 7</td>
<td>Virginia Crab</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>June 8</td>
<td>Grimes Golden</td>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

---

Fig. 3. Watersprout emerging from latent bud on a limb of Virginia Crab.
propagated in this manner as budding stocks.

Results similar to those reported by Gardner were obtained in many trials of rooting during the period of this investigation. When stem cuttings were made from tops of 1-year apple seedlings, the cuttings seldom failed to root. A typical rooted softwood cutting made from the tip of a 1-year apple seedling is shown in fig. 4.

SHOOTS FROM NODAL BUDS

Goebel (13) noticed that a reversion to the juvenile form in older plants occurred most often in shoots coming from near the base. He held the view, expressed by some other writers on the subject, that the root system and the lower portion of the plant may remain in the juvenile condition after other parts have passed into a mature type of growth. The experiments of Stewart (38) support this idea. He found, while working with stem and root cuttings of Acanthus, that shoots from the terminal buds on stems were in the mature growth phase, while those from nodal buds near the base showed evidences of juvenility. Shoots from adventitious buds on the internodes exhibited marked juvenility. These shoots all had a noticeable resemblance to those of seedlings.

While there is always the possibility of obtaining juvenile shoots from near the base of seedlings, there is much less possibility of obtaining them from grafted trees. The wood at the base of grafted trees is developed from the cion which is taken from shoots in the mature growth phase. In young seedling

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Fig. 4. Left: Softwood cutting made from top of 1-year apple seedling. Right: Root formation in a similar cutting.
TABLE 4. ROOTING OF CUTTINGS MADE FROM TOPS OF SHOOTS OF SECOND YEAR CUTBACKS OF WHITNEY CRAB SEEDLINGS.

<table>
<thead>
<tr>
<th>Date inserted</th>
<th>Date counted</th>
<th>Number of cuttings</th>
<th>Number rooted</th>
<th>Percentage rooted</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 28</td>
<td>July 23</td>
<td>100</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>May 29</td>
<td>July 23</td>
<td>50</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>June 15</td>
<td>Aug. 9</td>
<td>50</td>
<td>34</td>
<td>68</td>
</tr>
<tr>
<td>July 19</td>
<td>Sept. 10</td>
<td>50</td>
<td>17</td>
<td>34</td>
</tr>
</tbody>
</table>

trees, shoots capable of forming roots may often be obtained by heading the tree back in order to produce shoots at the base. On the other hand, if the trees are allowed to grow they lose much of the capacity for root formation early in the second season of growth. Gardner (10) found that rooting of cuttings made from tops of seedlings of apple was rather poor in the second year and was very meager in the third and fourth years.

In order to show that the capacity for rooting in the young seedling may be prolonged by cutting back, some seedlings of Whitney Crab planted early in the season of 1933 were cut back to the ground in the spring of 1934 before growth started. Shoots arose from near the ground, and cuttings were made from these. The amount of rooting obtained with these cuttings is shown in table 4.

Thus out of a total of 250 cuttings of this type, 137 were rooted, which is an average of 55 percent of the cuttings. The shoots used in these trials came from the nodal buds just above the crown, since the heading was not done severely enough to remove all nodal buds and thereby cause the production of adventitious buds on the root or crown.

Since these cuttings did not root as readily as those made from tops of young seedlings, it is possible that these cuttings were made from shoots which were intermediate between the juvenile and the mature forms. Some intermediate types apparently were obtained by Stewart (38) with Acanthus. The less abundant rooting with tops of cutback seedlings in the second and third years was noted by Gardner (10). The results obtained by these two experimenters suggest...
the presence of intermediate or transitional growth phases.

Another experiment with the apple species, *Malus Sargentii*, shows that the juvenile form may persist in seedlings. On Jan. 10, 1936, dormant seedling trees of this species were planted in a ground bench in a cool greenhouse. These trees had been started from seed planted in 1929. They were headed back to approximately 1 foot in height in order to encourage the production of abundant shoots. On March 11, 100 softwood cuttings were taken from these and were placed in sand on an open propagating bench. These cuttings had the round entire leaf characteristic of the juvenile form. On April 10 another lot of 100 cuttings was taken. These cuttings had deeply lobed leaves appearing at the tip, indicating the beginning of a change in growth phase. The rooting obtained with these cuttings is shown in table 5.

The change of growth phases is easier to follow in species of apple which have leaf dimorphism as one of the distinguishing characters of changes of growth phase, as do some of the species of Asiatic crab apples. The species *Malus Sargentii* possesses a deeply lobed leaf in the mature form, but the first leaves produced by juvenile forms tend to be entire or nearly so. The trees planted in the greenhouse on Jan. 10 produced both types of growth. At the time the first softwood cuttings were made on March 11, the shoots were all similar to those in fig. 5 and had entire leaves. One month later the leaf form had begun to change, and the cuttings made on April 10 were from shoots in which the lobed form of the leaf had begun to appear toward the tips of the stems. The lobed form is illustrated in fig. 6.

All those shoots which could not be clearly identified as be-

![Fig. 6. Shoots of Malus Sargentii showing the change from entire to lobed leaf form.](image)

<table>
<thead>
<tr>
<th>Date inserted</th>
<th>Date counted</th>
<th>Number of cuttings</th>
<th>Number of cuttings rooting</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 11</td>
<td>April 23</td>
<td>100</td>
<td>89</td>
</tr>
<tr>
<td>April 10</td>
<td>May 19</td>
<td>100</td>
<td>93</td>
</tr>
</tbody>
</table>
longing to either growth phase were avoided as much as possible. Only a relatively small amount of the experimentation was done with intermediate and transitional growth forms, so that no definite conclusions were drawn as to the capability of rooting of these forms.

SHOOTS FROM ADVENTITIOUS BUDS ON ROOTS

The ease of rooting of juvenile forms has been noted in certain conifers, particularly the Cupressineae, by Goebel (13). Assuming that the ease of rooting of cuttings from 1-year seedlings was due to the juvenility of the growth phase at that period, the conclusion is logical that if shoots possessing the juvenile form could be produced in mature apple trees, the cuttings made from them should likewise root very easily. After watersprouts were found to offer no possibilities for regeneration, the search for a method of producing rejuvenescent shoots in older trees was continued.

A careful comparison of a stem from an apple seedling with a shoot from the top of an older tree revealed several important external morphological differences. The leaves of the young seedling were thinner and the leaves and shoots did not have the pubescence evidenced in the leaves and shoots of mature trees. Anthocyanin production was often extremely abundant in the juvenile stage. In some cases, notably in some of the Asiatic crab apples, the leaf form varied. These characters were identical with those used by Balfour (4) in determining the time of persistence of the juvenile stage in rhododendron seedlings.

Undoubtedly many cases of change in growth phase in plants are unrecognized because the external characters of the two stages are closely similar. The examples discussed in the literature are those which are quite obvious because of the marked differences between the juvenile and mature forms. Thus some plants have alternate leaves in one stage and opposite leaves in another. Sometimes the shape of the leaf changes. In the bean, the first leaves are entire and opposite, while the later ones are compound and alternate. A plant may be a vine in the juvenile stage and a shrub in the mature. Diels (8) and Krenke (23) listed many plants in which such differences are known, including such genera as Hedera, Ranunculus, Bidens, Grevilles, Ailanthus, Cotinus, Euonymus, Pinus, Veronica, Rubus and Aristolochia. Laurie and Chadwick (24) mentioned the marked changes in Alisma, Sagittaria and many conifers, and stated that many of the nurseryman’s varieties of Thuja and Chamaecyparis are merely juvenile seedling forms which have been prolonged by propagation with stem cuttings. Michurin (27) noticed certain progressive changes in the appearance of shoots of fruit tree seedlings following heading back to the ground. Jeffrey (18)
has pointed out that seedlings and wound wood of stems sometimes have an anatomical structure of a very primitive type. He showed that such wood was a reversion to extinct ancestral types which grew in past geological ages. Goebel (13) has presented one of the most extensive treatments of juvenile forms and described several cases of reversion back to the juvenile type of growth. He mentioned that shoots of this type are most likely to occur at the base of the tree and often result from injuries or unfavorable growing conditions. He did not investigate the nature of the buds from which these shoots grew, but had some knowledge of the relation of the juvenile stage to ease of propagation. He mentioned that cuttings of certain conifers, such as the members of the Cupressineae, are much easier to root if they are taken from stems in the juvenile stage of growth rather than from those in the mature. The use of this principle for the propagation of plants difficult to multiply vegetatively has been neglected, probably because of the failure to recognize differences in growth phases unless they are accompanied by marked external changes.

The first shoots from older apple trees with noticeable juvenility were found in the summer of 1934. In one of the experiment station orchards a planting of trees of the Virginia Crab variety was made in 1917. This variety was root grafted on French Crab stocks, but cion rooting had taken place on these trees. After the trees were pulled out in 1929, numerous sprouts arose from the cion roots left in the ground. On May 17 a lot of 25 cuttings was made from those sprouts which were in the first season of growth. Unfortunately most of the shoots in this area had arisen in previous years and were obviously in the mature growth phase, so that larger lots of cuttings could not be used for lack of material. The cuttings used in this experiment apparently had many external characteristics in common with the young seedling stage, and this idea was supported by the fact that 14 of these cuttings rooted. At the same time, another lot of 25 cuttings was made from sprouts from 14-year-old trees in one of the fruit breeding orchards. The trees from which these cuttings were made were seedlings grown from various controlled crosses. In this lot of cuttings placed in sand in an outdoor propagating frame, 17 rooted.

The results obtained in this preliminary experiment were later confirmed by a greenhouse experiment during the same summer. The results have been reported previously by the author and associates (40). In these trials the cuttings were obtained from shoots grown from root cuttings about 6 inches long which were planted in soil in a ground bench in the greenhouse. It was found later that a different treatment of the root pieces gave far superior results. These cuttings were obtained
These preliminary experiments led to the view that reversion shoots of the juvenile stage can be produced in trees of any age if the shoots are grown from adventitious buds arising on the roots. These reversion shoots, in addition to having the external morphology of the seedling, would also root very readily when made into stem cuttings. This was true not only for the different varieties of apple, but also for various species. These theories,

### TABLE 6. ROOTING OF STEM CUTTINGS GROWN FROM ROOT PIECES.

<table>
<thead>
<tr>
<th>Date inserted</th>
<th>Date examined</th>
<th>Species or variety</th>
<th>No. of cuttings</th>
<th>No. of cuttings rooted</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 11</td>
<td>April 17</td>
<td>Malus Sargentii</td>
<td>100</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malus baccata</td>
<td>100</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>French Crab seedlings</td>
<td>100</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malus baccata</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virginia Crab</td>
<td>100</td>
<td>36</td>
</tr>
<tr>
<td>April 10</td>
<td>May 24</td>
<td>Malus Sargentii</td>
<td>100</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malus baccata</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Virginia Crab</td>
<td>100</td>
<td>28</td>
</tr>
</tbody>
</table>
which were developed in 1934, were tested and substantiated by experiments conducted during 1935 and 1936.

The following method was devised which invariably gave a very abundant production of adventitious buds and shoots on root pieces. The roots were cut in pieces and packed lightly in a 4-inch layer of moist sphagnum moss placed over sand or other moisture-holding material in the bottom of a closed propagating frame. The best results were obtained by using a bottom heat above 70° F. in the cases. Electrical heating was the most convenient, but good results were obtained with steam heating. A close control of the temperature was not required. Excellent results were obtained both in the greenhouse and also in outdoor frames such as nurserymen use for summer propagation. The results were inferior if the root pieces were planted in soil or if they were buried deeply in sand. The success of this method was probably due to the excellent aeration of the cuttings, the high humidity of the atmosphere surrounding them and the stimulation from the heat in the frames. This treatment has given excellent results with all of the apple varieties and species which have been tried. Upshall (47) has recently reported favorably on a treatment for root cuttings based on increased aeration in order to encourage shoot production. Root cuttings can be made at any time of the year with the assurance that they will produce adventitious buds very quickly if treated as outlined above. The production of shoots, however, was definitely smaller if the cuttings were taken from the tree during the short season of very active growth which culminates in June.

Stem cuttings were taken at the stage shown in fig. 7, which shows the shoots arising on some root pieces from own-rooted Virginia Crab trees. The young shoots were either pulled or cut from the root pieces and were put in a sand rooting medium, either on an open greenhouse propagating bench during the winter.
or in an outdoor propagating frame if the cuttings were rooted in the summer. The use of peat mixed in the rooting medium was undesirable.

Early in 1935 some tests were made in the greenhouse which proved that this method would work successfully with own-rooted apple varieties and true species. The roots of *Malus Sargentii* and *M. baccata* were obtained from 7-year-old nursery trees, while the roots of Virginia Crab were from trees planted in 1917. The results of these trials are shown in table 6. The shoots arising from roots of *Malus Sargentii* were exceedingly abundant. Sometimes as many as 30 or more shoots arose from a single root piece of this species. A typical root piece with shoots is shown in fig. 8. In *Malus baccata* exceedingly numerous minute buds were formed all over the small fibrous roots attached to the main root system. Root pieces of the Virginia Crab variety cut about 6 inches long frequently produced 10 or 12 shoots each, but the average production was about one shoot per inch of length of the cutting. After the shoots were removed, others continued to form on the stem as long as the root pieces remained in good condition. The effects of various factors on the abundance of shoot formation have not yet been determined. The treatment of the root pieces under a temperature of 70° to 80° F. while packed in moist sphagnum has been the most effective method. Several dozen 6-inch root pieces could be placed upon a square foot of area in the propagating frame. Crowding was detrimental only when excessive enough to prevent a good development of the shoots from which the cuttings were made.

The softwood cuttings taken from the root pieces likewise required very little space in the propagating bench. They were placed about 1 inch apart in rows spaced at intervals of 1 1/2 inches. The requirements of space and labor should not prove much greater for this method than are needed in many methods now used for producing transplanting stock of common ornamentals.

Some results with this type of softwood cutting were reported by the author and associates (41), using the Virginia Crab variety. This method of propagation was found to avoid the initial slow period of growth noticed with root cuttings by practically all of the previous investigators. Upshall (46) attributed this slow rooting effect to the vascular plugging in the root piece. In this modification of the method of propagation by root cuttings, a larger number of plants may be started than with older methods. In addition, no defective link in the root system of the new plant is present as a result of a poorly functioning conducting tissue of the original root cutting.

Cuttings made from shoots grown from an adventitious bud on a root or crown of an apple species or variety have never
failed to give at least a moderate amount of rooting of these cuttings. They also have the external characteristics of young seedlings as exhibited by their stem and foliage and permit drawing the conclusion that these shoots are true examples of reversion to the juvenile form. This state of juvenility is continued during the first season of growth. It is not possible to state definitely just when the mature stage is reached unless some purely arbitrary criteria are established, since the two growth phases grade imperceptibly into each other.

The following experiment demonstrates the contrast between the two phases of growth. During the summer of 1934, shoots up to 3 feet in height were grown from cuttings taken from shoots grown from adventitious buds on roots of Virginia Crab. Stem cuttings of these shoots taken as late as Sept. 21 were still rooting, and therefore it is assumed that the shoots were still juvenile in character. Bud sticks were made from these shoots in September, and the buds were set in actively growing trees of the Grimes and Delicious varieties, located in a ground bed of a cool greenhouse. The stock trees were about 6 feet high. The trees were kept dormant over the winter. The stocks started to grow in early February of 1935, and by pruning back the stems, enough of the buds were started into growth to permit a lot of 50 cuttings about 3 inches long to be made from them on April 10. All of the cuttings had failed to root during a period of 2½ months, although given favorable conditions. These shoots had every external characteristic of the mature growth stage from the start, although they were grown directly from a bud taken from a shoot in the juvenile stage. One of these shoots is shown in fig. 9. This photograph was taken shortly after the time of starting of growth in the bud, and the

Fig. 9. Shoot from bud of juvenile stem of Virginia Crab set in stem of Delicious tree. Note heavy pubescence indicative of the mature phase of growth.
heavy pubescence characteristic of the mature phase is clearly visible.

Sections of the cuttings made from these shoots showed that the wood was in an earlier stage anatomically than that of similar cuttings made in the previous season from stems of the juvenile form from which the buds had been taken. An interfascicular cambium had developed, but there was little lignification and an absence of fibers. This shows that the expression of either growth phase is not dependent upon the stage of internal development reached in the shoot.

**PETIOLES**

As was demonstrated in the rooting trials, the petioles from shoots in the mature growth phase had very little capacity for root formation. Petioles on all shoots in the juvenile stage of growth, however, formed roots very readily. Rooting occurred if the petioles were cut at any point between the base and the leaf blade.

Leaf-bud cuttings were made by cutting off the leaf in such a manner as to leave the axillary bud attached. These cuttings readily grew into young trees after rooting. On June 7, 1934,
200 leaf-bud cuttings of apple seedlings of a Brier x Mercer County cross were placed in outdoor propagating frames. In 6 weeks 183 rooted cuttings were obtained from 200 leaf-bud cuttings taken from sprouts from cutbacks of second year Whitney Crab seedlings. Some of the rooted leaf-bud cuttings from the seedlings of the Brier x Mercer County cross are shown in fig. 10.

Similar cuttings made from leaves on shoots grown from root pieces likewise had the capacity of rooting readily. On March 12, 1935, some leaf-bud cuttings were made to demonstrate this possibility. Out of 100 such cuttings of Malus Sargentii, 69 cuttings were rooted in 6 weeks. With 100 cuttings of Virginia Crab, 51 rooted cuttings were obtained.

**SHOOTS FROM ADVENTITIOUS BUDS ON STEMS**

Adventitious buds are produced readily from masses of callus on some trees, such as willow and poplar. Chandler (6) believed that these buds were impossible to find on apple trees, or at least very rare. According to some horticulturists, they occur at times on masses of callus on large pruning wounds in orchards. Sass (32, 33) has observed adventitious buds in callus masses about apple graft unions. They were not found occurring on any of the trees in the orchards of the Pomology Subsection of the

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The Mercer County Crab, which originated in Mercer County, Illinois, is one of the large forms of *Malus pumila*. There is a possibility that it may be hybrid in origin as the result of a cross with *Malus sylvestris*. 
Iowa Agricultural Experiment Station, although abundant pruning had been done in some of the orchards before this time.

The only instance in which adventitious buds were found on the trunks of orchard trees was in conjunction with sphaeroplasts on the bark of Virginia Crab trees. These trees had been pruned back very severely in the winter of 1934-35 in order to encourage the production of abundant watersprouts for experimental work.

Sphaeroplasts are small wood inclusions occasionally found in the bark of various species of trees, including apple and pear. In the orchards at Ames they can never be found on some varieties but are very common on others. They have been found most frequently on Iowa Blush, King David, Hutchins Red, Northwestern Greening, Virginia Crab and on seedlings from certain Jonathan x Delicious crosses in the fruit breeding orchards.

Sphaeroplasts possess a true cambium and continue growth over a period of years, forming annual rings. A cross section of one of these structures is shown in fig. 11.

The adventitious buds were found only on those trees which had received extremely severe pruning. Figure 12 shows a sphaeroplast bearing an adventitious bud which was swelling and about ready to start into active growth. Frequently these buds occurred in clusters of two or three. An anatomical study of these buds is presented in another section.
Fig. 13. (Upper left) Hypocotyl of young apple seedling showing transition from root to stem structure. Seedling 2 weeks from time of germination. X80

Fig. 14. (Upper right) Primary root of young apple seedling, 2 weeks from time of germination. Endodermis (E) is shown with large Casparian strips on radial walls of the cells. The pericycle (P) consists of large parenchymatous cells. Tetrarch structure of xylem. X80.

Fig. 15. (Lower left) Pericyclic origin of lateral root from primary root of apple seedling started from seed on March 9, 1934. Material killed April 16, 1934. X 160.

Fig. 16. (Lower right) Formation of periderm by a pericyclic phellogen (P) and abscission of primary cortex and endodermis in root of Whitney Crab seedling planted in nursery on April 20, 1933. Material killed May 27. Root was slightly over 5 inches from crown to tip. This section was taken at 1 inch from the tip. X 80.
During the summer of 1935 these buds produced rosettes of leaves but did not produce stems long enough to be used for making cuttings. These leaves resembled those of seedlings and apparently were in the juvenile stage of growth. Unfortunately, some construction work required the removal of all of these trees the following winter, and the growth phases of shoots from these adventitious buds could not be followed.

MORPHOLOGICAL INVESTIGATIONS

PRIMARY AND SECONDARY GROWTH IN THE APPLE TREE

ROOTS

In order to understand the structure of the stems used in the experiments which have been described, the progress of primary and secondary growth must be traced in the apple stem and root. There is a great lack of information on the anatomy of the apple tree, although the structure and development of the embryo and fruit have received considerable attention.

The young apple seedling has a very strong tendency to form a tap root system. In the seedling root the xylem is exarch. The primary root was tetraphyllous in all preparations which were made by the author, and this condition was found in slides of apple roots in the collection of the Department of Botany of Iowa State College. The tetraphyllous condition is shown in fig. 14.

The endodermis is prominent in the primary root when viewed in cross section because of the development of thick Casparian strips. The pericycle is a single layer of slightly smaller and more rounded cells. Lateral roots originate in the pericycle as shown in fig. 15.

The primary cortex and the endodermis are lost early in the roots of the apple. Krenke (23) mentioned that this condition exists in a number of genera of the Rosaceae. The loss of these tissues is gradual, and the time of initiation of pericyclic phellogen occurs toward the end of the first month, as evidenced by a thickening of the cell walls, by an increase in the density of the cytoplasmic contents of pericycle and by evidence of cambiform stratification in this region. The primary cortex and endodermis sometimes show evidences of tearing at only 1 inch from the root tip. This condition, which is shown in figs. 16, 17 and 18, continues up through an inch or more of the root. The outer tissues are practically entirely lost at 3 to 5 inches or less from the tip, as is shown in figs. 19 and 20. Sometimes the primary roots of a young seedling were observed to grow 2 or 3 inches before beginning to form a phellogen layer.

The point at which the primary tissues of the root begin to be lost can be seen by an external examination. The pure white region of the root extending back of the tip for a short distance
Fig. 17. (Upper left) Formation of pericyclic phellogen (P) and abscission of primary cortex and endodermis in root of Whitney Crab seedling. Seed planted in nursery on April 20, 1933. Material killed May 27. Section at slightly above 1 inch from tip and about 4 inches from crown. Abscission shown on lateral root also. X 80.

Fig. 18. (Upper right) Section at slightly over 2 inches from tip and 3 inches below crown. Primary cortex (C) and endodermis (E) are being lost due to the formation of periderm (P) by the pericyclic phellogen. X 80.

Fig. 19. (Lower left) Same root at 4 inches from tip and 1 inch below crown. Primary cortex and endodermis have been entirely lost, due to formation of periderm (P). X 80.

Fig. 20. (Lower right) Section of main tap root of plant from seed of a Salome x Jonathan cross, planted April 10, 1934, and killed June 21. Section at 1 inch below crown and 2 inches above root tip. X 80.
was found to be entirely primary tissue. The zone at which a brownish covering forms around the roots is the place where abscission of primary tissues occurs.

The pericyclic phellogen divides and forms the periderm, which consists of cork, phellogen and phelloderm.

The transition from the radial arrangement of xylem and phloem in the root to the collateral arrangement in the stem occurs in the hypocotyl. Figure 13 illustrates a cross section in the upper regions of this transition zone. The details of this root-to-stem transition were not studied.

**STEMS**

The stem of the young apple seedling has endarch xylem, and there are five procambial strands. This pentarch condition is noticeable even in stems of considerable age because of the five-angled pith.

Eames and MacDaniels (9) stated that the first phellogen is formed in the epidermis of *Malus*. Swingle (44), working with burr-knot varieties, confirmed this statement. The author has verified these observations, and an illustration of a typical example is shown in fig. 23. However, some exceptions were found. In stems of *Malus Sargentii* the phellogen often originates in the hypodermis, which is the much more common condition in woody stems. This is illustrated in figs. 21 and 22. Stems were found which had both types of phellogen. Probably this variability is without special significance and is an expression of the natural variability found in woody stem structures.

A deeper phellogen layer is shown in fig. 24. The succeeding phellogen layers begin to cut off more cortical and phloem tissue and thus form the characteristic bark of the apple trunk. The distinctive shape of pieces of bark of different sorts of trees is dependent on the manner in which these layers of cork form.

The protophloem and protoxylem elements and other early formed tissues remain in the stem for a long period after they are non-functional. Protophloem elements are difficult to locate in a cross section of an older stem and are crushed and possibly absorbed. The continued formation of periderm finally results in the complete loss of the primary cortex of the stem. According to Eames and MacDaniels (9) this does not occur in the apple until after 6 or 8 years.

**ADVENTITIOUS BUDS ON STEMS**

Careful examination of buds on the sphaeroplasts revealed that these are true adventitious buds, unlike the buds which produce watersprouts. Although the wood surrounding them was sectioned at various angles, no indication was found that the buds had existed previous to the season in which they appeared. These buds have a direct connection with the vascular system
Fig. 21. (Upper left) Phellogen formation in hypodermis (H). Shoot started from roots of *Malus Sargentii* brought into greenhouse Jan. 11, 1936, and placed in warm propagating case. From a softwood cutting inserted in sand Feb. 11. Material killed March 26. X 80.

Fig. 22. (Upper right) Portion of section shown in Fig. 21. X160.

Fig. 23. (Lower left) Phellogen formation in epidermis (E). Stem of young apple seedling started in greenhouse on March 9, 1934. Material killed April 16. X 160.

Fig. 24. (Lower right) Phellogen (P) formation in cortex (C) of young stem, together with formation of meristematic layers about masses of pericyclic fibres (F). Stem of *Malus Sargentii* from shoots grown from cutback tops of trees planted in the greenhouse on Jan. 10, 1936. Section from near base of young shoots. Material killed on April 14, 1936. X 80.
of the sphaeroplast. This is shown clearly in fig. 28, in which two buds are visible on a small sphaeroplast.

The origin of sphaeroplast has been misunderstood by some writers. The account given by Sorauer (36) is substantiated by the present investigations. They arise from a meristem which originates around a group of phloem fibers or pericyclic fibers. The formation of an active meristematic layer about a fiber strand was noticed near the cut surfaces of apple grafts by Sass (33). The same phenomenon has been observed frequently near the cut ends of softwood cuttings during the course of this investigation. A typical example is shown in figs. 24 and 25. Sphaeroplasts originate in the same way. Some exceedingly early stages were found in the bark of apple trees. As a rule the sphaeroplasts were found to grow more rapidly on the outer edge. This observation substantiates the report by Sorauer.

A young sphaeroplast is shown in fig. 26 and an older one is shown in fig. 27. Annual rings may be observed in the latter.

No other types of adventitious buds were found on stems of mature apple trees. This substantiates the observation of Chandler (6) regarding their rarity in apple trees.

**ADVENTITIOUS BUDS ON ROOTS**

An unfailing method of producing a juvenile shoot on a mature apple tree, discovered during these investigations, was to grow a shoot from an adventitious bud on a root. Shoots with this origin possess the external morphological characteristics of seedlings, and cuttings made from these shoots invariably root well. These shoots are in the juvenile growth phase. Some anatomical studies were made in order to find whether this behavior is related to the origin of these buds.

As has been demonstrated, the primary cortex and the endodermis of the root are both lost very early. In addition to forming a cork layer, phellogen lays down toward the inside of the stem a parenchymatous layer containing scattered groups of sclerenchyma. This layer of cells may be called the secondary cortex, although some anatomists have objected to this nomenclature without suggesting an acceptable alternate name. Secondary phloem and xylem are laid down by the stelar cambium in a manner so closely similar to that of the stem that often an older root in cross section cannot be readily distinguished from a woody stem, except by the absence of pith in the roots.

Roots in which adventitious buds were arising were sectioned serially, and these slides demonstrate that the buds originate in the parenchymatous cells of the secondary cortex. Examples are shown in figs. 30, 31 and 32. A typical example of a normal inactive root is shown in fig. 29. Adventitious buds originate in a zone well outside of the phloem and the masses of pericyclic
Fig. 25. (Upper left) Formation of meristem around groups of pericyclic fibers (F) near base of stem cutting of *Malus Sargentii* taken from cutback tops of trees planted in greenhouse on Jan. 10, 1936. Material killed on April 14, 1936. Sphaero­plasts arise in bark of mature trees in this manner. X 160.

Fig. 26. (Upper right) Small sphaeroplast in bark of mature Virginia Crab tree in orchard. X 13.

Fig. 27. (Lower left) Older sphaeroplast showing annual rings. From bark of Virginia Crab tree. X 10.

Fig. 28. (Lower right) Cluster of adventitious buds on sphaeroplast in bark of Virginia Crab tree. X 13.
fibers. Such bud primordia apparently do not arise at any definite points of location with respect to the system of vascular rays. The significant fact in the origin of these buds is that they originate entirely _de novo_ and are not derived from the cambial layer of the root.

**ROOT FORMATION IN JUVENILE STEMS**

A number of writers have discussed various phases of root formation in apple wood. Swingle (44) has treated the subject most fully from an anatomical point of view. His study was made with stems in the mature stage of growth. For this reason, a study of the rooting habits of stems in the juvenile stage of growth was considered desirable.

The preformed root primordia found in stems of the burr-knot varieties by Swingle (44) and others were not found in any of the stems of varieties sectioned regardless of the growth phase. Trees having burr-knots were avoided for the purpose of this study.

Smith (35), in reviewing the literature on the region of origin of roots in stem cuttings, found that this function had been ascribed to many different tissues by investigators using different species of plants. Although he worked with Begonia, his findings regarding the origin of roots are closely paralleled by the conditions found in apple stems.

All of the root primordia or roots found in cross sections of apple stem cuttings were found to be directly connected with vascular rays. This observation apparently was first made by Van der Lek (48) and was substantiated by Swingle (44), who noticed that the root primordia in burr-knot varieties occur opposite primary or secondary vascular rays, or else on leaf or branch traces. The same condition was found with the rooting of juvenile stems during the course of this investigation. Roots were sometimes found on leaf traces.

Van der Lek (48, 50) distinguished two general types of root formation; namely, morphological roots and wound roots. The roots which have just been considered belong in the first classification. They form within the stem tissues, ordinarily emerging at right angles to the vertical axis of the stem. The wound roots arise at cut ends of cuttings and sometimes apparently in masses of callus tissue. Examples of the wound root type of formation are shown in figs. 34 and 38. Roots which may be classified as morphological roots are shown in figs. 35, 36, 37 and 39. Some roots were found which were difficult to classify definitely.

Roots which originated directly on the cut ends of cuttings were found to be in direct alignment with the vascular system on the side of the stem on which they originated. The central axis of the new root was formed at the cambium of the stem of
Fig. 29. (Upper left) Cross section of root from mature tree of Virginia Crab planted in orchard in 1917. Material killed Dec. 31, 1934. X 13.

Fig. 30. (Upper right) Formation of adventitious bud (B) in secondary cortex of root taken from mature tree of Virginia Crab. Roots placed in warm case on Nov. 21, 1934. Material killed Dec. 31. X 13.

Fig. 31. (Lower left) Section from same lot of material showing formation of bud (B) in more advanced stage. Note swelling of root in region around the bud. Leaf primordia of the bud are beginning to form. X 13.

Fig. 32. (Lower right) Bud in advanced stage ready to break through the bark of the root. A number of leaves are well developed. X 13.
the cutting. Roots of this type may also apparently organize in callus masses, but the situation is not much different, for the callus cells retain a certain functional identity corresponding to that of the particular tissues from which they were formed. In large callus masses at the cut ends of cuttings, protoxylem conducting elements were observed in the callus in advance of any sign of root formation. A typical example is shown in fig. 33.

The morphological roots which arose within the stem tissues ordinarily emerge at right angles to the vertical axis of the stem. In the stem cuttings of apple, these roots originate in the cambium. The roots grow out through the cortex of the stem with considerable tearing and displacement of tissues, as shown in fig. 36.

The adventitious roots on stem cuttings of apple begin to lose the primary cortex and endodermis very quickly. This is shown in fig. 35. These roots pass through the same stages of development as those of the young seedling. The formation of roots on stems in the juvenile phase of growth does not differ in any way from the process as described by Swingle (44) for the mature phase of growth.

ANATOMICAL COMPARISON OF STEM STRUCTURE IN THE JUVENILE AND MATURE GROWTH PHASES

Studies were made in both the juvenile and mature growth phases in order to determine whether stem structure is related to the ease of rooting of juvenile stems. Priestley and his associates (29, 30, 31) have stressed anatomical explanations for the phenomena of root formation and have opposed the chemical theories of root formation first advanced by Sachs and the botanists of his school. The chemical theory of root formation, however, has received a great impetus recently because of recent discoveries in regard to plant auxins and root forming substances.

In the present investigation the sections were taken from seedlings, second year cut back seedlings, shoots from adventitious buds on roots, and in one instance from a shoot used in the budding experiment which has been described previously. Shoots from young seedlings are shown in figs. 42, 43 and 45. A shoot from a cut back seedling is shown in fig. 41, and shoots from adventitious buds on roots are illustrated in figs. 40, 44 and 46.

As demonstrated in the anatomical studies, the shoots are all nearly identical in their histological organization with respect to the primary and secondary tissues. Practically the only distinction which can be pointed out is that the mature phase stem of Virginia Crab contains more pericyclic fibers than are evident in similar stems of the juvenile form. This condition is illustrated in fig. 47. A stem from the budding experiment described previously, which exhibited the mature form, is shown in cross
Fig. 33. (Upper left) Formation of protoxylem elements (P) in callus at cut ends of cuttings made from second year cutbacks of Whitney Crab seedlings. Cuttings inserted in frame May 29, 1935. Material killed June 29. No rooting visible. X 160.

Fig. 34. (Upper right) Root formation at basal end of cutting from young seedling of Brier x Mercer County Crab. Cutting placed in outdoor propagating frame on July 9, 1935. Killed July 20. X 13.

Fig. 35. (Lower left) Adventitious root formed on cutting of Malus Sargenti taken from trees planted in greenhouse Jan. 10, 1936. Cutting inserted in propagating bench on March 11. Material killed April 14. Note beginning of abscission of primary cortex (C) in adventitious root. X 13.

Fig. 36. (Lower right) Section of same cutting. X 160.
Fig. 37. (Upper left) Rooting of cutting of Brier x Mercer County Crab seedling, placed in frames outdoors on July 9, 1935. Material killed July 20. The trees were grown from seed planted on April 13, 1935. X 13.

Fig. 38. (Upper right) Same lot of cuttings. One root is well developed and a root tip (T) is organizing in callus mass on other side of section. X 13.

Fig. 39. (Lower left) Formation of root primordium (P) in cutting of Virginia Crab, taken from shoot started from root piece placed in warm case in greenhouse on Nov. 21, 1934. Material killed Dec. 3. Note proliferation of vascular ray (R) in which the primordium developed. X 13.

Fig. 40. (Lower right) Cross section of stem of Malus Sargenti grown from root pieces placed in warm frame in greenhouse on Jan. 11, 1936. Killed March 26. Juvenile growth phase. X 13.
Fig. 41. (Upper left) Stem from second year cutbacks of Whitney Crab seedlings in field, planted in nursery April 20, 1933. Section at node. Juvenile growth phase. Killed May 29, 1934. X 13.

Fig. 42. (Upper right) Section from 1-year seedling of Whitney Crab, planted in nursery April 20, 1933. Section at one of upper nodes. Juvenile growth phase. Killed June 16, 1933. X 13.

Fig. 43. (Lower left) Section of stem of young Brier x Mercer County Crab seedling planted April 13, 1933. Section near node. Juvenile growth phase. Killed June 12, 1935. X 13.

Fig. 44. (Lower right) Cross section from stem grown from adventitious bud on root of seedling of Tolman Sweet, planted in greenhouse May 25, 1934. Section at node. Juvenile growth phase. Material killed June 18, 1934. X 13.
Fig. 45. (Upper left) Cross section of stem of young apple seedling planted in nursery on April 20, 1933. Killed May 27, 1933. Juvenile growth phase. X 13.

Fig. 46. (Upper right) Section through shoot grown from adventitious bud on root of seedling of Tolman Sweet planted in greenhouse May 23, 1934. Section between nodes. Killed June 15, 1934. Juvenile growth phase. X 13.

Fig. 47. (Lower left) Section through shoot from top of mature Virginia Crab tree in orchard. Note development of clusters of pericyclic fibers (P). Section at internode. Material killed June 29, 1934. Mature growth phase. X 13.

Fig. 48. (Lower right) Shoot grown from bud taken from juvenile stem grown from root piece of Virginia Crab. Bud was set on Jonathan tree in greenhouse in fall of 1934. The bud started into growth in early spring of 1935. Material killed May 17, 1935. Mature growth phase. X 13.
section in fig. 48. Cuttings from similar stems which exhibited a relatively undifferentiated condition anatomically nevertheless did not root.

DISCUSSION

An understanding of the distinctions between the juvenile and mature forms in apple trees is essential to a complete comprehension of the possibilities of regeneration in various types of apple wood. The existence of distinct growth phases has been entirely overlooked by the workers experimenting with vegetative propagation of the apple. This oversight has caused an extraordinary number of futile attempts to root stem cuttings of the mature growth phase. Failure to understand the changes which occur in the various types of apple trees has also caused some very inadequate interpretations of the data collected in past researches.

The failure to recognize phases of growth in apple trees in the past has probably been due to the slight and inconspicuous external differences between various growth phases in the ordinary commercial apple varieties. Marked leaf dimorphism is noticeable only in some of the species of Asiatic crab apples.

The work of Gardner (10) on the rooting of stems from young seedlings of woody plants suggests that all seedlings pass through distinct juvenile and mature growth phases. The idea that different phases of growth exist in plants only when they are evident to the eye must now be revised.

The results of the present study, together with some evidence from the literature, show that the juvenile growth phase may change to the mature form gradually through a series of transitional stages. In young seedlings the first noticeable change often occurs immediately after the first period of dormancy. The ability of stems to form roots begins to wane in young apple seedlings early in the second season of growth.

Buds produced on roots invariably grow into shoots which resemble seedling stems in external appearance and which can always be rooted easily as softwood cuttings. The petioles from such shoots likewise are capable of forming roots at any point.

In the production of these juvenile shoots two distinct anatomical factors may be operating together. The first factor is the fact that adventitious buds formed on roots have no connection with the primary meristematic tissue of the parent plant. The shoots growing from such buds repeat all of the seedling ontogeny and consequently might be expected to possess the juvenile form.
A second factor may also predispose such a shoot toward juvenility. Goebel (13) and other writers believe that the juvenile form tended to linger in the lower part of tree trunks and in the roots. The results obtained during this investigation on the rooting of cut back seedlings substantiate this view. Thus a shoot from a root is often, if not always, produced by a part of the parent plant which retains for an indeterminate length of time the juvenile character which it undeniably possessed at an earlier stage of growth.

In considering the possibilities of producing juvenile growth from older trees, a clear distinction must be made between grafted trees and seedlings. In grafted trees the cion is ordinarily made from wood of shoots in the mature growth phase. No part of the cion has ever passed through the juvenile condition. In the case of a seedling, the wood at the base was originally in the juvenile stage of growth. Even after the new growth is entirely mature in character, the juvenile condition may persist at the base. The growth from nodal buds near the base of seedlings may be in juvenile form. Practically all writers on the subject of juvenile forms have mentioned that juvenile shoots on mature trees are found most often near the base. Michurin (27) noticed that when one of his hybrid seedling fruit trees was headed back to the ground the shoots from the base had a habit of growth very different from that of the mature tree. He noticed that in time these sprouts from the base assumed the mature form. When a tree propagated by conventional vegetative methods was headed back in a similar way, the sprouts were always like the mature form. He thought that the juvenile shoots from the seedling trees were in some unknown manner a result of the process of hybridization and that the characteristics displayed were reversions to one of the parental forms. Michurin did not understand the fundamental differences between the two different types of trees in his experiments, or the conditions under which the different phases of growth are produced.

The methods used in this investigation for producing juvenile shoots on older trees are not to be considered as necessarily the only possible means for producing this result. Goebel (13) stated that environmental conditions, particularly those which are unfavorable, tend to cause the production of juvenile shoots. There is apparently good evidence to show that the duration of the juvenile forms of the lower vascular plants may be greatly prolonged by regulating the environment. No instances were noticed in apple trees of reversion from mature to juvenile phase which could be attributed to environmental conditions. Time and facilities did not permit a study of this phase of the
problem. It is quite possible that in the future such conditions as temperature, light and other environmental factors may be linked to the duration of the juvenile form. In the future, juvenility may possibly be produced artificially in mature plants.

The basis for the extreme ease of rooting of the juvenile shoots as contrasted with the usual difficulty of rooting of mature stems is still unexplained. The present investigation suggests that rooting is dependent on some unknown biochemical differences in the meristematic cells of stems in the various stages of growth rather than upon any feature of anatomical structure.

Differences in the anatomy of stems or roots of apple varieties may be measured by a statistical study. Beakbane and Renwick (5), using a photomicrographic technique for computing the relative percentages of various types of tissues in roots, were able to distinguish clone roots of a number of varieties from the roots of the East Malling IX rootstock. The roots of this stock had a large proportion of vascular ray tissue and a small proportion of vessels when compared with the varietal roots. There are several objections to the validity of such a method of study as applied to this problem. Apple stems and roots are in a condition of constant development and exhibit a succession of anatomical differences at various positions along the stem or root at any one time. Environmental conditions are also reflected by changes of internal anatomy. Nightingale (28) was able to cause marked differences in the internal structure of roots of both peach and apple by using variations of temperature alone. All of these factors complicate efforts to recognize the growth phase by means of the internal structure.

Several lines of evidence have been presented which show that the causes for the change of growth phase are not related to changes in anatomical structure within the plant. A twig may have only primary wood and be in the mature growth phase. At the apical meristems of twigs on a mature apple tree, the young growth at first has only the primary structures. Nevertheless, the character of the growth at these tips is always in the mature stage, and the stem cuttings which are made from these tips will not root.

Since the juvenile form is transitory, the stems in the mature stage of growth may have a much higher degree of lignification than can be found in any stem in the juvenile growth phase. With this one possible exception, the stems of the juvenile and mature growth phases are remarkably similar in internal structure. Furthermore, a stem in the juvenile phase may be much more advanced in internal development than one in the mature growth phase. The growth phases are not necessarily related to the stage of organization of tissues within the stem. There
are apparently no great differences in the relative proportions of the different types of tissues present in the two different types of stems.

The growth phase influences the external appearance of some plant species very greatly, while in others it does not have this effect. Possibly similar differences are found in the internal structure of the stems. In order to find any constant and significant distinctions between the different growth phases of the apple, a very detailed investigation of the physiological and biochemical conditions of the meristematic cells of the different types of stems may be necessary.

The occurrence of pre-formed root primordia and burr-knots in mature stems of some varieties, and also the success of Hitchcock and Zimmerman (17) in rooting stems of mature growth of a number of apple varieties by means of treatments with chemical root forming substances, both indicate that the necessary tissues and anatomical structures for root formation are always present and ready to act when the proper stimulus is given. The change of growth phase is possibly due to the formation of certain chemical substances which begin to be formed at a certain stage in the development of a new plant from a seed, or from a bud which has not originated directly from the cambium and vascular system of the mature plant. After a certain period these substances may cause the plant to pass through a stage of development corresponding to the change from adolescence to maturity in animals. According to Goebel (13), the juvenile stage, with the exception of a few conifers, cannot produce flowers and seed. This fact suggests that juvenility and the capacity for easy regeneration may be inhibited by biochemical agents which bring about the capability of reproduction. Investigators have not yet ascribed the development of sexual maturity to the action of hormones, but it is possible that a chemical compound of this type may be responsible for the change in growth phases and sexual maturity in plants.

Perhaps the greatest objection to a chemical theory of growth phase change is the extremely localized nature of the juvenile condition. Thus a part of the plant may remain juvenile long after the rest of the plant has become mature. If this effect is related to polarity and the substances are transported largely in one direction, however, a chemical explanation would still be possible. The tendency for the base of the stem to remain juvenile for a time is common in woody plants.

The principle developed in these studies in regard to the existence of two very different phases of growth in apple trees can be applied to the practical problems of vegetative propaga-
tion. Doubtless some of these applications cannot be visualized at present. Certain methods developed during this investigation are promising from a practical standpoint. The abundant production of juvenile shoots or root pieces enlarges the possibilities of using such juvenile shoots for propagation. The power of root formation also exists in petioles as well as in stems. The success of mound layering of certain vegetatively propagated rootstocks has probably been due, in part at least, to the numerous juvenile shoots which are doubtless produced by certain practices in layering. The above contributions to the vegetative propagation of the apple may be greatly augmented and advanced by recent developments in the use of chemical root forming substances.
LITERATURE CITED


Michurin, I. V. Itogi Shestidesiatilenikh Rabot. OGIS Selkhozgiz, Moscow. 368 p. 1934.


(49) Van der Lek, H. A. A. Overzicht over eenige oude en nieuwe publicaties betreffende de anatomische structure in verband met het bewortelingsvermogen. 16 p. Laboratorium voor Tuinbouwplantenteelt. Wageningen. 1928.


