VEGETATIVE PROPAGATION PROBLEMS WITH SUGAR MAPLE

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Both sexual and asexual propagation are essential procedures in the tree-improvement work being done with sugar maple at the Burlington Unit of the Northeastern Forest Experiment Station in cooperation with the Agricultural Experiment Station at the University of Vermont. During the past 6 years significant progress has been made in these fields. However, in the development of practical techniques for vegetative propagation, much remains to be done.

Asexual or vegetative propagation is an important and necessary tool in the study of inheritance of many sugar maple tree characteristics such as sap yield, wood quality, and flowering phenomena including dichogamy. The propagation techniques that offer promise include budding and grafting, air-layering, and the rooting of cuttings.

Our efforts have been concentrated on the latter two, principally because they do not involve the unknown but possible stock-scion interactions that could occur in grafting and budding. These interactions will eventually be determined, but at present we believe clues to the inheritance of such characteristics as percent of sugar in the sap can more readily be determined with clonal material growing on its own roots.

Nevertheless, we have done a limited amount of budding and grafting, In one small experiment, three out of six side grafts made in the crowns of 5-year-old sugar maple seedlings in mid-August were successful. One of the "takes" was obtained with succulent material; that is, both scion and stock were recent new growth. All leaves had been removed from the scion. The other two "takes" were with older, hardened material. In one of these, all leaves had been removed from the scion; in the other, two pairs of leaves had been left.

These successful side grafts in the crowns of young seedling maples suggest that it may also be possible to side-graft in the crowns of mature trees after heavy pruning to stimulate new growth. This technique might be employed in reciprocal grafting among mature trees of different flowering habits, thereby enabling us to learn more about the influence of environment upon flowering behavior.

We also have tried some budding, both in the field in late August and in the greenhouse in mid-May. Results have not been promising: one "take"out of 52 attempts in the greenhouse and one out of 24 in the field, We believe that the field budding failed because it was done too late in the season. In the greenhouse budding, the bud sticks may have been in poor condition: they had been collected March 12 and stored at 36°F; for 68 days in plastic bags before being used.

Considerable success has been achieved with air-layering of sugar maple at the Hopkins Experimental Forest at Williamstown, Massachusetts. Frank Cunningham and Dick Peterson have obtained 10 to 100 percent rooting by cutting

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a 1-inch-wide girdle, wrapping the girdle with sphagnum moss, and then covering the moss with clear polyethylene plastic. They have overwintered about 30 percent of the rooted air-layers by lining them out in the nursery under shade and mist soon after roots were observed inside the plastic wrapping. In this, as has been found in working with rooted greenwood cuttings, more attention to the details of treatment after rooting will be required for better survival.

Early results of experiments in propagating sugar maple by greenwood cuttings were reported by Gabriel, Marvin and Taylor[2] in 1961. Most of our research in vegetative propagation since that time has been a continuation and expansion of their initial work. The methods used and results obtained in our investigations were as follows.

EXPERIMENTS IN PROPAGATION

The physical set-up at the University of Vermont consists of an electronically controlled mist system in an outdoor rooting bed located between two greenhouses. The bed is completely covered with polyethylene plastic over a wooden framework, except for a 2-inch wide opening along each side between the inverted V-roof and the sides.

Incandescent 100-watt lamps are located 2 feet above the cuttings at 8-foot intervals down the center of the bed. These lamps are controlled by time-clocks to provide whatever photoperiod is desired.

In the past, 50-percent saran-cloth shade material has been placed over the plastic roof. However, after observing the successful rooting obtained on several shrub species under mist without shade by a horticulturist at the University of Vermont, we question the necessity of shading sugar maple cuttings.

Cuttings are usually collected from mature trees during the last 2 weeks of June, At this time, the terminal buds have formed and the leaves are fully expanded, The cuttings are transferred in plastic bags from the field to a headhouse adjacent to the rooting bed. There all leaves except the terminal three or four are removed and any hardwood that may be present on the base of the cutting is cut off. The basal end of each cutting is then wounded by removing strips of epidermis and phloem about an inch long on two opposite sides. After preparation, the cuttings are stuck in flats containing peastone gravel and perlite.

While in the rooting bed the cuttings are hand-sprayed twice a week with foliar applications of a complete, water soluble fertilizer. Future plans call for applications of nutrients intermittently through the mist system. The cuttings are illuminated for an hour in the middle of the dark period to delay the onset of leaf abscission until lateral roots have formed or until frosts occur. From our observations, it appears that neither extended day nor an interrupted dark period prevents the onset of leaf abscission once the cuttings have been subjected to one or two frosts.

Roots appear on the cuttings in 8 to 10 weeks, If the rooting medium is well drained, lateral or side roots will be present on most cuttings 2 to 3 weeks later. At that time, the shade is removed and the mist applications

[2] Gabriel, William J., James W. Marvin, and Fred H. Taylor. Rooting greenwood cuttings of sugar maple--effect of clone and medium. U. S. Forest Serv, Northeast, Forest Expt. Sta. Paper l24, 14 pp., illus., 1961.

are gradually reduced in frequency and duration over a 2-week period by changing the settings of control dials on the electronic mist-control box; then the applications are terminated. To keep the media moist, subsequent watering is done either with a hose or by manual control of the mist system.

The plastic sides of the rooting bed are removed when the mist system is shut off, and the plastic roof is removed soon after the first or second frost, when it begins to break up. The leaves then begin to show fall coloration and subsequently drop off. After leaf fall the cuttings are transferred in their flats to a walk-in refrigerator where they remain for at least 1,000 hours (about 42 days) at 33°F. The flats are watered occasionally during this period to keep the media moist.

After the chilling period, the cuttings are transferred to a greenhouse in which the temperature is usually maintained at 50° to 55°F. for a week and then gradually raised to 65° to 70°F. The cuttings that resume growth are lined out in the nursery under 50-percent shade in early spring, as soon as it is possible to work in the nursery.

THE MAJOR PROBLEM

At present, our major problem is not to root cuttings, but the failure of many rooted cuttings to resume growth after the chilling period. Unfortunately, all the material used in past overwintering studies had first been exhumed to observe the type of roots formed under different treatments in the rooting bed. This root disturbance may have been one of the contributing factors to overwintering failures.

To determine when lateral roots have formed without disturbing the cuttings, we are developing techniques for rooting them in perlite in perforated plastic bags. Last year, we found no significant difference in rooting percentage or type of roots between cuttings stuck in perlite in plastic bags and those stuck in flats in perlite and peastone gravel.

We do not believe that the failure of cuttings to resume growth after chilling was due to improper chilling temperature or to insufficient length of chilling period. Olmsted[3] and Kriebel and Wang[4] found that approximately 1,000 hours of chilling are sufficient to break bud dormancy in sugar maple seedlings. One of our studies indicated that such a period also is adequate for rooted cuttings.

In this study, two rooted cuttings of each of 15 sugar maple clones were transferred to a greenhouse from a 33 refrigerator after 21, 56, 75, 92, and 124 days of chilling. No cuttings resumed growth after less than 21 days (504 hours) of chilling. Maximum growth resumption occurred after 56 days of chilling (1,344 hours); longer chilling was of no benefit.

In another study cuttings were overwintered at four different temperatures: 15 to 25 rooted cuttings from each of 36 clones were stored at 20°, 28°, and 33°F, and 137 cuttings representing 7 clones were stored at 38°. As expected, no cuttings survived storage at 20°. The respective survival of cuttings

[3] Olmsted, C. E. Experiments on photoperiodism, dormancy, and leaf age and abscission in sugar maple. Bot. Gaz. 112 (4): 365-393, 1951.

[4] Kriebel, H. B., and Chi-Wu Wang. The interaction between provenance and degree of chilling in bud break of sugar maple. Silvae Genetica 11 (5/6): 125-130, illus., 1962.

stored at 28°, 33° and 38° were 5.8, 9.1 and 32 percent for cuttings with lateral roots, and 0.9, 0.9 and 0,0 percent for cuttings that had no lateral roots. Thus, the largest percentage survival was obtained with cuttings that possessed lateral roots and had been stored at 33°.

The importance of lateral roots is clearly shown again by the following cases. During the first week of September 1960, cuttings were examined in the rooting bed shortly after the mist system had been shut off. All cuttings were labelled as to whether or not they possessed lateral roots. The flats of cuttings remained outdoors until December 1. At that time the root systems of the cuttings were examined again. We found that 383 percent of the 391 cuttings that lacked lateral roots had died, as compared with only 17.9 percent of the 296 cuttings that had lateral roots.

Next the cuttings that had live root systems were stored at 33°F. for 89 days; then they were transferred to a greenhouse. After 39 days those cuttings that had resumed growth were tallied by root type. Twenty-two percent of the 240 cuttings with lateral roots had resumed growth, as compared to 4 percent of the 225 without such roots.

These findings show rather conclusively that we should try to induce the cuttings to develop lateral roots in the rooting bed. However, because only about 22 percent of these may resume growth, according to the above study, lateral roots evidently are but one of several requirements for successful overwintering. Additional research is needed to find how to increase survival percentages.

We believe there are many physiological factors undergoing change in the cuttings during the rooting, hardening-off, and chilling processes up to the time for growth resumption the following spring. These physiological factors will be the focal point of our attack in trying to solve the problem of over-winter survival in rooted sugar maple cuttings.

DISCUSSION[1]

SANTAMOUR - You presumably were dealing only with the northern ecotype of white ash, and probably encountered no polyploidy. Do you know of any work similar to yours that has been carried out on the southern and intermediate ecotypes?

HUNT - I assume that I worked only with the northern ecotype. These study areas are all within the range of the northern ecotype as described by Wright and all individuals seemed to fit his description. He also stated that this ecotype was diploid and fairly uniform over its entire range. As I made no attempt to study the chromosomes for polyploidy, this study should be applied only to our northern ecotype. Until someone analyzes the trees in my study areas for polyploids, I'd assume my work can only apply to diploids. Wright's work with seedling variation within the eastern portion of the natural range of white ash included a study of chromosome variation. However, polyploids were found only in the southern and intermediate ecotypes.

[1] Transcripts of the discussions were sent to each of the participants for editing with the specific request not to change the contents of their remarks.

SANTAMOUR - Well, I have one further observation to make on that I recently completed a study that has been published in the Bulletin of the Torrey Botanical Club, and I found that I could distinguish between polyploids and diploids on a morphological basis on the shape of the leaf scar, and I have called these polyploids Fraxinus biltmoreana,

HUNT - Is this a better means for determination than pubescence and the associated characteristics usually connected with biltmoreana?

SANTAMOUR - Pubescence is not considered a distinguishing character.

HUNT - The distinguishing character then appears to be polyploidy throughout the range of the ash?

SANTAMOUR - In the intermediate and southern ecotypes you can distinguish between polyploids and diploids on a morphological basis by visual examination.

HUNT - Fine, I have a question for Mr. Gabriel. What criterion do you use to estimate sap volume during the season? I realize that sap volume is dependent on many things, such as site, season and crown development, but throughout the season, no matter what reason given for a sap run, the tree, or that side of the tree that runs slowly, generally tests higher than the tap hole that runs rapidly. If this is the case, a sweet tree or tap hole can yield less sugar than an outstanding source but gives us the indication it is the outstanding tree. Would you care to comment?

GABRIEL - Yes I would. I would like to point out that the majority of the trees which we test usually flow at the same rate, or close to the same rate. This is helped by testing during periods when the sap is really running.

I will agree with you that slow running sap has a tendency to evaporate before it reaches the end of the spile. Therefore, there is a good chance of evaporation of water to occur and consequently you may get an erroneous reading if this is not taken into account.

In making our final selections we propose to test all of the taps around the tree if there are more than one, If there should be considerable variation from one tap to another on the same tree we will take an average of all of the taps, If we think the variation is too great we will drop this tree rather than put it into our catalog of selected trees:

In trying to maintain our testing procedure at the most uniform level possible, we also take the taps from the same side of each tree. In other words, if we are proceeding from south to north we would take sap from the taps on the south side of the trees.

As far as the correlation between rate of flow or sap volume and sweetness is concerned, I might say that we have had some very poor sugar producing trees which ran relatively slowly, and I would not say that trees which have a slow rate of sap flow are necessarily sweet. I might add that information on the correlation between sap flow and sap volume with sweetness may be forthcoming in the near future from the University of Vermont Agricultural Experiment Station.

HUNT - You mentioned various factors for slowdown, and I just wondered if you had run into bacteria.Could that be the reason for the other hole?

GABRIEL - Yes, the tapholes dry up near the end of the season since the bacteria has a tendency to clog them, The pores of the wood close up and consequently there is no sap flow. When this happens we go to the back side of the tree and drill our own holes. We have a piece of tubing with the same outside diameter as the drill bit and by inserting this tubing into the new hole we have a temporary spout which gives us a point from which we may collect the drop we need for testing.

Regarding pellets, it has been found that the use of paraformaldehyde pellets will prolong the life of the taphole by holding back the growth of bacteria. As far as the effects which the pellets have on the solids in the sap, we haven't been too concerned with this. Normally, if paraformaldehyde pellets are used they are used throughout the bush, and I would suspect that any error due to pellet would cancel out. Personally, I don't feel that the pellet contributes enough to the solids in sap to be of any significance.

FORD - Can you tell us anything more about the variation in volume of sap and percent of sugar in individuals over an entire season?

GABRIEL - Yes, our data shows that sugar content in sap drops from the beginning of the sugaring season to the end. As an example, one of our candidates for selection last year started off at 6.5 percent and in about 35 days dropped to 5.4 percent. Another tree started off at 8 percent and finished the sugaring season with the sap sugar content of 4.5 percent. Is this what you wanted to know?

FORD - Yes. You have no explanation for this, I presume.

GABRIEL - Probably Al Snow could answer this. Do you want to make any comments on this, Al?

SNOW - I would say, Bill, that the reason why the sugar content of the sap varies, or rather the exact correlations between temperature, time of day, environmental factors, and internal physiological factors, are unknown. For For sure, it varies during the day from the time the sap starts running to the end of the day; it also varies throughout the season. However, Bill's sampling technique, that he didn't get a chance to explain as such, allows him to take the sap over a very short period of time, so variation in day or season does not influence his results.

GABRIEL - One man can sample a bush in about two and a half hours, and two men can generally do it in about an hour and fifteen minutes. We feel that during this short period., any changes in sugar percentage, due to differences in time of day, are of no significance.

FUNK - It looks to me like you've got one advantage over those of us who are selecting on the basis of growth rate or form in that maples of all ages and sizes do have sap, and it might be an opportunity to do some early progeny testing, Do you plan any microtapping of young seedlings, or do you expect to find any difference?

GABRIEL - Probably this would be a good place to comment on our future plans for improving our technique for testing young trees. We've been trying to do testing of young seedlings out in the open, As you know, sap flow is influenced by temperature conditions and the younger the trees are the more susceptible they are to changes in temperature which leads to flow stoppage, You might begin testing a group of trees under ideal. conditions, then you get just a bit of a cold breeze and that's it. The sap flow stops and the entire testing procedure must start all over again.

We would like to control the sap flow environmental factors If we could eliminate cold winds this would prevent interruption of our testing for sugar content. We would also like to be able to cycle the young trees when we want to, perhaps three or four times a year, and get sap to flow when we want it rather than having to wait for nature to provide the proper natural conditions for this to happen. We might be able to do all this with a growth chamber, or something built along those lines.

Mr. Atkinson, our physiologist, has found that sap can be extracted from very thin stems using a hypodermic needle. When one of these fine needles is pushed into the stem one can get a drop of sap for testing from a year-old seedling. But you must have the proper conditions that will cause sap flow and here again we are dependent on natural conditions. As I have said previously a slight cooling breeze will shut these youngsters off at the drop of a hat.

WINIESKI How do you explain the effect of the age on selection?

GABRIEL We like to compare our candidates for selection with surrounding stand ards that are relatively uniform in size. We are assuming since these are forest grown trees, growing close together, that they are about the same age., I realize that this may not necessarily hold true. Granted, we do not always get the uniform stand in which to work.

As you saw in the last photograph, the trees are probably even-aged. They are located in one corner of the bush and they are all about the same size. I could have drilled them with an increment borer to get their exact age, but I would rather not get tied up with such measurements until the final screening comes up.

In our final screenings we may look into the effects of age by boring all the trees, that is, candidates for selection and their standards.

Since I dont seem to have a very reliable answer for your question on the effect of age on selection; Bob Morrow, how about commenting on the effect of age of a tree on sugar content, assuming that other factors that might influence sugar content are fairly uniform.

MORROW - Well, I dont know of any effects, Bill. And I don't know how you measure the age of all these trees anyway. It would be pretty difficult to do.

GABRIEL - Yes, I believe it would be rather time consuming and it would be questionable iether or not we would get anything of value from such a discussion at this time. I believe that there has been a study completed out in Michigan in which the effects of a whole raft of variables on sugar production were taken into consideration. I think that age was one of them. At this point it seemed to me that they found that age was not of too great importance.

LARSSON - Bill, have you got a special instrument, that is a refractometer for testing the sugar content of sap?

GABRIEL - Yes, we use a refractometer in making sugar tests. I believe it was borrowed from the chemists who use it in testing the density of liquids or solids in liquid.

MORROW - Bill, I have a few questions. I'm a little bit concerned about some of the sampling procedures. When you find a sweet tree, consider the origin of this tree. Would there not be likely to be a group of sweet trees from original sweet parents? How do you know when you have a group of four trees that are % and one tree that is 8% that these trees are not all genetically approximately the same and the 8% exists because of a difference in environment, I dont expect the answer to these questions at this time.

The second question is: assuming that there are groups of sweet trees, how is one going to locate them in the woods with this sampling along the contour? It is very likely that these groups are likely to appear anywhere.

GABRIEL - Group-wise selection, based on what we know about environmental effects on sap sugar, seems like a poor bet at this time. I would like to use an illustration to answer the first part of your question. Let us say that we were running a sample line through a sugar bush and we were getting readings between 2.5 and 3.0 percent sugar content. If we were to come into a cluster of trees, say 15 or so, that read 4.0 and 5.0 percent I don't think I'd get too excited about them. I would be suspicious of environment acting here to give us this increase.

With our present knowledge of effects of environmental factors on sap sugar, we can only hope to minimize these factors when making our selections. After doing this, we assume that most of the difference between a selected candidate and the surrounding standards is genetic in nature.

The second question you ask is in part answered by what I have just said. Right now, I'm not interested in groups that appear high in sap sugar, It's too difficult to assess the effects of environment, I would be more interested in tree-to-tree variation within such groups since here we could make such an assessment.

MORROW I appreciate this, but when you use the criterion of only 30%, this 30% may be due to environment as well as heredity.

GABRIEL - That depends on how you look at it. In our method of selection we try to minimize the effect of the environment. Looking at this photograph, the four standards surround the selection candidate which here is 70 percent sweeter than the average of the standards. Would you say this 70 percent advantage is mainly due to environment?

MORROW - Well, that wasn't what I said. Assuming a group of sweet trees runs 5% and one runs 30, 40, even 50% higher to 8%, you cannot be sure that this is due to heredity. I am sure that you're interested in this 8% tree, but I would also be interested in these 5% trees as compared to a 3% tree among 2% trees, That's the point I'm trying to make.

GABRIEL - Well, we were thinking about that too. Let us say that one sugar bush sample averages 3 percent, and a second averages 5 percent. If between-tree differences of the same magnitude are found in both bushes I would select in the sweeter stand which averages 5%„ I believe that it would be negative thinking to select a tree which is 50% sweeter than its standards in the 2% bush over another which is S0% sweeter than its standards in a 5% bush.

MORROW - I have a few other questions, Bill. Have you found some real sweet trees of 1045% that are not full of rot? So many reported sweet trees have rot. Is it possible that some sugar is produced by other than the tree itself? The last question is: how about sap flow? Economic studies show that sap flow is just about as important to the sugar producer as the sweetness, and this 15% sweet tree wouldn't be worth anything if it didn't produce any sap.

GABRIEL - To answer the first question on the influence of rot in trees on sugar percentage, I have some pictures which I had taken which unfortunately did not arrive in time for this meeting or I could have shown you that rot is not necessarily associated with sweetness, nor is a full crown. It is my belief

that if a tree is genetically not sweet, regardless of what sort of environment or treatment is given it, it will remain not sweet. There may or may not be a relationship between rot and sweetness but I don't believe enough data has been collected on this to decide one way or the other.

MORROW - Well, I just brought this out as important.

GABRIEL Regarding sap volume and sweetness, if we uring sap volume in the woods we would be glad to start investigating this possible relationship However, this measurement is difficult to make I believe the Vermont Agricultural Experiment Station is working on volume production at the present time and I think they are also looking into volumesweetness relations, as I mentioned earlier.
I' 11 grant you that a 15 percent tree isn't much good to the sugar makers if it produces only a few gallons of sap. However, I believe you would be premature in saying that unusually sweet trees produce significantly less sap

CONNOLA Have you looked into the possibility of root graft among these groups of sweet trees?

GABRIEL No we haven't formally, but there have been several studies done on natural root grafting. Within the pines, where most of this work has been done, there seems to be a lot of natural root grafting taking place. In our own nursery, we have found seedlings with good, solid root grafts that occurred naturally. If there is natural root grafting going on in our sugar bushes, and I don't see why there wouldn't be, then this would be of further help in minimizing environmental factors below ground since the selected candidate and the standards would be feeding on the same root system.

LARSSON I would like to ask Oscar a question., Is it advisable to establish a desirable hard maple on its own root system following budding on to nursery stock? We are working with high quality silver maple phenotypes and we are afraid that the growth of the budded trees might be effected by the root system of the grafted stock. To overcome this, we thought that it might be advisable to plant the budded trees sufficiently deep so that they will produce their own root system. I am curious if you get as good results with budding as with cuttings. The advantage of cuttings is that they are on their own root system whereas with budded stock they are on the root system of the grafting stock, Unfortunately we have had trouble with the functioning of the root system developed by silver maple cuttings.

ATKINSON Well, first of all, I would like to say that I've got to obtain better results in my budding before I can bud on to anything. Your technique might have possibilities, but I don't know whether or not new roots would form on the sides of stems of either silver or sugar maple while they already have a root system.

WILLITS How old is the oldest cutting on hand now?

ATKINSON I have some that were rooted in 198 that would be five years old. They are still growing well.