

UNCLASSIFIED

| |
|--|
| |
| |
| |
| AD NUMBER |
| AD867320 |
| NEW LIMITATION CHANGE |
| TO Approved for public release, distribution unlimited |
| FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; MAR 1970. Other requests shall be referred to Dept. of Army, Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701. |
| AUTHORITY |
| BDRL, D/A, 29 Sep 1971 |

THIS PAGE IS UNCLASSIFIED

AD 867320

AD

TECHNICAL MANUSCRIPT 588

A SOLUTION CULTURE METHOD FOR GROWING UNIFORM ASH AND MAPLE SEEDLINGS

Charles A. Vile
James W. Akerman
Robert W. Gesink
Woodland Hurtt

MARCH 1970

Reproduced by the
CLEARINGHOUSE
for Federal Scientific & Technical
Information, Springfield, Va. 22151

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

STATEMENT #2 UNCLASSIFIED
This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/T10, Frederick, Maryland 21701

DDC
RECEIVED
APR 13 1970
C

49598

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 538

A SOLUTION CULTURE METHOD FOR GROWING UNIFORM ASH
AND MAPLE SEEDLINGS

Charles A. Vile

James W. Akerman

Robert W. Gesink

Woodland Hurtt

Plant Physiology Division
PLANT SCIENCES LABORATORIES

Project 1B562602AD09

March 1970

ABSTRACT

This study evaluated methods and established procedures for growing seedling trees to a predetermined uniform size. White ash (Fraxinus americana L.) seed gathered in the fall from indigenous sources was placed in a cool-moist environment for 90 days to break embryonic dormancy. Red maple (Acer rubrum L.) and silver maple (Acer saccharinum L.) were similarly obtained in the spring but did not require a cold treatment. Seeds were germinated in vermiculite, a 1:1 peat-perlite mixture, and washed in sand in the greenhouse and in a growth chamber. Following emergence the seedlings were grown for various periods in a growth chamber in nutrient culture as well as in the above media in the greenhouse under supplementary light.

In contrast, dormant ash and maple seedlings were obtained commercially and exposed to various photoperiods. Daylengths of 16 and 20 hours were used in the growth chamber studies. Greenhouse photoperiods of 16, 20, and 24 hours were achieved by supplementing the natural day of 10 to 11 hours with increments of fluorescent or incandescent light.

The solution culture system is the only practical method that provides the opportunity for analyses or autoradiography of both foliage and roots. The optimum procedure evolved here was to germinate seed in vermiculite and transplant into individual pots of vermiculite, initially saturated with Hoagland's nutrient solution, when the seedlings were about 4 cm tall. After attaining a height of 20 to 25 cm, the seedlings were transferred to aerated solution culture and were considered ready for experimental use after 2 to 3 days of acclimatization. The environmental parameters were 25 ± 3 C, $60 \pm 15\%$ RH, and a 16-hour photoperiod of $1,000 \pm 300$ ft-c.

When grown from seed, ash and silver maple required 5 weeks to reach the optimum height of 20 to 25 cm; red maple required 8 to 9 weeks. We have successfully grown trees in solution culture in 1-quart pots for 2 months with no special requirements other than periodic replenishment of the solution. The only limitation to growth of larger trees would be the need for physical support.

CONTENTS

Abstract 2

I. INTRODUCTION 5

II. MATERIALS AND METHODS 6

 A. Seed Germination 6

 B. Dormant Seedlings 10

III. RESULTS AND DISCUSSION 11

 A. Seed Germination 11

 B. Dormant Seedlings 16

IV. RECOMMENDATIONS FOR GROWING ASH AND MAPLE SEEDLINGS 18

Literature Cited 23

Distribution List 31

DD Form 1473 33

APPENDIXES

A. Source of Materials 25

B. Greenhouse and Growth Chamber Environmental Conditions 27

C. Composition of Nutrient Solution 29

FIGURES

1. White Ash Growing in Six Nutrient Solutions in Controlled Environment Chamber 8

2. Red Maple in Half- and Full-Strength Nutrient Solution in Controlled Environment Chamber 8

3. White Ash in the Greenhouse Under Cool-White Fluorescent Lights 9

4. White Ash 3 Days, 1 Week, and 2 Weeks After Planting Seed in Vermiculite 12

5. Red Maple and Silver Maple 2 Weeks After Planting Seed in Vermiculite 12

6. Seed of White Ash 20

7. White Ash in Aerated Nutrient Culture 20

8. Seed of Red Maple and Silver Maple 21

9. Red Maple in Aerated Nutrient Culture 21

TABLES

| | |
|---|----|
| 1. Comparison of Effect of Seed Treatment and Planting Medium on Germination of White Ash Seed 2 Weeks After Planting | 13 |
| 2. Comparison of Height of White Ash Seedlings Growing in Nutrient Culture Solutions | 13 |
| 3. Growth of 8-Week-Old White Ash Seedlings in Four Nutrient Solutions | 14 |
| 4. Growth of Red Maple Seedlings After 45 Days in Two Concentrations of Hoagland's Nutrient Solution | 14 |
| 5. Comparison of the Height of White Ash Seedlings Growing in Two Propagation Media with Several Methods of Nutrition | 15 |
| 6. Time Required to Break Dormancy of Green Ash and Red Maple Under Various Photoperiod Regimes | 17 |

I. INTRODUCTION*

One of many problems involved in herbicide and plant growth regulator research with trees is the lack of suitable plants, especially during normal dormancy. For research, the use of uniform plants within a test population is very important to reduce inherent variability that could mask or attenuate any measured response resulting from an imposed treatment. Present methods of growing 1- to 2-year-old tree seedlings in soil under greenhouse conditions have not always produced desirable trees. Because many experiments require the utilization of the entire tree, including intact root systems, soil culture methods are not satisfactory.

A variety of soilless culture techniques have been described in the literature. Seedling trees have been grown in sphagnum moss soaked in nutrient solution,** perlite irrigated with nutrient solution,** and in aerated nutrient culture.^{1,2} However, we found each of these methods had certain limitations. Perry and Upchurch³ have described a method used for maple and ash. Seeds were germinated on wet paper towels, planted in a mixture of sand, peat, and soil (1:1:1), and transplanted to aerated nutrient culture after 8 weeks. Trees have been grown successfully under greenhouse conditions with supplementary light. A photoperiod exceeding 14 hours has been reported to be necessary for continued growth of hardwood species.⁴⁻⁶

Methods have been successfully developed for breaking dormancy for some forest trees. However, many of these methods are quite elaborate and complicated and often result in undesirable growth characteristics. Fergus⁷ has successfully broken dormancy by applying ethylene chlorohydrin to plants in a sealed container for 48 hours to break dormancy, but it had the undesirable characteristic of killing the terminal bud. Schoeneweiss¹⁰ found that certain growth regulating substances affected many plant species. Ethylene glycol or pure glycerol applied to leaf scars and buds after a cold period accelerated the breaking of dormancy by 2 to 3 weeks. However, chemical treatment often produced growth that was inconsistent with normal growth patterns. Dormancy was also broken in some hardwood species by a continuous photoperiod of at least 16 hours by extending the natural day with incandescent lights.^{4,11,12}

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** E. Hacskaylo, Forest Physiology Laboratory, U.S.D.A., Beltsville, Maryland 20705; personal communication.

Because our use of maple and ash seedlings frequently involves root-applied compounds, the solution culture technique appeared to be the only practical method that would allow us to analyze or autoradiograph intact seedlings without damage to or loss of the root systems. Although there are a number of methods for growing trees in various media, we experienced difficulties with all methods evaluated. Our principal problem, from an autoradiographic standpoint, was associated with attempting to obtain roots free of adhering particles of soil, peat, perlite, sphagnum moss, or other similar media. Thus, the objectives of this study were to evaluate methods and establish simple, reliable procedures for growing uniform tree seedlings in solution culture for laboratory investigations.

II. MATERIALS AND METHODS

Two representative genera, ash (Fraxinus) and maple (Acer), were selected for propagation on the basis of field response to various herbicides. Ash was selected for susceptibility to the phenoxy herbicides and resistance to picloram; maple was selected for resistance to the phenoxy herbicides and susceptibility to picloram.

Preliminary studies indicated that the ideal seedling for our study should have a single straight stem, five to six nodes, healthy buds and leaves, and a height of 20 to 25 cm. The two logical approaches to obtain such a seedling were to begin with seed or to begin with dormant seedlings. In either case, manipulation of the environmental parameters was requisite for obtaining seedlings that met the above criteria.

A. SEED GERMINATION

Seeds of white ash (Fraxinus americana L.) were gathered in October when mature and were air-dried at room temperature for 1 day. Because ash seed at this stage is considered to be in embryonic dormancy,¹³ a cool, moist stratification necessary to effect germination¹⁴ was accomplished by wetting the seed in a polyethylene bag with distilled water.* Wet paper towels were placed at the base, middle, and top of the bag to maintain a high relative humidity. The bags containing the seed were kept in the cold room at 4 C for a minimum of 90 days.

Media such as vermiculite, peat-perlite (1:1), and white silica sand are commonly used for plant propagation (Appendix A). An experiment was designed to determine the acceptability of these media. After 90 days in the cold room, ash seed that had received the cool-moist treatment described above and seed that had received only a cool treatment were sown in the

* Superintendent, Mont Alto Forest Nursery, Pennsylvania Department of Forests and Waters, Mont Alto, Pennsylvania; personal communication.

three media. In addition, seed with the terminal wing structure removed were placed on wet filter paper in petri dishes and exposed to 16 hours of continuous light at about 1,200 ft-c in a growth chamber. A total of 40 cool-moist and 40 cool-dry seed were used. Six-inch bulb pots were filled with vermiculite, peat-perlite (a 1:1 mixture of shredded peat moss and horticultural grade perlite), or white silica sand. The seed were planted at a depth of $\frac{1}{2}$ inch at rates of 25 or 75 per pot. One group was placed in the greenhouse under a 16-hour photoperiod. This photoperiod was obtained by extending the natural daylength with cool-white fluorescent lamps (Appendix B). Minimum light intensity at pot height was about 400 ft-c. The maximum light intensity obtained was about 3,000 ft-c and was a function of cloud cover and shading on the greenhouse. The temperature and relative humidity varied from 23 to 28 C and 40 to 50%, respectively. The second group was placed in the growth chamber under a 16-hour photoperiod of about 100 ft-c. Temperature and relative humidity were maintained at approximately 25 C and 50%, respectively. All pots were set in trays of water and kept moist by bottom watering as needed.

To determine the optimum concentration and pH of nutrient solution for seedling growth following germination, white ash seedlings were removed from the vermiculite seed bed 2 weeks after planting and placed in the four following Hoagland and Arnon's nutrient solutions¹⁵ (Appendix C): full- and half-strength at an unadjusted pH of 5.4 or 5.5 and full- and half-strength adjusted to pH 6.0 by 0.1 N KOH. Nine seedlings, each approximately 4 cm high and with one set of simple leaves, were selected for growth in each nutrient culture solution. Three seedlings were planted in each quart plastic pot of solution. After 2 weeks, each pot was thinned to the best two plants. The volume in the pots was brought up to the original volume every 2 days with the corresponding nutrient solution, and the entire solution was replaced at weekly intervals with fresh nutrient. Support for the individual plants was provided by inserting the stem into a split rubber stopper. This arrangement provided support without mechanically damaging the seedlings. The stoppers were inserted into precut holes in plastic lids designed for the pots. Aeration was provided by a glass tube inserted through a hole in the lid of the pot and connected to a source of air. Each aeration tube had a hosecock clamp for air adjustment. The seedling trees were grown in a growth chamber (Fig. 1) under a 16-hour photoperiod of about 1,200 ft-c. A similar experiment with red maple (*Acer rubrum* L.) was conducted under the same controlled environmental conditions (Fig. 2) using half-strength and full-strength Hoagland's nutrient solution at pH 6.0.



FIGURE 1. White Ash Growing in Six Nutrient Solutions in Controlled Environment Chamber. Half- and full-strength nutrient solutions at pH 5.5 and pH 6.9 and half- and full-strength nutrient solutions with NH_4NO_3 as nitrogen source.

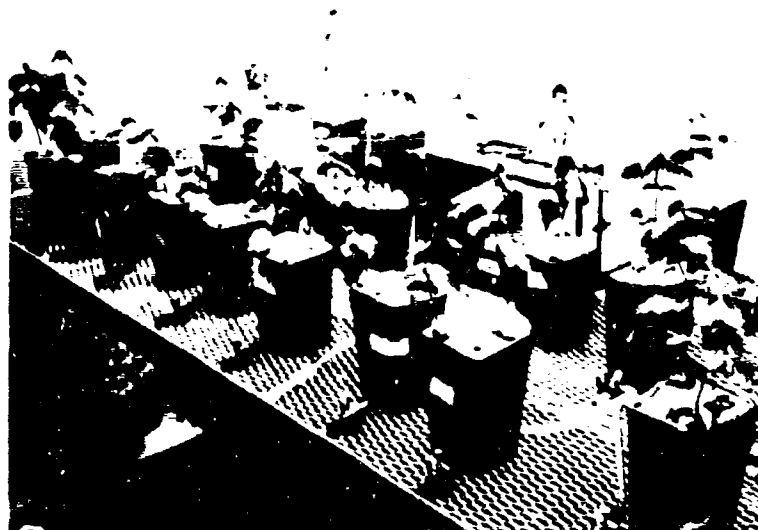


FIGURE 2. Red Maple in Half- and Full-strength Nutrient Solution in Controlled Environment Chamber.

Because it was not practical to grow all seedlings in a controlled environment chamber, an alternative method of growing trees in the greenhouse had to be developed. The objective of this series of studies was to raise seedling trees to the desired height (15 to 20 cm) and then to transplant them into nutrient culture in a controlled environment chamber for subsequent use. This method could be expected to provide a larger population from which to select uniform trees. Red maple seedlings germinated from seed in vermiculite, as previously described for white ash, were transplanted 4 weeks after planting to individual 1-quart pots filled with vermiculite or peat-perlite. Two-week-old seedling white ash and silver maple (*Acer saccharinum*) were handled in a similar manner. The trees were placed in the greenhouse under a 16-hour photoperiod (Fig. 3). Seedlings were top-irrigated with full- or half-strength nutrient solution and height measurements were recorded at weekly intervals.

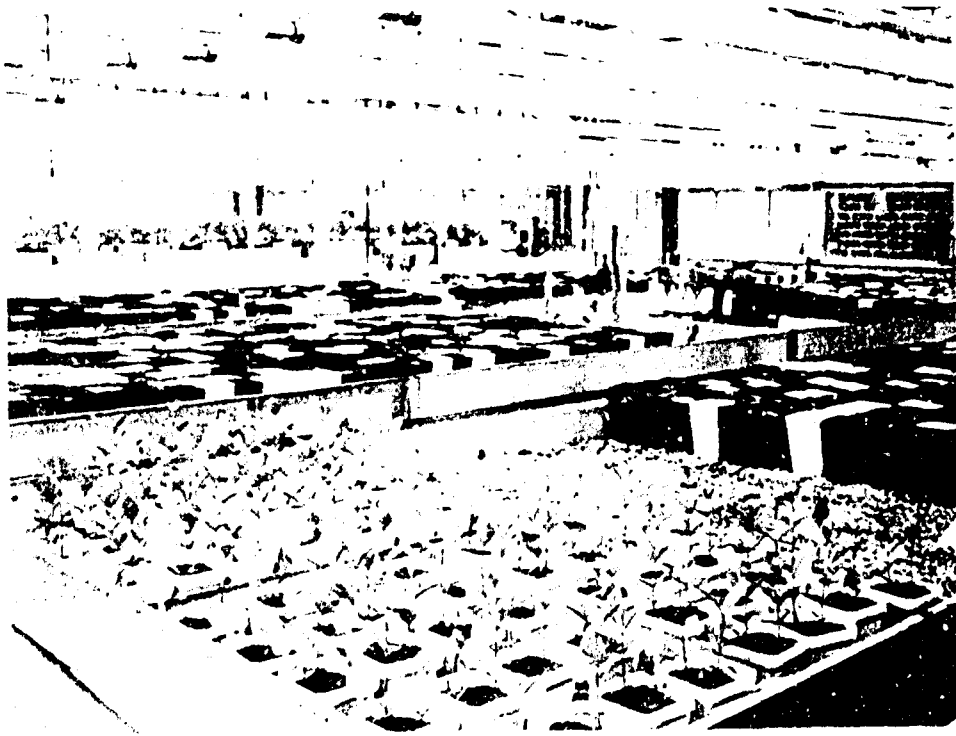


FIGURE 3. White Ash in the Greenhouse Under Cool-White Fluorescent Lights.

White ash seedlings were also grown to our optimum size by an additional technique. Vermiculite and peat-perlite were soaked in nutrient solution for 4 hours before transplanting seedlings in the following manner. One-quart pots containing the media were placed in trays (20 by 30 inches) in the greenhouse. Each tray accommodated 24 pots. Approximately 10 liters of either full- or half-strength nutrient solution were then added to the trays. After 4 hours, seedlings were planted two per pot and bottom-watered as needed. Weekly height measurements were recorded.

B. DORMANT SEEDLINGS

Investigations were conducted to determine the feasibility of developing uniform plants from dormant seedlings by breaking dormancy with photoperiodic manipulations.^{4,11,12} A series of experiments was initiated to determine the length of time required to break dormancy and to evaluate the subsequent new growth. Three light regimes of 16-hour duration were established: (i) ambient daylight in the greenhouse extended with incandescent lamps supplying about 60 ft-c, (ii) ambient daylight in the greenhouse extended with cool-white fluorescent lamps supplying about 800 ft-c, and (iii) fluorescent and incandescent light in a growth chamber supplying about 1,200 ft-c. In addition, a 20-hour photoperiod of about 1,400 ft-c was set up in another growth chamber with cool-white fluorescent and incandescent lamps. A 24-hour photoperiod was also established in the greenhouse by incandescent lamps, which provided about 350 ft-c at pot height. The purpose of the last two regimes was to ascertain if photoperiods longer than 16 hours would appreciably shorten the time required to break dormancy.

For these investigations, dormant green ash (Fraxinus pennsylvanica L.) and red maple seedlings were obtained from commercial nurseries. The trees arrived in December wrapped in moist sphagnum moss and were immediately placed in cold storage at 4 C for 2 months. Seedlings 20 to 25 cm high were removed from storage and potted in finely milled horticultural sphagnum moss soaked in half-strength nutrient solution. The seedlings were then placed in the photoperiods described above. Dormancy was considered broken when the first observable green leaves emerged. Observations were made daily.

III. RESULTS AND DISCUSSION

A. SEED GERMINATION

The ash seed from the cool-moist treatment germinated within 2 days on moist filter paper. After 1 week 92% had emerged. However, only 25% of the seed from the cool-dry treatment had germinated within 10 days. These results compared favorably with seed planted in the three propagation media (Fig. 4, Table 1). The seed from the cool-moist treatment in vermiculite germinated in 7 days, followed by those planted in peat-perlite and sand. Germination differences between seeds in vermiculite and in peat-perlite did not appear to be significant at either the low or high rate. Seed germination in sand was poor when compared with that in vermiculite or peat-perlite. A similar experiment using freshly harvested red maple and silver maple seed revealed the same general trend with respect to germination in vermiculite, peat-perlite, and sand (Fig. 5). Silver maple emerged within 1 week and red maple within 2 weeks. Two weeks after planting, the ash seedlings were 4 cm high with one set of simple leaves. This is an optimum size for transfer from the seed bed to individual containers. Silver maple required 2 weeks and red maple required 3 to 4 weeks to reach this size and stage of development.

Of the various media used for propagation, both vermiculite and peat-perlite were satisfactory for the germination of seed of all species. Vermiculite offered an additional advantage of being readily washed free of the root systems of the seedlings prior to transplanting into nutrient culture. Peat seemed to have an unusual tendency to adhere to the roots.

Table 2 represents the relative growth in height of the white ash seedlings in four nutrient culture solutions over a 5-week period. No significant differences in growth were detected. The seedlings were harvested after 8 weeks and fresh and oven-dry weight data were obtained in addition to their final height (Table 3). Again, no significant differences were obtained for any of the growth parameters recorded. Within 5 to 6 weeks, white ash seedlings were within our desired range of 20 to 25 cm high and were considered ready for experimental use. The experiment in which red maple was grown in full- and half-strength nutrient solution for 45 days also revealed no significant differences in height, fresh weight, or dry weight (Table 4). From these studies it was concluded that uniform white ash and red maple seedlings can be readily grown in our environmental chambers in half-strength nutrient solution. Adjustment of the pH of the nutrient solutions was an unnecessary precaution.



FIGURE 4. White Ash 3 Days, 1 Week, and 2 Weeks (Left to Right) After Planting Seed in Vermiculite.



FIGURE 5. Red Maple (left) and Silver Maple (Right) 2 Weeks After Planting Seed in Vermiculite.

TABLE 1. COMPARISON OF EFFECT OF SEED TREATMENT AND PLANTING MEDIUM ON GERMINATION OF WHITE ASH SEED 2 WEEKS AFTER PLANTING

| Planting Medium | Number of Seeds per Pot | Germination, % | | | |
|-----------------|-------------------------|--------------------------|----------------|------------------------|----------------|
| | | Cool-Moist ^{a/} | | Cool-Dry ^{b/} | |
| | | Greenhouse | Growth Chamber | Greenhouse | Growth Chamber |
| Vermiculite | 25 | 72 | 72 | 20 | 0 |
| | 75 | 83 | 92 | 16 | 4 |
| Peat-perlite | 25 | 92 | 64 | 0 | 4 |
| | 75 | 80 | 69 | 5 | 4 |
| Sand | 25 | 20 | 16 | 0 | 4 |
| | 75 | 31 | 79 | 15 | 11 |

a. Seed soaked with water and kept moist in a cold room at 4 C for 90 days.

b. Seed stored in a cold room at 4 C for 90 days.

TABLE 2. COMPARISON OF HEIGHT OF WHITE ASH SEEDLINGS GROWING IN NUTRIENT CULTURE SOLUTIONS

| Concn and pH of Hoagland's Nutrient Solution | Height, cm ^{a/} | | | |
|--|---|----------|----------|----------|
| | Weeks after Placing in Solution Culture ^{b/} | | | |
| | 2 | 3 | 4 | 5 |
| Half-strength, pH 6.0 ^{c/} | 8.9±0.6 | 14.7±1.2 | 21.5±2.5 | 28.4±3.8 |
| Full-strength, pH 6.0 ^{c/} | 8.8±0.9 | 14.2±1.2 | 20.9±1.4 | 27.8±1.9 |
| Half-strength, pH 5.4 | 8.7±0.4 | 14.3±1.4 | 21.4±2.0 | 28.4±3.0 |
| Full-strength, pH 5.5 | 8.8±1.2 | 14.4±2.0 | 21.3±2.5 | 28.4±3.4 |

a. Average height of nine trees per nutrient solution with 95% confidence limits.

b. Seedlings were 2 weeks old and 4 cm high when transplanted into solution cultures.

c. pH adjusted to 6.0 by addition of KOH.

TABLE 3. GROWTH OF 8-WEEK-OLD WHITE ASH SEEDLINGS
IN FOUR NUTRIENT SOLUTIONS

| Concn and pH of Hoagland's Nutrient Solution | Height, cm ^{a/} | Fresh Weight, g ^{a/} | Oven-Dry Weight, g ^{a/} |
|--|--------------------------|----------------------------------|-------------------------------------|
| Half-strength, pH 6.0 ^{b/} | 32.3±5.0 | 9.10±1.72 | 2.30±0.38 |
| Full-strength, pH 6.0 ^{b/} | 32.3±2.5 | 8.55±0.97 | 2.36±0.25 |
| Half-strength, pH 5.4 | 31.83±3.5 | 7.79±1.83 | 2.18±0.43 |
| Full-strength, pH 5.5 | 32.4±3.6 | 8.79±1.88 | 2.17±0.52 |

a. Average of nine trees per nutrient solution with 95% confidence limits.

b. pH adjusted to 6.0 by addition of KOH.

TABLE 4. GROWTH OF RED MAPLE SEEDLINGS AFTER 45 DAYS IN TWO CONCENTRATIONS
OF HOAGLAND'S NUTRIENT SOLUTION^{a/}

| Concn of Nutrient | Height, cm ^{b/} | Fresh Weight, g ^{b/} | Oven-Dry Weight, g ^{b/} |
|-------------------|--------------------------|-------------------------------|----------------------------------|
| Half-strength | 13.2±2.5 | 2.91±0.57 | 0.71±0.15 |
| Full-strength | 13.6±3.0 | 3.69±1.04 | 0.94±0.27 |

a. There were 26 replications in half-strength solution and 20 replications in full-strength solution.

b. Plus and minus values give 95% confidence limits on the mean.

White ash and silver maple seedlings grown in vermiculite and peat-perlite in the greenhouse grew quite satisfactorily and reached our pre-determined size of 20 to 25 cm within 5 to 6 weeks. Red maple attained this size in 8 to 9 weeks. Silver maple and red maple grew equally well in both media. White ash, as judged by height after 3 weeks in the propagation media, also grew at an almost equal rate in both vermiculite and peat-perlite when watered from the top with half-strength nutrient solution at weekly intervals (Table 5). However, in all other treatment combinations white ash grew significantly better in vermiculite than in peat-perlite. The two concentrations of nutrient solution were statistically indistinguishable from each other with respect to growth of the seedlings in peat-perlite, whether irrigated weekly or presoaked in the nutrient. In vermiculite, however, half-strength nutrient was superior when the presoaking technique was employed. This apparent superiority of half-strength nutrient over full-strength nutrient developed between the 2nd and 3rd week for those seedlings growing in presoaked vermiculite. Thus, a 20-cm white ash seedling could be obtained for transplanting into nutrient culture in 5 weeks from the time of planting the seed when vermiculite presoaked in half-strength nutrient solution was used as the propagation medium.

TABLE 5. COMPARISON OF THE HEIGHT OF WHITE ASH SEEDLINGS GROWING IN TWO PROPAGATION MEDIA WITH SEVERAL METHODS OF NUTRITION

| Method of Supplying Hoagland's Nutrient Solution | Concentration of Nutrient | Height, cm ^{a/} Weeks after Transplanting into Propagation Media ^{b/} | | |
|--|------------------------------|---|----------|----------|
| | | 1 | 2 | 3 |
| <u>Peat-perlite</u> | | | | |
| Weekly irrigation ^{c/} | Half-strength | 11.4±0.7 | 14.2±0.8 | 16.4±0.9 |
| | Full-strength | 11.6±0.7 | 13.0±0.8 | 15.6±0.9 |
| Presoaked ^{d/} | Half-strength | 9.7±0.7 | 11.0±0.7 | 11.9±0.8 |
| | Full-strength | 9.6±1.0 | 11.3±1.1 | 12.3±1.2 |
| <u>Vermiculite</u> | | | | |
| Weekly irrigation | Half-strength | ND ^{e/} | 12.5±0.8 | 16.7±0.9 |
| Presoaked | Half-strength | 11.0±0.7 | 15.7±0.8 | 20.7±1.1 |
| | Full-strength | 10.1±0.6 | 14.3±0.9 | 15.8±1.1 |

- a. Plus and minus values give 95% confidence limits on the mean.
- b. Seedlings were 2 weeks old and 8.0±1.0 cm high when transplanted.
- c. Irrigated from the top once a week with nutrient solution.
- d. Propagation media were soaked in nutrient solution for 4 hours before transplanting seedlings. Seedlings were irrigated from the bottom with water as needed.
- e. Not done.

B. DORMANT SEEDLINGS

The results of the photoperiod experiments (Table 6) indicate that dormancy can be broken by a 16-hour photoperiod utilizing supplemental low-intensity light. Ash under a 16-hour light regime produced flushes of new growth from all viable dormant buds in 5 to 6 weeks. Red maple required about twice that length of time to reach the same stage of development. Later experiments with vermiculite, perlite, and peat-perlite under the same conditions showed similar results. Trees of the desired size can be grown either from seed or from dormant seedlings. However, the use of dormant seedlings is not preferable to the use of seed for several reasons. The source of dormant seedlings was not dependable and their seed source was unknown. Ash and maple seedlings became etiolated in the cold room within 9 months. Red maple seedlings from commercial nurseries were undesirable because many of the trees were not single stemmed. Although the terminal bud on ash usually broke dormancy first, the lateral buds of maple broke before the terminal buds, resulting in too many new shoots. Often the terminal bud did not break dormancy. The ash seedlings tended to be cumbersome and a large amount of greenhouse space was required to produce a population from which to select uniform candidate specimens. Variation in height among individuals was greater in dormant trees than in those grown from seed.

In our investigations with dormant red maple, the type of growth was dependent upon the planting media. Peat-perlite produced well-formed healthy leaves. With vermiculite, marginal necrosis and slight chlorosis of the leaf occurred. Erratic and undesirable growth from previously dormant red maple made it difficult to obtain sufficient numbers of uniform trees. The peat-perlite and dormant red maple combination, while capable of producing some desirable trees, did not equal the rate of growth and quality of seedling produced from the seed-initiated method.

TABLE 6. TIME REQUIRED TO BREAK DORMANCY OF GREEN ASH AND RED MAPLE
UNDER VARIOUS PHOTOPERIOD REGIMES

| Photoperiod, hours | Light Source and Approx Intensity | No. of Days Until 50% of the Green Ash Broke Dormancy | No. of Days Until 50% of the Red Maple Broke Dormancy |
|-----------------------|--|---|---|
| 16 | Daylight + 50 ft-c Incandescent ^{a/} | 13 | 18 |
| 16 | Daylight + 800 ft-c fluorescent ^{a/} | 18 | 30 |
| 16 | Growth chamber: 1,200 ft-c Incandescent and fluorescent ^{b/} | 14 | 27 |
| 20 | Growth chamber: 1,400 ft-c Incandescent and fluorescent ^{b/} | 14 | 33 |
| 24 | Daylight + 350 ft-c Incandescent ^{a/} | 17 | 21 |

a. Ambient day length of 10 to 11 hours extended as indicated. Intensity varied from about 3,000 ft-c to indicated base line as a function of cloud cover and time of day. Greenhouse temperature and relative humidity varied from 23 to 28 C and 40 to 50%, respectively.

b. Seedlings were grown at 25±3 C and 60±15% relative humidity.

IV. RECOMMENDATIONS FOR GROWING ASH AND MAPLE SEEDLINGS

1) Gather three to four 8- by 12-inch polyethylene bags of ash seed (Fig. 6) and 10 bags of maple seed (Fig. 8) from local trees. Collect the maple seed in May and the ash seed in October after it turns brown. Silver maple matures about a week before red maple.

2) Allow seed to dry on laboratory bench for 1 to 2 days.

3) To insure seed viability for at least 1 year, place the seed in sealed containers and store at 4 C.

4) A cool-moist storage of 90 to 120 days at 4 C is necessary to break embryonic dormancy of ash seed. This may be done by placing seed in a container with wet paper towels and periodically moistening with distilled water. This method increases percentage germination for maple seed; however, maple will germinate without the prescribed cool-moist period.

5) Six weeks prior to an experiment, plant 75 ash seeds ($\frac{1}{2}$ inch deep) per 6-inch bulb pot of moist vermiculite, place in greenhouse or growth chamber, and bottom water when necessary. Maintain temperature and relative humidity at 23 to 28 C and 40 to 50% RH, respectively. Follow the same procedure when planting maple seeds, but use only 25 seeds per pot for silver maple and 50 seeds for red maple. Red maple should be planted 9 weeks prior to an experiment rather than 6 weeks as with ash and silver maple.

6) Within 3 weeks candidate ash and silver maple seedlings can be selected for transplanting. Red maple requires an additional 1 to 2 weeks. These seedlings will have one set of simple leaves in addition to the cotyledons.

7) Transplant seedlings (one or two per pot) to 4-inch pots of vermiculite which have been presoaked with half-strength Hoagland's nutrient solution. Place pots on trays, bottom water as needed, and provide a 16-hour photoperiod. Plants can also be grown in the greenhouse if supplemental illumination is provided from September through May. Gro-lux illumination is not recommended.¹⁸

8) When the seedlings are 20 to 25 cm tall, select test specimens for transfer to nutrient culture. This should be 5 weeks from the initial planting date for ash and silver maple, 8 weeks for red maple.

9) Seedlings are easily removed from the vermiculite if they are not watered 48 hours prior to transplanting. Any particles of vermiculite clinging to the root system can be easily removed by rinsing with water.

10) Place one or two seedlings per 1-quart pot of half-strength Hoagland's nutrient solution (Fig. 7 and 9). The same apparatus (rubber stoppers, aeration tubes and lines) used for bean culture can be utilized for growing tree seedlings. Recommended growth room conditions are: 1,300 ft-c of light, 16-hour photoperiod, 25 C, and 50% RH.

WHITE ASH

Fraxinus americana

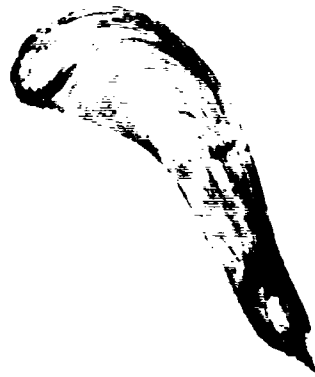


FIGURE 6. Seed of White Ash. Approximately twice actual size.



FIGURE 7. White Ash in Airtight Nutrient Culture.

RED MAPLE
Acer rubrum



SILVER MAPLE
Acer saccharinum

FIGURE 8. Seed of Red Maple and Silver Maple.
Actual size.



FIGURE 9. Red Maple in Actual Nutrient Culture.

LITERATURE CITED

1. HacsKaylo, J.; Finn, R.F.; Vemmerstedt, J.P. 1969. Deficiency symptoms of some forest trees. Res. Bull. 1015, Ohio Agr. Res. Develop. Center, Wooster
2. Ingestad, T. 1957. Studies on the nutrition of forest tree seedlings: I. Mineral nutrition of birch. *Physiol. Plant.* 10:418-439.
3. Parry, P.W.; Upchurch, R.P. 1968. Growth analysis of red maple and white ash seedlings treated with eight herbicides. *Weed Sci.* 16:32-37.
4. Kramer, P.J. 1936. Effect of variation in length of day on growth and dormancy of trees. *Plant Physiol.* 11:127-137.
5. Nitsch, J.P. 1957. Growth responses of woody plants to photoperiodic stimuli. *Proc. Amer. Soc. Hort. Sci.* 70:512-525.
6. Nitsch, J.P. 1957. Photoperiodism in woody plants. *Proc. Amer. Soc. Hort. Sci.* 70:526-544.
7. Roberts, R.H.; Struckmeyer, B.E. 1938. The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. *J. Agr. Res.* 56:633-677.
8. Vaartaja, O. 1954. Photoperiodic ecotypes of trees. *Can. J. Bot.* 32:392-399.
9. Fergus, C.L. 1955. A note about breaking dormancy of oak in the greenhouse. *Plant Dis. Rep.* 39:873.
10. Schoeneweiss, D.F. 1963. Methods for breaking dormancy of oak seedlings in the greenhouse. *Proc. Amer. Soc. Hort. Sci.* 83:819-824.
11. Olmsted, C.E. 1951. Experiments on photoperiodism, dormancy, leaf age and abscission in sugar maple. *Bot. Gaz.* 112:365-393.
12. Wareing, P.F. 1953. Growth studies in woody species: V. Photoperiodism in dormant buds of Fagus sylvatica L. *Physiol. Plant.* 6:692-706.
13. Steinbauer, G.P. 1937. Dormancy and germination of Fraxinus seeds. *Plant Physiol.* 12:813-824.
14. Fowells, H.A. 1965. Silvics of forest trees of the United States. Agricultural Handbook No. 271, Forest Service, U.S. Dept. of Agriculture, Washington, D.C.

15. Hoagland, D.R.; Arnon, D.I. 1950. The water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. 347 (Rev. ed.). 32 p.
16. Marquis, D.A. 1965. Artificial light sources differ in effect on birch seedling growth. U.S. Forest Serv. Res. NOTE NE-35. N.E. Forest Exp. Sta., Upper Darby, Pa.

APPENDIX A

SOURCE OF MATERIALS

The following materials were utilized in the various propagation experiments:

Vermiculite - Zonolite brand plaster aggregate vermiculite. Aggregate conforms with the provision of ASTM specifications C35-57T. Zonolite Division, W.R. Grace & Company.

Perlite - Peri-Lome, Horticultural Perlite, Atlantic Perlite Company, Washington, D.C.

Peat-moss - Milburn Peat Company, Otterbein, Indiana.

Sand - White silica sand, fine grade, purchased from Frederick Brick Works, Frederick, Maryland.

Sphagnum Moss - Horticultural sphagnum moss, finely milled. Approximately 2.5 lb. of sphagnum moss were soaked in 8 liters of half-strength Hoagland's nutrient solution for 24 hours.

APPENDIX B

GREENHOUSE AND GROWTH CHAMBER ENVIRONMENTAL CONDITIONS

1. Light - All light readings were determined by a Weston illumination meter Model 756 with quartz filter.

a. Greenhouse - This 16-hour photoperiod consisted of natural daylength supplemented by cool-white fluorescent lamps (G.E. T10 reflector lamps, 200-watt). A minimum intensity of about 400 ft-c was provided.

b. Growth Chamber - The 16-hour photoperiod in the growth chamber was provided by 18 high-output cool-white fluorescent lamps and eight 75-watt incandescent bulbs. Light intensity during the course of these studies varied from about 1,000 to 1,300 ft-c at pot height.

2. Temperature and Relative Humidity

All temperature and relative humidity data were recorded by Hygro-thermographs, manufactured by Friez Instrument Division, Bendix Aviation Corporation, Baltimore, Maryland.

a. Greenhouse

The temperature and relative humidity in the greenhouse ranged from 23 to 28 C and 40 to 50%, respectively, October through May. During the summer months the temperature often exceeded 38 C and the relative humidity exceeded 90%.

b. Growth Chamber

The temperature and relative humidity were normally maintained at 25 C and 50%, respectively. However, over the entire period in which these studies were performed the ranges of temperatures and relative humidities encountered were 25 ± 3 C and $60 \pm 15\%$, respectively.

APPENDIX C

COMPOSITION OF NUTRIENT SOLUTION

The following table gives the composition of the nutrient solution used for growing trees. It is a slight modification of the Hoagland and Arnon nutrient solution No. 1 in that a chelated source of iron is used rather than ferric tartrate or citrate.

| <u>Stock Solutions</u> | <u>Moles per Liter Stock Solution</u> | <u>Milliliters per Liter of Final Volume for a Half-Strength Solution</u> |
|---|---|---|
| 1. KNO_3 | 1 | 2.5 |
| 2. $\text{Ca}(\text{NO}_3)_2$ | 1 | 2.5 |
| 3. MgSO_4 | 1 | 1.0 |
| 4. KH_2PO_4 | 1 | 0.5 |
| 5. Micro elements (one solution): | <u>Grams per Liter Stock Solution</u> | 0.5 |
| H_3BO_3 | 2.86 | |
| $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ | 1.81 | |
| $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ | 0.22 | |
| $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ | 0.08 | |
| $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}^{\text{a}/}$ | 0.02 | |
| 6. Source of iron: | | |
| Sequestrene 330 ^{b/} | 24.0 | 0.5 |
| a. Assaying 85% MoO_3 . | | |
| b. Geigy Chemical Corp., Sawmill River Road, Ardsley, New York. This solution should be autoclaved after preparation. | | |

Unclassified
Security Classification

| DOCUMENT CONTROL DATA - R & D | | |
|---|-----------------------|---|
| <i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i> | | |
| 1. ORIGINATING ACTIVITY (Corporate author) | | 25. REPORT SECURITY CLASSIFICATION |
| Department of the Army Fort Detrick, Frederick, Maryland, 21701 | | Unclassified |
| | | 26. GROUP |
| 2. REPORT TITLE | | |
| A SOLUTION CULTURE METHOD FOR GROWING UNIFORM ASH AND MAPLE SEEDLINGS | | |
| 3. DESCRIPTIVE NOTES (Type of report and inclusive dates) | | |
| 4. AUTHOR(S) (First name, middle initial, last name) | | |
| Charles A. Vile Robert W. Gesink James W. Akerman Woodland (NMI) Hurtt | | |
| 5. REPORT DATE | 72. TOTAL NO OF PAGES | 73. NO OF REFS |
| March 1970 | 34 | 16 |
| 22. CONTRACT OR GRANT NO | | 24. ORIGINATOR'S REPORT NUMBER(S) |
| A. PROJECT NO 1B562602AD09 | | Technical Manuscript 588 |
| C. Task-Work Unit N.A. | | 23. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) |
| D. DD 1498 Agency Access. N.A. | | CMs 6651 AMXFD-AE-T 49598 |
| 10. DISTRIBUTION STATEMENT | | |
| Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized. | | |
| 11. SUPPLEMENTARY NOTES | | 12. SPONSORING MILITARY ACTIVITY |
| Plant Physiology Division | | Department of the Army Fort Detrick, Frederick, Md., 21701 |
| 13. ABSTRACT | | |
| <p>This study evaluated methods and established procedures for growing seedling trees to a predetermined uniform size. White ash (<u>Fraxinus americana</u> L.) seed gathered in the fall from indigenous sources was placed in a cool-moist environment for 90 days to break embryonic dormancy. Red maple (<u>Acer rubrum</u> L.) and silver maple (<u>Acer saccharinum</u> L.) were similarly obtained in the spring but did not require a cold treatment. Seeds were germinated in vermiculite, a 1:1 peat-perlite mixture, and washed silica sand in the greenhouse and in a growth chamber. Following emergence, the seedlings were grown for various periods in a growth chamber in nutrient culture as well as in the above media in the greenhouse under supplementary light.</p> <p>In contrast, dormant ash and maple seedlings were obtained commercially and exposed to various photoperiods. Daylengths of 16 and 20 hours were used in the growth chamber studies. Greenhouse photoperiods of 16, 20, and 24 hours were achieved by supplementing the natural day of 10 to 11 hours with increments of fluorescent or incandescent light.</p> <p>The solution culture system is the only practical method that provides the opportunity for analyses or autoradiography of both foliage and roots. The optimum procedure evolved here was to germinate seed in vermiculite</p> | | |

DD FORM 1473 1 NOV 66
REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ALL USE

Unclassified
Security Classification

Security Classification

and transplant into individual pots of vermiculite, initially saturated with Hoagland's nutrient solution, when the seedlings were about 4 cm tall. After attaining a height of 20 to 25 cm, the seedlings were transferred to aerated solution culture and were considered ready for experimental use after 2 to 3 days of acclimatization. The environmental parameters were 25 ± 3 C, $60 \pm 15\%$ RH, and a 16-hour photoperiod of $1,000 \pm 300$ ft-c.

When grown from seed, ash and silver maple required 5 weeks to reach the optimum height of 20 to 25 cm; red maple required 8 to 9 weeks. We have successfully grown trees in solution culture in 1-quart pots for 2 months with no special requirements other than periodic replenishment of the solution. The only limitation to growth of larger trees would be the need for physical support.

14. Key Words

Tree seedlings
Nutrient solution
Hydroponics
Ash seedlings
Maple seedlings
Fraxinus
Acer
Dormancy (embryonic)
Germination
Photoperiod

UnclassifiedSecurity Classification