MODERATOR STOUTEMYER: Our next speaker is a native Californian and has been at UCLA quite a long time. He's a "green thumb" man, a natural propagator; but I think he should have been a professor, because if you have ever had a chance to talk to him for a while, you find he is just brim full of ideas. He should be directing experiments in propagation as well as carrying them out. It gives me great pleasure to introduce Mr. Edward F. Frolich -- all of us at UCLA know him as "Ted" --

Etiolation and the Rooting of Cuttings

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Webster defines the term, etiolation, as "to blanch by the exclusion of sunlight". This is hardly an adequate definition when applied to this particular discussion. A better title might be "the inhibiting effect of light on the production of root initials". This effect differs somewhat from those in the preceding discussions in that here we are interested only in the light falling on the actual tissue that is to produce the root initials. This, in ordinary nursery practice, would be the tissue at or near the base of the cutting.

There are statements in the literature that mention was made of the beneficial effects of darkness on rooting of apples as early as 1537 (2). Since that time there have been several papers describing the use of this technique for rooting cuttings of several different kinds of plants. Plants vary widely as to their ability to produce root initials when exposed to light. Many plants, of course, must be not at all, or only slightly, inhibited by light in the rooting process. We have all seen aerial roots on such things as ivy, Philodendron, Ficus, and tomatoes. Many plants can be rooted in a jar of water or in a humid atmosphere with the entire cutting exposed to light. The production of root initials in many other plants, however, is inhibited unless light is excluded from the tissue involved.

Sachs in 1865 (9) reported on an experiment with <u>Cactus speciosus</u>. He found that cuttings of this cactus kept in the dark for several weeks formed adventitious roots, while cuttings kept in the light for the same length of time did not. Cuttings of <u>Tropaelum majus</u> and <u>Veronica speciosa</u> behaved in a similar manner. In 1931 Mevius (4) worked with species of <u>Tradescantia</u>. He found rooting of <u>T. fluminensis</u> and <u>T. purpusi</u> was inhibited when the bases were exposed to light. Mevius pointed out that there are genetic differences involved. <u>T. fluminensis</u> var. <u>myrtifolia</u> was much less inhibited by light than was <u>T. fluminensis</u>. Once roots formed in these materials, however, they grew perfectly well in the light.

The simplest example of this practice is what we all do in our everyday work. We place the cuttings in a more or less opaque rooting medium which excludes light from the tissue where we wish roots to form. This undoubtedly would not be necessary with all materials but it is one of the factors contributing to successful rooting with many of them. I do not know of any extensive survey to determine which materials root better with light excluded from the base of the cutting.

A slightly more involved application of the same thing is illustrated by the process of mounding soil around the bases of shoots before the cuttings are taken. This is one of the effects of stooling. Regel (7) in 1853 reported better rooting of rose cuttings by this practice. A modification of the same thing is wrapping a section of the stem with black paper for a period of time before taking the cutting. Reed (6) in 1922 and Blackie, Graham, and Stewart (1) in 1926 found this method effective for rooting a difficult clone of Camphor. Smith (10) in 1924 reported that rooting was improved in a species of Clematis by this same method. In general it was the opinion of these workers that some growth of the tissue must take place either in length or thickness in the darkened area before the cuttings are detached.

The root formation of some other plants is even more strongly inhibited by light than those mentioned above. With these more difficult subjects we have to actually grow what is to be the base of the cutting in the dark. This generally is what is referred to as etiolation. Many of you have, no doubt, seen or read about the method employed with certain plums in England. They plant their stock out in such a way that it is possible to lay the branches down flat on the ground so they can cover them with soil before growth starts in the spring. The shoots grow up through the soil and into the light where they form normal leaves. By the end of the season most of these shoots develop roots in the soil and can be detached for planting in the nursery row. Gardner (3) in 1937 rooted cuttings of commercial apple varieties by a modification of this method. He covered his trees with an opaque box before growth started in the spring. When the trees had made about an inch of growth he removed the box and wrapped black tape around the base of the shoots. These shoots were allowed to grow naturally and the next winter they were detached and used as hardwood cuttings. Roots grew out from the area which had been covered by the tape.

Some years ago workers in avocado rootstock research at UCLA needed rooted cuttings of avocado (Persea americana) for certain experiments. We could always root cuttings of very young avocado seedlings and we had a very few old varieties that could be rooted from normal green cuttings, but with the great majority of varieties we could get absolutely no rooting. These cuttings would survive for two to three years with prolific callus formation but regardless of any "hormone" treatment used we could not initiate roots. A trial with the etiolation technique proved to be quite successful for producing experimental material although it requires too much labor for a commercial operation.

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We first grow an avocado seedling in a small container and graft it with the variety we wish to root. For some unknown reason the rooting of shoots from plants in small containers is much better than shoots from larger containers so we have to assume that etiolation is not the only factor involved. The plants are cut back to near the original scion and when the buds show signs of pushing the plants are placed in a dark room maintained at 70° - 75°F. At higher temperatures the new shoots are affected with what seems to be a physiological breakdown. Tissues in the dark will not stand as high a temperature as the same tissues in the light. At lower temperatures growth is slower and we also get water condensing on the shoots which favors fungal attack. The etiolated shoots do not form a true epidermis and are far more subject to invasion by fungi than are normal green shoots. The plants are left in the dark room until the shoots are about 3" long at which time they are brought out into the light (Fig. 1). A paper cylinder is placed around the shoots and filled with vermiculite or some other material to exclude the light. The tips of the shoots are left exposed and in a few weeks normal leaves develop (Fig. 2). At this time the shoots can be girdled near the base (Fig. 3) and the collar and vermiculite replaced or the shoots can be detached and rooted as cuttings in a propagating case. Girdling weakens the stock plants more than detaching the shoots and does not allow using them over again as many times. The plants are put back in the dark room to grow new shoots again. After two or three times the stock plants get so weak they have to be discarded. It is interesting that the weaker growing shoots root more readily than do stronger shoots but if they become too weak they will not elongate in the dark. For satisfactory rooting it is necessary to work in the area between the two extremes.

Rooted cuttings of the avocado are difficult to handle in the initial stage. They apparently have a higher soil oxygen requirement than do avocado seedlings. However, once they reach a certain size they grow well and develop into satisfactory trees (Fig. 4).

Some attempts were made to find out more about what etiolation is doing. If a collar is placed around a cut-back plant and filled with vermiculite as the buds grow so that just the tip of the shoot is visible but no stem area is exposed to light the shoot will root as well as if the shoot were grown entirely in the dark for the same period.

There seems to be no transmission either up or down from an etiolated section of stem. It is possible to grow a shoot with a ring of tissue from which light has been excluded and when such a shoot is put in to root the initials form only in the area which has had the dark treatment.

Ryan (8) ran some trials at UCLA with both Hass avocado shoots and mung bean seedlings. The Hass was used because it is one that will not root without etiolation and the mung bean because it has been used as a test plant in other rooting work. Ryan showed with both avocado and mung bean that reduction in rooting was dependent on the total light period for a given intensity (Tables 1 and 2). He also showed that with the avocado, inhibition of rooting was not limited to

light of a certain color (Table 3). The earlier in the development of a shoot a certain quantity of light is applied, the greater is the inhibition of subsequent rooting (Table 4).

Priestly and his co-workers (5) showed in their anatomical studies of etiolated shoots of broad bean that an endodermis and starch sheath formed which they associated with increased root production. Ryan, however, could find no endodermis in etiolated avocado shoots and no striking anatomical difference between shoots that would root and those that would not. In the etiolated mung bean shoots he did find an endodermis but the endodermis was still present in tissues that had been exposed to light for a sufficient length of time to reduce root formation.

There has been little progress on the basic reason for light inhibition of rooting in the past few centuries. Perhaps with some of the new techniques and equipment Dr. Piringer has described someone may be able to discover the mechanism involved.

Literature Cited

- 1. Blackie, J. J., R. D. T. Graham, and L.B. Stewart. 1926. The propagation of camphor. Royal Bot. Gardens Bul. Misc. Inf.
- Edbjerg, Niels. 1930. Earliest reference to etiolation for layering: apple trees propagated in Denmark. Rept. & Proc. IX Inter. Hort. Cong. Roy. Hort. Soc. 197.
- 3. Gardner, E. F. 1936. Etiolation as a method of rooting apple stem cuttings. Proc. Amer. Soc. Hort. Sci. 34:323-329.
- 4. Mevius, W. 1931. Licht und adventivworzelbildung bei commelinaceen. Ztscher. f. Bot. 23:481-509. Abs. in Bot. Centrbl. 160:325-326.
- 5. Priestley, J. H. and C. F. Swingle. 1929. Vegetative propagation from the standpoint of plant anatomy. U.S.D.A. Tech. Bull. 151.
- 6. Reed, Oona. 1922. Camphor by cuttings. Trans. Bot. Soc. Edin-burgh 28:184-188.
- 7. Regel, E. 1853. Vermehrung der neuen englischen Stockrosen aus Stecklingen. Gartenflora 2:123-124.
- 8. Ryan, G. F. Effects of light on root initiation in etiolated shoots. (Unpublished)
- 9. Sachs, J. 1864. Ueber die Neubildung von adventivewurzeen durch dunkelheit. Verhandlungen des naturhistorischen Vereines der preussischen Rheinlande und Westphalens pp. 110-111. Abs. in Bul. Soc. bot. de France 12: pt. 2. p. 221. 1865.
- 10. Smith, Edith P. 1924. The anatomy and propagation of Clematis. Bot. Soc. Edinb. Trans. & Proc. 29:17-27.

Table I

Effect of light exposure on subsequent rooting of layers of etiolated Hass Avocado shoots, 1958. Shoots grown in dark to height of 3" then exposed to light for period indicated after which they were handled as illustrated in Figures 1, 2, 3.

Light exposure 12 hr. days 600-700 f.c.	No. of Shoots	Percent rooted	Mean number of roots
0	12	92	7.1
1	11	64	4.3
3	11	45	4.4
5	12	42	4.4
7	12	33	1.0

Table II

Effect of duration of light exposure on rooting of etiolated mung bean (Phaseolus aureus) hypocotyls. Seedlings grown in dark for five days, then exposed to light for the period indicated, after which they were detached and rooted in darkness.

Hours of Light	Number of Roots	Percent of dark controls
0	31.6	
1.5	23.9	75
3	22.4	70
6	16.4	52
12	14.4	46
24	7.7	24
36	5.5	17
48	2.8	9

Table III

Hass Avocado etiolated shoots, 14 12-hour days, 300-350 f.c. Shoots grown in dark to height of 3" then exposed to colored light as indicated, after which they were handled as illustrated in Figures 1, 2, 3.

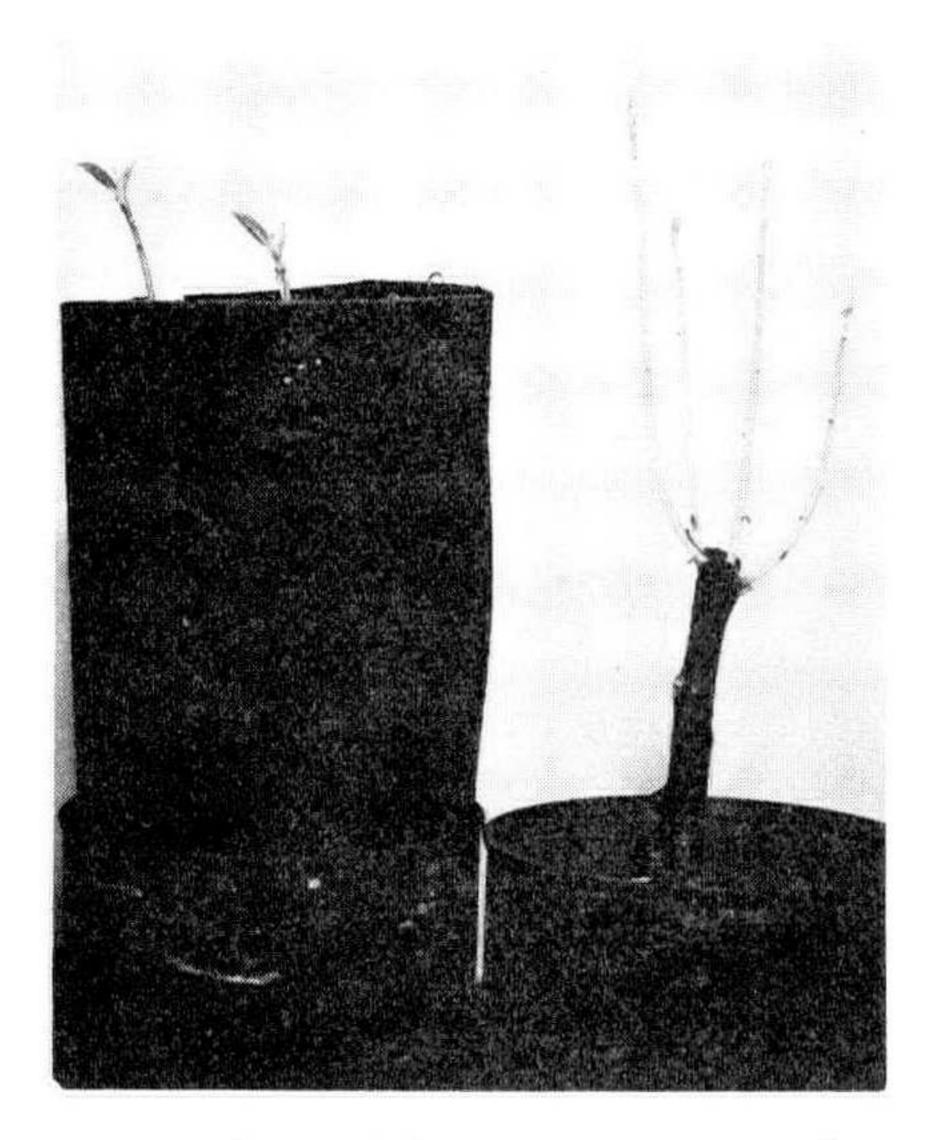
Color of light	No. of Shoots	No. rooted	No. of roots per cutting
Green	4	0	0
Blue	5	0	0
Red	3	0	0
White	3	0	0
No light	4	3	7

Table IV

Effect of time of interruption of etiolation by light exposure on subsequent rooting of layers of Hass avocado shoots. Shoots were grown in darkness to a height of 3", then treated as illustrated in Figure 1. The collars were removed at times indicated and the bases of the shoots were exposed to 600-700 f. c. of light for 7 12-hour days. The plants were then handled as illustrated in Figures 1, 2, 3.

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Light exposure	Percent	Mean number of roots per shoot	Mean number of roots per rooted shoot
Before layering	22	0.4	2.0
After start of layering			
1 week	38	1.7	3.0
2 weeks	67	2.0	3.0
3 weeks	67	2.7	4.0
4 weeks	88	1.8	2.0
5 weeks	86	1.7	2.0
No light	100	7.0	7.0



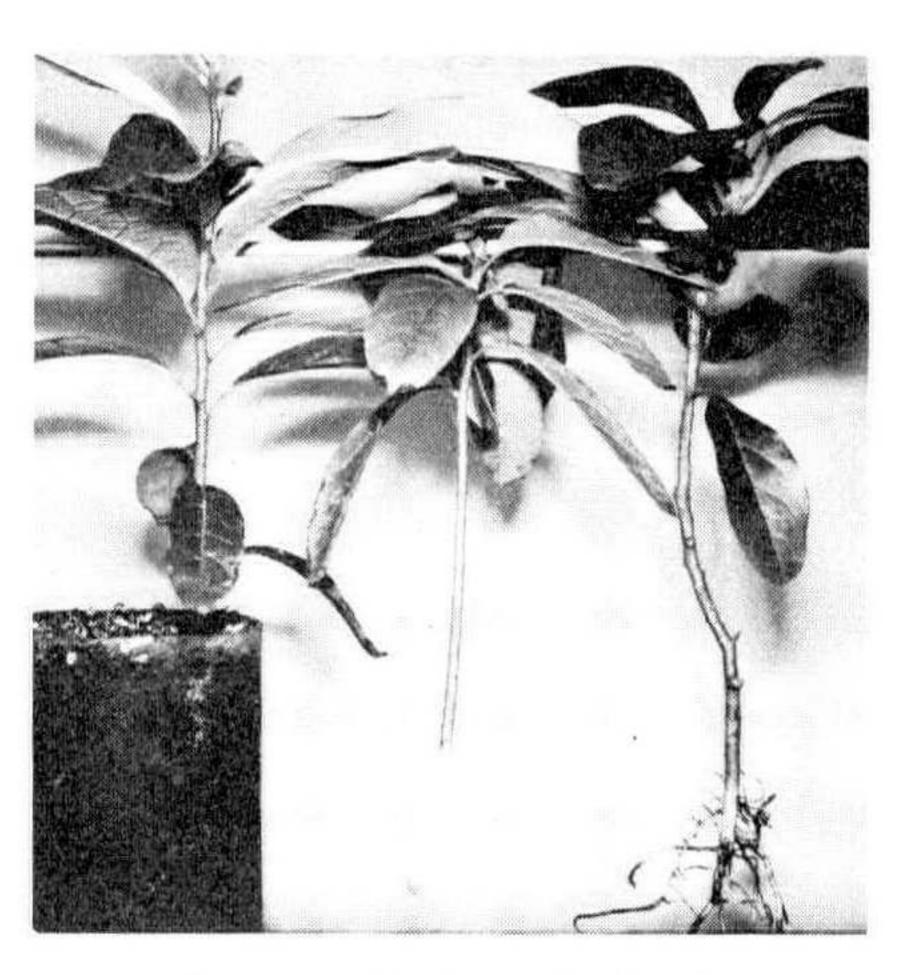
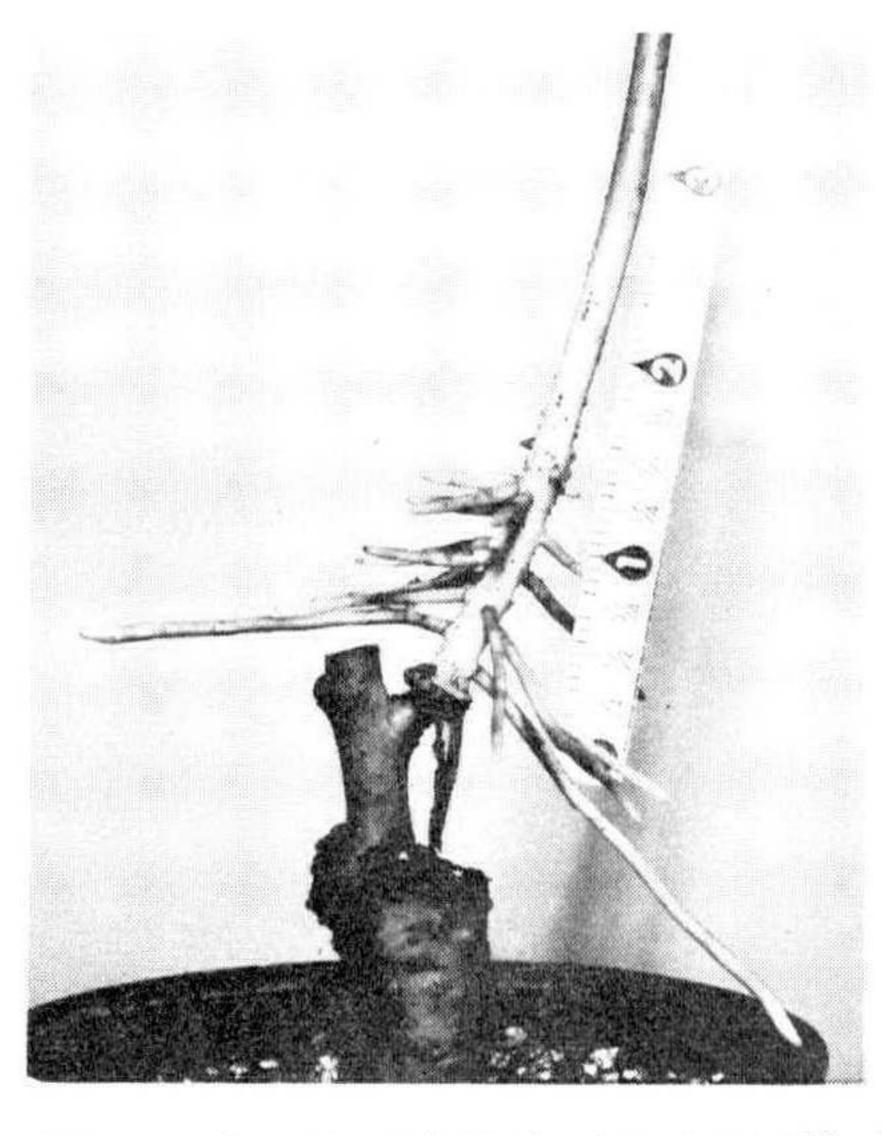


Figure 1. Right -- Hass avocado at time of removal from dark chamber.

Left -- Vermiculite-filled collar placed around base of etiolated shoots.

Figure 2. Left -- Shoot ready to detach. Middle -- Detached shoot showing etiolated base. Right -- Detached shoot after 6 weeks in propagating case.



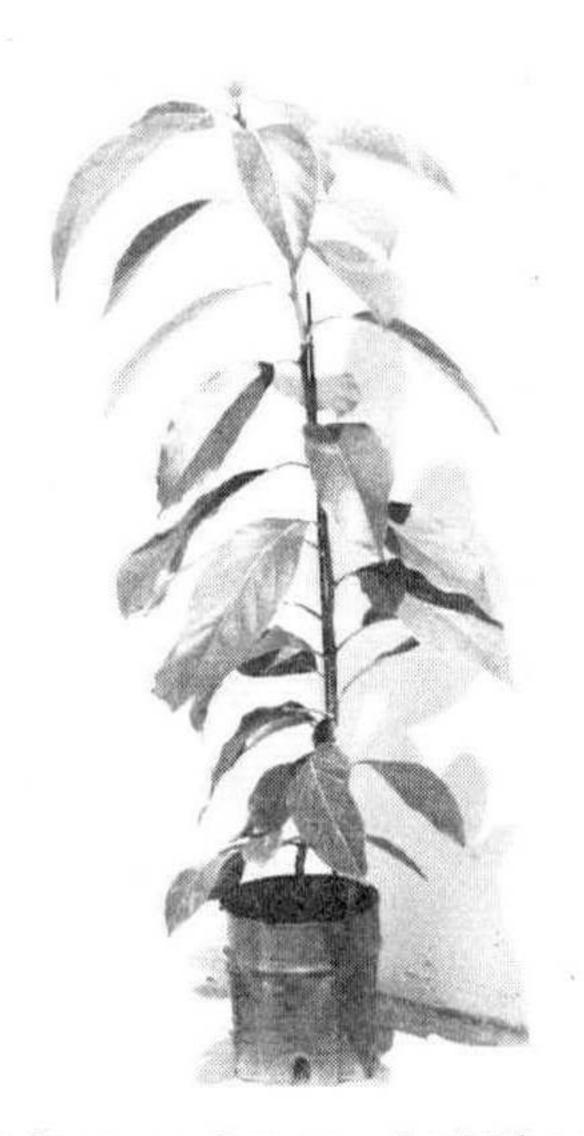


Figure 3. Etiolated shoot girdled near base and rooted while still attached to stock plant.

Figure 4. Established rooted cutting in gallon can.