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Propagation of Hybrid larch by summer and winter cuttings

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Summary

Summer and winter cuttings from young Hybrid larch [*Larix* × *eurolepis* (HENRY)] were rooted at high levels (> 90%). The rate of rooting of summer cuttings was increased by indole butyric acid treatment though the final level was unaffected. Rooting decreased when the cuttings were collected later in the growing season. Cold storage of winter cuttings increased rooting whereas warm storage of more than three weeks resulted in extensive callus development but a large reduction in subsequent rooting. Extended warm storage followed by cold storage drastically reduced rooting.

Key words: Rooting, Hybrid larch, Summer cuttings, Winter cuttings.

Zusammenfassung

Sommer- und Winterstecklinge von Lärchenhybriden [*Larix* × *eurolepis* (HENRY)] bewurzelten sich mit Erfolg (> 90%). Behandlung mit Indolbuttersäure erhöhte das Bewurzelungsvermögen der Sommerstecklinge, blieb jedoch ohne Einfluß auf den Gesamterfolg. Die später in der Vegetationsperiode geschnittenen Stecklinge wiesen geringere Bewurzelung auf. Kühlraumlagerung während der Wintermonate begünstigte das Bewurzelungsvermögen; dagegen trat nach Warmlagerung von mehr als drei Wochen beträchtliches Calluswachstum an der Schnittfläche der Stecklinge auf, jedoch auf Kosten des darauffolgenden Bewurzelungsvermögens. Längere Warmlagerung mit nachfolgender Kühlraumlagerung verminderte das Bewurzelungsvermögen ganz erheblich.

Introduction

Larix × *eurolepis* (HENRY) is frequently superior in the F_1 generation to the two parent species in terms of growth (HOLST, 1974), form (ELIASSON and CARLSON, 1963), drought resistance (KEIDING, 1962) and canker resistance (KIELLANDER, 1958). It has been planted extensively in the United Kingdom and demand has always exceeded the supply of seed (BIGGIN, 1977). Production of seed has been low due mainly to differences in the flowering times of the two parent species in seed orchards and the irregularity of good seed years. It is envisaged that, in the short term and until production from seed orchards can be increased, the demand for Hybrid larch seedlings might be met by vegetative multiplication from seedlings of superior genotypes.

The vegetative propagation of plants from stem cuttings has been used extensively in the horticultural industry for both herbaceous and woody species. The production of rooted stem cuttings of conifers has become important in recent years for producing clonal plants for experimental purposes (BURDEN and SHELBOURNE, 1974), for the testing of genetically superior stock produced by tree improvement programmes (RAUTER, 1974) and for commercial forestry (KLEINSCHMIT, 1974).

Larix spp. have been propagated vegetatively from cuttings although the levels of rooting achieved have tended to be low. ISIKAWA (1969) demonstrated that cuttings from young *Larix kaempferi* clones rooted best but rooting declined with increasing lignification of the cuttings and other workers found that the level of rooting also declined with the age of the donor clone (SAKAMOTO, 1972; OKADA, 1968). CHANDLER (1959) achieved 40% rooting with cuttings collected in August from *Larix* clones under four years old. Rooting at other periods of the summer, and from older ortets, was lower, although it could be increased by treatment of the cuttings with plant hormone solutions or commercially available rooting powders.

Hybrid larch is deciduous and two very different types of cutting are available at different times of the year, ie softwood, leafy summer cuttings and hardwood, leafless winter cuttings. This paper describes the propagation of both types of cutting.

Materials and Methods

a. Leafy, softwood summer cuttings.

Current season, first order lateral shoots, 10 cm long, were collected from nursery grown, 1½ year old seedlings (Hybrid larch seed orchard NT 10) on 8, 22 and 29 August and 5 and 12 September 1977. The bases of the cuttings in the earlier collections were greenish-brown with little lignification and extending apices whereas those in the later collections were browner with more lignification and had set bud.

The cuttings were transported moist in large polythene bags. The basal 0.5 cm was trimmed from each cutting and the cuttings were basally soaked for 2h in an aqueous solution of 10 or 100 pm indole butyric acid (IBA) with deionised water as control. The control and two treatments were randomised as split plots within each of six blocks for each of the five collection dates. There were 20 cuttings per split plot.

b. Leafless, hardwood winter cuttings.

First order lateral shoots, 10 cm long were collected from nursery grown 1¾ year old seedlings (NT 10) on 25 November 1976. The basal 0.5 cm was trimmed from each cutting and the cuttings were basally soaked for 6h in an aqueous solution of 50 ppm IBA with deionised water as control or basally dipped for 1 sec in a solution of 1000 ppm IBA in absolute alcohol. After IBA treatment, the cuttings were subjected to a period of warm storage followed by a period of cold storage, ie the cuttings were packed in polythene bags with a small amount of moist peat and treated with a factorial of 0, 3, 6 and 9 weeks warm storage at 25⁰ in the dark followed by 0, 3, 6 and 9 weeks cold storage at 2⁰ in the dark. The IBA treatments were randomised as split plots of 20 cuttings within each storage treatment, with the storage treatments randomised within each of three blocks.

c. Propagation

After treatment, both summer and winter cuttings were inserted to a depth of 3 cm into a 50-50, vol-vol mixture of fine peat and sand (2—5 mm) in seed trays at 3.5 cm spacing. The cuttings were maintained in a mist house daylength being supplemented to 18hrs with mercury vapour lamps (G·G wm^{-2}) where necessary, with an air temperature of 18—24⁰ and a propagation medium temperature of 17—20⁰. Watering was carried out by a pressure mist system controlled by an electronic leaf.

All cuttings were assessed for rooting and viability at intervals after insertion into the propagation beds until 98 days. Cuttings were considered to have rooted when the first root to emerge was longer than 1 cm, adjudged to be dead when the base showed definite signs of being water soaked with dying leaves and the remainder assessed as alive and unrooted. The data was subjected to arcsin transformation for proportions before statistical analysis, ie sine $^{-1}\sqrt{x}$ (SNEDECOR and COCHRAN, 1967).

Winter cuttings were visually assessed for signs of development during their period of warm and cold storage and sample cuttings were taken, hand sectioned, stained with potassium iodide/iodine solution and inspected microscopically.

Results

a. Leafy, softwood summer cuttings.

The level of rooting varied both with the time of collection of the cuttings and with the IBA treatments. In the earlier collections, such as 22 August, most of the cuttings had rooted by 8 weeks with little additional rooting after this time (*Fig. 1*). By contrast, the rooting of cuttings from the last collection, 12 September, was slower initially and the final level achieved was lower. Statistical analysis of the data demonstrated that the rooting of cuttings collected on 8 August was significantly increased by 100 ppm IBA treatment initially although at later assessments rooting

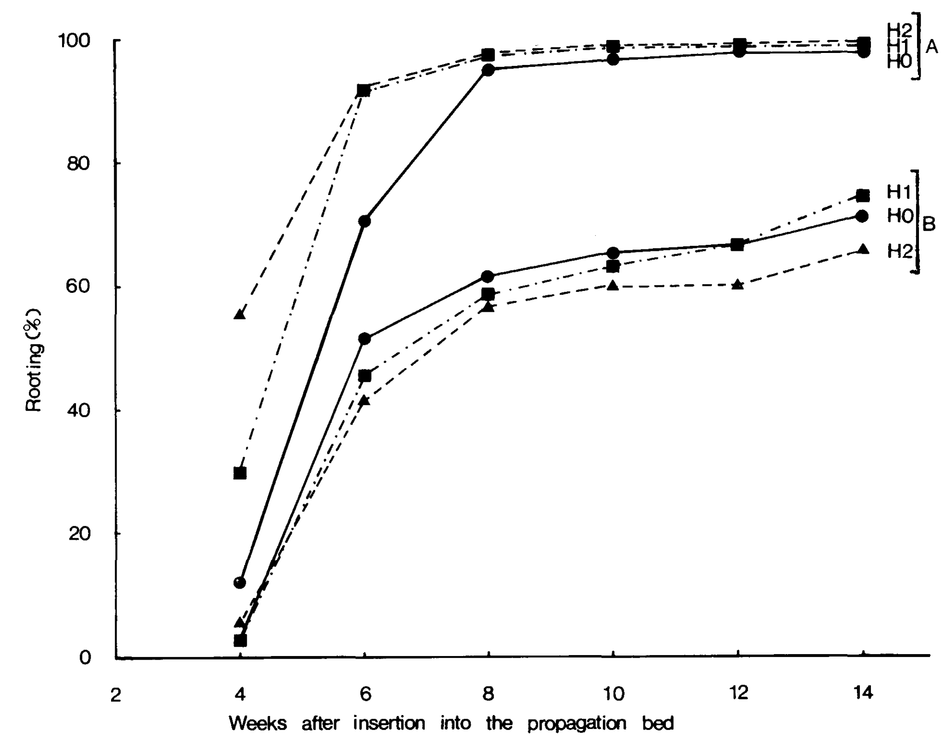

Figure 1. — The rooting of summer cuttings collected 22 August 1977 (A) and 12 September 1977 (B) after treatment with Oppm IBA (H0), 10ppm IBA (H1) and 100ppm IBA (H2).

Table 1. — The rooting of summer cuttings collected 8 August 1977. The percentage data has been back-transformed from arcsin.

Weeks After Insertion into Propagation Bed.	Treatment Control arcsin	% rooted	10ppm IBA arcsin	% rooted	100ppm IBA arcsin	% rooted	LSD (5%) arcsin
4	6.0	1.1	6.5	1.3	26.4	19.8	9.4
6	47.5	54.3	46.0	51.7	63.6	80.1	11.0
8	64.2	81.0	62.7	79.0	73.0	91.4	7.6
10	73.0	91.4	74.8	93.2	81.9	98.0	7.7
12	76.5	94.5	77.9	95.6	81.9	98.0	8.7
14	77.4	95.2	77.9	95.6	81.9	98.0	8.5

Table 2. — Increased rooting of summer cuttings as a result of IBA treatment. Statistically significant differences at 5% (*), 1% (**) and no significant difference (-) are represented.

Collection Date	Weeks After Insertion into Propagation Bed 4	6	8	10	12	14
8 August	**	**	*	-	-	-
22 August	**	**	-	-	-	-
29 August	**	-	-	-	-	-
5 September	**	-	-	-	-	-
12 September	-	-	-	-	-	-

Table 3. — Analysis of rooting for each assessment date between collections, and between IBA treatments for summer cuttings. Statistical differences at 5% (*), 1% (**) and no significant difference (-) are represented.

Source of Variation	Weeks After Insertion into Propagation Beds 4	6	8	10	12	14
Collections	**	**	**	**	**	**
IBA Treatment	**	-	-	-	-	-
Interaction	*	**	-	-	-	-

was not significantly affected *(Table 1)*. In the later collections, early rooting was also promoted by IBA treatment although the differences were not maintained, and the effectiveness of IBA treatment was reduced when cuttings were collected later in the year *(Table 2)*.

There were significant differences between the collection means at each assessment *(Table 3)*. There was also a significant IBA treatment effect at 4 weeks and an interaction between collections and IBA treatment at 4 and 6 weeks. When the data were meaned across hormone treatments within each collection, cuttings collected on 22 August and 5 September were found to root at a significantly higher level than the other collections after 4 and 6 weeks *(Table 4)*. After 14 weeks there was no significant difference between collections except that those collected 12 September rooted at a signficantly lower level throughout. The 12 September collection was found to have more cuttings alive and unrooted, and cuttings that had died, compared to the other collections after 14 weeks *(Table 5)*.

b. Leafless, hardwood winter cuttings.

The development of the cuttings during warm storage followed a similar trend in the different hormone treatments. After 1 week of warm storage, the cuttings were beginning to swell slightly at the base and by 2 weeks the first traces of callus were visible as a ring in the region of the cortex, with little or no apical die back. After 3 weeks, most cuttings were callused and callus completely covered the bases of 50% of the cuttings. The bases of the cuttings were considerably swollen by 4 weeks and fungal infection was found to be present in some instances after 5 weeks and increased with the duration of storage. Roots development on a few cutting bases after 6 weeks, though this was not correlated with any particular IBA treatment. The development of callus and roots at the base of the cuttings was halted when they were transferred to cold storage. The cuttings that were cold stored without previous warm storage showed neither basal development nor any visible trace of fungal infection.

The untransformed data of percentage rooting with time, when plotted before statistical analysis, gave rise to a number of different types of curve for the IBA treatments within each of the storage treatments. Some examples are given in *Fig. 2*. Generally the data for cold storage, without previous warm storage, give curves that rise sharply to 10 weeks followed by a levelling off. The control, no storage curves, show a linear increase in rooting with time to 14 weeks, whereas the curves for warm storage alone show levels of rooting after 6 weeks that barely increase with time. Combinations of warm and cold storage result in curves that have some similarities to the two types of storage alone. The shapes of the individual curves have not been analysed statistically.

Statistical analysis of the data after arcsin transformation demonstrated that more than 3 weeks of warm storage was, by itself, sufficient to cause a significant decrease in the level of rooting after 14 weeks *(Table 6)*. Cuttings that had been warm stored or cold stored for up to 3 weeks rooted at a higher level than unstored cuttings and cold storage for up to 9 weeks without previous warm storage further increased the level of rooting. These effects were common to all three IBA treatments. Any warm storage prior to cold storage reduced the promotion of rooting attributable to cold storage alone. Indeed, when the two types of storage were used for extended periods, 9 weeks each for example, rooting was reduced almost to zero. The deleterious effect of warm storage produced vast responses that made it difficult to give proper emphasis to the statistically significant interactions between warm and cold storage, between warm storage and IBA treatment and between warm storage, cold storage and IBA treatment. There was neither a significant effect of IBA treatment nor an interaction between hormone and cold storage. Even though not all the cuttings that were unrooted at 14 weeks were dead, it was found that the longer the warm storage period, the greater the

Table 4. — The effect of collection date on the rooting of summer cuttings. Means include all IBA treatments. The percentage data has been back-transferred from arcsin.

Collection date	Weeks After Insertion Into Propagation Beds											
	4		6		8		10		12		14	
	arcsin	% rooted	arcsin	% rooted	arcsin	% rooted	arcsin	% rooted	arcsin	% rooted	arcsin	% rooted
8 August	13.0	5.0	52.3	62.7	66.6	84.2	76.6	94.6	78.7	96.2	79.1	96.4
22 August	33.7	30.8	69.2	87.4	82.9	98.5	84.7	99.1	85.4	99.3	85.4	99.3
29 August	15.1	6.8	56.0	68.7	75.7	93.9	77.7	95.4	78.2	95.8	78.2	95.8
5 September	26.8	20.3	66.5	84.1	80.2	97.1	81.9	98.0	82.6	98.3	82.8	98.4
12 September	8.5	2.2	42.9	46.3	50.6	59.8	53.9	65.4	54.9	66.9	58.9	73.3
LSD (5%)	8.9		12.6		7.0		7.4		7.5		7.0	

incidence of basal death after insertion into the propagation beds (*Table 7*).

Inspection of the iodine stained, hand cut sections demonstrated that stored carbohydrate in the form of starch gradually disappeared from the winter cuttings with increased duration of warm storage. Starch was found to be present in large quantities in the rays, phloem parenchyma and cortex of unstored cuttings. The amount of starch was drastically reduced in the cortical tissues and had disappeared from the basal 3 cm of the cuttings after 3 weeks warm storage and the only traces that could be found after 6 weeks were minute amounts in the rays in the apical region of the cuttings. Cold storage alone resulted in no observable reduction in the amount of starch present in the cuttings.

Discussion

The results demonstrate that stem cuttings taken from Hybrid larch seedlings under two-year-old can be rooted easily if taken during the summer or the winter.

The summer cuttings were collected over a period of time and it was only in the collection near the end of the growing season that a decline in rooting was observed, associated with a slower rate of rooting and a higher incidence of basal death. It has been found that an increase in lignification at the base of summer cuttings can result in a small decrease in rooting (unpublished) even though the roots do not arise in the lignified tissue of the cuttings (John, 1978). Treatment of the cutting bases with IBA resulted in an increase in the rate of rooting during the early stages of the rooting process but the treatment did not significantly alter rooting after 14 weeks in any of the different collections.

The winter cuttings differed from the summer cuttings in that growth had ceased and they were more lignified and leafless. The cuttings remained leafless during the warm and cold storage periods and bud break occurred only after insertion into the propagation beds. The two types of storage had very different effects in that cold storage resulted in virtually no basal development whereas warm storage resulted in extensive anatomical changes, with the formation of roots in some instances. When the two types of storage were used in combination, basal development during treatment stopped when the cuttings were transferred from warm to cold storage.

The warm storage resulted in extensive basal development but subsequent rooting was poor if the storage was extended beyond 3 weeks. Bartoloni and Briccoli-Bati (1975) observed similar effects when they stored peach hardwood cuttings in polythene bags and found that short periods of warm storage (15 days) resulted in good rooting and establishment (57%) whereas extended warm storage resulted in a reduction of rooting and no establishment. It is probable that, after warm storage, the Hybrid larch cuttings were so depleted of reserves that they were unable to flush properly, produce photosynthate and grow normally, with the result that the cuttings died soon after insertion into the propagation beds.

The effectiveness of cold treatment alone in promoting subsequent rooting was probably due to the cuttings being

Table 5. — Summer cuttings that were alive and unrooted, or dead after 14 weeks in propagation beds. The Arcsin data has been back transformed to percentages.

Collection Date	Alive		Dead (by difference)
	arcsin	%	%
8 August	8.0	1.9	1.7
22 August	1.0	0.1	0.6
29 August	4.6	0.6	3.6
5 September	3.4	0.4	1.2
12 September	23.5	15.9	10.8

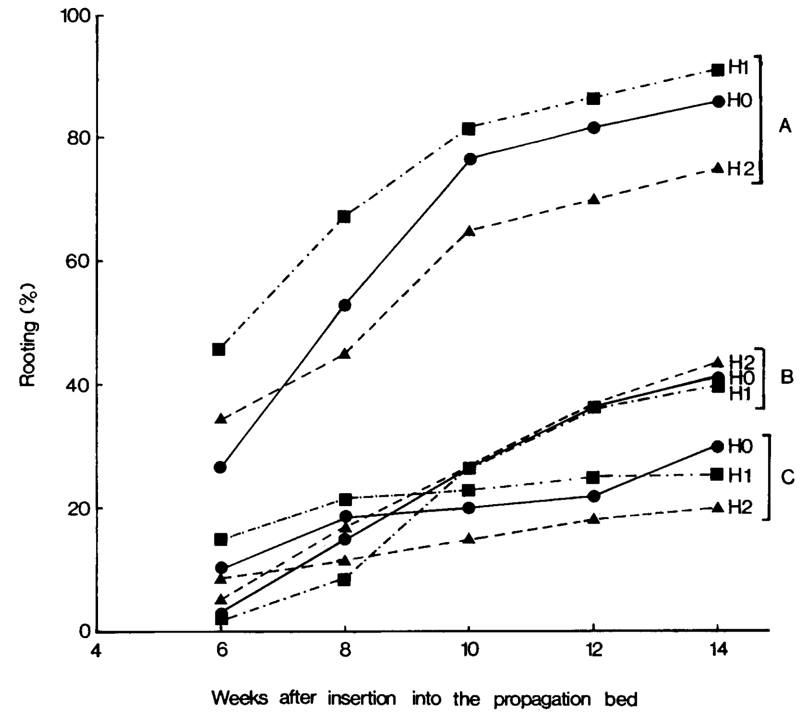

Figure 2. — The rooting of winter cuttings after storage of 0 weeks warm and 6 weeks cold (A), 0 weeks warm and 0 weeks cold (B) and 6 weeks warm and 0 weeks cold (C) and treatment with 0ppm IBA (H0), 50ppm IBA (H1) and 1000 ppm IBA (H2).

Table 6. — Rooting of winter cuttings after treatments with IBA warm and cold storage and 14 weeks in the propagation beds. The Arcsin data (standard error 4.91°) has been back transformed to percentages

Cold Storage (weeks)	IBA treatment (ppm)	Warm Storage (weeks) 0 Arcsin	0 % Rooted	3 Arcsin	3 % Rooted	6 Arcsin	6 % Rooted	9 Arcsin	9 % Rooted
0	0	40.2	41.6	60.6	75.9	26.7	20.2	13.2	5.2
	50	36.1	34.8	48.8	56.7	22.3	14.4	18.1	9.6
	1000	41.1	43.6	58.0	71.9	33.9	31.0	0.0	0.0
3	0	58.9	73.2	39.0	39.7	20.5	12.2	4.3	0.6
	50	52.4	62.8	49.8	58.4	16.5	8.0	0.0	0.0
	1000	55.0	67.1	41.2	43.3	30.7	26.0	8.6	2.2
6	0	71.4	89.8	52.4	62.8	20.8	12.6	0.0	0.0
	50	76.3	94.3	60.1	75.1	4.3	0.6	0.0	0.0
	1000	60.1	75.1	59.7	74.6	10.5	3.3	4.3	0.6
9	0	64.8	81.9	38.0	37.9	25.2	18.1	4.3	0.6
	50	70.5	88.9	40.8	42.7	0.0	0.0	0.0	0.0
	1000	72.0	90.4	39.8	41.0	14.8	6.5	0.0	0.0

Table 7. — The effect of storage of winter cuttings on rooting and death. The data give examples only before Arcsin transformation and statistical analysis.

Storage (weeks) Warm	Cold	% Rooted	% Dead	% Alive Unrooted (by difference)
0	0	40.0	2.2	57.8
0	9	86.7	1.1	12.2
9	0	6.1	90.6	3.3
9	9	0.6	99.4	0.0

transformed from a dormant to a post dormant state, *Larix* being a genus that requires a period of chilling for effective bud break and growth (SIMAK, 1970). The possible reduction of inhibitory substances during cold storage (WAREING and PHILLIPS, 1970) with no reduction in nutrient reserves could have resulted in the cuttings being in the right physiological condition for rooting (BIRAN and HALEVY, 1973). Conversely it can be argued that the reduction in rooting in the last summer collection was due to an increase in inhibitory substances.

The present breeding programme for Hybrid larch in the United Kingdom is aimed at combining the best attributes of the two parent species to produce plants that are of good form, canker resistant and vigorous. The demand for Hybrid larch for afforestation exceeds the amount of available seed by far and vegetative propagation is seen as a technique that can make good the present shortage. It has been demonstrated that, by using seedlings grown under nursery conditions as a cutting source, a multiplication of 10 times can be easily achieved over a period of four years (Biggin unpublished). It is estimated that intensive greenhouse management could increase this to 500 times. However the technique envisaged requires the continuous input of juvenile clones for cutting stock plants since Hybrid larch ramets are particularly prone to cyclophysis and topophysis and cuttings taken from mature stock plants remain plagiogeotropic. Orthotropic growth can only be achieved readily if cuttings are taken from stock plants that are under four-year-old.

The use of untested clones for large scale propagation could introduce an element of risk, although it has been demonstrated that clones derived from NT10 seed orchard seed are comparable in growth to a known, superior hybrid cross (Faulkner *et al.*, 1976). Clones not demonstrating the desired characteristics could be selected out during stock tree production.

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