PRACTICAL VALUE OF EMBRYO CULTURE IN NURSERY PRACTICE

WALTER E. LAMMERTS
Horticultural Research Division of Germain's
Livermore, California

INTRODUCTION

If, by practical value, we mean the germination of seed for routine growth of nursery stock, I might as well say right now that embryo culture has none. However, most larger nurseries by now realize the value of at least some plant selection as a means of improving their general line of nursery items and finding varieties better adapted to specific localities. The very fine work of the Saratoga Horticultural Foundation, Saratoga, California, is an outstanding example.

Also, many nurseries are realizing the need of breeding work involving actual cross pollination in order to combine a desirable trait such as unusually lovely foliage with exceptional flower or fruit quality. Unfortunately, in many genera the percentage of germination obtainable by routine methods is rather low, varying from 10 per cent to 65 per cent. Also, and even more important, the really desirable combination of characteristics is often found in that percentage of seeds which do not germinate!

Finally, in most shrubs and trees such as the camellia, peach, nectarine, and cherry a number of years elapse from the time a seed is harvested until fruit is produced, if one follows routine nursery practice. The question then is: Granted that it does pay nurseries to carry on at least modest research programs, just what are the advantages of embryo culture?

A brief resume of the history of this technique and some examples of its successful application is probably the best way to answer this question. Also, suggestions for research leading to simplification and large scale adaptation will be made.

HISTORY OF PRACTICAL APPLICATION

H. B. Tukey, working at Geneva, New York Agricultural Experiment Station, was one of the pioneers in this technique. His paper, "Artificial Culture Methods For Isolated Embryos of Deciduous Fruits," (1) is a classic and should be read by everyone contemplating this sort of research. He quite correctly credits O. W. Davidson and Florence Flemion of the Boyce Thompson Institute as also being pioneers. The basic formulas described by Tukey are the ones which we still use. I have found them applicable for use with cherry, peach, nectarine, plum and camellia. Tukey also reports successful use of embryo culture with pear and apple seed. Undoubtedly many other types of shrubs in various genera which have embryos encased in thick-walled seed coats, taking three to six months to germinate in the usual manner, would also respond. My own work which made it possible for me to make such rapid headway in rose, peach and nectarine breeding at Armstrong Nurseries from 1935 to 1940 was reported in the American Journal of Botany under the title of "Embryo

Culture, an Effective Technique for Shortening the Breeding Cycle of Deciduous Trees and Increasing Germination of Hybrid Seed." (2)

In describing the material and methods, I recommended several improvements over the Tukey technique, such as sterilization of the peach pits with mercuric chloride at 1 to 1000, use of hot water to make the calcium hypochlorite disinfecting solution, and disinfection at 110 to 115° F. The use of a good surface tension reducer, at the rate of one gram per liter, was most helpful if the coats or testas were badly infected. By use of this technique a much higher percentage of germination resulted from my very extensive apricot, peach and nectarine crosses made in 1937. About 10,500 flowers were emasculated and pollinated which resulted in 2536 fruits, an average of 24 per cent; 69 percent of these were successfully embryo-cultured. Many were from crosses involving very early fruiting parents, seeds of which do not germinate at all following usual stratification methods. The very successful Robin variety of peach was from one of these 1937 crosses, that is, a cross of Babcock peach with the early-fruiting Mayflower. From the 1938 cross pollinations, 76 per cent of the seed were germinated. A comparison of normal stratification with embryo culture involving four different crosses, gave the following comparative percentage:

Stratified Seed	Embryo Cultured Seed
44%	67%
26	100
50	80
23	95
44	78

The culturing technique was particularly satisfactory and helpful with crosses involving early types, such as Early Imperial or Mayflower, which characteristically have many abortive embryos which do not respond to stratification.

In 1939, 81 per cent of the 1092 embryos from 1114 fruits were successfully cultured.

Similarly, I found in my work at Descanso Gardens that a very great increase in germination of camellia seeds was effected by this technique. This was particularly true when crosses were made involving species such as *Camellia japonica* x *C. reticulata* and *C. cuspidata*. Seeds of these crosses planted in the usual manner simply do not germinate because the embryos are partially aborted. Embryo culture readily effected their germination, though considerable extra care was necessary until the very young seedlings became established.

The most important part of this work involves a combination of the use of embryo culture with the proper photoperiod, as shown in my article, "Effect of Photoperiod and Temperature on Growth of Embryo Cultured Seedlings," (3) published in 1943 in the American Journal of Botany. Many of the late fruiting varieties, such as Muir, usually remain very dwarf-like, even following germination by embryo-culturing. When exposed to a continuous 24-hour photoperiod, they could be grown to 3 -3 ½ feet in height during the winter and

spring following germination in the fall. When transplanted to the field in April, following hardening-off and exposure to six weeks cold storage in a dark room at 40° F., these seedlings made rapid growth and flowered abundantly the second spring after pollinations were made. Peach, nectarine, cherry and other deciduous tree hybrids, having a shorter chilling requirement, responded even better. A combination of embryo-culture immediately in the fall after harvest, followed by continuous-light treatment and transplanting to the field in the spring was most effective.

In camellias, as reported in the 1949 Camellia Yearbook, under the title, "The Effect of Continuous Light, High Nutrient Level, and Temperature on Flowering of Camellia Hybrids," (4) it was possible to grow seedlings from embryos cultured in October, 1946, to a height of over six feet and have them set flower buds by January of 1948. Thus, these hybrid camellia seedlings were brought into flower one year and four months after germination! The hybrids which grew the fastest tended to be the slowest in flowering. Even with the handicap of nine months under normal day-length prior to continuous light treatment, seedlings were all in flower by April of 1949. It is then possible to shorten the breeding cycle of the camellia from a period of four to eight years to about one and one-half years.

The flowers resulting were typical enough to be indexed for color, petal number, and size. I might add that a weekly feeding with a high nutrient level, balanced plant food, containing 420 ppm of nitrogen, 120 ppm of phosphorus and 120 ppm of potash was used. This nutrient solution also contained a small amount of sulfur, calcium, iron and traces of manganese and magnesium and other minor elements.

Embryo culture technique was usually employed by me only when moving from one breeding location to another. It was most useful when I began the plant breeding program at Armstrong Nurseries and again when I started my general ornamental shrub and fruit tree breeding work at the University of California at Los Angeles in 1940. It was of particular value when I transferred my work to what was then known as Rancho del Descanso in 1945. Here time was certainly the essence since Mr. Howard Asper and I had the problem of making our general nursery operation pay for the cost of the breeding work. My successful development of the Daily News Series of double flowering peaches would not have been possible without the use of this technique at that time.

In my rose breeding I use this technique only when I am beginning some unusually new line of work where genetic considerations make it quite clear that there is no hope of any commercially desirable hybrid until at least two or three generations of cross pollinations, or back crosses, have been grown.

POSSIBLE FUTURE DEVELOPMENTS

At present we are working on the use of various antibiotics in the hope that they may greatly reduce the possibility of contamination. This has always been a limiting factor in the use of embryo culture

since transfer of the embryos after they have been sterilized in the calcium hypochlorite solution always involves considerable risk of contamination even under the most careful and clean laboratory conditions. A high concentration of penicillin used in the form of Crysticillin & Mycostatin does not harm the embryos of roses or peaches. The percentage of infection is considerably reduced. It is hoped that by the proper combinations of antibiotics we may eventually be able to use less tedious methods of embryo culture, which, of course, would tremendously increase its practicality.

Thus, if we could use large Stender dishes and transfer embryos at the rate of 100 or so per dish, insuring them against contamination by the use of a high concentration of antibiotics, it might well be possible to use this technique even in general nursery practice for the production of seedlings otherwise difficult to obtain by use of germination methods.

LITERATURE CITED

1. Tukey, H. B, 1934. "Artificial Culture Methods for Isolated Embryos of Deciduous Fruits." Proceedings of the American Society for Horticultural Science. 32:313-322.
2. Lammerts, Walter E, 1942. "Embryo Culture, An Effective Technique for Shortening the Breeding Cycle of Deciduous Trees and Increasing Germination of Hybrid Seed." American Journal of Botany. 29:166-171.
3. Lammerts, Walter E., 1943. "Efeect of Photoperiod and Temperature on Growth of Embryo-cultured Seedlings." American Journal of Botany. 30:707-711.
4. Lammerts, Walter E., 1949. "The Effect of Continuous Light, High Nutrient Level and Temperature on Flowering of Camellia Hybrids." American Camellia Society Yearbook. 1949:53-56.

MODERATOR BATCHELLER: Are there any questions for Dr. Lammerts?

VOICE: What type of disinfectant do you use in your embryo culture work?

DR. LAMMERTS: First, place the embryos in 8 hydroxy-quiniline sulphate solution, 1-20,000, for botrytis and bacteria control.

Then transfer to ortho-phenyl-phenol sodium salt (1-2000). This is very important for control of parasitic fungi. The latter treatment is quite important since otherwise, if only the first disinfectant is used, unusual parasitic fungi which are not killed by the first disinfectant will become established and kill the embryos.

VOICE: What type of lights were used for increasing the growth of your peach seedlings and at what distance were they placed above the plants?

DR. LAMMERTS: In the 16-hour photoperiod experiments, 200-watt type C Mazda lamps placed about three feet above the plants were used to supplement the normal day length, i.e. they automatically went on during the winter at about 5:00 p.m. and were turned off by the clock at 10:00 p.m.

Experiments now going on indicate that the recently developed Gro-Lux lamps, developed by Sylvania, are even more effective than 200-watt C Mazda lamps.

MODERATOR BATCHELLER: Thank you, Dr. Lammerts. Our next speaker on the program, Mr. C. J. Eden, will talk to us on the subject of conifer seeds. Mr. Eden.