ETIOLATION AS AN AID IN PROPAGATION

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Etiolation results from the exclusion of light from plants or plant parts. In this discussion we are concerned only with the effect of excluding light from that portion of the stem from which roots may develop. Effects of light, or absence of light, on chlorophyll formation or other changes in the leaves are not a part of this discussion, because in the use of etiolation for propagation, leaves are allowed to develop normally in the light above the etiolated portion of the stem.

One of the externally visible effects on etiolated stems is blanching, resulting from the disappearance or lack of chlorophyll. *Etiolation is sometimes defined in terms of this blanching effect*, but in relation to propagation, the presence or absence of chlorophyll in the stem probably is of no significance.

Etiolation is generally thought of in relation to deliberate exclusion of light during stem development, or for a period of time prior to the rooting process, but exclusion of light in the normal procedure of placing the base of the cutting in a rooting medium probably also is a factor in the rooting of many kinds of plants. For other plants an opaque rooting medium is not essential. The primary function of the rooting medium is to support the cutting in an environment with a favorable balance of moisture and aeration, and rooting of many plants can be accomplished if the cuttings are suspended in a suitable atmosphere without excluding light. Aerial roots on such plants as philodendron, and the juvenile form of ivy, are examples of the capacity of some plants to initiate and grow roots in the presence of light.

On the other hand, a number of observations have been made of the inhibiting effect of light on rooting. Sachs (17) reported that cuttings of *Cactus speciosus* (sic) kept in the dark for several weeks formed adventitious roots, while cuttings kept in the light for the same length of time did not. He made similar observations on cuttings of *Tropaeolum majus* and *Hebe speciosa* (*Veronica speciosa*).

Galston (4) cultured asparagus stem tips in nutrient agar containing indoleacetic acid (IAA) and found that they rooted only in darkness. Hackett (7) noted that shoot tips of the adult form of ivy (*Hedera helix*) rooted in the presence of IAA in low light (50 f.c.) or darkness but not in high light intensity (500 f.c.). Rooting of juvenile tips was increased by reduction or exclusion of light. In all of these instances, the entire cutting was in either darkness or light, which may involve a different effect than exclusion of light from only a section of stem.

Mevius (12) reported that rooting of *Tradescantia* cuttings was inhibited when the bases were exposed to light. Once the

roots formed, however, they grew well in the light. The adult form of ivy normally does not produce aerial roots, but when light was excluded from a portion of stem, numerous roots were formed (7).

Excluding light from the stem for a period of time before taking cuttings influences rooting of some plants. Regel (14) reported this effect on rose from mounding soil around the bases of the shoots for some time prior to taking cuttings.

Wrapping a portion of stem of clematis with black paper 10 days to 3 weeks before taking cuttings resulted in roots appearing at the nodes instead of only internodally, and more rapid rooting in the internodal region (19).

Herman and Hess (8) studied the effect of excluding light from stems of red kidney beans for 3 weeks before taking cuttings and reported over 5 times as many roots from etiolated as from non-etiolated cuttings after 4 days, and nearly twice as many after 8 days. After treatment with indolebutyric acid (IBA), the difference between etiolated and non-etiolated cuttings was even greater.

The greatest response to exclusion of light is from etiolation during the early stages of shoot development. This is the response that has been of the greatest value in propagating plants that are difficult from ordinary cuttings.

In the stool method of rooting fruit tree rootstocks, established plants of the desired variety are cut to the ground after one season of growth, and as shoots grow in the spring, soil is mounded around their bases. The most shoots are produced when the crown of the plant is left exposed to light until the shoots have made some growth, but rooting is best if the plant is covered lightly with soil before bud break and more soil is added at intervals as the shoots grow. In this way the basal portion of the shoot, the portion from which roots will develop, is never exposed to light. This procedure is essential for success with most plum stocks, vigorous quince varieties, and with pears and cherries. Most apple stocks will root well if the shoots are allowed to grow in the light and mounded up later (9).

The etiolation method of trench layering was developed at the East Malling Research Station in England for propagating those stocks that do not root well in severely pruned stool beds (9). An essential step in this method is the covering of layered stems with 1-2 inches of soil just prior to bud break in the spring, with more soil added as the shoots emerge.

Lambourne (10) used layering for a number of plants in Malaya, but found that covering the buds with soil before they began to grow was fatal to many of the evergreen tropical species. He therefore made the first application of soil when the new shoots were 4-6 inches high, covering them to half their height. Even this delayed exclusion of light was beneficial, as was noted earlier for rose, clematis and bean.

A different version of the etiolation principle was used

by Gardner (6) for rooting cuttings from 'McIntosh' apple trees. He wrapped black tape as close as possible to the growing tips of shoots on the tree so that light was excluded from the differentiating stem. Cuttings taken the following spring rooted from the eitolated portion. Herman and Hess (8) increased rooting of 3 hibiscus varieties by the same procedure using black plastic as a wrap. Gardner later developed a procedure of covering the shoot tip with a tube of black paper through which the shoot grew, leaving the basal portion in darkness.

Blackie *et al.* (1) used similar techniques for rooting camphor cuttings. Reid (15) enclosed branches of a camphor tree in an opaque bag and found that rooting was accelerated on cuttings taken after 2 to 4 weeks.

Working with avocado, Frolich (2) developed an etiolation method using plants in containers. The procedure as finally developed consists of placing the plants in a dark chamber until new shoots grow to a length of about 3 inches. They are then moved to the glasshouse, and a tar-paper collar is placed around the shoots and filled with vermiculite to exclude light. After the shoots grow out and develop normal leaves in the light, they can be cut off and placed in a cutting bed to root, or girdled and left to root in the vermiculite-filled collar. The method has been used extensively for propagating avocado varieties and rootstocks for experimental work.

Several studies have been conducted in efforts to explain the effect of etiolation on root initiation. Gardner (6) working with apple and Frolich (3) with avocado both determined that the shoo tip could be exposed to light without interfering with the etiolation effect as long as the stem immediately below the tip was in darkness. If the apple shoots were taped only to within an inch of the tip instead of as close as possible, there was a reduction in rooting.

Exposure of etiolated avocado shoots to 12 hours of light reduced the per cent rooting and the number of roots per rooted shoot, but in one experiment a third of the shoots still rooted after 7 daily exposures to 12-hour periods of light, with 1 root per shoot (11). Rooting was reduced by delaying the exposure of etiolated shoots to light as long as 5 weeks after the start of the rooting period, but the greatest inhibiting effect was from exposure at the start or after 1 week. The time of girdling the shoot was considered the start of the rooting period. By microscopic examination of stem sections, evidence of root initiation was seen 3 weeks after the start of the rooting period, and counts of root initials indicated initiation was completed by the end of 8 weeks (11).

Exposure to light reduced the effect of IAA on rooting of cuttings from etiolated pea (5, 21) and mung bean seedlings (16). In some of our studies with mung bean, exposure for 30 minutes to red light at 100 f.c. measurably reduced the number of roots.

Galston's studies with asparagus stem tips indicated the effect of light could not be attributed either to lack of absorption of IAA in light or to light-activated destruction of IAA (4).

Asparagus stem tips repeatedly subcultured in the dark without IAA lost their capacity to root when IAA was supplied, suggesting that an "accessory substance" necessary for root initiation was depleted. Addition of various materials to cultures of "depleted" stems in the presence of IAA did not restore the rooting capacity, although some of them greatly increased stem growth. The materials tested included ammonium sulfate and arginine, which van Overbeek *et al.* (20) reported were effective in combination with IBA in promoting rooting of defoliated hibiscus cuttings.

Rooting ability was restored only by exposing stem tips to light for a week or longer, after which they would root in darkness. Apparently something essential for rooting was produced in the light, but the actual root initiation process was inhibited by light.

Naturally-occurring auxins were slightly higher in etiolated than non-etiolated bean and hibiscus stems, and in some cases higher levels of rooting "cofactors" were found in etiolated stems. However, the etiolation effect on rooting was not attributed to either of these differences. The presence of unknown substance(s) which act synergistically with auxin were postulated (8).

Hackett found no more methanol-extractable rooting cofactor in etiolated than non-etiolated tissue of either juvenile or adult ivy, and there was no rooting response of adult shoot tips to extracts from etiolated shoots (7). He suggested that a suitable extraction solvent had not been found, or that possibly the factors controlling rooting are in a fraction of the cell which is not readily extractable or transmissible.

Frolich (3) found no evidence for translocation of the etiolation response. When a shoot was grown with light excluded from only a marked section of stem, roots developed in that section but not in adjacent areas above or below. Priestley and Ewing (13) had earlier noted that etiolated portions of plants show etiolation effects even though other parts of the same plant are not etiolated.

In a study of anatomical differences between etiolated and non-etiolated shoots, Priestley and Ewing (13) observed the presence of an endodermis in etiolated shoots of *Vicia faba*. They attributed the etiolation effect on rooting to stimulation of meristematic activity by a restricting influence of the endodermis, resulting in root initiation. On the other hand, an endodermis was not observed in etiolated avocado shoots (11) nor in etiolated hibiscus or bean stems (8). Less mechanical tissue and less lignification were seen in etiolated than non-etiolated shoots (8, 11, 18, 19), but these mechanical tissues did not increase when etiolated stems were later inhibited

from rooting by exposure to light (11). Several other anatomical differences were observed but none was thought to be responsible for the rooting response (8).

Etiolated tissues generally contain less starch than normal tissues (8, 11, 19). Smith postulated that the reduced starch level resulted in a carbohydrate-nitrogen ratio more favorable for meristematic activity and root initiation than in the mature non-etiolated stem (19).

It is clear that more research is needed to explain the effect of etiolation on rooting. Studies to determine how exclusion of light promotes rooting may also contribute to an understanding of the factors involved in ease or difficulty of rooting in general, and thereby help to improve the efficiency of our propagating methods.

In the meantime, with our present knowledge of the rooting response to exclusion of light, the propagator can continue to make use of the etiolation effect on otherwise difficult to propagate plants without knowing why it is so effective.

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PRESIDENT KRAUSE: We now have time for questions and answers. We have a floor microphone this year. When you have a question we would appreciate your giving your name and to whom you would like to address the question. Do we have some questions? Yes.

AUSTIN KENYON: I would like to ask Dr. Hackett two questions. One, I noticed that with catechol and IAA, you just merely increased rooting up to the level obtained with NAA or IBA. Did you try the other two rooting hormones in this same test? In other words, with etiolation and red light?

DR. HACKETT: Are you asking whether we used catechol in combination with red light?

MR. KENYON: No. It seemed to me that in your comparison of the three hormones IAA was the worst, but combined with catechol you increased rooting up to about the same as the other two. So then did you try the other two in other tests, such as the IAA — catechol combinations?

DR. HACKETT: In one slide I showed the use of catechol in combination with NAA, but there catechol gave no increase in rooting over NAA alone.

MR. KENYON: Right. I understood that, but what I was getting at is that it seemed like NAA and IBA without catechol were equal to IAA with catechol.

DR. HACKETT: That's right.

MR. KENYON: And so I wondered, did you try the other two in the same experiments without catechol, and I wondered too, if maybe they would react better under etiolation and with red light than IAA plus catechol.

DR. HACKETT: We used NAA in the etiolation experiments; NAA was always superior to IAA as far as root initiation was concerned, even when the plants are grown in the dark. The answer to your question is — yes; NAA is a preferable auxin to use, even with etiolation for ivy shoot tips.

MR. KENYON: One more question; in your light experiments how did you obtain the red light — what wavelength was it — and what type of light was used?

DR. HACKETT: We used fluorescent tubes and then used a cellulose acetate, (red cellophane) film to wrap the lamps in.

DON DILLON: Another question for Dr. Hackett. What was the wavelength you obtained with the red light?

DR. HACKETT: About 650 millimicrons. This is more a characteristic of the fluorescent lamps then of the cellophane.

The lamps we used were Grolux fluorescent and their red peak comes at about 650 millimicrons.

ED SCHULTZ: Dr. Hackett, can you convert milligrams per liter to parts per million?

DR. HACKETT: Milligrams per liter is the same as parts per million.

VOICE: Dr. Leiser, has foliar analysis been used as an established technique for determining the levels of nutrition in rooting cuttings?

DR. LEISER: Foliar analysis has been used but the problem is: what is standard? Normal levels vary considerably from variety to variety in a similar nutrient regime, whether it is chysanthemums or azaleas. Most foliar analyses with ornamentals therefore become meaningless. The figures are there but they don't mean much. As a side light, you might be interested to know, there has been organized just this year a Council on Soil and Plant Analysis to which individuals will be invited to subscribe or join. It will attempt to determine standards of analysis for particular plants. Through this Council we finally may arrive at some standards which will be meaningful.

Referring to the previous question on parts per million vs. milligrams per liter, this is really a "plug" for the metric system as opposed to the English system of weights and measures. One reason we like to use the metric system is that milligrams per liter equals parts per million. It is an easy switch back and forth.

JIM BROWN: I have a question for Dr. Leiser. You said that calcium is essential for new cell division in meristematic tissue. I was wondering if this would be a pH relationship or a nutrient relationship?

DR. LEISER: Calcium is essential for the middle lamella — calcium pectate — which is the adjoining line between two cells. Calcium is an essential element in this part of the "building blocks", the structure of cell formation; whether there are other relationships or not, I don't know. Calcium is just an essential nutrient for proper building of the tissue structure. The cell wall has a lot of calcium in it. We might make an analogy of the child who needs good calcium to build strong bones. It is not a pH factor — it is a nutrition factor as far as I know.

LES CLAY: Another question for Dr. Leiser. I understand you to say something about sodium salts being detrimental to the initiation of roots. What would be the effect of the concentration of sodium salts in the water supply?

DR. LEISER: This adverse effect of sodium probably doesn't concern you in British Columbia, western Washington, or western Oregon, like it does us in California. Levels of sodium in the water supply that are detrimental to rooting are found in much of California. For example, the Los Angeles basin gets a lot of their water from the Owens Valley and

much of it is rather high in sodium. At Davis, California, we have some well water which is rather high in sodium — high enough to inhibit rooting.

DR. HACKETT: I might add to that. Some city water systems may be softening their water. If you are in a city that is softening the water for general use, then the sodium in that softened water is going to be high enough to be detrimental to root initiation in cuttings.

DON DILLON: On the same point — how high is high; what levels are we talking about, Andy?

DR. LEISER: We have no trouble getting 100 parts per million at Davis, California. Certainly the sodium in your home water softener, if it happened to be hooked up wrong, and you were getting it through your cold water line, into your propagation area, would give you trouble. I think some people may toy with the idea of a water softener unit to avoid the calcium buildup in their misting nozzles. This could be disastrous. You could offset it considerably by just using gypsum in the existing beds, because calcium is held on peat more strongly than sodium. It will displace the sodium, and the sodium will leach on through. Certainly the prevention of calcium buildup on mist nozzles by the use of a table salt rejuvenated water softener is very bad. If you use ion exchange beds you are all right. This is quite different than the usual water softeners.

RICHARD THOMPSON: Dr. Hackett, did you make any attempt to locate any inhibitor for root initiation rather than a root promoter in the initiation of roots in the plants that were grown in the light as compared to those under etiolation?

DR. HACKETT: If you will recall from our graphs, our untreated control plants rooted quite poorly. When we used IAA at 10 parts per million as the auxin, the control plants had only approximately 1 root per cutting. So this left little leeway to assay for inhibition of rooting. Our assay really was not the kind of assay to detect inhibition of rooting so I don't think I could comment whether or not we had rooting inhibitors.

BILL HALL: Dr. Leiser, I just wondered about your statement of using mineral nutrients in the mist system in rooting of cuttings. Does this disprove the old theory that you get best results in either clean river washed sand or peat — perlite or vermiculite, or combinations of these?

DR. LEISER: I think one of the reasons for use of "clean river-washed sand", and so on has been in regard to disease control. Certainly, with nitrogen in the rooting medium, if pathogens — fungi or bacteria — exist, there will be a great increase in these diseases.

PRESIDENT KRAUSE: Thank you very much. We must cut off our question and answer period now. For those of you who have further questions to address to these gentlemen, take advantage of our Question Box.