Prospects for Vegetative Propagation in the Genus *Castanea*

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ABSTRACT.—Literature concerning the vegetative propagation of chestnut by grafting, rooting cuttings, layering, and tissue and organ culture is reviewed. It is concluded that attempts to develop a system of propagating desirable chestnut clones should be continued. The most promising techniques are concluded to be nut grafting, rooting cuttings and tissue and organ culture.

In 1925, the United States Department of Agriculture at Beltsville, Maryland, instituted a program to breed chestnut trees which would be resistant to the blight fungus (*Endothia parasitica* [Murr.] P. J. and H. W. And.) (Saucier, 1973). Four years later the Brooklyn Botanical Garden started a similar program, which was later continued at the Connecticut Agricultural Experiment Station at Hamden, Connecticut (Jaynes, 1972). Meanwhile, a search for resistant American chestnut (*Castanea dentata* [Marsh] Borkh), was being conducted by various state and federal agencies. While neither program has been completely successful, there are a small number of residual trees which may have genes for partial resistance, and there are several hybrids which show some resistance and have good form. Success in either of these programs will necessitate the development of a practical technique for vegetative propagation. The fortunate gene combination will rarely occur and the genus *Castanea* is generally self-sterile (Clapper, 1954), so the establishment of pure breeding lines would be difficult or impossible. Therefore vegetative propagation is the only alternative for the multiplication of desirable trees.

Vegetative propagation can be accomplished by grafting, rooting cuttings, layering, or the relatively new procedure of growing plantlets through tissue culture.

Grafting

Grafting has generally been more successful as a means of propagating chestnut than layering or rooting cuttings. Grafting consists of placing a twig (scion) from the resistant plant into another seedling or tree (stock plant). The two grow together after a period of time, creating a new plant. Splice, whip, cleft, and side grafts have been used successfully in bench grafting, or grafting in the greenhouse (Fig. 1). The splice graft is simplest and seems to be the most effective (Nienstadt and Graves, 1955). This type of graft can also be used in field grafting such as is done on stock plants in the seedbed or the seed orchard (Jaynes, 1972). Mature trees can be topworked using the veneer crown graft (Nienstadt and Graves, 1955) (Fig. 2) or bark graft (McKay and Jaynes, 1969).

The best time for scion collection is February or March (Nienstadt and Graves, 1955). The scion should be cut to 12 inches in length and stored in nearly dry peat moss at 35-36 F in sealed plastic bags (Jaynes, 1969). Field grafting should be done when the leaves of the rootstock are mature to avoid frost damage (Nienstadt and Graves, 1955).

Nienstadt and Graves (1955) recommend grafting onto well-established stock plants. Park (1967), however, grafted onto juvenile tissue of Japanese chestnut (C. *crenata* Sieb. & Zucc.) with some success. The rootstock consisted of newly germinated seeds. The graft was made onto the epicotyl (stem) which had at least four mature leaves. The

scions were either newly elongated shoots of mature trees or epicotyls with four mature leaves.

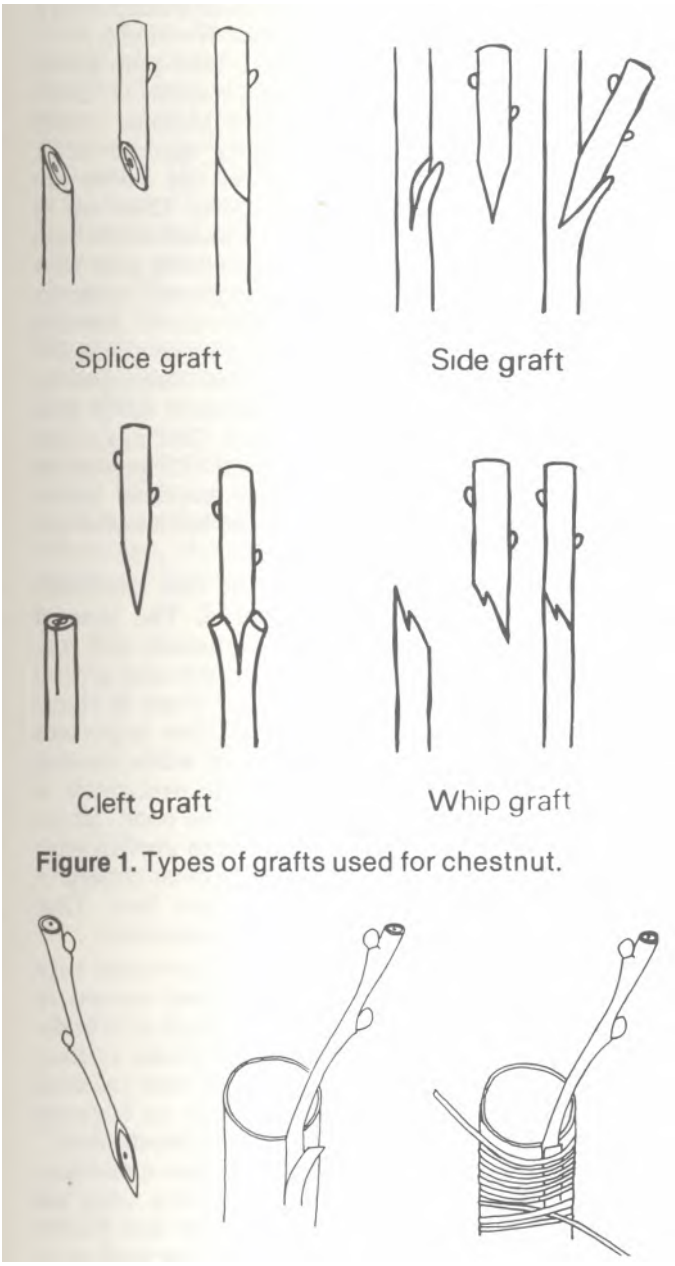

Figure 1. Types of grafts used for chestnut.

Figure 2. Veneer crown graft. (Taken from Nienstadt and Graves, 1955).

A major problem associated with the grafting of chestnuts is incompatibility between the rootstock and scion. This is caused when the scion does not make a completely successful union, and can be compared to the rejection of a transplanted organ in humans. This problem can be alleviated by choosing the proper rootstock. McKay and Jaynes (1969) recommend using seed collected from the variety being grafted as a source of stock plants for Chinese chestnut *(C. mollissima* Bl.). The degree of success in grafting hybrid scions was affected by the species of rootstock, with no apparent pattern to compatibility (Stairs, 1964). Morris reported that chinquapin would accept almost any species of chestnut, but appears to have a dwarfing affect on the scion (Kains and McQuesten, 1967). Incompatibility is probably genetically controlled, as indicated in Park's (1967) study. In three trials using Japanese chestnut rootstock and scions of three clones, success was 0, 10, and 70 percent.

Another problem with grafting is its prohibitively high cost. This factor alone eliminates grafting as a means of mass-producing desirable clones. Only in a situation where the final product yields a rapid return and high price (ie., commercial nut production), or the goal is preservation of desirable germ plasm (ie., seed orchards), would grafting be economically feasible.

One technique which might alleviate these two problems is nut grafting. Moore (1963) first described such a technique which he called nurse seed grafting. In this method a seed is allowed to germinate and grow until only the hypocotyl has emerged. Then the seed is cut so that the hypocotyl and radicle are removed. A knife is inserted into the cotyledons and the scion, cut to a wedge on one end, is inserted into this slit (Fig. 3). Jaynes (1965) reported 60-80 percent rooting success within 21 days in 453 grafts. Losses during a 2-week hardening-off period reduced this figure to 38 percent success. Of 4,384 grafts, success averaged 43 percent with some scion-nut combinations ranging from 45-80 percent success (Jaynes and Messner, 1967). The main advantages of this technique are the reduced time span since rootstock need not be grown, timing is not critical, and less skill and time are involved in the actual grafting.

Figure 3. Nurse seed graft. a) Two views of scion prepared with wedge-shaped cut. b) Germinating nut with a dashed line indicating cut that will go through petioles. c) Shoot and root primordia removed, with dashed line where knife-blade is inserted into nut. d) Knife blade inserted in nut. e) Two views of completed graft. (Taken from Jaynes, 1965.)

A similar technique,. called the inverted radicle graft, was described for Japanese chestnut by Park (1968). The seeds are allowed to germinate and the radicle tip is cut off prior to root hair formation and epicotyl emergence. Then the scion and radicle are grafted as a normal cleft graft would be made (Fig. 4). Near 100 percent survival was reported in greenhouse trials. Field success of 288 grafts averaged

55.2 percent when planted 7-8 cm deep. Survival after the first growing season was not reported. Obviously, this technique would require more skill and time than nurse seed grafting.

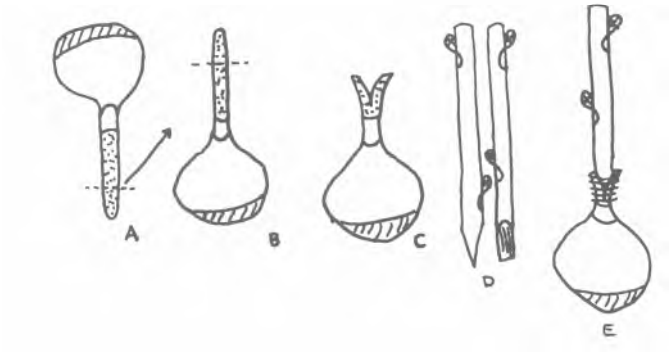

Figure 4. Inverted radicle graft. a) The optimum stage of the radicle to be used as stock. b and c) The prepared radicle and stock. d and e) The scion is prepared with a wedge-shaped cut. f) The completed graft. (Taken from Park, 1968.)

Rooting Cuttings

Rooting cuttings, or the forcing of root formation on severed stem sections, would be a more practical method of propagating chestnut than grafting since the amount of labor would be less, the labor need not be as skilled, and the time spent growing rootstock is avoided. Unfortunately, chestnut is generally difficult to root, apparently due to two factors. Vieitez *et al.* (1964) found that European chestnut *(C. sativa* Mill.) and *C. mollissima* contained little or no endogenous indole-3-acetic acid (IAA), a hormone which stimulates root development. In addition, *C. sativa* was found to contain salicylic and hydroxyaliphatic acids, which appear to inhibit rooting (Vieitez *et al.,* 1967). The quantity of any endogenous IAA-like compounds decreases with age of the tree while the levels of rooting inhibitors increase (Vieitez *et al.,* 1966). Hence, younger plants tend to root more easily. Vieitez also found that placing chestnut cuttings under running water for a period of five months allowed these inhibitors to be leached out and stimulated rooting (Jaynes, 1972). This extended time period, however, could be a drawback in a mass-production system.

Experimentation with various hormone treatments and collection times have met with varied results. Pease (1953) ran separate experiments in a rooting bed and in a cold frame. In the rooting bed, softwood cuttings of Chinese and American chestnut (collected in the summer) which had been soaked in a 60 ppm indole-3-butyric acid (IBA) solution for 24 hours rooted 80 percent in 70 days. Cuttings which had been clipped in IBA: talc (1:200) or left untreated failed to root. Cuttings collected on June 9, July 24, and August 19, and treated with the IBA soak, rooted 75, 100, and 67 percent, respectively. In cold frame trials using cuttings collected August 20 from ten- and three-year-old Chinese and three-year-old American chestnuts, rooting was 54.5, 50.0, and 20.0 percent, respectively. Doran (1957) treated cuttings collected in late June from a ten-year-old Chinese chestnut with Hormodin No. 2 (300 ppm IBA), Hormodin No. 3 (800 ppm IBA), and no hormone. Rooting success was 25, 17, and 0 percent, respectively. Jaynes and Messner (1967) report an effective method using sprouts of C. *dentata.* These are taken just as the leaves are nearly or fully expanded and cut to 12-20 cm in length. The cuttings are lightly wounded at the base and dipped for 1-2 seconds in 5,000-8,000 ppm IBA in 95 percent ethanol. These are placed under an intermittent mist in peat: perlite (3:1). Rooting success of certain clones can be 75 percent in 3-8 weeks. Huff slightly modified this technique by using a 3-4 second dip in a solution of 5,000 ppm Rootone in 70 percent isopropanol. Cuttings taken from a hybrid in September rooted 92.3 percent in eight weeks. Cuttings from the previous year's growth rooted 66.7 percent and the buds leafed out (Jaynes, pers. comm.).

From these reports it is evident that treatment with rooting hormones is essential. The time of collection, method of applying hormones, and conditions of the rooting environment are also critical factors. Jaynes (1976) reports that there is clonal variation in rooting and survival. One important point about these reports is that, while rooting success may be good, the ability to overwinter is either not mentioned or is said to be poor. Moore (1963) reported that forcing buds into growth after rooting was a problem. The cuttings must undergo a dormant period in which many are lost. Thor (pers. comm.) reported a similar phenomenon.

Various other rooting techniques have met with limited success. Bretz (1949) reported success in limited trials of rooting leaf-bud cuttings of hybrids. These cuttings consisted of the leaf blade, axillary bud, and a shield of stem tissue. They were collected in May and June and received either no hormone treatment or treatment with a "hormone dust." Once again, dormancy after rooting was a problem.

Trials with root sections of *C. sativa* were not successful. Landaluce (1952) reported bud formation on 2 percent of the sections. Another method he tried was to girdle a stem at ground level. This stimulated adventitious bud formation. These buds were then broken off with pieces of the roots. This method, however, would destroy the parent plant and so is not very practical.

A similar technique used in Europe with *C. sativa* and tried with success in Connecticut with C. *dentata* is stooling. This method consists of cutting the parent plant to the ground in winter, and covering the shoots which emerge in the spring to half their height with soil. When the mound is 6-8 in. high, no additional soil is added. The following winter the shoots are cut from the parent, whether rooted or not, and treated as nursery stock (Nienstadt and Graves, 1955). The European method is different in that a steel wire is loosely fastened to

the sprouts. Then sandy loam soil mixed with peat moss and of pH 5 is mounded to 6 in. above the wire. As the sprout grows, the wire girdles it and roots form above the girdle. If treated properly, the parent plant can be stooled for several years. The disadvantages of stooling are its high cost and strong clonal response to rooting (McKay and Jaynes, 1969; Solignat, 1964).

Another technique used with American chestnut is the buried-inarch. In this method a 6-in.-deep hole is dug around a well-established tree. About 2 in. above the ground, upward diagonal cuts are made in the tree. The scions are cut to 6-8 in. and wedged on the top. The wedged end is then fitted into the tree, the graft union is wrapped and waxed, and the lower end of the scion is covered with soil. At least one bud is left exposed on the scion (Fig. 5). Due to a drought year, initial rooting results of only 36 percent were reported, but rooting of 50 percent might be expected. This method is costly and dependent on favorable climatic conditions. It has an advantage, though, in that the age of the scion appears to be less important (Jaynes, 1962).

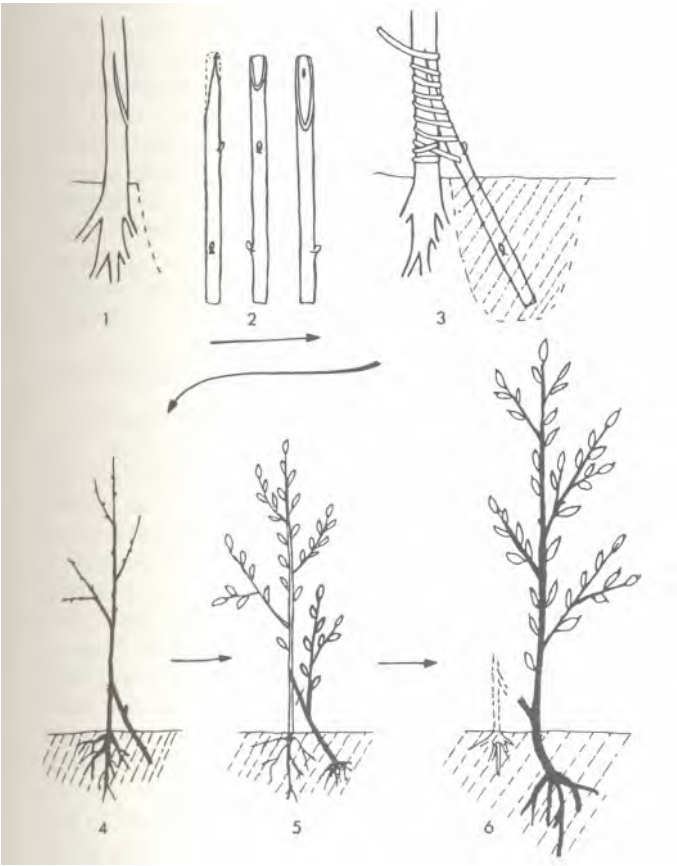

Figure 5. Buried-inarch technique. 1) Upward diagonal cut made in the stock plant. 2) Scion cut to a wedge on top. 3) Graft union is formed and scion is buried. 4-6) Scion is removed after roots and shoots form. (From G. Bazzigher. 1968. Die selektion Endothia-resistenter Kastanien and ihre Vermehrung. Schweitz. Beitr. Dendrol. 16/18:29-38.)

Layering

Ground and air layering (forcing root formation on stems while on the parent plant) have also been tried with chestnut with varying results. Landaluce (1952) reported negative results using a ground layer on young plants of European chestnut. Sprouts on older stumps responded with 20 percent rooting success. Girdling and hormone applications were not beneficial.

In later work, Vieitez (1953) reported successful air layering of European chestnut. Carrying out the operation in the spring is best. Hormones were applied in a lanolin paste. The branch was then covered with moist sphagnum moss, and the layer was covered with plastic and tied at both ends. Of the hormone treatments tested 10mg/g IBA, 4mg/g IAA and 2,4-dichlorophenoxyacetic acid (2,4-D), and 5mg/g IAA and NAA with 1mg/g 2,4-D resulted in the best rooting. Care was recommended in using 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), since toxic responses were noted. The roots formed were thick and non-fibrous, except in the IAA-2,4-D treatment, and generally not suitable for transplanting.

In another study using ground layers of European chestnut, Vieitez (1955) reported very good success in rooting. Once again the hormones were applied in a lanolin paste. Then the branches were covered with moist sphagnum moss and soil. The best time for layering was from the end of May to the beginning of June. Moderately fast growing plants formed more roots on vertical branches than branches bent to the ground. The best hormone treatments were 10mg/g IBA and 5mg/g IBA and NAA with lmg/g of the dimethylamine of 2,4-D. Both of these treatments yielded 100 percent rooting and the roots were fibrous and of good quality. Using the dimethylamine of 2,4-D reduced the toxicity of this hormone. Vigorously growing stump sprouts responded favorably to all hormone treatments. Fibrous root production in 100 percent of the layers occurred using 12mg/g IBA with or without 0.1mg/g 2,4-D or its triethanolamine. The major emphasis of this research was on the response to hormones, so attempts to transplant these layers were not reported.

As with grafting, the major drawback of layering for mass production of desirable chestnut clones is the high cost due to the numerous man-hours required. Also, a large number of stock plants would be required to produce an adequate number of offspring. Loss during transplanting might also be a problem.

Tissue and Organ Culture

The use of tissue and organ culture as means of vegetatively propagating tree species is presently in the basic stage of research. These techniques are now being used for limited commercial production of many horticultural plants. However, extending the technique to the commercial production of hard-to-root tree species may be more difficult.

Basically, this method involves the aseptic removal of a piece of tissue (cambium) or an organ (apical meristem, epicotyl, etc), which is then placed in a sterile environment and supplied with all the necessary minerals, carbohydrates, and vitamins. Growth and differentiation can be controlled by the use of the proper levels of nutrients and hormones, and the proper light and temperature regimes (Fig. 6).

The trend has been to start cultures on a medium containing adequate nutrients plus a cytokinin (a hormone which stimulates bud formation) with or without an auxin (a rooting hormone). Then the culture is transferred to fresh medium with a limiting nutrient content and either lacking hormones or with a cytokinin. This technique has resulted in plantlet formation in the poplars *(Populus)* (Venverloo, 1973; Winton, 1970, 1971; Wolter, 1968), pines *(Pinus)* (Sommer, *et al.,* 1975), spruces *(Picea)* (Campbell and Durzan, 1976), Douglas-fir *(Pseudotsuga menziesii) (Mirb.)* Franco (Cheng, 1975), hemlock *(Tsuga)* (Cheng, 1976), and American elm *(Ulmus americana* L.) (Durzan and Lopushanski, 1975).

There have been few reports concerning the tissue culture of chestnut. Jacquiot (1947) was first to report successful culture of *C. vesca* Gaertn. Gautheret (1959) states that Jacquiot also reported that myo-inositol stimulated "bud" formation in chestnut tissue cultures. Chestnut has also been cultured

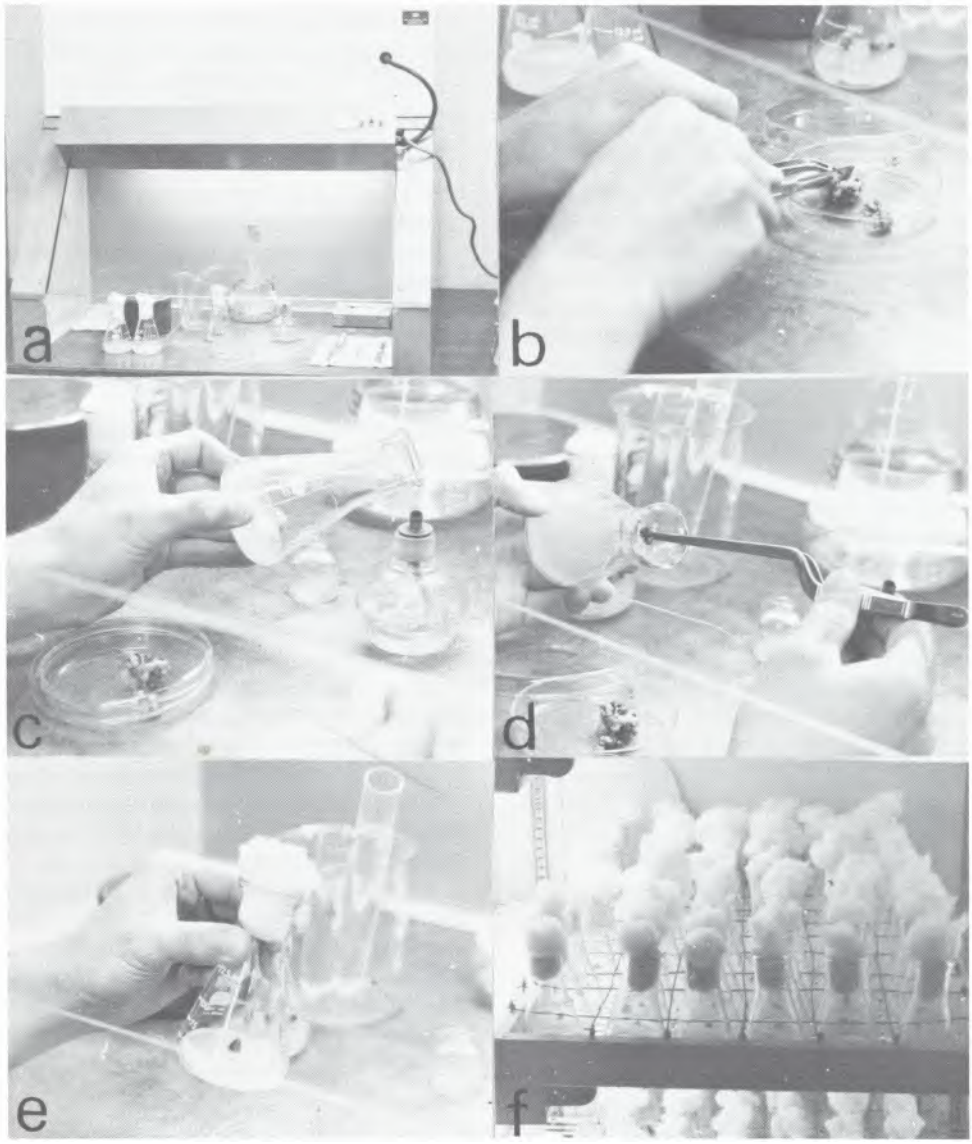

Figure 6. Tissue culture method. a) Sterile culture hood. b) Desired tissue is excised. c) Culture flask is flamed for sterilization. d) Tissue is placed in culture flask. e) Flask is plugged with cotton. f) Cultures are grown in a growth chamber in controlled temperature and light.

for the purpose of studying host-pathogen interactions (Durbin, pers. comm.; Van Alfen, pers. comm.). Hu (1977) reported the effects of various levels of kinetin, a cytokinin, on the morphology of callus tissue derived from stem apices of *C. dentata.*

Attempts to vegetatively propagate American chestnut in tissue culture are being made at the Division of Forestry, West Virginia University. In callus derived from cambial explants of mature stems, possible "meristematic" regions developed but failed to form shoots (Keys, 1977). Later attempts using epicotyl tissue from seedlings which were grown in darkness (etiolated) were promising. "Bud-like" growths developed on these sections, but failed to develop into shoots.

If successful, the major disadvantages of this method of propagation is the difficulty in transferring the plantlets from agar culture to soil. The root systems on many plantlets are of very poor quality. A second disadvantage is the large initial investment in supplies, equipment, sterile facilities, and growth chambers, since most places are not properly equipped for tissue culture work. Trained personnel are also required.

However, the advantages of tissue culture, if a workable system is developed, far outweighs the disadvantages. Theoretically, thousands of plantlets of a desirable clone could be produced from small amounts of tissue. Therefore harm to the parent plant is kept to a minimum. The time span for plantlet production would be shorter and the space required would be less than for grafting or layering. In addition, there is chance for mutation in culture (which could be advantageous or detrimental).

CONCLUSION

A review of the literature reported here suggests that grafting, inarching, stooling, and layering are not suitable techniques for commercially propagating chestnuts. The number of plants, time, and trained personnel required prohibit their use.

The most promising methods at this time are nut grafting, rooting cuttings, and tissue culture; however, each method has problems which must be overcome if they are to be practical. Results with nut grafting have been good. The skill required is less than for other techniques, and clonal effect is less important. The major problem now is the difficulty in successfully transplanting from the propagation frames, or overwintering in outdoor frames. Rooting cuttings is an even more desirable method, but dormancy after rooting has been a problem. Perhaps the use of the various hormone treatments used by Vieitez on ground layers, or the application of a cytokinin, would overcome this problem. If a workable system can be developed, tissue and organ culture appears to us to be the most desirable method. Large numbers of plantlets could be produced in a relatively short time. Differentiation into plantlets must be achieved, however, before this work can proceed any further.

In considering the commercial propagation of chestnuts, it is important to consider the potential market as well as the potential techniques. Nut growers, homeowners, and possibly wildlife managers would be interested in such trees. But convincing public and private foresters of the economic advantages of replanting chestnut on large acreages now occupied with other valuable species may be more difficult. Many people would like to see chestnut trees thriving once again in our forests. But such replanting would be difficult and costly. Therefore, replanting programs would be most feasible on marginal quality sites such as strip mine spoils or poorer sites which are unoccupied or where only low-value species are now growing. Chestnut would probably do as well or better than most species which could be planted on such sites, since it is known to grow well on poor sites. Small landowners who have an interest in chestnut may want to replant their land with this species. Many such landowners have expressed an interest in such a program.

As was previously stated, some system of vegetatively propagating chestnut must be developed if any of the research being done to develop disease resistance is to have any value to the public. As in any research of this type, there are problems which need to be overcome. The situation with the propagation of chestnuts is certainly not hopeless, and may even border on the promising side. Therefore, attempts to develop a propagation technique for chestnut should be continued.

LITERATURE CITED

Bazzigher, G.
1968. DIE SELEKTION ENDOTHIA-RESISTENTER KASTANIEN UND IHRE VERMEHRUNG. Schweitz. Beitr. Dendrol. 16/18:29-38.

Bretz, T. W.
1949. LEAF-BUD CUTTINGS AS A MEANS OF PROPAGATING DISEASE-RESISTANT ELMS. Plant Dis. Rep. 33(11): 434-436.

Campbell, R. A. and D. J. Durzan.
1976. VEGETATIVE PROPAGATION OF *PICEA GLAUCA* BY TISSUE CULTURE. Can. J. For. Res. 6:240-243.

Cheng, T. Y.
1975. ADVENTITIOUS BUD FORMATION IN CULTURE OF DOUGLAS FIR *(PSEUDOTSUGA MENZIESII* [MIRB.] FRANCO). Plant Sci. Letters 5:97-102.

1976. VEGETATIVE PROPAGATION OF WESTERN HEMLOCK *(TSUGA HETEROPHYLLA)* THROUGH TISSUE CULTURE. Plant and Cell Phys. 17:1347-1350. Abstract in Plant Tissue Cult. Abstracts 1(1):24.

Clapper, R. B.
1954. CHESTNUT BREEDING TECHNIQUES AND RESULTS. J. Hered. 45:107-114.

Doran, W. L.
1957. PROPAGATION OF WOODY PLANTS BY CUTTINGS. Univ. of Mass., Exp. Sta. Bull. No. 491:25.

Durzan, D. J. and S. M. Lopushanski.
1975. PROPAGATION OF AMERICAN ELM VIA CELL SUSPENSION CULTURES. Can. J. For. Res. 5:273-277.

Gautheret, R. J.
1959. LA CULTURE DES TISSUS VEGETAUX. Masson et Cie, Paris, France.

Hu, C. Y.
1977. EFFECT OF KINETIN ON CALLUS MORPHOGENESIS FROM STEM APEX EXPLANTS OF AMERICAN CHESTNUT *(CASTANEA DENTATA)* WINTER BUDS. Ohio State Univ. Fourth Ann. Coll. of Biol. Sci. Coll., In print.

Jacquiot, C.
1947. EFFET INHIBITEUR DES TANNINS SUR LE DEVELOPPEMENT DES CULTURES *IN VITRO* DU CAMBIUM DE CERTAINS ARBRES FRUITIERS. C. R. Acad. Sci. 225:434-436.

Jaynes, R. A.
1962. BURIED-INARCH TECHNIQUE FOR ROOTING CHESTNUT CUTTINGS. Proc. 9th N. E. For. Tree Imp. Conf.,' pp. 14-16.

1965. NURSE SEED GRAFTS OF CHESTNUT SPECIES AND HYBRIDS. Proc. Am. Soc. Hort. Sci. 86:178-182.

1969. LONG-TERM STORAGE OF CHESTNUT SEED AND SCIONWOOD. North. Nut Growers Assoc. 60th Ann. Rep., pp. 38-42.

1972. GENETICS OF CHESTNUT. USDA For. Ser. Res. Pap. WO-17.

1976. NEW DEVELOPMENTS IN CHESTNUT RESEARCH. Proc. 23rd N. E. For. Tree Imp. Conf., pp 36-41.

_____ and G. A. Messner.
1967. FOUR YEARS OF NUT GRAFTING CHESTNUT. Proc. Int. Plant Prop. Soc., pp. 305-310.

Kains, M. G. and L. M. McQuesten.
1947. PROPAGATION OF PLANTS. Orange Judd Pub. Co., Inc., New York, p. 367.

Keys, R. N.
1977. TISSUE CULTURE OF AMERICAN CHESTNUT, *CASTANEA DENTATA* (MARSH) BORKH. M.S. Thesis, West Virginia Univ., Morgantown, W. Va. 93 pp.

Landaluce, P. V.
1952. MULTIPLICACION ASEXUAL DE CASTANOS. An. de Invest. Agron. 1(2): 317-323.

McKay, J. W. and R. A. Jaynes.
1969. HANDBOOK OF NORTH AMERICAN NUT TREES. R. A. Jaynes, ed., North. Nut Growers Assoc., Knoxville, Tenn.

Moore, J. C.
1963. PROPAGATION OF CHESTNUTS AND CAMELLIA BY NURSE SEED GRAFTS. Proc. Int. Plant Prop. Soc. 13 : 141-143.

Nienstadt, H. and A. H. Graves.
1955. BLIGHT RESISTANT CHESTNUT CULTURE AND CARE. Conn. Agr. Exp. Sta. Circ. No. 192.

Park, K. S.
1967. NEW METHOD OF JUVENILE TISSUE GRAFTING OF SOME SPECIAL-USE-TREES. I. STUDIES ON THE JUVENILE TISSUE GRAFTING OF SOME CROP-TREE SPECIES (WALNUT, CHESTNUT, GINGKO, AND OAK). Res. Rep. Inst. For. Gen. 5:75-84.

1968. STUDIES ON THE JUVENILE TISSUE GRAFTING OF SOME SPECIAL-USE-TREES. II. AN EXPERIMENT ON INVERTED RADICAL GRAFTING OF CROP-TREE-SPECIES. Rep. Kor. J. Bot. 11(3): 8-17.

Pease, R. W.
1953. ROOTING CHESTNUTS FROM SOFTWOOD CUTTINGS. Am. Hort. 32:259-264.

Saucier, J. R.
1973. AMERICAN CHESTNUT . . . AN AMERICAN WOOD. USDA For. Serv. FS-230.

Solignat, G.
1964. ROOTING CHESTNUT TREES. Ann. Rep. North. Nut Growers Assoc. 55:33-36.

Sommer, H. E., C. L. Brown, and P. P. Kormanik.
1975. DIFFERENTIATION OF PLANTLETS IN LONGLEAF PINE *(PINUS PALUSTRIS* MILL.) TISSUE CULTURES *IN VITRO.* Bot. Gaz. 136(2):192-200.

Stairs, G. R.
1964. HETEROPLASTIC GRAFTING OF FOREST TREES. State Univ. of New York, Coll. of For., Syracuse, N.Y. Dept. of Silvic. Ser. 64-1, 54 pp.

Vieitez, E.
1953. ESTUDIOS SOBRE LA REPRODUCCION VEGETATIVA DEL CASTANO. I. ENRAIZAMIENTO EN EL ACODO ALTO MEDIANTE EL EMPLEO DE FITOHORMONAS. An. de Edafol. Y Fisio. Veg. 12(4):337-373.

1955. EL EMPLEO DE SUSTANCIAS DE ACCION HORMONAL EN EL ENRAIZAMIENTO DEL CASTANO POR ACODO BAJO. An. de Edafol. Y Fisio. Veg. 14(9/10):483-518.

_____, E. Seoane, M. D. V. Gesto, J. Mendez, M. C. Mato, and A. Vazquez.
1964. THE FIRST STEPS IN THE ISOLATION OF PLANT HORMONES ASSOCIATED WITH THE ROOTING CAPACITY OF THE WOODY CUTTINGS. An. de Edatol. Y Agrobiol. 23(11/12):777-798.

_____,A. Vazquez, and M. L. Areses.
1966. ROOTING PROBLEMS OF CHESTNUT CUTTINGS. Univ. de Liege.

_____ , E. Seoane, M. D. V. Gesto, A. Vazquez, J. Mendez, A. Carnicer, and M. L. Areses.
1967. GROWTH SUBSTANCES ISOLATED FROM WOODY CUTTINGS OF *CASTANEA SATIVA.* Phytochem. 6:913-920.

Venverloo, C. J.
1973. THE FORMATION OF ADVENTITIOUS ORGANS. I. CYTOKININ-INDUCED FORMATION OF LEAVES AND SHOOTS IN CALLUS CULTURES OF *POPULUS NIGRA.* Acta. Bot. Neerl. 22(4):390-398.

Winton, L. L.
1970. SHOOT AND TREE PRODUCTION FROM ASPEN TISSUE CULTURE. Am. J. Bot. 57(8):904-909.

1971. TISSUE CULTURE PROPAGATION OF EUROPEAN ASPEN. For. Sci. 17(3):348-350.

Wolter, K. E.
1968. ROOT AND SHOOT INITIATION IN ASPEN CALLUS CULTURES. Nature 219:509-510.