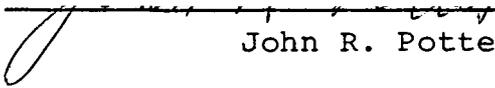


AN ABSTRACT OF THE THESIS OF

Tim D. Davis for the degree of Master of Science
in Horticulture presented on April 24, 1980

Title: The Effects of Light on Photosynthesis and Rooting
of Leafy Cuttings of *Pisum sativum* L.

Abstract approved:


John R. Potter

The effects of light incident on leafy cuttings is quite complex because light influences several factors which may affect root formation. One effect of light is to influence the carbohydrate status of the cutting by influencing photosynthesis. The role of current photosynthate in the rooting of leafy cuttings is uncertain. Accordingly I investigated the extent to which the supply of current photosynthate influences root formation on leafy cuttings of *Pisum sativum* L. When net photosynthesis was reduced to zero by maintaining the cuttings at the CO₂ compensation point or by blocking CO₂ exchange with an antitranspirant, rooting was reduced to about 50% of the controls. In addition to reducing rooting these treatments significantly reduced sucrose and glucose levels in the basal portion of the cuttings compared to controls. These experiments suggest that the supply of current photosynthate can limit root formation. The above conclusions

were supported by experiments using different photosynthetic photon flux densities and photoperiod lengths during rooting.

Defoliation experiments also supported the role of current photosynthate in root formation. Defoliation of cuttings reduced rooting and carbohydrate levels in the base of the cuttings. Light incident on defoliated stems increased rooting and sucrose levels in the base of the stems. Dipping the defoliated cuttings in an antitranspirant blocked CO_2 fixation and almost completely inhibited root formation. These data suggest that stem photosynthesis also contributed to root formation.

These experiments suggest that one of the major effects of light incident on leafy pea cuttings is to influence the amount of photosynthate available for root formation. It is difficult to establish whether current photosynthate influences rooting directly or indirectly by affecting some other factor(s) which control rooting. Whatever the case, treatments which influence photosynthesis influence rooting in a parallel manner.

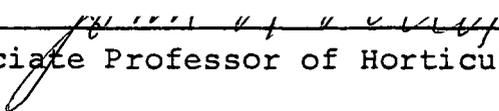
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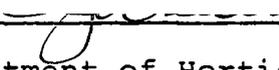
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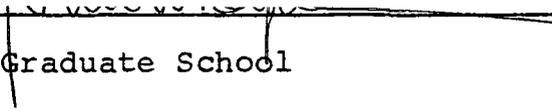
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Typed by Patti F. and Tim D. Davis for Tim D. Davis

DEDICATION

This thesis is dedicated to Patti and Lance.

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Note: This thesis is presented as a literature review followed by two papers written in the format required by the Journal of the American Society for Horticultural Science. Citations in the literature review are numbered to correspond to the numbering in the Bibliography. Citations in the papers are numbered to correspond to the literature cited at the end of each individual paper.

THE EFFECTS OF LIGHT ON PHOTOSYNTHESIS AND ROOTING
OF LEAFY CUTTINGS OF PISUM SATIVUM L.

LITERATURE REVIEW

Introduction

The rooting of leafy cuttings is dependent upon a complex interaction between internal and environmental factors. Light is one factor which has recently received attention (9,18,25,44,61). Light incident on cuttings may affect several factors which influence rooting such as carbohydrate levels (33,35,44), hormonal status (31,32,38), water status (25,44), and anatomy (7,8,32). In addition, the influence of light incident on cuttings is dependent upon which portion of the cutting is illuminated. For instance light incident on the zone of root initiation decreases rooting (23,32,38,42) while light incident on leaves may promote rooting (18,35).

Because light can affect several different factors which influence rooting, investigators often reach different conclusions about the effects of light levels on rooting. Some report that rooting of some leafy cuttings increases with increasing light levels incident on leaves (18,35). Other investigators found that rooting decreases with increasing light incident on leaves (44,61) while still others report that light incident on leaves has no

effect on rooting (29,64). It is clear that experiments based on different light levels during rooting are often difficult to interpret and often lead to seemingly contradictory statements.

This review will discuss the literature pertaining to the various effects of light incident on leafy cuttings. The effect of light incident on the zone of root initiation will be discussed first after which the effects on the upper portion of the cutting will be considered in relation to the effects on: 1) carbohydrate levels, 2) auxins, 3) water potential, and 4) temperature.

Effects of Light Incident on the Zone of Root Initiation

Light incident on the zone of root initiation reduces root formation on cuttings. Krul (42) found that no roots formed on Phaseolus vulgaris L. hypocotyls when the basal portion of the cuttings was illuminated, however when the area of initiation was kept in the dark roots formed readily. Similarly Stromquist and Eliasson (61) reported that rooting of Picea abies L. cuttings is almost completely inhibited when the base of the cuttings is irradiated at 8 or 40 W m⁻². Reduced rooting of cuttings of several other species has also been reported when basal portions of cuttings were not etiolated (23,32,38). The inhibitory effect of light on the area of initiation

appears to be exerted through changes in both the hormonal and anatomical status of the cutting.

Kawase (38) has studied the hormonal aspects of etiolating the basal portion of Phaseolus aureus Roxb. and Salix alba L. cuttings. Rooting is reduced in cuttings of both these species when the basal portion of the cutting is not kept in the dark. Kawase found that light incident on the basal portion of the cutting was associated with a rapid decrease in a growth promoting factor which he believed was IAA. From this work Kawase proposed that etiolated cuttings retained relatively high levels of IAA at the site of etiolation during the root initiation process and that the increased level of IAA then stimulated root initiation.

Herman and Hess (32) also reported that light incident on the area of initiation affected the hormonal status of cuttings. They found that etiolated portions of Phaseolus vulgaris and Hibiscus rosa-sinensis L. cuttings rooted better than non-etiolated segments.

Light incident on the zone of initiation may also alter the anatomy of the cutting. Beakbane (7) reported that the ability of stems to form roots is related to the anatomical structure of the stem and found that tissues characterized by a high degree of sclerification generally do not root well. Beakbane found that differentiation of the primary phloem parenchyma cells into

fibers and sclereids was retarded by shading and partial etiolation of the stock plants.

The fact that light may alter the anatomy of stems is further illustrated by work of Yamagisawa (71) who reported that the fiber content in Linum usitatissimum L. stems is reduced in plants grown under heavy shade. Similarly reduced lignin levels in Fagus sylvatica L. grown under heavy shade have also been reported (39).

Etiolated stems of Hibiscus rosa-sinensis and Phaseolus vulgaris cuttings had more parenchyma cells, more tissues in a less differentiated state, reduced cell wall thickness, reduced cell wall deposits, and less mechanical strengthening tissues than non-etiolated stems (32). Cuttings taken from Dahlia variabilis Willd. stock plants in which the zone of initiation was shaded by wrapping stems with aluminum foil rooted much better than cuttings taken from stock plants in which stems were not wrapped (8). The proposed effect of shading the basal portion of the stem was to promote the herbaceous character of the rooting region.

It is clear that light incident on the zone of root initiation reduces rooting of cuttings. In studying the effects of light on rooting, therefore, it is important to account for the effects of light on the area of initiation and to ensure that the basal portion of cuttings is not inadvertently illuminated during rooting studies.

Effects of Light Incident on the Leaves of Cuttings

Effects on Carbohydrate Status

One important effect of light incident on leaves of cuttings is to influence the carbohydrate status of the cutting. Carbohydrates are considered necessary for root formation (30) but their role in the process is obscure (29). It may be that they function only as a source of energy and carbon skeletons for the root formation process.

The role of carbohydrates in rooting is confounded by their possible involvement in auxin transport or synthesis. It has been suggested that auxin synthesis (5) or transport (34,65) is tightly linked to the supply of photosynthate. These ideas are based on the observation that when Nicotiana tabacum L. plants were grown in an environment lacking CO₂, auxin levels were greatly reduced (5). Avery et al. proposed that the reduction was due to reduced synthesis of the hormone. Later their data was reinterpreted and the reduced auxin levels were attributed to decreased transport rather than decreased synthesis (34). Vardar (65) has proposed that the products of photosynthesis are necessary for auxin transport and that auxin transport is reduced in environments lacking CO₂ because there is no photosynthesis. Maintaining plants in an

environment lacking CO₂ may cause senescence (68) or could influence ethylene activity (1). Therefore it is questionable whether reduced photosynthesis in an environment lacking CO₂ is directly responsible for reduced auxin transport. That photosynthesis is not always necessary for polar auxin transport is illustrated by work of Koevenig and Jacobs (41). They reported that green Coleus blumei Benth. stems were able to transport IAA in a polar direction when exposed to monochromatic light of 730 nm wavelength. Because monochromatic light of this wavelength cannot drive photosynthesis, it does not appear that photosynthesis was necessary for polar auxin transport.

If auxin levels are tightly linked to the supply of current photosynthate, it may be difficult to separate photosynthetic effects from those of auxin on rooting. For instance if any treatment which alters photosynthesis also alters auxin metabolism, it would be difficult to exclude the possibility that the apparent effect of photosynthate on rooting was due to auxin. The following consideration of the effects of carbohydrates on rooting is made bearing this in mind.

Correlations between carbohydrate content and root formation have been variable. Some investigators have found that carbohydrate levels in cuttings are

positively correlated with root formation (35,60,69) while other investigators have reported a negative correlation (29,44). Hansen and coworkers (29) reported that cuttings of Pinus sylvestris L. with relatively high carbohydrate levels rooted poorly compared to cuttings with low carbohydrate levels. They proposed that high carbohydrate levels within cuttings may be supraoptimal for root formation. Loach and Gay (44) have also suggested that carbohydrates may reach supraoptimal levels in cuttings of Weigela florida (Bunge) and Forsythia X intermedia Zab. This hypothesis is difficult to test, however, because any treatment which stimulates the accumulation of carbohydrate within cuttings may also influence other factors which might also affect root formation such as auxin levels or water status. The effects of these factors on rooting will be discussed later.

Ali and Westwood (2) working with three Pyrus species found that the carbohydrate content of the cuttings was not related to root formation on the cuttings. They concluded that rooting in these species was limited by some factor which was not a carbohydrate. This factor may have been a developmental or anatomical characteristic which could override more subtle characteristics such as carbohydrate levels. Brandon (11) working with a Rosa species and Steponkus and

Hogan (59) working with Abelia grandiflora Rehd. also found no correlation between carbohydrate levels and root formation. Nanda and coworkers (51) found that seasonal influences overrode the influence of carbohydrate levels on the rooting of Populus nigra L. cuttings thus indicating that factors other than carbohydrates were limiting root formation. If factors other than carbohydrates are limiting then a positive correlation between carbohydrate levels and rooting would not necessarily be expected as suggested by Haissig (28).

Just as the significance of carbohydrates in rooting is obscure the importance of photosynthesis to the rooting of leafy cuttings is unclear. Several investigators have provided evidence that photosynthesis by leafy cuttings influences root formation (18,23,33,35,45,48,57,61) while others have suggested that current photosynthate is of little or no importance to rooting (12,25,29,64).

Support for the involvement of current photosynthate in root formation on leafy cuttings comes from several lines of investigation including experiments using: 1) CO₂ enrichment during rooting (48), 2) exogenous carbohydrate treatments (18), 3) different light treatments during rooting (18,23,35), or 4) a

combination of environmental conditions during rooting favorable to the accumulation of photosynthate (33,57).

CO₂ enrichment of the atmosphere surrounding cuttings has been reported to increase rooting of cuttings from various woody species (48). This would suggest that increased photosynthate due to stimulation of photosynthetic rates by CO₂ levels improved rooting.

The treatment of cuttings with exogenous sucrose supports the involvement of current photosynthate in root formation. Eliasson (18) reported that exogenous sucrose supplied to cuttings rooted at low irradiances improved rooting. This suggests that rooting was carbohydrate limited and that exogenous sucrose could substitute for current photosynthate in supplying carbohydrates for root formation. It should be pointed out that exogenous sucrose may affect factors other than carbohydrate levels. For example, Lovell et al. (46,47) and Moore et al. (49,50) reported that exogenous sucrose caused senescence of Sinapis alba L. and Raphanus sativus L. cotyledon cuttings.

Eliasson (18), Howard (35), and Foster (23) increased the amount of light incident on cuttings and obtained increased rooting. They attributed this increased rooting to increased accumulation of photo-

synthate within the cuttings. In addition to affecting photosynthesis, however, light also influences the auxin status of cuttings (31,32,38). It is therefore difficult to determine whether increased rooting in these experiments was due to increased photosynthesis induced by higher light levels, improved auxin status of the cuttings, or other unknown factors.

In some cases a combination of conditions which were favorable for the accumulation of photosynthate within cuttings have been favorable for root formation. Hess and Snyder (33) increased photosynthesis sevenfold by rooting cuttings in relatively high light under mist. Increased rooting accompanied this increase in photosynthesis. In addition to increasing photosynthesis, the water potential of the cuttings was increased. Similarly Scott and Marston (57) improved the rooting of Saintpaulia ionantha Wendl. cuttings by using a mist system during propagation. They attributed the increased rooting to increased photosynthesis and reduced transpiration under mist. In both of the above investigations, conditions which were favorable for the accumulation of photosynthate, also would be expected to raise the water potential of the cuttings. Since increased water potential can result in increased rooting (25,44) it is not possible to determine whether improved rooting in these studies was related to

increased photosynthesis, higher water potential, or other unknown factors.

Other lines of investigation suggest that current photosynthate is of little or no importance in root formation on leafy cuttings. Early work by van Overbeek et al. (64) and a more recent study by Breen and Muraoka (12) indicate that rooting of some leafy cuttings apparently is not influenced by the supply of current photosynthate. Van Overbeek et al. reported that leafy Hibiscus rosa-sinensis cuttings rooted equally well in both darkness and moderate light while Breen and Muraoka reported that current photosynthate remained in the upper portion of leafy "Marianna 2624" plum (Prunus cerasifera X Prunus monsoniana) cuttings until after roots formed. Apparently both the Hibiscus and the plum cuttings contained adequate carbohydrate reserves to serve the demands of root formation.

Hansen et al. (29) reported that different irradiances during the rooting period had no effect on root formation in Pinus sylvestris cuttings. They further suggested that the carbohydrate status of the stock plant had more influence on rooting than carbohydrates formed during the rooting period. Their suggestions are based on experiments in which two different light treatments during rooting were used. Since light affects other factors as previously discussed it is

difficult to determine the extent to which carbohydrate levels influenced rooting in their system.

It has been suggested that leafy cuttings of Cornus alba L. used carbohydrate reserves for root formation rather than current photosynthate (25). This argument is based solely on the observation that the leaf conductance values of the cuttings during rooting were low. Neither photosynthetic rates nor carbohydrate levels were measured. However, significant photosynthetic rates may occur even with low leaf conductance.

In summary, the role of carbohydrates in rooting is unclear and is confounded by their possible influence on auxin synthesis or transport. Light levels during rooting may influence carbohydrate levels within cuttings, but the extent to which current photosynthate limits root formation is not clear.

Auxins and Light

Auxin-carbohydrate interactions. Auxins promote rooting of many different types of cuttings (30). The mode by which auxin promotes rooting is unknown although several ideas have been proposed. Several investigators have suggested that interactions between carbohydrates and auxins regulate root formation (22,26,29,52,53). As mentioned earlier, carbohydrates may influence the synthesis or transport of auxins.

In contrast to the above hypothesis that carbohydrates influence auxin transport, some investigators have suggested that auxins may influence carbohydrate transport. One proposed effect of auxins on root formation is to increase the transport of assimilates to the site of root formation. For instance, IBA may increase the movement of photosynthate to the base of Pinus radiata D. cuttings (13). Similarly it has been suggested that IAA promoted rooting of Phaseolus vulgaris cuttings by increasing sugar availability to the zone of root formation (3). Auxins may mobilize reserve food materials in Populus nigra cuttings by enhancing the activity of hydrolytic enzymes (55). Several investigators have reported that auxins increase the activity of hydrolytic enzymes (55,66,70).

In contrast to the above reports, other investigators reported that auxins had no effect on the basal transport of assimilates to the site of root formation. Application of IBA to the base of plum cuttings stimulated rooting but did not influence sugar levels in the mid and basal portions of the cuttings until after roots formed (12). Similarly, IAA reportedly had no effect on the basal transport of sucrose in Pinus lambertiana Dougl. cuttings (26).

Interactions between carbohydrates and auxins have been suggested to influence rooting of Populus robusta Schneid. cuttings. Nanda et al. (54) reported that a proper balance between auxin and assimilates is needed for organogenesis. High sucrose to auxin ratios led to phloem and shoot production while low sucrose to auxin ratios led to xylem production which is necessary for vascularization of root primordia.

Protein and nucleic acid synthesis are involved in root formation and a carbon source is important for the biosynthesis of these molecules (28,30,37). Nanda et al. (52) have further suggested that auxin may act as a regulator at the transcriptional level and carbohydrates as a source of energy regulate the synthesis of specific enzymes that are required for root formation.

The effect of light on auxin. The effect of light on endogenous auxin levels in plants is quite complex because light may regulate several aspects of auxin metabolism including (65): 1) synthesis, 2) transport, 3) interconversion of free and bound forms, and 4) inactivation.

Light during rooting influences auxin levels in cuttings. Leaf cuttings of Begonia X cheimantha Everett contained much higher levels of endogenous

auxin when they were rooted in the light than when rooted in the dark (31). Also the rooting capacity of the leaves maintained in the light was much greater than that of leaves rooted in the dark. Of course the carbohydrate status of the cuttings rooted in the light may have been at least partially responsible for increased rooting, a point left unaddressed by Heide. Light levels during rooting may also influence the effect of exogenously supplied auxin. Hackett (27) reported that light intensity during rooting of Hedera helix L. altered the effects of exogenous IAA on rooting.

Light may also influence auxin levels by activating the auxin destroying enzyme IAA oxidase. Light has been shown to stimulate the destruction of ^{14}C labelled IAA (21) and in addition, light increased the activity of IAA oxidase (24).

Effects on Water Status

The amount of light incident on a cutting can influence the water potential of the cutting. A high level of energy can raise the leaf temperature and increase the water potential gradient between the leaf and air thereby accelerating transpiration and reducing the water potential. A lowered water potential

may in turn reduce root formation. The mechanism by which water potential affects rooting is unclear.

Rooting of Weigela florida and Forsythia X intermedia cuttings was inversely related to the amount of light incident on the foliage (44). Cuttings rooted under high light had lower moisture content compared to cuttings rooted under low light, and the moisture content was positively correlated with root formation. Carbohydrate levels were highest under high light thus reduced rooting in this case was not due to insufficient carbohydrate. Positive correlations between rooting and water potential have also been reported for cuttings of Rhododendron, Ceanothus, and Hebe (43). It has been suggested that high light during rooting of Picea abies cuttings may adversely effect the water status of the cuttings and thus reduce rooting (61). High light may also reduce carbohydrate levels if the water potential becomes low enough for photosynthesis to decrease.

Gay and Loach (25) reported that environmental conditions which allowed Cornus and Rhododendron cuttings to remain turgid produced fastest rooting. Similarly, reduced transpiration in cuttings of Saintpaulia ionantha due to misting (57), and several woody species due to application of an antitranspirant (67) improved rooting.

It may be that rooting is not always reduced by water stress. Argles (4) suggested that water stress during the first stages of initiation might be beneficial to rooting. He proposed that in relatively mature cutting material water stress might encourage callusing and thus lead to increased rooting. In most cases callus formation and rooting are independent of each other, however (30). Adventitious roots have been found to form in callus tissue of only a few species.

Effects on Temperature

Light may also influence leaf temperature of cuttings (33,44). Cameron and Rook (14) have suggested that the temperature of shoots has an important effect on rooting. High temperatures hastened root development but also favored fungal disease. They suggest an optimum temperature regime to balance both effects.

It has been suggested that low leaf temperatures, in addition to reduced transpiration, have been at least partially responsible for increased rooting of Saintpaulia ionantha cuttings under mist (57). High leaf temperatures may increase respiration rates more

than photosynthetic rates and thus accelerate the depletion of carbohydrates which may reduce rooting (33,35).

CURRENT PHOTOSYNTHATE AS A LIMITING FACTOR
IN THE ROOTING OF LEAFY CUTTINGS OF PISUM SATIVUM L.

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compensation point, antitranspirant

Abstract. The role of current photosynthate in the rooting of leafy cuttings is uncertain. Accordingly I investigated the extent to which the supply of current photosynthate influences root formation on leafy cuttings of Pisum sativum L. When net photosynthesis was reduced to zero by maintaining the cuttings at the CO₂ compensation point or by blocking CO₂ exchange with an antitranspirant, rooting was decreased to about 50% of the controls. In addition to decreasing rooting these treatments lowered sucrose and glucose levels in the basal portion of the cuttings compared to controls. Increasing the photosynthetic photon flux density or lengthening the photoperiod during rooting tended to improve rooting and increase carbohydrate levels. These experiments suggest that the supply of current photosynthate can limit root formation.

Introduction

The importance of photosynthesis to the rooting of leafy cuttings has not been determined. Several investigators have suggested that photosynthesis by leafy cuttings is important for root formation (5,7,11,12,14, 15,17,18) while others have found evidence to the contrary (2,8,9,21).

Support for the involvement of current photosynthate in the rooting of leafy cuttings comes from several lines of investigation including experiments using: 1) CO₂ enrichment during rooting (15), 2) exogenous carbohydrate treatments (5), 3) different light levels during rooting (5,7,12), and 4) a combination of environmental conditions during rooting which were favorable to the accumulation of photosynthate (11,17).

Other lines of investigation suggested that current photosynthate is of little or no importance to root formation on leafy cuttings. Experiments using ¹⁴CO₂ to follow the accumulation of current photosynthate (2) and those in which cuttings rooted equally well in both moderate light and in the dark (21) indicate that some leafy cuttings do not require current photosynthate for root formation. Some investigators have suggested that leafy cuttings use carbohydrate reserves rather than

current photosynthate in root formation (8,9) but their evidence is equivocal because factors other than photosynthate may have been varied in their treatments.

It has been suggested that the supply of current photosynthate can limit root formation on pea cuttings (5). The objective of the present investigation was to test this hypothesis and to determine the extent to which the supply of current photosynthate influences root formation on leafy pea cuttings.

Materials and Methods

Plant material. Pea (Pisum sativum L. cv. Alaska) stock plants were grown from seed in flats of Jiffy Mix (George Ball Pacific) for 10 days at a photosynthetic photon flux density (PPFD) of $350 \mu\text{E m}^{-2} \text{s}^{-1}$ (measured 10 cm above the mix surface) provided by mixed fluorescent ($340 \mu\text{E m}^{-2} \text{s}^{-1}$) and incandescent ($10 \mu\text{E m}^{-2} \text{s}^{-1}$) light. The stock plant photoperiod was 14 hr and day/night temperatures were 21/19°C. In one experiment stock plant growth conditions were altered so that PPFD was $600 \mu\text{E m}^{-2} \text{s}^{-1}$ and day/night temperatures were 25/20°C.

Ten days after sowing, cuttings were made by excising the plants just above ground level and the

basal 2-3 cm inserted in Jiffy Mix. Cuttings consisted of 6-9 cm of stem, 2 nearly fully expanded leaves and a terminal meristem. The rooting environment was the same as stock plant growth conditions except PPFD was $280 \mu\text{E m}^{-2} \text{ s}^{-1}$. The number of roots per cutting was counted after 10 days.

Photosynthesis and dark respiration measurements.

For photosynthesis measurements, a 5 cm pot containing 2 cuttings was placed in a semi-closed assimilation chamber (1) with the following environment: PPFD= $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ at the plant apex; temperature= 21°C ; relative humidity= 75%. The pot was sealed to prevent mixing of rooting media gases with gases inside the chamber. Photosynthesis and dark respiration rates were determined using infrared gas analysis to measure the time required for a known amount of leaf area to change the CO_2 concentration $30 \mu\text{l/l}$ (between 305 and 335 $\mu\text{l/l}$) while the system was closed. Cuttings remained in the assimilation chamber until a steady state photosynthetic or respiration rate was obtained (usually 1-2 hr for photosynthesis and $\frac{1}{2}$ -1 hr for dark respiration).

Light compensation point and light saturation for the unrooted cuttings were determined by varying the level of PPFD incident on the cuttings with a white cheesecloth filter.

Changes in dry weight and leaf area of the cuttings during rooting were also measured. Dry weights were determined by drying to constant weight at 70° C. Leaf areas were measured by a Li-Cor Model LI-3000 portable leaf area meter (Lambda Instruments).

The total mass of photosynthate produced during rooting was estimated from the CO₂ exchange rate data where the ratio of the mass of photosynthate to the mass of CO₂ was assumed to be 0.68 (6). The mass of photosynthate was calculated daily based on the leaf area and unit leaf area CO₂ exchange rates.

Rooting experiments. Cuttings were rooted at several different PPFd provided by mixed fluorescent and incandescent light, where the ratio of these light sources was the same as for stock plant conditions. An exception to this was the CO₂ compensation experiment in which the light source was entirely incandescent. PPFd was adjusted using black polypropylene shade fabric.

Cuttings were also rooted under 4 different photoperiods where the PPFd was 280 $\mu\text{E m}^{-2} \text{s}^{-1}$ for all treatments.

In the CO₂ compensation experiments, the CO₂ levels during rooting were controlled by infrared gas analysis. In these experiments two different CO₂ levels during rooting were used.

The antitranspirant (Wilt pruf, Nursery Specialty Products), a polyvinyl chloride emulsion, was applied by dipping the entire cutting (except the basal 3 cm) in a mixture of 1 ml per 4 ml distilled water immediately after excision.

Carbohydrate analysis. In selected experiments the starch, sucrose, and glucose content of the basal 3 cm of cuttings was determined. Sampling was usually on day 7 of the rooting period, the day when roots began to emerge. The basal 3 cm of some cuttings was weighed fresh and again after drying to determine fresh to dry weight ratios in order to express carbohydrate data on a dry weight basis.

The stem segments used for carbohydrate analysis were weighed fresh and then homogenized with a mortar and pestal in 15 ml of 80% ethanol. The homogenate was then boiled for 2-4 minutes, centrifuged at 12,000 g for 5 minutes and the supernatant decanted. The ethanol extraction and centrifugation were repeated twice. The pellet was dried at 60° C and the supernatants were combined.

The combined supernatant containing the soluble sugars was dried at 100° C and dissolved in 4.0 ml of 0.1 M acetate buffer (pH 4.7). The glucose content of 30 μ l aliquots of the soluble sugar fraction was determined with Statzyme (Worthington Biochemical) which

uses the Trinder reaction (19) to couple the peroxide formed in the breakdown of glucose by glucose oxidase with a chromophore. The dye formed by this reaction has an absorption maximum at 500 nm. The sucrose content of the soluble sugar fraction was determined by digesting 30 μ l of the soluble sugar fraction with 10 μ l of a suspension of invertase (37 units per mg, Grade V, Sigma) containing 1.0 mg/ml of 0.1 M acetate buffer (pH 4.7). Digestion was for 90 minutes at 55°C. The glucose content of the digest was determined with Statzyme, as before, and the sucrose content was calculated by subtracting the absorbance due to the glucose content before digestion. Glucose and sucrose were used as standards for their respective assays.

After drying, the pellet was dispersed in 10 ml of 0.1 M acetate buffer (pH 4.7) and boiled for 2-4 minutes. Starch was digested with 20 mg of amyloglucosidase (10 units/mg, Grade III, Sigma) at 55°C for 90 minutes (3). The digest was centrifuged at 12,000 g for 5 minutes, and 30 μ l aliquots of the supernatant were analyzed for glucose using Statzyme. Starch suspensions were used as standards and the moisture content was accounted for.

Results

Photosynthesis and respiration. Cuttings maintained measurable photosynthetic rates throughout the rooting period when exposed to a PPFD of $280 \mu\text{E m}^{-2} \text{s}^{-1}$ (Figure 1), although rates were lower than the $8\text{-}10 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ recorded for intact 10 day old pea seedlings. After excision, photosynthetic rates gradually dropped to a minimum level on about day 6 after which they increased. This photosynthetic rate increase began at the time roots emerged from the cutting. Dark respiration rates were fairly constant throughout the rooting period (Figure 1). The light compensation point for the cuttings remained at about $50 \mu\text{E m}^{-2} \text{s}^{-1}$ throughout the rooting period. Photosynthesis was light saturated at $350\text{-}450 \mu\text{E m}^{-2} \text{s}^{-1}$.

The estimated dry weight increase per cutting during rooting as calculated from photosynthetic and dark respiration rates was about 42 mg (Figure 1). The observed increase in dry weight of the cuttings during the rooting period was about 60 mg. The average dry weight per cutting at the beginning of the rooting period was about 65 mg thus the cuttings nearly doubled in dry weight during rooting when rooted at $280 \mu\text{E m}^{-2} \text{s}^{-1}$. Leaf area of the cuttings increased by about 20-30% during the rooting period and resulted

from the formation of a new leaf rather than the expansion of older leaves.

Rooting and carbohydrate analysis. Rooting of cuttings increased with increasing PPF_D during rooting (Figure 2). At a PPF_D slightly below the light compensation point rooting was still greater than in the dark where cuttings formed only about two roots each.

Starch, sucrose, and glucose levels in the basal 3 cm of cuttings rooted in the dark were considerably lower than levels in cuttings rooted at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ (Table 1). Also the dry weight of the basal 3 cm of cuttings rooted in the dark was less than half that of cuttings rooted at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$. As in the experiment described in Figure 2, the cuttings at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ rooted significantly better than those in the dark which produced only about 2 roots each. By comparing the data in Table 1 to values obtained from stock plants grown at $350 \mu\text{E m}^{-2} \text{ s}^{-1}$ (Table 2) it is apparent that the basal portions of pea cuttings accumulate carbohydrates during the first 7 days of the rooting period when rooted at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$. This accumulation did not occur in the dark.

Cuttings made from stock plants grown at a relatively high PPF_D ($600 \mu\text{E m}^{-2} \text{ s}^{-1}$) also rooted poorly in the dark. Growing the stock plants at 600

$\mu\text{E m}^{-2} \text{ s}^{-1}$, however, increased both sucrose and glucose levels in the basal 3 cm of stems at the time cuttings were excised compared to plants grown at $350 \mu\text{E m}^{-2} \text{ s}^{-1}$ (Table 2). Since very little starch was present its analysis was omitted on this and some of the following experiments.

Increased rooting was obtained with increasing photoperiod length (Figure 3). Also, basal portions of cuttings rooted under a 22 hr photoperiod had larger dry weights and higher levels of sucrose and glucose than did cuttings rooted under a 4 hr photoperiod (Table 3).

Cuttings rooted at the CO_2 compensation point (approximately $100 \mu\text{l/l}$) formed only about one half as many roots as cuttings rooted at $330 \mu\text{l/l CO}_2$ (Table 4). Starch, sucrose, and glucose levels in the basal 3 cm of the cuttings were highest in the cuttings rooted at $330 \mu\text{l/l}$. At the end of the rooting period the dry weight of the cuttings rooted at $330 \mu\text{l/l}$ was nearly twice the dry weight of cuttings rooted at $100 \mu\text{l/l}$.

Rooting was reduced by roughly 50% when photosynthetic rates were slowed by dipping cuttings in an antitranspirant following excision (Table 5). Sucrose and glucose levels were also lower in dipped cuttings compared to controls. Net photosynthesis of cuttings

dipped in the antitranspirant remained about ± 0.5 $\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ throughout the rooting period.

Discussion

This investigation strongly suggested that the supply of current photosynthate can limit root formation on leafy pea cuttings. Regardless of how net photosynthetic rates were reduced, both rooting and carbohydrate levels were decreased. Net photosynthesis was reduced to 0 by 3 independent methods: 1) reducing the PPFD, 2) lowering the ambient CO_2 level, and 3) partially blocking the stomata with an antitranspirant. Because each of the above treatments reduced rooting to about one half that of controls and further reduction of the PPFD nearly eliminated all root formation, I infer that at least 50% of the rooting under the conditions used in this investigation was associated with the supply of current photosynthate.

Pea cuttings fixed appreciable amounts of CO_2 during the rooting period. Despite the decline in photosynthetic rate during the first 6 days of the rooting period, the cuttings fixed appreciable amounts CO_2 before the roots emerged. Similarly, others have reported that the formation and development of roots on cuttings was associated with a rise in photosynthetic rate (13,20).

Photosynthesis by the cuttings resulted in a substantial increase in dry weight during the rooting period. The estimated value of dry weight increase calculated from photosynthetic and respiration rates was below the observed increase in dry weight. The reason for this is not clear. Handling of the cuttings (i.e. transport from growth chamber to assimilation chamber) exposed the cuttings to low light and low humidity which may have caused stomates to close and lowered photosynthetic rates. Initial low rates of photosynthesis could have led to an underestimation of the daily photosynthetic rates.

In studying the influence of current photosynthate on root formation, ideally one need a system in which photosynthesis can be varied independently of all other factors which might influence rooting. The method of varying photosynthetic rates by varying CO_2 levels or by blocking CO_2 exchange with an antitranspirant as used in the present investigation seems to provide a method by which endogenous carbohydrate levels can be regulated without the complicating influence of varying light levels. These methods reduced net photosynthesis to 0 but did not completely inhibit photosynthesis.

Other lines of evidence support the hypothesis that current photosynthate was important to root formation. Root formation increased with increasing PPF D from 0 to $280 \mu\text{E m}^{-2} \text{s}^{-1}$. The highest PPF D used for rooting experiments was $280 \mu\text{E m}^{-2} \text{s}^{-1}$ because prolonged higher PPF D caused the cuttings to wilt. Photosynthesis was light saturated at $350\text{-}450 \mu\text{E m}^{-2} \text{s}^{-1}$. These values, however, were determined by measuring photosynthesis for only 1-2 hr at a given PPF D and $280 \mu\text{E m}^{-2} \text{s}^{-1}$ may have saturated photosynthesis during most of the rooting period.

Cuttings rooted in the dark formed roots very poorly, an observation supported by others (5). The basal portion of my cuttings rooted in the dark contained very low levels of sucrose and glucose compared to cuttings rooted at $280 \mu\text{E m}^{-2} \text{s}^{-1}$ indicating that root formation in the dark may have been carbohydrate limited. However, it is also possible that darkness reduced the auxin levels in the cuttings (10,16).

To test the hypothesis that the cuttings used in these experiments were deficient in carbohydrates at the beginning of the rooting period, stock plants were grown at $600 \mu\text{E m}^{-2} \text{s}^{-1}$ instead of $350 \mu\text{E m}^{-2} \text{s}^{-1}$ in an effort to raise initial carbohydrate levels. Cuttings taken from stock plants with high carbohydrate levels rooted no better in the dark than did

cuttings taken from stock plants with low carbohydrate levels. The carbohydrate status of the stock plants, therefore, did not alter the rooting of cuttings in the dark. This apparently is not in agreement with other experiments in this investigation which show that increased carbohydrate is associated with increased rooting. The carbohydrate levels in the base of the $600 \mu\text{E m}^{-2} \text{s}^{-1}$ stock plants on day 0, however, were low compared to levels measured on day 7 in cuttings rooted at $280 \mu\text{E m}^{-2} \text{s}^{-1}$ (compare carbohydrate data in column 2 of Table 2 to column 1 of Table 1). Carbohydrate levels in cuttings taken from $600 \mu\text{E m}^{-2} \text{s}^{-1}$ stock plants were not determined on day 7, however. It is therefore unknown whether carbohydrate levels in cuttings taken from the $600 \mu\text{E m}^{-2} \text{s}^{-1}$ stock plants were higher during the first seven days than levels in the cuttings taken from the $350 \mu\text{E m}^{-2} \text{s}^{-1}$ stock plants.

Results from rooting experiments using different photoperiod lengths during rooting supported the hypothesis that current photosynthate may limit root formation. Rooting as well as sucrose and glucose levels were higher under a long rather than a short photoperiod.

It should be noted that rooting between experiments was quite variable for unknown reasons. It is

therefore necessary to use caution when comparing rooting data between experiments.

My conclusions are not consistent with those of several other investigators (2,8,9,21) who have reported that current photosynthate is of little or no importance to root formation. One reason for the differential effect of current photosynthate on the rooting of my cuttings compared to other species may be because shoots on pea cuttings continue to elongate and produce new leaf area during the rooting period. As pointed out by Eliasson (5) this type of growth may compete with the stem base of cuttings for assimilates which are necessary for root formation. For example, growing shoots on Populus tremula L. cuttings are reported to be strong carbohydrate sinks which out-compete roots for available carbohydrate (4).

Another possible reason for the difference between my results and those of several others may be related to the carbohydrate status of the stock plant material. Early work by van Overbeek (21) and later by Breen and Muraoka (2) indicated that some cuttings had sufficient carbohydrate reserves for root formation and current photosynthate had no effect on root formation. When attempting to evaluate the importance of current photosynthate in rooting, therefore, one must be aware of

the initial carbohydrate status of the cutting material. In addition to the carbohydrate status of the stock plant, the length of the rooting period may be important in determining the importance of current photosynthate. Even cuttings with substantial carbohydrate reserves may require additional carbohydrates for root formation if the rooting period is quite long (18). The carbohydrate demand for root formation among species may also be extremely variable.

Rooting of pea cuttings apparently is influenced by the supply of current photosynthate. From the present investigation it is difficult to establish whether current photosynthate influenced rooting directly by affecting some other factor(s) which control rooting. Whatever the case, treatments which influenced photosynthesis (CO_2 levels, light levels, photoperiod lengths, antitranspirant dip) influenced rooting in a parallel manner.

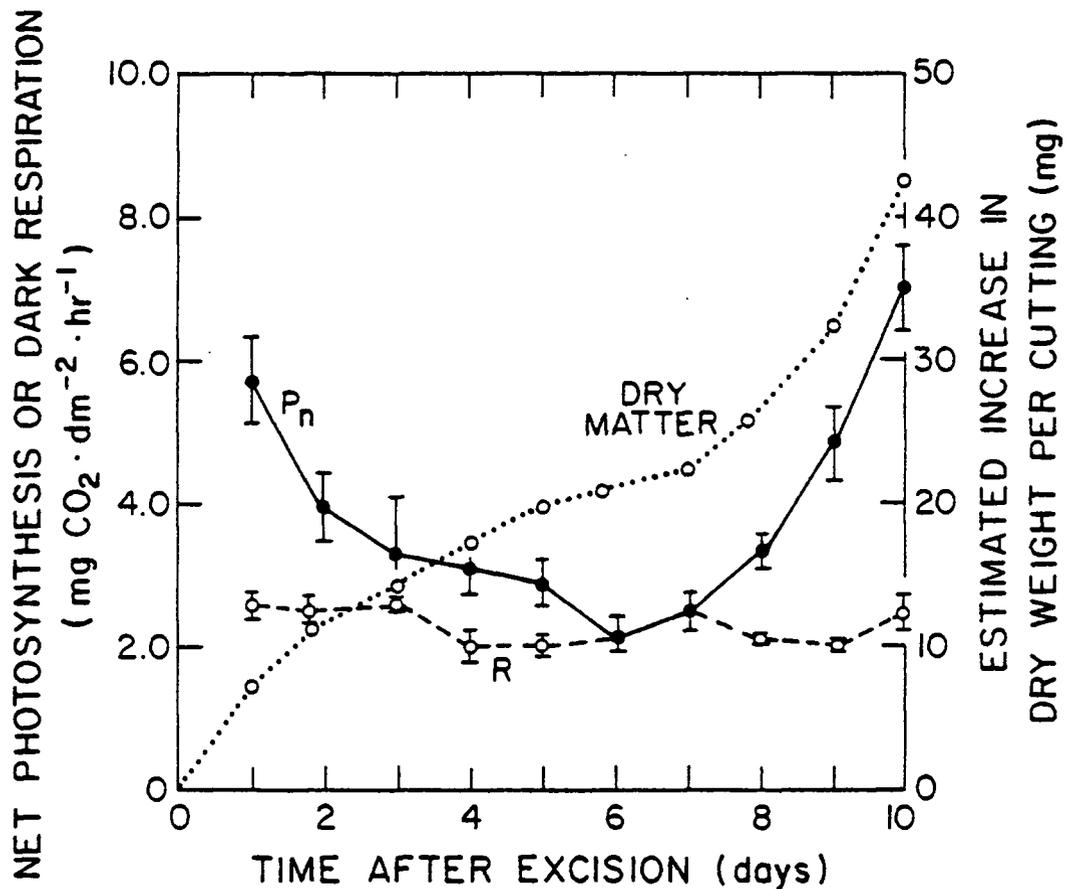


Figure 1. Net photosynthesis (P_n), dark respiration (R), and estimated dry weight accumulation of pea cuttings during the rooting period. Bars indicate \pm standard error of the mean ($n=6$ for P_n data, $n=4$ for R data). Photosynthetic photon flux density during P_n measurements was $280 \mu\text{E m}^{-2} \text{s}^{-1}$. Dry weight increase was calculated daily based on the leaf area and unit leaf area CO_2 exchange rates.

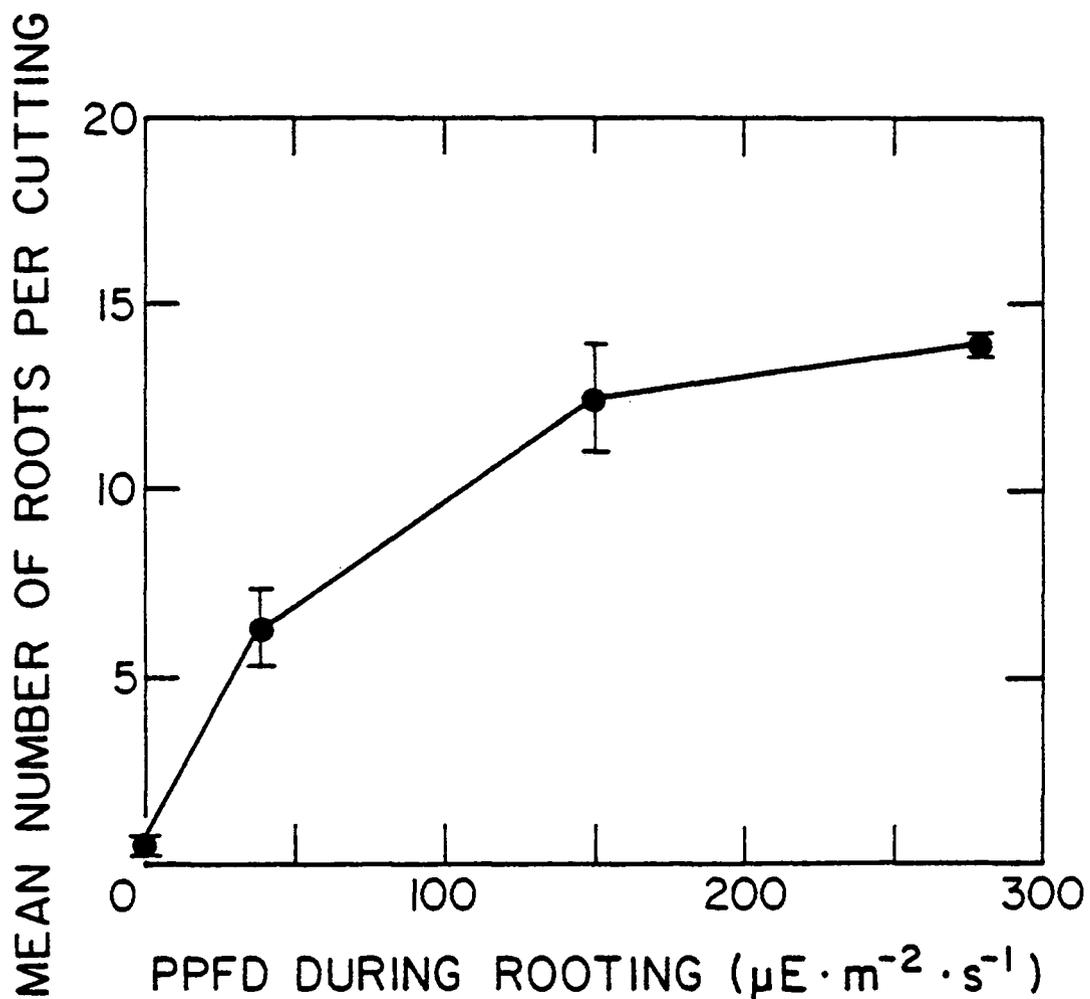


Figure 2. Root formation on pea cuttings rooted under different photosynthetic photon flux densities (PPFD). Bars indicate \pm standard error of the mean ($n=14$). The number of roots per cutting was counted 10 days after excision.

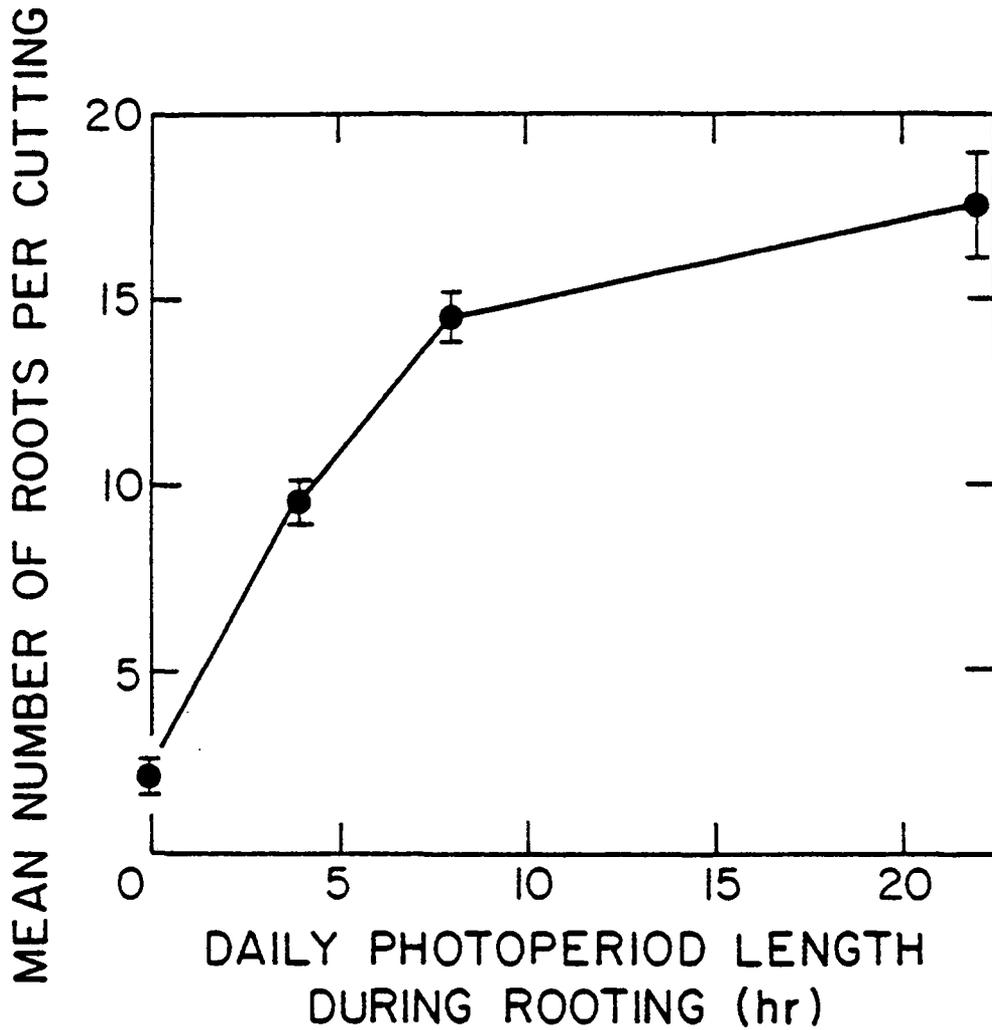


Figure 3. Root formation on pea cuttings rooted under different photoperiod lengths. Bars indicate \pm standard error of the mean ($n=15$). Photosynthetic photon flux density during photoperiods was $280 \mu\text{E m}^{-2} \text{s}^{-1}$. The number of roots per cutting was counted 10 days after excision.

Table 1. Comparison of rooting, dry weight, and carbohydrate levels in the basal 3 cm of pea cuttings rooted at 2 photosynthetic photon flux densities (PPFD). The analysis was on the seventh day of the rooting period.

	PPFD during rooting ($\mu\text{E m}^{-2} \text{s}^{-1}$)	
	280**	0
	<u>Mean number of roots per cutting</u>	
	13.2	2.2
	<u>Dry weight (mg)</u>	
	17.4	7.1
	<u>Carbohydrates (mg)</u>	
Starch	0.07	0.001
Sucrose	1.78	0.18
Glucose	1.08	0.14
	<u>Carbohydrates (% of dry weight)</u>	
Starch	0.40	0.01
Sucrose	10.4	2.5
Glucose	6.4	1.7

**all $280 \mu\text{E m}^{-2} \text{s}^{-1}$ means are significantly higher than the corresponding $0 \mu\text{E m}^{-2} \text{s}^{-1}$ means at the 1% level of probability as determined by unpaired t test (n=13 for rooting data, n=4 for starch data, n=8 for dry weight and sugar data)

Table 2. Rooting and carbohydrate levels of the basal 3 cm of pea cuttings taken from stock plants grown at 2 different photosynthetic photon flux densities (PPFD). Carbohydrates were analyzed at the beginning of the rooting period and the number of roots per cutting was counted 10 days after excision.

	Stock plant PPFD ($\mu\text{E m}^{-2} \text{s}^{-1}$)	
	350	600
	<u>Dry weight (mg)</u>	
	8.3	9.7**
	<u>Number of roots per cutting when rooted in the dark</u>	
	1.5	1.2
	<u>Carbohydrates (mg)</u>	
Sucrose	0.16	0.31**
Glucose	0.28	0.52**
	<u>Carbohydrates (% of dry weight)</u>	
Sucrose	2.0	3.2**
Glucose	3.4	5.3**

**600 $\mu\text{E m}^{-2} \text{s}^{-1}$ means are significantly higher than the corresponding 350 $\mu\text{E m}^{-2} \text{s}^{-1}$ means at the 1% level of probability as determined by unpaired t test (n=10).

Table 3. Comparisons of rooting, carbohydrate levels, and dry weight of the basal 3 cm of pea cuttings rooted under 2 different photoperiods. The analysis was conducted on the seventh day of the rooting period. Photosynthetic photon flux density during the light periods was $280 \mu\text{E m}^{-2} \text{s}^{-1}$.

	Daily photoperiod length (hr)	
	22*	4
	<u>Number of roots per cutting</u>	
	15.1	9.4
	<u>Dry weight (mg)</u>	
	17.9	12.4
	<u>Carbohydrates (mg)</u>	
Sucrose	1.45	0.30
Glucose	0.68	0.27
	<u>Carbohydrates (% of dry weight)</u>	
Sucrose	8.4	2.4
Glucose	4.0	2.3

*all 22 hr means are significantly higher than the corresponding 4 hr means at the 5% level of probability as determined by unpaired t test (n=8).

Table 4. Rooting, dry weight, and carbohydrate levels in the basal 3 cm of pea cuttings rooted at 2 different CO₂ levels. The number of roots per cutting and the dry weight of the whole cuttings was determined 10 days after excision. Carbohydrate levels and dry weight of the basal 3 cm were determined 7 days after excision. Photosynthetic photon flux density during rooting was 280 $\mu\text{E m}^{-2} \text{s}^{-1}$.

	CO ₂ levels during rooting ($\mu\text{l/l}$)	
	330*	100
	<u>Mean number of roots per cutting</u>	
	13.8	7.3
	<u>Dry weight of whole cutting (mg)</u>	
	116.0	62.8
	<u>Dry weight (mg)</u>	
	16.5	13.0
	<u>Carbohydrates (mg)</u>	
Starch	0.065	0.015
Sucrose	1.23	0.33
Glucose	0.99	0.26
	<u>Carbohydrates (% of dry weight)</u>	
Starch	0.40	0.11
Sucrose	7.7	2.5
Glucose	6.1	2.0

*all 330 $\mu\text{l/l}$ means are significantly higher than the corresponding 100 $\mu\text{l/l}$ means at the 5% level of probability as determined by unpaired t test (n=23 for rooting and whole cutting dry weight data, n=4 for starch data, n=8 for sugar and basal 3 cm dry weight data)

Table 5. The effect of an antitranspirant dip on rooting, dry weight, and carbohydrate levels in the basal 3 cm of pea cuttings. The cuttings were dipped in the antitranspirant immediately after excision. The number of roots per cutting was counted after 10 days. Carbohydrate levels and dry weights were determined 7 days after excision. Photosynthetic photon flux density during rooting was $280 \mu\text{E m}^{-2} \text{s}^{-1}$.

	Treatment	
	Control**	Antitranspirant dip
	<u>Mean number of roots per cutting</u>	
	9.9	4.9
	<u>Dry weight (mg)</u>	
	14.0	9.1
	<u>Carbohydrates (mg)</u>	
Sucrose	1.22	0.29
Glucose	0.86	0.35
	<u>Carbohydrates (% of dry weight)</u>	
Sucrose	8.8	3.1
Glucose	6.2	3.7

**all control means are significantly higher than the corresponding antitranspirant means at the 1% level of probability as determined by unpaired t test (n=63 for rooting data, n=8 for carbohydrate and dry weight data)

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THE EFFECTS OF LIGHT AND DEFOLIATION ON
THE ROOTING OF LEAFY CUTTINGS
OF PISUM SATIVUM L.

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Additional index words. pea, root formation, sucrose, glucose, leaves

Abstract. The influence of light on cuttings is quite complex because light may affect several factors which influence root formation. This investigation was conducted to determine the extent to which the supply of photosynthate of pea (Pisum sativum L.) cuttings, altered by defoliation, darkness, or timing of light exposure, influences rooting.

Light incident on defoliated stems increased rooting and sucrose levels in the base of the cuttings. Dipping the defoliated stems in an antitranspirant blocked CO₂ fixation and almost completely inhibited root formation. These data suggest that stem photosynthesis influences root formation.

Defoliation of the cuttings reduced rooting in the light and dark suggesting that leaves supply factors other than current photosynthate for root formation. The

leaves stimulated rooting much better in the light than in the dark, but the nature of the factors which leaves supplied in the dark is unknown. Light incident on leaves during the first half of the rooting period stimulated rooting more than light incident during the second half of the rooting period. This suggests that light incident on leaves influences root initiation. Light incident on the zone of initiation reduced rooting, however.

Introduction

The rooting of leafy cuttings is dependent on a complex interaction between internal and external factors. The light level during rooting is one factor which has recently received attention (3,6,9,15,17). Light incident on the area of root initiation was inhibitory to root formation (13,14,17) while light incident on leaves had variable effects.

Some investigators have reported that increasing light incident on leaves of cuttings increased root formation (6,12,13). However, others have reported that increasing light incident on cuttings during rooting resulted in decreased root formation (3,15,17) while still others reported that light levels during rooting had little effect (9,18).

Light incident on cuttings may affect several factors which influence root formation including carbohydrate levels (12,15), water status (8,15,16), hormonal status (10,11,13), and anatomy (1,2,11). The reason investigators using different rooting systems often reach conflicting conclusions about light levels during rooting may be because light affects so many different parameters within cuttings. The rooting response to light levels will depend, therefore, on the nature of the factors limiting root formation.

This investigation was conducted to determine the extent to which the supply of current photosynthate of pea (Pisum sativum L.) cuttings, altered by defoliation, darkness, or timing of light exposure, influences rooting. Previous work has suggested that the supply of current photosynthate can limit the rooting of pea cuttings (p. 19-34).

Materials and Methods

Pea (Pisum sativum L. cv. Alaska) stock plants were grown from seed in flats of Jiffy Mix (George Ball Pacific) for 10 days at a photosynthetic photon flux density (PPFD) of $350 \mu\text{E m}^{-2} \text{s}^{-1}$ (measured 10 cm above mix surface) provided by mixed fluorescent ($340 \mu\text{E m}^{-2} \text{s}^{-1}$) and incandescent ($10 \mu\text{E m}^{-2} \text{s}^{-1}$) light. The stock plant photoperiod was 14 hr and day/night temperatures were 21/19°C.

Ten days after sowing, cuttings were made by excising the plants just above ground level and the basal end inserted 2-3 cm deep in Jiffy Mix unless stated otherwise. Cuttings consisted of 6-9 cm of stem, 2 nearly fully expanded leaves, and a terminal meristem. The rooting environment was the same as stock plant growth conditions except that light levels were varied. The number of roots per cutting was counted after ten days.

Several different rooting experiments were conducted. Four different PPF regimes during rooting were used. Cuttings were also rooted in 100 ml beakers (7 cuttings per beaker) containing distilled water. Half of the beakers were wrapped with aluminum foil to exclude light from the basal portion of the cuttings. The PPF normal to the external surface of the beakers was $80 \mu\text{E m}^{-2} \text{ s}^{-1}$. A small amount of light entered the wrapped beakers where the cuttings were inserted through the aluminum foil. This represented less than $1 \mu\text{E m}^{-2} \text{ s}^{-1}$.

Various defoliation treatments were used as follows: 1) cuttings were defoliated on either day 0, 2, 5, or not at all, where day 0 was the day of excision, 2) the leaf at the first or second node above the scale leaves was removed on day 0, 3) defoliated and leafy cuttings were rooted in the dark.

Defoliated stems were rooted with and without an anti-transpirant (Wilt pruf, Nursery Specialty Products). The

antitranspirant (1 ml per 4 ml distilled water) was applied by dipping all but the basal 2-4 cm of the stems immediately after excision. Carbon dioxide exchange from the dipped and non-dipped defoliated stems was measured using infrared gas analysis as described previously (p. 22)

The sucrose and glucose contents of the basal 3 cm of cuttings from some of the above treatments were measured on day 7 of the rooting period. Methods of analysis are described elsewhere (p. 24).

Results

Cuttings rooted at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ for all 10 days rooted better than cuttings rooted at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ for either the first half or second half of the 10 day rooting period (Table 6). When cuttings received light for only the first 5 days they rooted significantly better than when they received light for only the second 5 days. Cuttings receiving the latter treatment still rooted better than those maintained in the dark for the entire rooting period. Roots first became visible on the seventh day of the rooting period, but were not counted until the tenth day.

Maintaining the basal portion of the cuttings in the dark increased rooting. When light was excluded from the

basal portion of the cuttings 13.6 roots per cutting were formed compared to 8.7 roots per cutting when the basal portion was exposed to $80 \mu\text{E m}^{-2} \text{ s}^{-1}$.

Complete defoliation of the cuttings decreased root formation regardless of the presence of light (Table 7). Rooting was similarly suppressed when the leaf at the first or second node above the scale leaves was removed. Rooting, however, was still greater than in cuttings which were completely defoliated (Table 7).

Cuttings which were defoliated and rooted in the dark formed almost no roots while leafy cuttings in the dark formed only about 2 roots per cutting (Table 7). Defoliated cuttings which were rooted at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ formed more roots and contained more sucrose in the basal 3 cm than leafy cuttings rooted in the dark. The longer the leaves remained on the cuttings the more roots were formed (Figure 4).

Defoliated stems dipped in an antitranspirant and rooted at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ formed only 0.3 roots per cutting which was less than their non-dipped counterparts which formed about 2 roots each (data not presented in tables). Carbon dioxide efflux from non-dipped defoliated stems at $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ was 75% of the CO_2 efflux from the same stems in the dark (0.036 vs. 0.048 mg CO_2 efflux per cm of stem length per hour). When defoliated stems were dipped

in the antitranspirant, CO_2 efflux was the same at either $280 \mu\text{E m}^{-2} \text{ s}^{-1}$ or in the dark.

Cuttings rooted in the dark contained low levels of sucrose and glucose regardless of the presence of leaves (Table 7). Dry weight of the basal end of cuttings rooted in the dark was also low, but it was slightly higher in leafy cuttings as compared to defoliated ones.

Discussion

Previous work has suggested that leaves on pea cuttings provided photosynthate which stimulated root formation (p. 19-34). The present investigation suggests that stem photosynthesis also contributes to root formation. Defoliated stems rooted better and had more sucrose in the basal 3 cm when rooted in the light compared to the dark. When defoliated stems were dipped in an antitranspirant, photosynthesis and root formation were almost completely inhibited. These results suggest that although stems evolved CO_2 in the light, stem photosynthesis was still enough to increase the sucrose levels in the basal portion of the cuttings compared to levels in defoliated cuttings rooted in the dark. This increase in sucrose was accompanied by the formation of several more roots per cutting than when stem CO_2 fixation was blocked with an antitranspirant. Dipping the defoliated

stems in the antitranspirant decreased rooting to the same extent as did continuous darkness.

In addition to supplying current photosynthate for root formation as suggested by previous work (p. 19-34), the present investigation suggests that leaves on pea cuttings may have another role in root formation. The leaves apparently were able to perform part of their function in the dark because leafy cuttings rooted better than defoliated ones even when both were rooted in the dark. From the present study it is difficult to ascertain the function that leaves on pea cuttings performed in the dark. It is possible that some carbohydrates were mobilized from the leaves to the basal portion of the cuttings even in the dark. Sucrose and glucose levels in leafy cuttings rooted in the dark were not significantly different from defoliated cuttings rooted in the dark, however. Dry weight of the basal 3 cm was slightly higher in the leafy cuttings rooted in the dark. It is possible that leaves supply factors other than carbohydrates which stimulate rooting (4,5,18).

Van Overbeek et al. (18) reported that leaves on Hibiscus rosa-sinensis cuttings performed their root-forming function equally well in the light and in the dark. In the present study, however, leaves promoted rooting much better in the light. The difference between my cuttings and those of van Overbeek et al. may be that

rooting of mine was influenced by the supply of current photosynthate while theirs apparently were not.

Removing either the leaf at the first or second node above the scale leaves had the effect of similarly reducing rooting indicating that both of these leaves were equally important in supplying factors which enhance root formation. This sensitivity of rooting to the removal of a single leaf indicated that the factor(s) the leaves supplied were at or below some critical threshold value.

Light incident on leafy cuttings during the first half of the rooting period promoted rooting much better than light during the second half of the ten day rooting period. This suggests that one role of light incident on leaves may be to somehow influence root initiation rather than just sustaining roots after initiation. In this context, Eriksen (7) reported that root initiation in Alaska pea cuttings occurred in the first 4 days of the rooting period. In addition to affecting the initiation process, however, it appears that light is also important for the growth and development of initials because cuttings which received light for only the second half of the rooting period still rooted better than cuttings which remained in the dark for the entire rooting period.

Light does not always promote the rooting of pea cuttings, however, as its effect is dependent upon which

portion of the cutting is illuminated. Light incident on the area of initiation was inhibitory to root formation in the present study. This observation is supported by the results from a number of other studies using different species (11,13,14,17) and indicates the importance of ensuring that the basal portion of cuttings are not inadvertently illuminated during rooting studies. The mechanism by which light on the zone of initiation reduces rooting is not known although effects on auxin (11,13) and anatomy (1,2,11) have been suggested as being at least partially responsible.

The results of this investigation support the idea that current photosynthate influences rooting of leafy pea cuttings since defoliation reduced rooting and carbohydrate levels. The present study indicated that stem photosynthesis can also influence root formation. In addition to supplying current photosynthate, leaves supplied other unknown factors which influenced rooting.

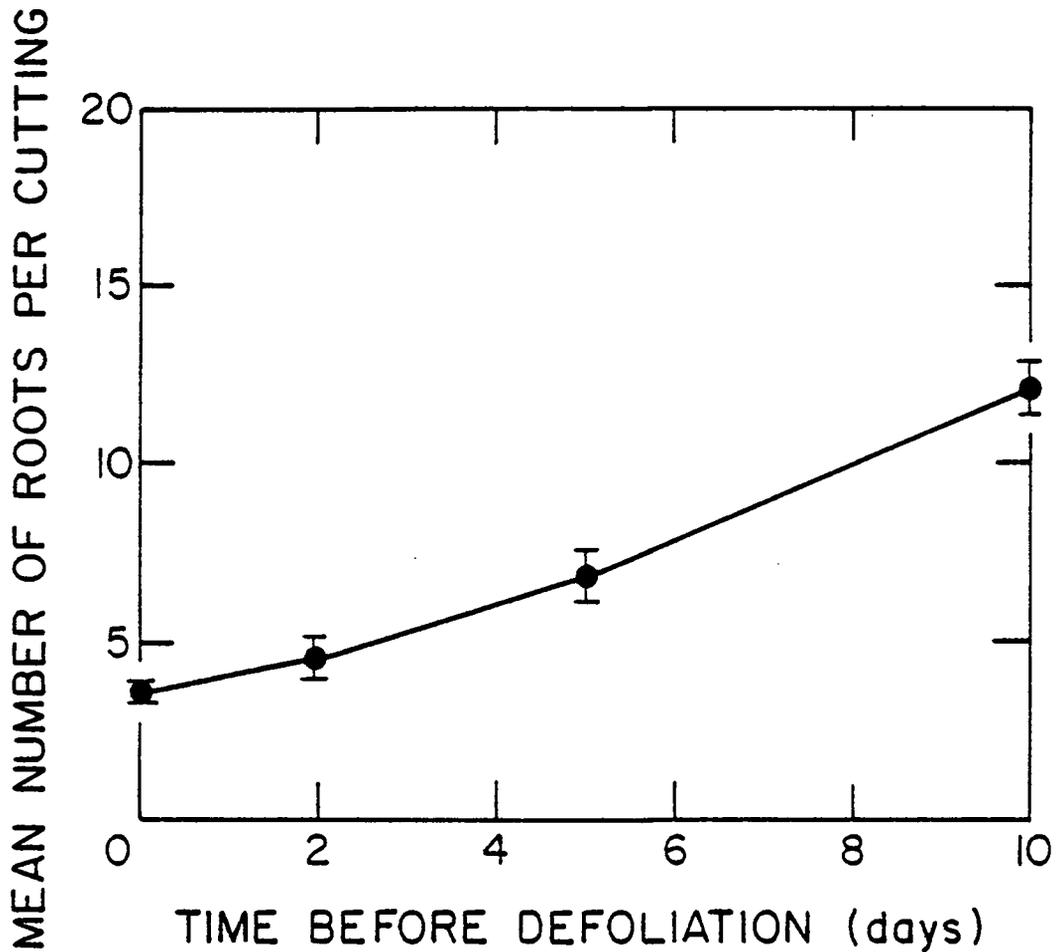


Figure 4. The effect on rooting of defoliation of pea cuttings at different times during the rooting period. Bars indicate \pm standard error of the mean ($n=32$). PPFD during rooting was $280 \mu\text{E m}^{-2} \text{s}^{-1}$. The number of roots per cutting was counted 10 days after excision.

Table 6. Effect on rooting of light incident on pea cuttings for the first or second 5 days of the rooting period. The number of roots per cutting was counted 10 days after excision. Photosynthetic photon flux density during light periods was $280 \mu\text{E m}^{-2} \text{s}^{-1}$. Daily photoperiod length was 14 hr.

Period during rooting in which light was incident on the cuttings

	<u>entire rooting period</u>	<u>first 5 days of the rooting period</u>	<u>second 5 days of the rooting period</u>	<u>darkness for the entire rooting period</u>
Number of roots per cutting + standard error of the mean	11.9 _± 1.1	8.7 _± 0.9	3.4 _± 0.5	1.4 _± 0.3

--all means are significantly different from one another based on all possible unpaired t tests (n=23)

Table 7. Effects of light and defoliation on rooting, dry weight, and carbohydrate levels in the basal 3 cm of pea cuttings. Photosynthetic photon flux density during light periods was $280 \mu\text{E m}^{-2} \text{s}^{-1}$. Carbohydrate levels and dry weights were measured 7 days after excision. The number of roots per cutting was counted 10 days after excision. Plus/minus values indicate standard error of the mean (n=29 for rooting data in the first 4 columns, n=17 for rooting data in the last two columns, n=8 for dry weight and carbohydrate data). Means within a row with common lower case letters are not significantly different at the 5% level of probability.

	Treatment					
	<u>light + leaves</u>	<u>light + defoliated</u>	<u>dark + leaves</u>	<u>dark + defoliated</u>	<u>second node leaf removed</u>	<u>first node leaf removed</u>
number of roots per cutting	12.6+1.0a	5.1+0.4c	2.5+0.3d	0.1+.05e	9.4+0.8b	9.2+1.0b
dry weight, mg	15.0+0.6a	7.4+0.5bc	7.6+0.5b	6.2+0.3c	--	--
mg sucrose	1.29+0.1a	0.22+.03b	0.04+.04c	0.03+.03c	--	--
sucrose, percent of dry weight	8.7+0.6a	3.1+0.4b	0.50+0.07c	0.41+0.1c	--	--
mg glucose	0.77+.05a	0.13+.02b	0.12+.02b	0.10+.01b	--	--
glucose, percent of dry weight	5.3+0.3a	1.7+0.2b	1.6+0.2b	1.7+0.2b	--	--

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APPENDICES

The Time Course Of Adventitious
Root Appearance on Pea Cuttings

To determine the time course of root formation on leafy pea cuttings made from 10 day old pea seedlings, the number of roots per cutting was counted 7,9,10, and 11 days after excision. Stock plant growth conditions were: PPF_D= 350 $\mu\text{E m}^{-2} \text{s}^{-1}$; photoperiod= 14 hr; day/night temperature= 21/19 C; and the soil media was Jiffy Mix (George Ball Pacific). The rooting environment was the same as stock plant growth conditions except the PPF_D was 280 $\mu\text{E m}^{-2} \text{s}^{-1}$. Roots began to appear 7 days after excision (Figure 5). By the 11th day of the rooting period the appearance of new roots had nearly reached a plateau and most of the roots had appeared by the 10th day. Experiments presented in this thesis, therefore, were scored ten days after excision.

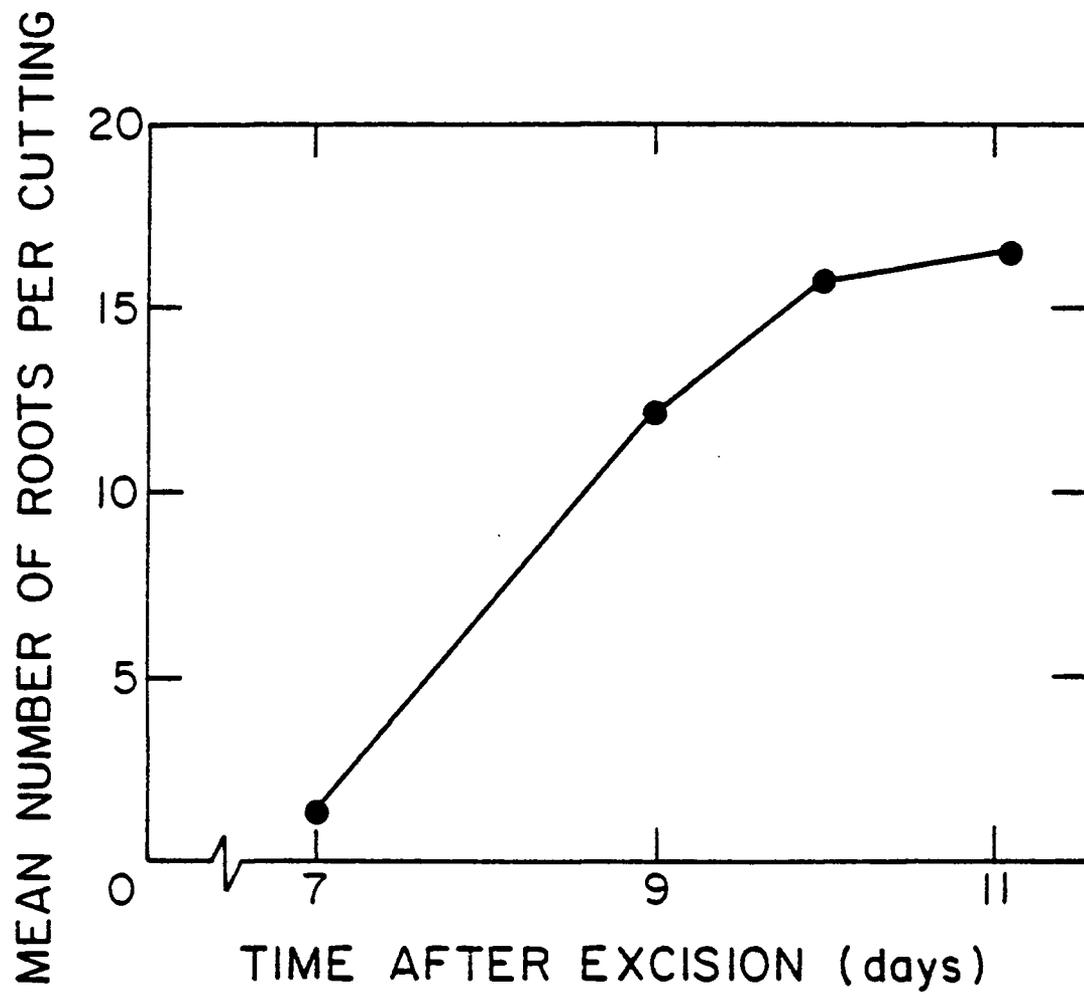


Figure 5. The time course of the appearance of roots during the rooting of pea cuttings.

The Pathway of Water Uptake by Pea Cuttings

It has been suggested that cuttings take up water through the cut end of the stem during rooting (14). In the present study, to determine the importance of water uptake through this pathway, lanolin was applied over the cut surface of pea cuttings. Lanolin, a non-phytotoxic, hydrophobic substance, should block this pathway of water uptake. Stock plant and cutting growth conditions were the same as described elsewhere (p. 67). Lanolin-treated cuttings wilted severely with an apparent loss in turgor and suffered severe desiccation. Rooting was almost completely inhibited as lanolin-treated cuttings formed only 0.5 roots per cutting compared to controls which formed 12.0 roots per cutting. This experiment indicated that pea cuttings take up water through the cut end of their stems and that blockage of this pathway increases desiccation and greatly reduces rooting.

Carbohydrate Accumulation
in the Basal End of Leafy Pea Cuttings
During the First Seven Days of the Rooting Period

The extent to which carbohydrates accumulate in the basal 3 cm of leafy pea cuttings was investigated. Stock plant growth conditions and rooting conditions were the same as described elsewhere (p. 67). Starch, sucrose, and glucose levels in the basal 3 cm were analyzed on the day of excision and again 7 days later. Methods of analysis are described on p. 24-25. Levels of all three carbohydrates increased greatly during the first 7 days of the rooting period on both a total weight and percent of dry weight basis (Table 8). Starch was a very small component of the carbohydrate content. Dry weight of the basal 3 cm increased threefold over the first 7 days. Roots first appeared on the cuttings 7 days after excision. These results suggest that the basal end of pea cuttings is a strong carbohydrate sink during root formation.

Table 8. Carbohydrate accumulation in the basal 3 cm of pea cuttings during the first 7 days of the rooting period. The photosynthetic photon flux density during rooting was $280 \mu\text{E m}^{-2} \text{s}^{-1}$ and the daily photoperiod length was 14 hr.

	Time when component was analyzed	
	at excision	7 days after excision*
	<u>Dry weight (mg)</u>	
	6.1	20.0
	<u>Carbohydrates (mg)</u>	
Starch	0.007	0.057
Sucrose	0.06	1.87
Glucose	0.18	1.3
	<u>Carbohydrates (% of dry weight)</u>	
Starch	0.10	0.29
Sucrose	1.0	9.5
Glucose	3.0	6.4

*all 7 day means are significantly higher than the corresponding at excision means at the 5% level of probability as determined by unpaired t test (n=4)

Comparison of the Rooting of Cuttings of
Begonia foliosa and Coleus blumei in Light and Dark

It has been reported that leafy pea cuttings rooted poorly in the dark (18) whereas leafy Hibiscus rosa-sinensis cuttings rooted equally well in moderate light or in the dark (64). To better understand the generality of rooting in the dark, cuttings of Begonia foliosa HBK and Coleus blumei Benth. cv. Princeton were rooted in moderate light and in the dark. B. foliosa cuttings were 5-8 cm long and had 6-10 leaves. C. blumei cuttings were 5-8 cm long and had 4-6 leaves. Rooting conditions were: PPF_D = 225 or 0 $\mu\text{E m}^{-2} \text{s}^{-1}$ for B. foliosa and 125 or 0 $\mu\text{E m}^{-2} \text{s}^{-1}$ for C. blumei; day/night temperatures = 21/19°C; photoperiod length = 14 hr; rooting media was Jiffy Mix (George Ball Pacific). Cuttings were taken from stock plants grown in a greenhouse at an approximate temperature of 22°C. The number of roots per cutting was counted 14 days after sticking for B. foliosa and 12 days after sticking for C. blumei.

The rooting of cuttings of both species was greatly reduced in the dark compared to moderate light (Table 9). Light incident on the upper portion of cuttings of these species apparently was necessary for good root formation. These species responded more like pea cuttings than

. Hibiscus cuttings when they were rooted in the dark.

Hence, the poor rooting of pea cuttings in the dark is not unique.

Table 9. Rooting of Begonia and Coleus cuttings in moderate light and darkness.

The number of roots per cutting

<u>Photosynthetic photon flux density during rooting</u>	<u>Begonia foliosa</u>	<u>Coleus blumei</u>
225 $\mu\text{E m}^{-2} \text{s}^{-1}$	28.1**	--
125 $\mu\text{E m}^{-2} \text{s}^{-1}$	--	16.3**
0 $\mu\text{E m}^{-2} \text{s}^{-1}$	1.3	0.0

**means significantly higher than corresponding 0 $\mu\text{E m}^{-2} \text{s}^{-1}$ means at the 1% level of probability as determined by unpaired t test (n=8)