BARD

FINAL REPORT
PROJECT NO. US-806-84

**Use of Rejuvenation Techniques to Increase
the Rootability of Developmentally Mature
Clones of Tree Species**

W. P. Hackett, A. H. Halevy

1989

Final Report

BARD Project 1985-88

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**Use of Rejuvenation Techniques to Increase
the Rootability of Developmentally Mature Clones
of Tree Species**

Investigators:

Wesley P. Hackett and Abraham H. Halevy

Cooperators:

Carl Mohn

Emily Chesick

Kathryn Louis

Joseph Riov

Mirit Hamburger

Standard BARD Cover Page for Scientific Reports

Date 6/6/89

BARD
P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. U.S. 806-84

Title of Research Project:

Use of rejuvenation techniques to increase the rootability of
developmentally mature clones of tree species

Names of Investigators:

Principal Investigator: Wesley P. Hackett

Cooperating Investigator(s): Abraham H. Halevy

Name and Address of Affiliated Institutions:

Principal Institution: University of Minnesota, St. Paul, MN 55108

Cooperating Institution(s): Hebrew University, Rehovot

Project's starting date: 9/16/85

Type of Report: 1 Annual 2nd Annual Final X

Signatures:

Principal Investigator

Institution's Authorizing Official

Wesley P. Hackett

Mitch Pearl

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c. Abstract

White and jack pine (Pinus strobus and P. banksiana)

Two approaches were emphasized in efforts to induce rejuvenation in these species:

1. Induction of interfascicular bud formation on mature trees.
2. In vitro culture of buds from mature trees.

The results reported show that interfascicular buds can be induced in large numbers on mature white pine trees if expanding buds are pruned when needles are half fully expanded and pruned expanding buds are treated 4 times with cytokinins thidiazuron at 50 or 100 mg/l or benzyladenine at 1000 mg/l over a three week period. Unpruned buds did not respond to a one time treatment with 1000 mg/l BA but a 4 time treatment was not used and should be tried in future experiments to eliminate time required for pruning. The cytokinin multi-treatment protocol also works for induction of interfascicular buds from mature jack pine trees but the numbers formed are much lower.

Rooting of cuttings from interfascicular and natural shoots of mature white pine trees was poor (17-25%) and was not affected by IBA treatment.

Optimal protocols for disinfestation and in vitro survival of excised buds from mature trees of these species were determined experimentally. Many disinfestation agents were tested. The two most important steps in disinfestation protocol for these pines are: 1) the removal of resin which harbors micro-organisms and impedes wetting by and penetration of disinfestation agents and 2) disinfestation. Ethanol is the best resin solvent taking into account phytotoxicity. With the optimal disinfestation protocols 80% sterile viable buds could be obtained with white pine and 50-75% sterile viable buds with jack pine.

Experiments with plant growth regulators indicate the auxin IBA is more important for bud elongation than is the cytokinin BA. A combination of IBA at 10^{-5} M with BA at 10^{-5} M also gave excellent elongation and resulted in a few buds forming interfascicular buds. These findings suggest that in addition to treatments involving combinations of IBA and BA to promote multiplication via interfascicular bud formation, sequences of growth regulators involving IBA followed by BA should be tried.

Hedera helix:

The leaf cutting grafting data provides strong evidence that cells with mature characteristics can be physiologically transformed to have juvenile characteristics by a translocatable substance(s). These results provide strong support for continued work on graft induced rejuvenation of pines. These results also provide a strong rationale for trying to isolate and characterize the translocatable substance(s). The in vitro debladed mature petiole bioassay system provides a means to detect biological activity as the isolation and purification procedures are carried out.

Aleppo pine: (Pinus halepensis)

Rooting ability in P. halepensis, was low and declined rapidly with age. Rooting of cuttings taken from 2- and 4-year-old trees was 30 and 10-15%, respectively.

An anatomical study indicated that mature tissues in the base of cuttings are able to proliferate and form callus which then differentiate single tracheary elements. The reason mature cuttings fail to root is probably the inability to initiate root primordia in the callus formed.

Three main strategies were conducted in order to improve rooting of cuttings:

1. Treatments given to cuttings.
 2. Using cuttings from interfascicular (brachyblast) shoots which were induced to elongate.
 3. Grafting mature scions on juvenile rootstocks in order to achieve rejuvenation.
- Preferable seasons for rooting were winter and spring, which correspond to the period of shoot elongation in mother plants.

Eight-tenths percent IBA combined with 10% sucrose, both given as powder, improved rooting of cuttings. Treatment with 10% sucrose powder without auxin also improved rooting in certain experiments.

Sucrose given in solution with IAA to hypocotyl cuttings inhibited auxin uptake and upward movement to cotyledons. Sucrose increased significantly IAA accumulation in the basal part of the hypocotyl, where roots are regenerated.

Removal of the terminal bud from 4 year-old trees resulted in formation of interfascicular buds followed by their elongation. These shoots were suitable for rooting. Treatment of decapitated shoot with benzyladenine (50 and 200 ppm) augmented the number of interfascicular shoots, decreased their length and caused formation of numerous axillary buds.

Cuttings from interfascicular shoots induced on 4-year-old plants, showed high rooting ability in a few experiments (up to 60%). Methods to utilize this ability have not been determined.

Success in grafting 4-year-old scions on juvenile rootstock was ca 50%. Graft incompatibility was not observed during the first 2 years after grafting. Success in grafting 60-year-old scions was 30% and was followed by invigoration and temporary juvenile-like morphology of the mature scions. Repeated grafting augmented graft success, invigoration and juvenile-like growth. Morphological or physiological markers that indicates improved rootability are not yet known. Therefore, appearance of juvenile-like growth indicated rejuvenating effects of different treatments in this work. This happened in interfascicular shoots which burst after removing the terminal bud, in elongating shoots of rooted cuttings and in mature scions that were grafted on juvenile rootstock. In all these cases, the duration of the juvenile-like morphology was no longer than 7 months.

Therefore, we recommend that further work with P. halepensis should concentrate in 2 main subjects: rejuvenation and improving of rooting. Serial grafting (4 repeated grafts per year) should be done for rejuvenation. Improvement of rooting percentage can be achieved by auxin

and sucrose treatments.

d. Objectives

The main objective of this project was to develop rejuvenation techniques for increasing the rooting potential of mature trees of Pinus halepensis (aleppo pine), P. banksiana (jack pine) and P. strobus (eastern white pine). A secondary objective was to develop a model experimental system for studying the basis of rejuvenation using juvenile and mature Hedera helix (English ivy).

e. Body of Report

Description of the Problem

Propagation of forest tree species is currently almost exclusively from seed. Genetic improvement is pursued to a limited extent by systematic hybridization, but to a greater extent through collection and utilization of seed from selected high productivity trees in natural stands and plantations. Unlike fruit tree species where quality and quantity of productivity can be estimated relatively early in development, the productivity (and perhaps other desirable characteristics) of forest trees can be determined only after many years (5-10 years or longer) when trees are developmentally mature.

Utilization of sexual (seed) propagation to capture genetic gain from breeding and/or selection programs for forest tree species is not very efficient because of heterozygosity and variability of progeny. However, genetic gain can be increased substantially if selected trees are cloned by propagating vegetatively. When cloning is used in conjunction with sexual breeding and/or selection programs, productivity gains of 25-50% per selection generation have been reported for forest tree species in Europe. The increase in productivity by cloning is immediate whereas with seed propagation several generations are required.

However, cloning of forest tree species of sufficient age and size to have demonstrated their silvicultural potential is often difficult or impossible with traditional methods of vegetative propagation such as cuttage and graftage, and equally or more difficult with newer in vitro tissue culture methods. This difficulty of vegetative propagation is related to the general loss of regeneration potential as woody plants age and mature during phasic development from seed. That is young (juvenile) seedlings up to some species-related size or age have high regeneration potential while older or larger (mature) plants begin to lose such potential and at some age or size will have a very low regeneration potential. For jack pine cuttings are very difficult to root when ortets reach the age of 5 to 7 years.

Evidence is accumulating that problems with vegetative propagation of mature trees can be reduced in some forest tree species by reversing the phasic developmental process so that they behave physiologically more like seedlings once again(3). Such so-called rejuvenated individuals are relatively easy to propagate by stem cuttings, an efficient and economically feasible method of cloning. Although successful methods of rejuvenation are not well

characterized and their physiological and developmental bases are entirely unknown, they have great potential for increasing our ability to clone mature trees using traditional and emerging cell and tissue culture methods of propagation.

Aleppo pine is one of the most important forest trees in Israel. Until 1973 this was the main forest tree planted. It is considered as a "pioneer tree" that can sustain and grow in conditions under which most other trees will not grow. It's main properties are adaptation to drought and alkaline soil. In the mid-seventies a severe attack by Matsucoccus josephi caused death of aleppo pines in wide area of Israel. This was the main reason for the great decrease in aleppo pine planting in recent years. Furthermore, P. halepensis is considered to be an inferior timber tree. However, one can find among the native populations of this tree specimens resistant to the pest and those that are superior in their timber qualities.

Vegetative propagation of these genotypes may enable the resumption of wide spread planting of P. halepensis. The superior qualities of the trees can be distinguished only in mature trees, by that time the tissue has lost its rooting ability. Rejuvenation of mature pine trees may enable us to vegetatively propagate these genotypes.

Jack pine and white pine are important forest species in north central USA and are major sources of pulpwood and lumber. Jack pine exhibits great genetic diversity of silvicultural significance. White pine is genetically diverse but susceptible to white pine blister rust disease which limits its use and future importance. However, rust resistant genotypes have been identified. The high economic value, genetic variability and disease resistance of selected genotypes make these species prime candidates for use of clonal methods of propagation.

We have taken three approaches to rejuvenation of pines: a) induction of interfascicular bud formation, b) graftage of mature scions on juvenile root-stock, and c) in vitro culture of mature buds.

Materials and Methods

White and jack pine

Induction of interfascicular bud formation: P. banksiana (jack pine) mother plant trees at Cloquet, Minnesota were 8-10 years old from seed depending on the year of treatment. They were from four genetically distinct populations, two with relatively high rooting potential when rooting was assessed at age 5 years. P. strobus (white pine) and P. banksiana mother plant trees at Rosemount, Minnesota were approximately 10-12 years old depending on the year of treatment.

Individual expanding buds were treated, with branches 3 to 6 feet off the ground serving as the experimental treatment unit. Five to ten buds on a branch unit were treated. Treatment time was varied based on fascicle needle length (May, June, and July). Plant growth regulators (benzyladenine, BA; naphthylphthalamic acid NPA, thidiazuron, TDZ) were applied as a spray in 50% (white pine) or 95% (jack pine) ethanol solution or a 0.2% dimethylsulphoxide (DMSO) in 95 or 50% ethanol solution (TDZ) to individual buds on the branch unit. Individual buds were pruned with a razor so that 0.5 to 1.0 cm of the tip was removed. Data were taken by counting

interfascicular buds on treated branches in September and October.

Rooting of cuttings: Cuttings from pruning and BA induced interfascicular (brachyblast) shoots and from shoots formed naturally as a result of branching at the beginning of a growth flush were stuck in fine textured sand on intermittent mist bench in a greenhouse at a minimum night temperature of 18°C. The intermittent mist was activated for 10 sec every 10 min. Cuttings were treated with 4000 ml/l of indolebutyric acid (IBA) in 50% ethanol or 50% ethanol alone. Rooting data was taken 12 weeks after sticking.

Grafting: With white pine grafting methods developed for Aleppo pine were used as a base. All grafts were done on containerized seedlings (12-15 months old) in a greenhouse.

In vitro bud culture: Dormant buds were obtained from 10-12 year old white pine and jack pine mother plant trees growing at Rosemount, Minnesota. Dormant buds from such a field source present difficult disinfestation problems. Initial experiments were carried out to find a treatment to obtain sterile explants with little injury to their tissues. In these experiments, Mercuric chloride, calcium hypochlorite, ethanol, sodium hypochlorite, iodine, hydrogen peroxide, antibiotics, flaming and ultra-sonication were used both alone or in combination. Treatments were applied to intact buds and to buds with scales partially or completely removed. Four standard tissue culture media were evaluated using excised white pine embryos: Schenk and Hildebrandt (4) Murashige and Skoog (5), Gresshoff and Doy (4), Durzan and Gupta (2) and Le Poivre (1). All were supplemented with 3% sucrose solidified with 0.6% agar and adjusted to pH 5.5. Various concentrations of BA and IBA have been tested to promote bud growth and development using LP medium. Cultures were grown in growth chambers with a 16 hour photo-period at 25/20 C day/night temperatures. The photon flux density was 50-80 E S⁻¹ m⁻² from fluorescent lamps.

Hedera helix:

Grafting: Grafting experiments with Hedera helix were carried out with excised fully expanded leaves. Four reciprocal graft combinations of juvenile and mature leaf petioles and laminae (Fig. 1) were made using either of two grafting techniques. In one, the scion or donor leaf was prepared by making sloping cuts into the adaxial and abaxial sides of the petiole in a direction away from the lamina resulting in a 20 mm tapered wedge shape end. The stock leaf was prepared by making a downward sloping incision approximately 15 to 20 mm in length into the adaxial side of the petiole starting 10 mm below the lamina. The scion was inserted into the incision in the stock petiole with the abaxial side of the lamina facing the adaxial side of the stock lamina. In the second grafting technique, a cut about 1 cm long was made along the long axis of each petiole so as to remove a slice of tissue with maximum thickness of one third the diameter of the petiole. The cut was positioned approximately equidistant between the base of the petiole and the lamina. The cut surfaces of the petioles were brought together. In both techniques, the graft was carefully wrapped with Parafilm to facilitate contact between the cut petioles surfaces and prevent the grafted area from drying out. Treatments include non-grafted juvenile and mature controls, juvenile scion/juvenile stock, juvenile scion/mature stock, mature scion/mature stock and mature scion/juvenile stock. Grafted and control petioles were stuck in a perlite-vermiculite medium (1:1 v/v) in flats and placed into an intermittent mist bench with bottom heat (27°C). Fifteen days after grafting, at which time the graft union was

anatomically complete, the cutting was removed from the propagation medium and the additional lamina and/or petiole removed with a scalpel.

The experiments were conducted as a completely randomized design with subsampling including at least 10 cuttings per treatment. Experiments were repeated several times and the results presented are from a representative experiment. Means were transformed when the variances were not homogeneous and separated by the least significant differences test (LSD) or Tukey's HSD multiple range test.

In vitro leaf petiole rooting system: The lamina was removed from the petiole of fully expanded leaves from the juvenile and mature phase of *H. helix*. Petioles were aseptically cut to a length of 2.3 cm and 5 petioles were placed in 25 ml Erlenmeyer flasks containing 1 ml of liquid medium at pH 5.8. An aluminum foil cap was placed over each flask prior to autoclaving. Root formation was evaluated after 18 days culture. A full description of the procedure can be found in Geneve et al, 1988.

Aleppo pine:

Grafting: One year old seedlings having juvenile growth appearance were normally used as rootstocks. In spring 1986 we used also 2-year old seedlings. As scion we used plant material from trees 4, 20, 35 and 60 years old collected from various locations. The grafted plants were grown either in a saran net house (15% shade) under ambient conditions, or in a greenhouse with 25-30°C day and 10-15° night temperatures during November-February and 25-32° day and 15-20° night during March-April. In one experiment, one lot was lighted throughout the night with cool white fluorescent lamps providing illuminance of 5000 lux at plant level.

Grafting was by cleft-grafting. The rootstock was pruned at a height of 5-15 cm, leaving a few viable branches at the base of the stock. The scion was cut 5 cm from the tip, and the needles cut to half their length (see Fig. 2). The grafting area was sprayed with fungicides, and the graft union was tied with parafilm. The scion and grafting area were covered with polyethylene bag and tied below the graft. A paper bag was placed on top of the plastic bag. The bags were gradually removed from the plants with successful grafts. First the plastic bag was perforated, then it was removed and finally the paper bag was removed. As the grafted scion grew, the rootstock branches were gradually removed, until only the scion was retained.

Promotion of interfascicular bud formation and sprouting of dormant buds: Most experiments were conducted with 4 year old plants. Some of the plants had been grafted 2.5 years before. Plants were in 5 l plastic bags with loamy soil and grown in greenhouse as above. Pinching was done by cutting the terminal bud with 1 to 5 cm of the stem below the bud.

The cytokinin BA was sprayed on the entire plant to run-off. Plants were sprayed 4 times at one week interval. The first spray was applied immediately after pinching. The spray solution included in addition to BA also the spreader L-77 (0.1%), 10 drops/l of KOH 0.25 N, and 1.5 ml/l of ethanol. The spreader was added first with constant stirring, BA was dissolved in ethanol and then KOH was added before pouring slowly into the spreader solution.

Rooting of cuttings and their development: Plant material for rooting was taken from various kinds of plants. Seedlings of different ages were obtained from KKL nurseries or from seeds germinated by us. Seeds were rinsed under running water for 48 hr, treated with Captan for 10 minutes and germinated at 22°C.

Cuttings were taken either from 3-4 year-old forest trees at Bert-Oren or from the plants used for the experiments to promote bud sprouting (see above). The interfascicular buds were removed together with a piece of the mother branch. The cutting was recut at the base of the interfascicular bud, at the point of its connection to the branch. Woody or semiwoody cuttings, 10-15 cm long were normally used. When interfascicular buds served as cuttings they were semiwoody, 5-18 cm long. The rooting substances were applied either as power or as solution. The solution was applied in a growth chamber with 18 h light at 25° and 6h dark at 20°. Four main rooting treatments were tested: a) control-talcum powder + 0.25% Captan, b) T₈ - a commercial rooting preparation containing 0.72% IBA and 3% TBZ in talcum, c) T₈ + sucrose (90% T₈ as above) plus 10% sucrose, d) sucrose (10% sucrose in talcum + 0.25% Captan).

The cuttings were rooted in various media in a rooting bench, using bottom heat during the winter months to 27 ± 2°C. A plastic tent was placed 1.2 m above the bench. Mist was applied during the day, twice a day for 10 minutes in the first year and every hour for 2 seconds in the later years.

In some cases in which the base of the cuttings rotted, the base was recut and rooted again.

Rooted cuttings were transferred to pots with vermiculite-peat-volcanic scoria (1-1-1 v/v), watered by drip twice a day with liquid fertilizer. Two months later they were repotted in 0.5 l plastic bags with local loamy-sand soil with Osmocot (2 g/l) and watered by overhead sprinklers.

Results & Discussion

White and jack pine.

Induction of interfascicular bud formation: Tables 1 and 3 show that for both white and jack pine pruning or decapitation of the expanding buds is required to induce needle fascicle bud formation. Results shown in these tables and Table 2 indicate that time of pruning in relation to fascicle needle development is also important. Early (bud elongation but no needle elongation) to mid (needles 50% of final length) development appear to be the best time of treatment for jack pine while mid to late (needles fully elongated) development is best for white pine. Tables 1, 2 and 3 also show that the growth regulators BA and NPA had little effect on either the number or size of fascicles buds formed on pruned or unpruned expanding buds even when applied at the optimal developmental stage. Based on these results 1986, subsequent experiments in 1987 and 1988 were performed only with expanding buds that were pruned at the middle stage of development (needles 50% elongated).

Because BA at 500 or 1000 mg/l did not promote fascicle bud formation in either species in 1986, a higher concentration was also used in 1987. Tables 4 and 5 show that there was little promotion of fascicle bud formation even by the higher concentration of BA in either species

when applied to pruned, expanding buds at the optimal stage of development.

These results suggested that either BA was not very active for inducing fascicle bud formation or that the timing or duration of its application was more important than we had thought. These possibilities led us to use TDZ, a much more active cytokinin and the use of multiple applications in 1988. Table 6 and 7 show that TDZ at 50 or 100 mg/l is more active than BA at 1000 mg/l but that for either TDZ or BA to be effective in promoting fascicle bud formation in white pine, it must be applied 4 times over a period of 3 weeks. Four time treatment with TDZ or BA was 7 to 10 times as effective as a one time treatment. From these results it is not possible to tell whether timing or duration of treatment is more important. Table 6 indicates that even a 4 times treatment with TDZ is not very effective for jack pine. These experiments in 1988 were carried out in the second year of a drought cycle and therefore may not reflect maximum activity for the treatments given.

In summary, these results show that interfascicular buds can be induced in large numbers on white pine if expanding buds are pruned when needles are half fully expanded and TDZ at 50 or 100 mg/l or BA at 1000 mg/l is applied 4 times over a 3 week period. Interfascicular buds can also be induced on jack pine by the same regime but the numbers formed are much lower.

Rooting of Cuttings: Rooting of cuttings from interfascicular and natural shoots of mature white pine trees was poor (17-25%) and was not affected by IBA treatment (Table 8). Poor rooting percentages were partially a result of rotting of cuttings particularly those from interfascicular shoots.

In vitro bud culture: Over 40 experiments involving surface disinfestation procedures for white and jack pine were carried out. These involved various combinations of the disinfestation chemicals listed in the Materials and Methods section and the use of intact buds and those with partial or complete removal of needle scales. Of the disinfestation agents tested, ethanol and calcium hypochlorite were most promising.

White and particularly jack pine buds are coated with resin or pitch. Ethanol is the best solvent, taking into account phytotoxicity considerations, for removing the resin which harbors micro-organisms and impedes wetting and penetration of disinfestation agents. Once the resin was removed, calcium hypochlorite was the most effective disinfestation agent taking into account phytotoxicity.

For white pine the best disinfestation and bud survival was obtained with the following sequence of treatments:

1. Wash in running water 10 min.
2. Agitate in Captan (1 gm/200 ml) 30 min.
3. Sterile tap water rinse.
4. Ethanol (50-70% depending on bud stage) 90 sec.
5. Sterile tap water rinse.
6. Agitate in filtered calcium hypochlorite solution (20 gm/l + 4 drops of Tween 20) 10-12 min.
7. Rinse 5 times in sterile distilled water.

8. Dissect away outer needle fascicle scales on wet paper towels.
9. Re-cut base of bud.

This procedure resulted in about 80% sterile, viable buds as evaluated 30 days after implantation (Table 9).

For jack pine the best disinfestation and bud survival was obtained with the following somewhat different sequence of treatments:

1. Wash in running water 10 min.
2. Ethanol (50%) 10 min.
3. Sterile tap water rinse.
4. Dissect away outer needle fascicle scales on wet paper towels.
5. Agitate in filtered calcium hypochlorite (20 gms/l + 1 ml Tween 20/l of water)
6. Rinse in sterile tap water.
7. Agitate in Captan 2.5 gm/l plus Alconox 1gm/l water.
8. Rinse 3 times in sterile distilled water.

This procedure resulted in 50-75% sterile, viable buds 30 days after implantation.

Following the successful definition of disinfestation procedures, experiments were established to define the culture medium and growth regulators needed for successful culture of white pine buds. Initially, only LP culture medium was used for dormant buds based on a screening of media for optimal growth of white pine embryos. Table 9 shows that viability of buds is low unless IBA or BA or a combination of the two growth regulators is included in the medium. The highest concentration of BA (10^{-3}M) alone was toxic while the highest concentration of IBA (10^{-5}M) alone gave the highest viability rate. BA alone at 10^{-4} and 10^{-5}M gave viability rates nearly as high as 10^{-5}M IBA. Combinations of 10^{-5}M BA with 10^{-7} or 10^{-6}M IBA also gave near maximal viability rates.

In a subsequent experiment, the growth regulator concentrations were modified to optimize bud growth and development. The results in Figure 3 show that buds elongate best using IBA alone at 10^{-5} or 10^{-4}M . IBA at 10^{-5}M in combination with BA at 10^{-5}M also gave excellent elongation but there was a tendency for basal callusing with this combination. This treatment resulted in a few buds forming interfascicular buds. There was also a tendency for basal callus with IBA at 10^{-4}M alone.

These results indicate that the auxin IBA is more important for bud elongation than is the cytokinin BA. Combinations of IBA and BA tend to give basal callusing at least at the concentrations tried so far. These findings suggest that in addition to treatments involving combinations of IBA and BA to promote multiplication via interfascicular bud formation, sequences of growth regulator treatments should be tried. It appears that an IBA treatment to obtain bud elongation followed by a BA treatment to induce fascicle bud formation holds potential for successful in vitro multiplication.

Work with jack pine has not progressed far enough to give conclusive results.

Grafting: Using methods developed for Aleppo pine as a base (see below), 150 scions from mature white pine trees were grafted to containerized seedlings in the greenhouse. Optimal time for grafting was just prior to bud expansion in early spring (late March in Minnesota). Scions developed very slowly so that there hasn't yet been enough material for serial grafting experiments. Grafting success with mature scions of jack pine has been very low, possibly related to the large amount of resin bleeding from wounds in spring. Fall grafting has not been tried.

Hedera helix

Grafting: The four reciprocal graft treatments of H. helix leaf cuttings shown in Figure 1 were designed to test whether juvenile lamina would promote rooting of difficult-to-root mature petioles and whether mature lamina would inhibit rooting of easy-to-root juvenile petioles. The results in Table 10 show that mature laminas do not inhibit rooting of juvenile petioles. However, juvenile lamina do promote rooting of difficult-to-root mature petioles but the rooting response occurs only during the period between 35 and 45 days when considerable callus has been formed at the base of the mature petioles. Histological examination shows that root initials originate from the callus cells only. This indicates that promotion of root initiation by juvenile laminas can occur only in cell populations resulting from auxin induced cell divisions. Mature petioles with mature laminas form much callus but few roots. This is strong evidence that cells with mature characteristics can be physiologically transformed by a translocatable substance so that they have juvenile characteristics (rejuvenated) and is strong support for continued work on graft induced rejuvenation of mature conifers. These results also provide a strong rationale for trying to isolate and characterize the translocatable substance(s) from juvenile lamina. The in vitro debladed mature petiole bioassay system provides a means to detect biological activity as the isolation and purification procedures is carried out.

In vitro leaf petiole rooting system: We have discovered that de-laminated juvenile and mature H. helix leaf petioles can be cultured in vitro and that mature petioles display the same low rooting ability as mature detached intact leaf cuttings or stem cuttings (Table 11). The conditions for their in vitro culture and the advantages of their use as an in vitro assay for rejuvenation activity are described in papers attached.

Aleppo pine:

Grafting: Pine trees and especially resin-rich species such as P. halepensis are difficult to graft. We started, therefore, with grafting scions from 4-year old plants and then used scions from much older plants. A total of about 650 graftings were performed. Over 50% of the grafts were successful. Grafting success was assessed by 4 criteria: a) percent graft take of total plants grafted, b) length of scion growth above the graft, c) number of shoot tips in the scion and d) percent tips with juvenile-like growth of total tips in the scions.

1. The effect of grafting-season.

In plants grafted in autumn (Nov-mid Dec), graft take could be observed about 3 months after grafting, growth started at end of February to early March. In the spring (March) - grafted

plants graft-take and scion growth was observed 5 weeks after grafting. Table 12 summarizes the differences between autumn and spring graftings. Much higher percent of rejuvenation was observed in the spring as compared to the autumn.

2. Effect of scion age.

Table 13 summarizes results of many grafting experiments using material from plants of various ages. It can be seen that even scions from 20-year old plants had high graft success rate, with relatively high percent of juvenile growth. These results also demonstrate that graft-success can be obtained even with 60-year old plants (see Fig. 4).

3. Repeated graftings.

In repeated graftings the scion was taken from a plant that have been grafted before. Table 14 presents data comparing first grafting to two types of repeated graftings. It is clear that repeated grafting greatly enhanced the graft success over first grafting. Repeated grafting after one year gave significantly better results in rejuvenation and number of growing tips. It seems that repeated graftings should be performed as soon as possible after the previous one.

4. Growth conditions of the grafted plants.

Plants grafted in the autumn and spring were divided into two lots, one grown under uncontrolled conditions in a saran net-house and the other in the greenhouse. Results of Table 15 show that better graft success was obtained in the greenhouse than in the net-house. In the spring graftings also much more rejuvenation and longer shoots were obtained in the greenhouse.

5. Effect of photoperiod.

Under natural conditions there is a great reduction of growth rate in the autumn, this may be related to the decrease in day-length at that period. Four year old grafted plants, one year after grafting, were illuminated throughout the night during October, prior to removal of the growth tips for repeated grafting. Although lighted plants produced somewhat more tips, growth and rejuvenation rate was smaller than those of non-lighted plants (Table 16).

Promotion of interfascicular bud formation and sprouting of dormant buds: The purpose of these experiments was to enhance sprouting of dormant buds and obtain young interfascicular shoots of juvenile-like properties that will serve as cuttings for rooting.

1. Effect of pinching.

Pinching the terminal bud, induced sprouting of inhibited lateral buds. Swelling of interfascicular buds was observed at the base of needles 3 weeks after pinching and the swollen buds developed into small interfascicular shoots (Fig. 5). The new buds close to the pinching site were the first to sprout and elongate and they became the longest shoots (Fig. 6).

In addition to the interfascicular buds, two other type of buds sprouted occasionally: a) "Whorl buds" - 2 to 5 buds sprouting from the same region, b) "Compound buds" - in addition to the central bud, one or two additional buds sprouted forming a cluster of buds.

2. Effect of pinching and treatment with benzyladenine (BA).

In plants treated with BA, a higher percentage of compound buds was observed. They were of two kinds, first order and second order compound buds. First order compound buds appeared soon after sprouting of the interfascicular bud, the bud split at the base, forming 3 short branches (Fig. 7 and 8). Each of these branches later produced later second order sprouted buds, creating a "cone"-like structure of secondary buds (Fig. 7). Most of these "cones" dried up and died one year after the BA spray.

In the first season BA sprays at 50 and 200 mg/l were tested following pinching. The effect on number of sprouted buds and their characteristics are presented in Table 17. BA increased the number of sprouted buds and the percentage of compound buds, but decreased bud growth.

In the next season we used lower concentrations of BA in hope to reduce the incidence of the undesirable compound buds. BA even at 25 mg/l increased both bud sprouting and the number of compound buds. A surprising result was the significant promotion of bud sprouting by water and detergent spray without BA (Table 18).

3. Bud sprouting in grafted plants.

The response of ungrafted plants to plant grafted 2.5 years prior to pinching and BA treatment was compared. Plants of the two groups behaved similarly in all aspects, except percent interfascicular buds produced (Table 19). The percent interfascicular buds was considerably higher in grafted plants.

4. Effect of photoperiod.

Photoperiodic light was applied to plants throughout the night during the whole month of October. One month after the start of the light treatments the plants were pinched and treated with BA and continued to be lighted for additional 5 months, when measurements were taken. The main effect of light was on bud growth (Table 20). Lighted sprouts were significantly longer than those grown under natural photoperiod.

5. Response of buds at various locations on the tree.

Significant differences were found in the response of buds at various whorls along the plant axis in 3 parameters of bud sprouting: number of sprouted buds, length of sprouted buds and percent interfascicular buds (Table 21). More buds sprouted at the top of the plant, they were more elongated with higher percentage of interfascicular buds than in the lower part of the trees.

6. Bud sprouting in grafted plants

In the experiments described above, the new shoots reached a suitable size for use as cuttings in April, a season too late for optimal rooting (see below). We therefore tried pinching grafted plants at various times and compared the results to pinching in November (Table 22). Grafted plants responded more to pinching in producing elongated shoots than nongrafted plants of the same age. More buds sprouted in November pinch, but elongation was better in April.

Results of Table 22 are from plants grown in net-house. To test the effect of greenhouse conditions and lighting on sprouting, similar plants to those used for the experiment of Table 22 were pinched in November and grown in greenhouse with and without supplemental photoperiodic light. The number of buds sprouted per branch 4 months later (10.9) was only half that obtained in the plants grown in the net house, but they were longer (7.9 cm vs. 4.9 cm). Lighting had little effect on bud sprouting, but considerably reduced bud growth (4.9 cm vs. 7.9 cm).

Rooting of cuttings and their development:

1. The rooting process.

Swelling of the cutting base was observed in most cuttings 3 to 4 weeks after placing the cuttings in the rooting media, but only a small percentage of these swollen bases developed roots later.

Two rooting flushes were normally observed, depending on the age of the mother plants. In seedlings, up to 2 month old, rooting started after 3 weeks and the second rooting flush was after 6-7 weeks. In cuttings from plants up to 1 year, first rooting flush was after 5 weeks and the second after 10 weeks. In cuttings from plants at the intermediate stage between juvenile to mature plants up to 4 years, first rooting flush was after 6-7 weeks and the second after more than 13 weeks.

A detailed anatomical study of the cuttings (Hamburger, 1988) indicated that mature tissues at the base of cuttings are able to proliferate and form callus, which then differentiate single tracheary elements, but only a small percentage developed roots. The reason mature cuttings often fail to root is probably their inability to initiate root primordia in the callus.

There are probably many factors both endogenous and environmental, which affect the rooting of cuttings. More than 50 rooting experiments have been carried out with various cuttings, rooted at different periods, in different media and rooting substances. It is, therefore, normally not easy to separate one factor from the many variable in a specific experiment. Furthermore, results were often not consistent, therefore only general trends can be presented.

2. Effect of harvesting season of cuttings.

Generally, autumn and spring were the best seasons, and summer the worst. It is, however, difficult to define more clearly these seasons and to determine which of the two seasons (autumn or spring) is more favorable for rooting.

3. Effect of plant age and form.

Cuttings taken from seedlings up to 18 month old normally have a high percentage of rooting

(Fig. 9). When cuttings from older plants were used, a gradual decline in rooting ability was obtained with cuttings from 1.5 to 4 year-old plants. Cuttings from 60 year-old trees did not root (Fig. 10).

In several experiments, cuttings taken from regular shoots were compared to those taken from shoots that developed from interfascicular buds. In most cases, no clear difference in rooting ability was observed between cuttings of the two types. In one case, however, with cuttings harvested from 4 year-old plants and rooted in May, 60% rooting was obtained with interfascicular cuttings as compared to only 25% with regular cuttings.

4. Treatments to promote rooting.

Treatments of cuttings with IBA (T8), sucrose or T8+sucrose generally promoted rooting. The effect varied, however, at the various periods of rooting (Fig. 11) and with cuttings of various ages (Fig. 12). In several (but not all) experiments, the addition of sucrose to the auxin enhanced rooting more than auxin alone.

Recutting the base of cuttings that failed to root, 3 or 5 weeks after the initial treatment with T8, greatly enhanced their rooting (Fig. 13). Repeating the auxin treatment after 5 weeks without recutting the base, decreased rooting.

5. Development of rooted cuttings.

Rooted cuttings originated from 4-year old plants, were transplanted from the rooting bed to pots in March. Six weeks later they were transplanted again to larger containers. Three months after the transfer from the rooting bed, about 80% of the plants survived and the average growth increment was 20 cm.

Two groups of plants could be distinguished: a) those having at least one viable bud which started growing immediately after transplanting and continued with uninterrupted growth. b) those that did not have an apparent viable bud. They did not grow for 3 to 5 months and then a new bud (often interfascicular) sprouted, and started to grow.

6. Effect of sucrose on IAA absorption and movement.

Results presented above indicate that in several cases, addition of sucrose to the auxin treatment enhanced the promotive effect of the auxin on rooting of cuttings and sometimes even sucrose alone enhanced rooting.

We have studied the effect of sucrose on the absorption and movement of labeled IAA in cuttings of one month old seedlings. Seedlings were cut at the junction of the root and the hypocotyl, pulsed through the base of cuttings with IAA or IAA + sucrose for 24, 48 or 72 h and then divided into 3 sections: the lower half of the hypocotyl, the upper half of the hypocotyl and the cotyledons.

The pulsing solution contained $5 \times 10^{-5} \text{M}$ cold IAA, chloramphenicol $100 \mu\text{g/l}$, and 400,000 dpm of ^{14}C -2-IAA. Sucrose was added at 100 mM. Pulsing was carried out in a growth chamber with 18h light at 25° and 6 h dark at 20° .

Sucrose reduced the total amount of IAA absorbed by the cuttings (214,200 dpm in IAA and

113,100 dmp in IAA+sucrose after 72 h pulse). However, sucrose greatly increased the proportion of IAA retained at the basal part of the cutting (Table 23). The effect of sucrose on the distribution of IAA and its accumulation at the base of the cutting may explain the promotive effect of sucrose on rooting.

Table 1. Interfascicular bud formation on jack pine trees at Cloquet treated once at different times in the growing season. 1986.

Treatment	Treatment time			total
	early	middle	late	
control	2	2	0	4
pruned (P)	153	57	3	213
EtOH	9	1	0	10
EtOH + P	134	68	0	202
500 BA	19	0	0	19
500 BA + P	193	87	3	283
1000 BA	21	0	0	21
1000 BA + P	207	56	13	276
100 NPA	17	0	0	17
100 NPA + P	183	66	3	252
500	19	0	0	19
500 NPA + P	188	55	1	244
total	1145	392	23	1560
number of trees	36	16	18	

Table 2. Data from treatments in Table 1 involving pruned branches summarized by treatment time, family, and growth regulator treatment.

<u>Treatment time</u>	% branches w/ interfascicular buds	# interfascicular buds/branch	<u>bud length (mm)</u>
Early	86	6.0	4.8
Middle	88	6.4	3.8
Late	19	0.5	2.9
<u>Family</u>			
8376	50	2.1	5.2
8336	65	3.8	4.5
8382	69	6.7	4.8
8337	97	5.2	3.7
<u>Growth Regulator</u>			
<u>Treatment</u>			
none	73	4.5	4.0
EtOH	72	4.2	4.7
100 NPA	63	4.6	4.5
500 NPA	67	5.2	4.8
500 BA	63	4.9	4.4
1000 BA	79	5.4	4.2

* Data collected from pruned branches, 5 pruned tips per branch. Only buds greater than 0.5 mm were measured.

Table 3. Total number of interfascicular buds formed per treatment at Rosemount. 1986.

Treatment	White pine				Jack pine			
	treatment time			TOTAL	treatment time			TOTAL
	early	middle	late		early	middle	late	
control	0	1	0	1	1	0	1	1
pruned	62	103	74	239	89	85	24	198
EtOH	0	3	0	3	0	0	6	6
EtOH + P	49	368	69	486	89	107	43	239
500 BA	0	0	0	0	11	1	3	15
500 BA + P	65	113	142	320	66	95	20	181
1000 BA	0	16	8	24	1	9	12	22
1000 BA + P	28	420	178	626	68	120	54	242
TOTAL	204	1024	471	1699	325	417	163	905

P = pruned

Table 4. Number of interfascicular buds formed per treatment on white trees at Rosemount. 1987.

Treatment	TREE NUMBER										Total
	1	2	3	4	5	6	7	8	9	10	
Pruned	19	15	19	16	17	34	11	12	20	18	181
EtOH + P	37	16	20	12	36	46	10	9	13	18	217
500 BA + P	33	14	13	16	21	12	8	10	10	10	147
1000 BA + P	35	48	37	17	60	16	12	12	42	13	292
2000 BA + P	28	54	33	13	45	47	21	27	24	19	311
TOTAL	152	147	122	74	179	155	62	132	109	78	1148

P = pruned

Table 5. Number of interfascicular buds formed per treatment on jack pines at Rosemount. 1987.

Treatment	TREE NUMBER										Total
	1	2	3	4	5	6	7	8	9	10	
Pruned	32	17	18	7	20	14	2	6	24	10	150
EtOH + P	24	15	15	6	11	12	19	9	18	2	131
500 BA + P	16	16	21	14	15	10	15	6	17	10	140
1000 BA + P	25	4	13	5	18	0	17	2	5	10	99
200 BA + P	19	2	9	6	25	13	13	8	14	18	127
TOTAL	116	54	76	38	89	49	66	31	78	50	647

P = pruned

Table 6. Interfascicular buds formed on trees at Rosemount after multiple treatments of pruned branches with BA and TDZ. 1988.

Treatment	white pine trees					jack pine trees				
	1	2	3	4	TOTAL	1	2	3	4	TOTAL
P 50 BA 4x	18	4	12	13	47	2	1	4	0	7
P 500 BA 4x	26	28	21	8	83	1	27	3	2	33
P 50 TDZ 1x*	16	16	20	5	57	0	2	5	0	7
P 50 TDZ 4x	11	18	30	13	72	6	13	6	5	30
P 100 TDZ 1x*	24	7	12	3	46	8	5	46	2	28
P 100 TDZ 4x	31	40	46	42	159	12	13	7	1	33
P 500 BA 1x^	19	3	4	1	27	0		0	0	0
P 50 BA 1x	2	1	1	2	6	0	0	0	2	2
P 50 TDZ 1x	4	9	9	14	36	8	4	0	0	12
P 100 TDZ 1x	8	7	8	10	33	0	9	5	5	19
TOTALS	159	133	139	163	566	34	74	43	17	171

* Branches, treated 6/6/87 midstage of needle development.

^ branches treated 6/29/87

4x treatments - 1st treatment 6/6/87 followed by 3 more treatments at weekly intervals

Table 7 Number of interfascicular buds formed per treatment on pruned branches of white pine trees at Rosemount. 1988.

tree	EtOH 1x	EtOH 4x	DMSO+ EtOH 4x	DMSO+ 1000 1x	BA 1000 1x	BA 1000 4x	TDZ 50 1x	TDZ 50 4x	TDZ 100 1x	TDZ 100 4x	TOTAL
1	0	0	0	2	9	55	2	101	23	124	316
2	5	11	4	4	6	76	12	42	1	54	225
3	4	7	-	6	-	33	3	75	1	44	173
4	4	0	4	1	4	2	8	57	0	48	128
5	4	3	1	1	8	17	11	54	20	36	155
6	10	3	5	7	5	97	6	178	6	117	434
7	2	5	8	1	5	1	16	37	4	61	140
8	10	13	3	3	2	3	10	54	2	108	208
TOTAL	39	42	25	35	39	284	68	588	57	592	1769

1x treatments - at midstage of needle development

4x treatments - 1st treatment at midstage of development followed by 3 more treatments at weekly intervals

Table 8. The influence of cutting type and IBA treatment on rooting of cuttings from mature (9-11 year old) white pine trees. Data taken 12 weeks after sticking.

	% of cuttings rooted	number of roots/cutting
Interfascicular shoots	19 ± 5	2.3 ± 1.1
Interfascicular shoots plus IBA (4000 mg/l)	25 ± 6	2.2 ± 1.2
Natural shoots	17 ± 4	1.9 ± 1.3
Natural shoots plus IBA (4000 mg/l)	23 ± 7	1.9 ± 1.2

Table 9. Viability and contamination of dormant buds from mature white pine trees cultured on LP medium containing various concentrations of IBA and BA. Twenty-five buds per growth regulator treatment. Data taken 30 days after implantation.

IBA conc. (M)	Status of Buds	BA Concentration (M)			
		0	10^{-5}	10^{-4}	10^{-3}
0	Dead	9	4	5	18
	Alive	13	19	20	7
	Contam.	3	2	0	0
10^{-7}	Dead	8	3	5	22
	Alive	15	21	17	1
	Contam.	2	1	3	2
10^{-6}	Dead	4	1	8	18
	Alive	17	22	16	3
	Contam.	4	2	1	4
10^{-5}	Dead	3	6	3	15
	Alive	21	17	21	5
	Contam.	1	2	1	5

Table 10. Influence of juvenile or mature lamina scion on rooting of juvenile or mature petiole rootstock. Data as percent of petioles rooting and mean number of roots per petiole.

	Time after grafting		
	24 days	35 days	45 days
J/J no IBA:	50% 2.7 ± 3.2ab*	100% 10.0 ± 3.3b	100% 11.1 ± 3.2e
J/J 2000 ppm IBA	90% 10.8 ± 6.6cd	100% 13.4 ± 6.0b	100% 16.8 ± 5.9e
M/J no IBA	40% 2.6 ± 3.5ab	100% 9.8 ± 4.0b	100% 11.1 ± 5.6e
M/J 2000 ppm IBA	100% 12.8 ± 3.6cd	100% 15.3 ± 5.2b	100% 17.1 ± 5.7e
J/M no IBA	0%	40% 0.9 ± 1.2a	60% 2.0 ± 2.0c
J/M 2000 ppm IBA	0%	60% 2.9 ± 2.9a	100% 7.7 ± 6.4d
M/M no IBA	0%	0%	10% 0.3 ± 0.9ab
M/M 2000 ppm IBA	0%	0%	30% 0.8 ± 1.3ab

* Tukey (HSD) multiple range test at 5% level. Mean root number within a column followed by the same letter were not significantly different.

Key:

J/J = juvenile/juvenile graft combination

M/J = mature/juvenile graft combination

J/M = juvenile/mature graft combination

M/M = mature/mature graft combination

Table 11. Rooting response of de-bladed juvenile and mature *H. helix* petioles treated with IAA and NAA cultured on a modified Romberger medium (2% sucrose).

Auxin conc. (μ M)	No. of roots per petiole			
	<u>Juvenile</u>		<u>Mature</u>	
NAA	IAA	NAA	IAA	
	0	0	0	0
0.1	0	0	0	0
1	0	0	0	0
10	0.5c ²	1.2c	0	0
50	1.3c	7.5b	0	0
100	6.4b	9.4a	0	0
500	4.6b	0.8c	0	0
1000	0	0	0	0

² Means followed by the same letter are not significantly different at the 0.05 level.

Table 12. P. halepensis: A comparison of graft success between autumn (Nov-Dec 1985) and spring (March 1986) graftings. Data was taken in July 1986 (4 months after the spring grafting and 8 months after the autumn graftings). The rootstocks were one year old seedlings, the scions taken (as repeated grafting) from 4-year old plants, first grafted 2 1/2 years before.

Season	Graft success (%)	Length of scion (cm)	No. of tips per plant	Juvenile tips (%)
Autumn	68.3 $\pm$ 7.4	23.6 $\pm$ 2.8	1.0	14.8 $\pm$ 5.2
Spring	66.2 $\pm$ 2.6	17.1 $\pm$ 1.0	1.0	39.9 $\pm$ 17.2

Table 13. Aleppo pine: A Comparison of graft success of scions taken from trees of different ages. Scions 4 and 60 years old were grafted in the autumn and those 20 and 35 years old in spring. Data was taken in July. Plants were grown in nethouse.

Scion age years	Graft success %	Length of scion (cm)	Juvenile tips %
4	39.9	18.3	5.8
20	40.9	11.8	22.2
35	17.5	14.9	0
60	28.6	11.3	0

Table 14. Aleppo pine: A comparison of graft success between first graftings and repeated graftings. First graftings were scions from 4 year old plants. Repeated graftings were done with scions from 4-year old plants, grafted one or 2 1/2 years before. All grafts were done in the autumn on 1-year old rootstocks and data taken 8 months later.

	Graft success (%)	Length of scion (cm)	No. of tips per plant	Juvenile tips (%)
First grafting	39.9 ± 11.5	18.3 ± 2.2	1.0	5.8 ± 3.8
2nd grafting (after 2.5 years)	68.3 ± 7.4	23.5 ± 2.8	1.0	14.8 ± 5.2
2nd grafting (after 1 year)	74.7 ± 3.4	17.4 ± 2.3	3.9	22.9 ± 0.7

Table 15. Aleppo pine: Effect of growing conditions (greenhouse or nethouse) of grafted plant on graft-success, scion growth and rejuvenation. Experiment was done with scions from 35-year old plants grafted in the spring and measured 4 months later and with scions from 60-year old plants grafted in the autumn and measured 8 months later.

	Graft success (%)	Length of scion (cm)	No. of tips per plant	Juvenile tips (%)
Spring grafting (35 yr old)				
Greenhouse	22.9 ± 2.6	14.2 ± 2.0	2.8 ± 0.3	0
Net-house	17.5 ± 3.9	14.9 ± 3.6	4.1 ± 0.6	0
Autumn grafting (60 yr old)				
Greenhouse	31.1 ± 9.5	16.4 ± 1.3	2.4 ± 0.6	15.3 ± 7.9
Net-house	28.6 ± 13.6	11.3 ± 1.3	1.5 ± 0.2	0

Table 16. Aleppo pine: Effect of lighting at night on graft-success of scions taken from the plants for repeated grafting. Measurements were recorded 6 months after grafting.

	Length of scion (cm)	No. of tips per plant	Juvenile tips (%)
Natural day	19.6 ± 1.5	3.5 ± 0.3	23.3 ± 6.3
LD, lighted	15.1 ± 2.3	4.4 ± 0.7	13.8 ± 2.5

Table 17. Aleppo pine: Effect of pinching and spray with BA on bud sprouting and properties. Four-year old trees were pinched and sprayed 4 times at one week intervals in November 1985. Data was collected in April 1986.

Treatment	No. of sprouted buds/plant	No. of sprouted buds/branch	Length of sprouted bud (cm)	Brachyblasts (%)	Compound buds (%)
Pinch	52.2 ± 7.2	8.1 ± 1.1	7.1 ± 0.6	61.6 ± 4.6	28.4 ± 4.5
Pinch + BA 50 mg/l	102.2 ± 26.6	12.3 ± 2.6	3.2 ± 0.4	79.4 ± 7.0	54.5 ± 8.0
Pinch + BA 200 mg/l	138.0 ± 27.9	15.9 ± 3.2	1.7 ± 0.2	72.1 ± 6.3	72.5 ± 7.7

Table 18. Effect of pinching and spray with solution lacking BA or with BA (25 mg/l) on bud sprouting and bud characteristics of Aleppo pine. Four-year old trees were pinched and sprayed 4 times at one week intervals in November 1986. Data was collected in April 1987.

Treatment	No. of sprouted buds/tree	No. of sprouted buds/branch	Length of sprouted bud (cm)	Interfascicular buds (%)	Compound buds (%)
Pinch	107.5 $\pm$ 2.1	4.9 $\pm$ 0.8	1.8 $\pm$ 0.2	28.4 $\pm$ 0.1	4.3 $\pm$ 2.5
Pinch+Spray without BA	249.0 $\pm$ 27.9	6.4 $\pm$ 1.1	3.7 $\pm$ 0.7	44.7 $\pm$ 8.3	8.9 $\pm$ 5.5
Pinch+BA (25 mg/l)	221.8 $\pm$ 28.1	7.5 $\pm$ 1.4	1.9 $\pm$ 0.2	54.3 $\pm$ 8.8	12.2 $\pm$ 3.9

Table 19. Percent interfascicular buds sprouted on non-grafted 4-year old trees of Aleppo pine and those grafted 2 1/2 years prior to pinching and spray with BA (200 mg/l). Data recorded 5 months after treatment.

Treatment	Non-grafted plants	Grafted plants
Pinch	46.2 $\pm$ 9.2	69.3 $\pm$ 3.2
Pinch + BA	56.8 $\pm$ 8.0	82.3 $\pm$ 7.0

Table 20. Aleppo pine: Effect of photoperiodic light prior to and after pinching and spray with BA (25 mg/e), on length of sprouts 5 months after treatment.

Treatment	Sprout length (cm)	
	Natural day	LD (lighted)
Pinch	1.8 ± 0.2	2.9 ± 0.3
Pinch & BA	1.9 ± 0.2	3.9 ± 0.7

Table 21. Effect of bud location on branches along the plant axis of Aleppo pine on bud sprouting parameters. Data collected on 4-year old plants 5 months after pinching and spraying with BA.

Bud location on Plant	No. of sprouted buds/branch	Length of bud (cm)	Interfascicular buds (%)
Top	11.0 ± 1.4	4.5 ± 0.5	63.6 ± 7.4
First whorl	4.6 ± 0.6	3.9 ± 0.4	47.6 ± 7.1
Second whorl	3.9 ± 0.6	3.7 ± 0.4	51.3 ± 6.6
Lower whorls	7.4 ± 0.8	2.6 ± 0.3	23.6 ± 4.8

Table 22. Effect of pinching time on bud sprouting and growth of Aleppo pine. Four-year old plants grafted a year or more before the experiment were used. Plants were held in a net-house. Data measured 4 months after pinching.

Pinching time	Measurements time	No. of buds sprouted/ branch	Length of bud (cm)
Nov.	Apr.	21.0 $\pm$ 5.0	4.9 $\pm$ 0.9
Apr.	Aug.	10.3 $\pm$ 1.0	9.8 $\pm$ 0.4
July	Nov.	8.6 $\pm$ 0.9	4.1 $\pm$ 0.5

Table 23. Absorption of labeled IAA in 3 sections of Aleppo pine seedling cuttings after 3 periods of pulsing, as affected by sucrose (100 mM) added to the IAA solution.

Treatment	% IAA accumulated of total absorbed		
	24h	48h	72h
<u>Basal hypocotyl</u>			
IAA	49.9	38.8	18.6
IAA & sucrose	82.5	67.4	69.7
<u>Upper hypocotyl</u>			
IAA	27.7	34.8	10.6
IAA & sucrose	9.9	24.1	12.3
<u>Cotyledons</u>			
IAA	22.4	26.4	70.9
IAA & sucrose	7.6	8.5	18.0

Figure 1. Schematic representation of *Hedera helix* leaf approach grafting procedure used to obtain cuttings with reciprocal combinations of juvenile and mature petioles and juvenile and mature lamina.

		BA Concentration (M)		
IBA Concentration (M)		0	10^{-5}	10^{-4}
	10^{-5}
	10^{-4}
10^{-3}

Figure 3. Excised buds from mature white pine trees after 3 months culture on L.P. medium with various combinations of BA and IBA.

Figure 2. An example of grafted Aleppo pine plant.

Figure 4. The upper part of a grafted Aleppo pine plant. Scion was taken from 6- year-old tree and grafted on one year-old plant. The basal needles are original, the new upper growth is of a juvenile-like brachyblast.

Figure 5. Sprouted buds on an Aleppo pine plant 9 weeks after pinching.

Figure 6. Sprouted buds on an Aleppo pine plant 19 weeks after pinching.

Figure 7. A compound bud structure ("cone") 19 weeks after pinching and spraying an Aleppo pine plant with BA (200 mg/l).

Figure 8. Compound buds, 19 weeks after pinching and spraying an Aleppo pine plant with BA (200 mg/l).

Figure 9. Effect of Aleppo pine seedling age on rooting ability of the cuttings. All cuttings were treated with the rooting powder T_8 . Results are from 6 rooting periods:

- - December; ○ - February;
- - March; □ - April;
- ▲ - June; △ - August

Figure 10. Percent rooting of cuttings harvested from Aleppo pine trees of various ages. All cuttings were treated with the rooting powder T_8 . Results are from rooting experiments conducted at various periods:

- - December; ○ - February;
- - March; □ - April;
- △ - June;

Figure 11. Percent rooting of cuttings from Aleppo pine of an intermediate age, 1.5 to 2.5 years old rooted at various periods and treated with various rooting substances.

○ - December; ▲ - May; △ - June

Figure 12. Percent rooting of cuttings harvested from Aleppo pine plants of various ages and at various periods in response to treatments with various rooting treatments:

▲ - 4 months, June;
 ■ - 7 months, August;
 ● - 14 months, December;
 ○ - 18 months, December.

Figure 13. Percent rooting of cuttings harvested from Aleppo pine plants at an intermediate age. All cuttings were initially treated with auxin (T_8).

○ - control;

△ - retreated after 3 weeks;

▲ - retreated after 5 weeks.

Literature Cited

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f. Description of Cooperation

Although different species were worked on in the U.S. and Israel, a cooperative approach was taken in that development of grafting procedures was undertaken in Israel and development of in vitro bud culture procedures was undertaken in the U.S. Investigators in both countries worked on procedures for induction of interfascicular bud formation. The discovery that multiple treatments with BA enhanced bud formation in Aleppo pine made possible induction of large numbers of buds in white pine which had been recalcitrant with single treatments with BA. Unfortunately, protocols for in vitro culture of white pine buds was not developed soon enough to be extended to Aleppo pine. The Aleppo pine grafting procedures worked well with white pine but not jack pine.

g. Evaluation of research achievements

Progress in developing rejuvenation procedures for the temperature zone pines, white and jack, was slow because they flush only once a year even in the greenhouse. However, a good foundation for future work with these species has been laid. White pine is much easier to work with experimentally and therefore it should be used for future work. Both in vitro bud culture and induction of interfascicular buds show promise for rejuvenation with this species but no evidence for rejuvenation was obtained. Development of rejuvenation techniques to enhance rootability would probably take another 2 to 3 years.

Progress with rejuvenation techniques for Aleppo pine was more rapid because of its multi-flushing capability. Cuttings from interfascicular shoots induced on 4 year-old plants showed high rooting ability in a few experiments (up to 60% rooting). Methods to utilize this rooting ability have not been determined. Repeated or serial grafting enhanced graft success, shoot vigor, and juvenile-like foliage morphology. These characteristics were not necessarily correlated with rootability. The duration of the juvenile-like foliage morphology was never longer than 7 months. Development of successful rejuvenation procedures to enhance rootability would take at least another year.

Perhaps the most significant discovery resulting from this project came from the grafting experiments with leaf cutting from juvenile and mature H. helix and the rooting experiments done with de-bladed petioles of juvenile and mature leaves of this species. The leaf cutting grafting data provides strong evidence that cells with mature characteristics can be physiologically transformed to have juvenile characteristics by a translocatable substance(s). These results provide strong support for continuing work on graft induced rejuvenation of pines. These results also provide a strong rationale for trying to isolate and characterize the translocatable substance(s). The in vitro debladed mature ivy leaf petiole bioassay system provides a means to detect biological activity as the isolation and purification procedures are carried out. Such a substance could be very important in improving rooting of cuttings from mature trees which have low rooting potential.

h. Publications

Geneve, R.L., W.P. Hackett, and B.T. Swanson, 1988. Adventitious root initiation in de-bladed petioles from the juvenile and mature phases of English ivy. J. Amer. Soc. Hort. Sci. 113:630-635.

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