# TISSUE CULTURE OF PECAN, OAK, AND OTHER WOODY PLANT SPECIES

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Developing Plant Reproduction International, Inc. and moving from the academic world to industry has been a challenge — a challenge that has increased my respect for all industry-related people. In the industry one must not only keep up with new technology, but one must also be creative, stubborn, a good business person, a leader, and have the mental and physical capacity to prevail. To start and develop a business takes a lot of pioneer spirit with the tenacity to succeed.

At P.R.I., Inc. our main emphasis is on quality and our present objectives are:

1. To produce herbaceous and woody plants through tissue culture. At present all production work is pre-contracted.

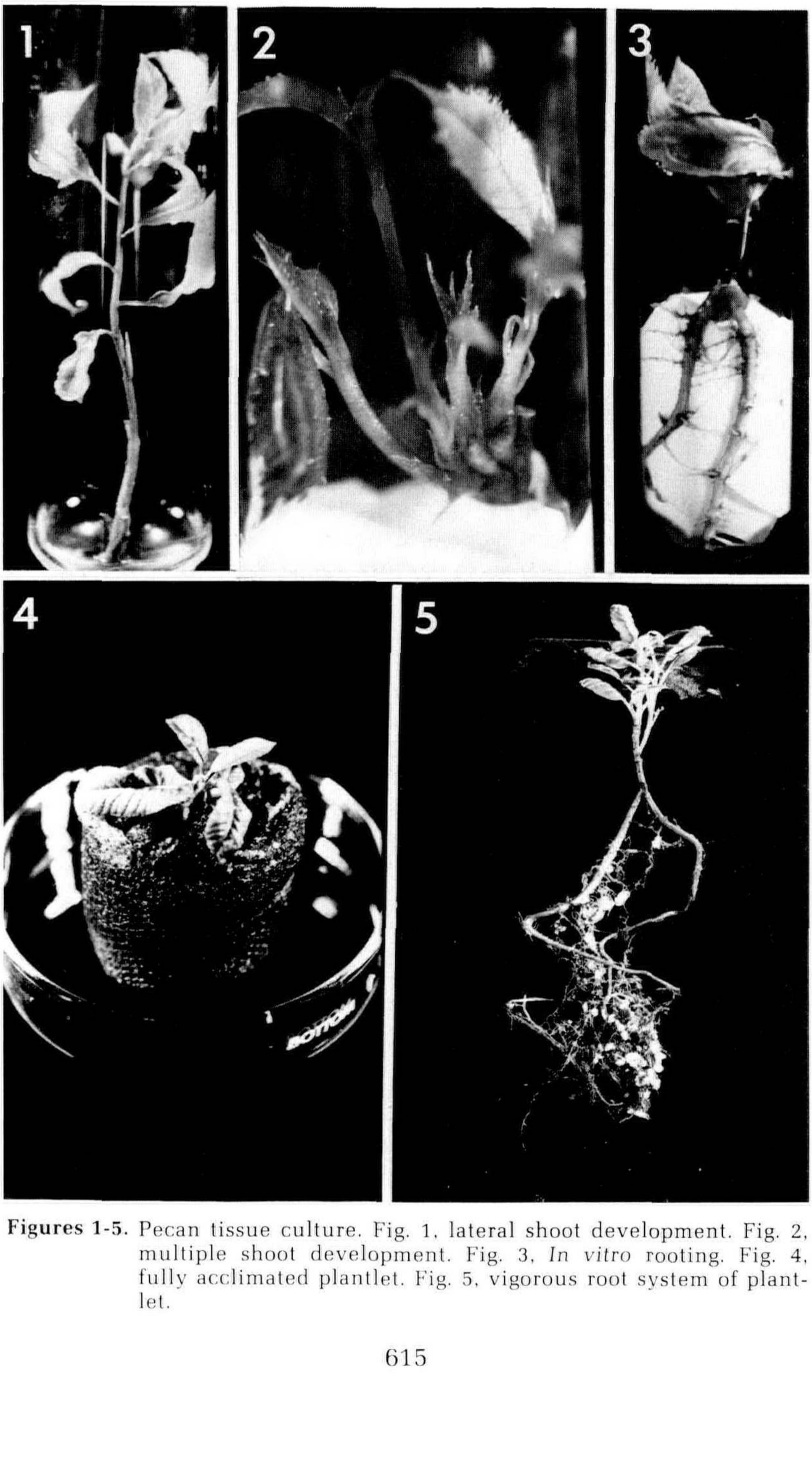
, 2. To research for procedures and better production systems to propagate plants via tissue culture. This research is either contracted or of our own interest.

3. To offer consulting services in plant propagation and production, especially in the field of tissue culture.

A field of major concern to us is woody plant tissue culture, because it is in a developmental state and there is much to be accomplished. However, working in the commercial world one finds that it is important to keep a constant production of quality plants to satisfy the industry needs. The development of efficient crop production systems and better quality of stock plants are the most important problems in woody plant tissue culture. To solve these problems only one thing is required — TIME. It takes time to develop new procedures, to increase the number of stock plants in culture and to develop new selections and varieties.

It is for these reasons that we are producing herbaceous ornamentals such as gerbera, syngonium, spathiphyllum, dieffenbachia and ferns, while developing new production techniques for woody plant species.

Two years ago at the 1981 meeting of the Southern Region of the International Plant Propagation Society in Houston, Texas, I presented a complete procedure for tissue culture production of thorny blackberries and preliminary informaton for the *in vitro* shoot multiplication of pecans. Today I would like to




**Figures 1-5.** Pecan tissue culture. Fig. 1, lateral shoot development. Fig. 2, multiple shoot development. Fig. 3, *In vitro* rooting. Fig. 4, fully acclimated plantlet. Fig. 5, vigorous root system of plantlet.

present the complete procedures for tissue culturing pecans. This work was completed in June, 1982, at Texas A & M University, and will be published in *HortScience* with Keith Hansen as senior author.

A production system for clonal pecan rootstock would be advantageous, since at present all pecan rootstocks are seedlings with great genetic variability (3,4,6). Stem and root cuttings have been used to propagate pecans, but only with root cuttings has limited success been achieved. Major drawbacks are poor rooting and survival after transplanting (1). Another method is mounding. However, the rate of multiplication is limited and seasonal.

Attempts at pecan tissue culture were reported by Smith in 1977 (5) and Knox in 1980 (2). However, neither was successful in obtaining well-rooted explants, and plantlets did not survice transplanting.

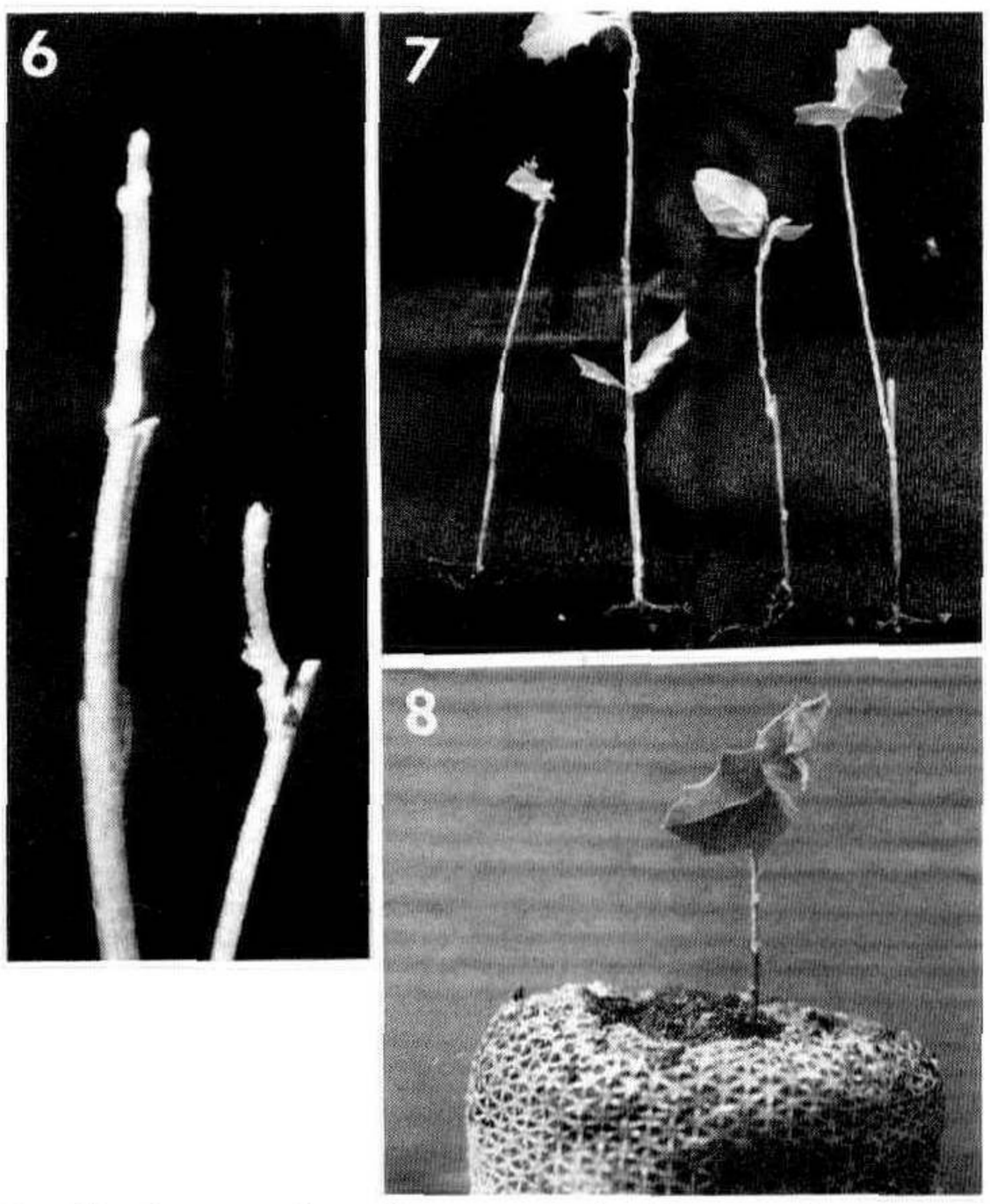
Since we were interested in developing needed procedures for rootstocks, we used explants or plant material from 2-month-old seedlings of the cultivar 'Desirable'. The seedlings were grown under two conditions: 16-hr photoperiod in a greenhouse, and in complete darkness.

**Shoot Multiplication.** Explants consisted of stem cuttings or nodal cuttings, washed with 1% Liquinox® and sterilized with 0.525% NaOCl for 10 min. Explants were placed in test tubes containing Woody Plant Medium (WPM) modified with 2% glucose. All cultures were placed in darkness for the first 2 weeks and under 16-hr photoperiod for the remainder of the experiment. Best shoot break and multiplication was obtained using 3 mg/liter benzyl amino purine (BA) (Figure 1). Etiolated stock plant explants had better bud break and elongation at the beginning, but after 6 to 8 weeks there was no difference between etiolated and non-etiolated stock plants. It was with 3 mg/liter BA that we obtained more than one shoot per node, and in some cases 10 shoots per node were counted (Figure 2). It is important to remember that 'Desirable' has 4 to 5 buds per node, but normally the most apical or primary is the only one that breaks.

**Rooting.** Rooting was accomplished *in vitro* in test tubes and *ex vitro* in peat pellets. For *in vitro* rooting, excised *in vitro* developed shoots were placed in test tubes containing WPM plus 2% glucose. Best rooting was observed using 3 mg/liter indolebutyric acid (IBA) for a 10 day dip (Figure 3). For *ex vitro* rooting, *in vitro*-derived shoots were excised and placed in test tubes containing WPM plus 2% glucose and 10 mg/liter IBA for 10 days. Shoots were then transplanted to peat pellets, watered with half-strength WPM minerals and covered with

plastic cups. Fifteen days after insertion, the plastic cups were perforated with two 5 mm holes to begin acclimatization. Two months after initial treatment with IBA, plantlets were well-rooted and fully acclimated to greenhouse conditions, where they grew vigorously (Figure 4). Acclimated plants had functional and vigorous root systems with profuse lateral branching from primary roots (Figure 5).

During the present meeting we have discussed plant species that could be propagated more conveniently with tissue culture. Many of them are difficult to multiply due to a limited number of propagules. Others, such as oaks, can only be propagated from seed, which results in genetic variability. We have been working on procedures to tissue culture live oaks for the last 12 months and recently have had excellent results for shoot break and mutiplication (Figure 6). Preliminary experiments on rooting have been also very encouraging (Figure 7). We have obtained oaks rooted *in vitro* and they were successfully acclimated to greenhouse conditions (Figure 8). This is just preliminary, and we are planning to do final experiments this coming spring. We are also working on Texas pistachio, *Ilex vomitoria*, and *Hibiscus* spp. and also hope to get other new or difficult-to-propagate woody plants in culture. Tissue culture techniques can add greatly to the number of cultivars available to the woody plant industry in the years ahead.




**Figures 6 to 8.** Preliminary development in live oak tissue culture. Fig. 6, lateral shoot development. Fig. 7, *In vitro* rooting. Fig. 8, fully acclimated plantlet.

## LITERATURE CITED

1. Brutsch, M. O., P. Allan and B. N. Wolstenholme. 1976. The anatomy of adventitious root formation of adult-phase pecan (*Carya illinoensis* (Wang.) K. Koch) stem cuttings. *Hort. Res.* 17:23-31.
2. Knox, C. A. 1980. Histological and physiological aspects of growth responses and differentiation of pecan, *Carya illinoensis* (Wang.) Koch. tissues *in vitro*. Ph.D. Dissertation, Texas A & M University, College Station.
3. McEachern, G. R. and J. B. Storey. 1972. Pecan clonal rootstock propagation techniques. *Pecan Quart.* 6(3):5-7.
4. McEachern, G. R. 1973. The influence of propagating technique, the rest period phenomenon, and juvenility on the propagation of pecans, *Carya illinoensis*, stem cuttings. Ph.D. Dissertation, Texas A & M University, College Station.
5. Smith, M. W. 1977. Shoot meristem and callus tissue culture of pecans, *Carya illinoensis* (Wang.) K. Koch. Ph.D. Dissertation, Texas A & M University, College Station.
6. Wolstenholme, B. N., and P. Allan. 1975. Progress and problems in pecan clonal propagation by stem cuttings. *Gewasproduksie/Crop Production* IV:29-32.


# ESTABLISHING TISSUE-CULTURED PLANTS IN SOIL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It has been almost 9 years since Oglesby Nursery, Inc. ventured into the plant tissue culture business. In that time, our facility has grown from a small laboratory with one technician and 120 $ft^2$ of culture space to a modern production laboratory with over 3500 $ft^2$ of culture space and about 40 employees, plus a separate research and development facility with 250 $ft^2$ of culture space and 2 employees. The demand for tissue-cultured plants is such that our laboratory is in continuous operation, 24 hours a day, Monday through Friday. An additional shift also operates on Saturday. We have, over the years, successfully propagated through tissue culture more than 400 kinds of plants including bananas, pineapples, plantains, gerbera daisies, spathiphyllums, daylilies, caladiums, and many other ornamental species (2). Included among current research projects are tissue-culture propagation of avocados, nandinas, heliconias, araucarias, various spices, and numerous other plants.

Because of our considerable expertise in tissue culture propagation of plants, we are often asked many questions concerning all stages of the process. One of the most common