Botanical Journal of the Linnean Society (1996), *122:* 183–241. With 56 figures

Orchid micropropagation: the path from laboratory to commercialization and an account of several unappreciated investigators

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Received August 1995, accepted for publication June 1996

A commonly held view is that the ideas and basis for the practice of orchid micropropagation arose *de novo* in 1960 from the work of Georges Morel in France. In this paper we argue that the crucial developments in micropropagation were made by Gavino Rotor in 1949 in the USA and Hans Thomale in 1957 in Germany, and that Morel's work needs to be seen in the context of a long line of research achievements in the *in vitro* culture of a wide range of explanted tissues and organs from plants of many species. A critical, historical, analysis of the events as they relate to clonal orchid multiplication is offered here. Two important technical innovations for orchid micropropagation — the use of activated charcoal to darken nutrient media and the adoption of liquid culture environments for part of the process — are examined in detail. In addition, an unusual US patent claiming invention of 'a method for rapidly reproducing orchids', especially cattleyas, is analysed. The origin of the micropropagation process claimed in this patent, said by the nominal inventor to go back as far as 1950, is discussed, but the claim remains unsubstantiated. Finally, consideration is given to the problems of adjudicating unequivocal priority for 'discovery' of a process as complicated and as broad as micropropagation.

ADDITIONAL KEY WORDS: — activated charcoal – 'mericloning' – propagation *in vitro* – stem tip culture – tissue culture.

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0024–4074/96/011183 + 59 $25.00/0

INTRODUCTION

> It is hardly possible to state any truth strongly without apparent injustice to some other (E. Mach, 1838–1916, quoted in Gaffron, 1969).

Micropropagation, mass rapid clonal propagation of plants *in vitro* by a technique commonly referred to as tissue culture[1] (all notes are in the Appendix, pp. 225–228), is currently used world-wide with numerous species ranging from ferns to trees. Over the years, a large body of literature dealing with basic science and practical applications has been generated for this important area of plant biotechnology. Since a *Cymbidium* orchid was the first plant to be propagated commercially using this method, it is appropriate to attempt a reconstruction of the research process which led to it. Our hope is that by doing so we will convey some of the flavour of the events associated with the path from laboratory bench to commercialization, and place in perspective the role played by key participants. This account will also make apparent the fact that methods of this sort do not emerge suddenly and fully formed in 'Eureka' fashion. Rather, they result from the findings of many who may have had little concern for fame or financial reward. In fact, some may not have appreciated partially, much less fully, the implication of their work.

ORCHID SEED GERMINATION AND THE FIRST *IN VITRO* CULTURE OF ORCHIDS

In 1891 a brief notice was published in *L'Orchidophile* that a grower named Perrenoud (no first name given) who saw reports in so-called 'journaux anglais', placed sections of *Phalaenopsis* roots in humid enclosures and obtained a plant (Anonymous, 1891). This is reminiscent of micropropagation, and while no details are available, it is known that *Phalaenopsis* roots can produce buds and plants

Figures 1–11. Historical figures in research on orchid seed germination and micropropagation, plant hormone studies and tissue culture research. Fig. 1. Professor Lewis Knudson (Arditti, 1990; photograph courtesy Department of Botany, Cornell University; signature courtesy Cornell University Archives). Fig. 2. Professor Wilhelm Pfeffer (Wittrock, 1897–1903). Fig. 3. Dr Gavino Rotor (photograph courtesy of Dr Gavino Rotor; signature from a letter to J.A.). Fig. 4. *Phalaenopsis* floral-stalk node cultures (Rotor, 1949). Fig. 5. Professor John T. Curtis (Skoog, 1951). Fig. 6. Professor Gottlieb Haberlandt (White, 1943). Fig. 7. Professor Hans Fitting (photograph from a Kodachrome slide taken by Ms. Brigitta H. Flick, J.A.'s technician, *c*. 1970; signature from letter to J.A.). Fig. 8. Professor Kenneth V. Thimann (photograph from Skoog, 1951; signature from an autographed copy of Went & Thimann, 1937 owned by J.A.). Fig. 9. Professor Frits W. Went (photograph and signature from Went, 1990). Fig. 10. Professor Johannes van Overbeek (photograph from Skoog, 1951; signature from letter to J.A.). Fig. 11. Professor Albert F. Blakeslee (Skoog, 1951).


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(Churchill, Ball & Arditti, 1972); this account, therefore, could perhaps be described as being part of its pre-history.

Modern orchid micropropagation began in 1949, when "a new [tissue culture or *in vitro*], simple and practical method for vegetative [clonal] propagation of *Phalaenopsis* [orchids] was developed at Cornell [University]" (Rotor, 1949) five years before the first published report of orchid stem tip cultures and thus any suggestion that these methods could be used for micropropagation (Thomale, 1956, 1957). The nutrient medium used for these cultures was 'Knudson C' formulated for the asymbiotic germination of orchid seeds by Lewis Knudson (1884–1958; Fig. 1),[2] Professor of Plant Physiology at Cornell University (see Arditti, 1990 for a biography of Knudson).

Knudson's first solution, a modification of a formulation devised by the German plant physiologist Wilhelm Pfeffer (1845–1920; Fig. 2) known as 'Pfeffer's Solution', was his medium B ('Knudson B'). It was, and still is, a reasonably good medium but Knudson improved it and published his solution C ('Knudson C', KC) in 1946 (Knudson, 1946). This is still used widely for the germination of orchid seeds (Arditti *et al*., 1982) and the micropropagation of some orchids (for a review see Arditti & Ernst, 1993).

Gavino Rotor Jr. (Fig. 3) was born in Manila on 26 March 1917.[3] His mother was an avid orchid enthusiast and by the time Gavino entered high school he knew the scientific names of the major Philippines species. He went on to major in agriculture at the University of the Philippines where he received his B.S. in Agriculture in 1937. Rotor was waiting to go abroad for further study when World War II broke out. This caused him to delay but not cancel his plans. He "chose Cornell University for several reasons, the most important ones being Dr Knudson's presence there and its impressive reputation in the horticultural sciences". After receiving his M.S. degree in 1947 and "hearing Dr Kenneth Post's lectures on the effects of day length and temperature on the growth and flowering of various florist crops … [Rotor] … decided to focus on the responses of orchids to temperature and day length" for his doctorate at Cornell University. His major professor was the floriculture crop physiologist Kenneth Post (1904–1955), whereas Knudson was a member of his doctoral thesis committee.

Rotor conceived the idea of propagating orchids while attending a lecture by Knudson on the role of sugars in plant growth. He cut *Phalaenopsis* inflorescences into segments and placed nodal sections, each with a bud, on KC in the hope that they would produce plants. The buds became swollen and leaves appeared after 14–60 days. Roots were produced after 2–3 leaves were formed (Fig. 4). Only seven of 65 buds failed to develop (Rotor, 1949). Knudson's eyes brightened when Rotor showed him the first successful propagation and told him how he got the idea from Knudson's lecture (Arditti, 1990:48).

There is no question that Gavino Rotor invented micropropagation of orchids and was the first to publish a scientific report on clonal multiplication of a higher plant *in vitro*.[4] Rotor's method involved a nutrient culture medium, aseptic techniques and explants. Some might argue that his procedure was not 'true micropropagation' for it only produced one shoot from a given explant with a pre-existing bud. Certainly his procedure did not involve callus formation or proliferation (for a more extensive discussion see Arditti & Ernst, 1993). However, neither multiple plantlet production nor callus proliferation are part of the definition or requirements for 'micropropagation'.[1]

Rotor's method was not widely noticed or appreciated at the time.[5] One reason for this may have been its publication in the *American Orchid Society Bulletin*, a hobbyist publication. Orchid growers who read it may have failed to grasp its importance and probably found the procedure difficult and complicated.[6] Scientists who might have appreciated Rotor's method probably did not read the *American Orchid Society Bulletin*. When Rotor's discovery was finally noticed, other claims of priority had become widely accepted. However, it seems clear that *in vitro* clonal propagation (or whatever other term is used to describe the process for any higher plant in aseptic culture) was first achieved by Gavino Rotor Jr. in 1949 at Cornell University. The number of plants which can be produced by Rotor's method is not large, but it has practical significance.

During the same period, Professor John T. Curtis (1913–1961; Fig. 5) and co-workers in the Department of Botany at the University of Wisconsin described in detail the formation of many growing points on proliferating callus of *Cymbidium* and *Vanda* (Curtis & Nichol, 1948). They used the word 'calloid' to describe protuberances which developed from young asymbiotically germinated protocorm stage seedings after treatment with barbiturates. They observed that these tissue masses often had a capacity for continued growth into complete plants (Curtis & Nichol, 1948). Their appreciation of the *potential* for clonal multiplication is apparent in the statement "the practical ability to produce clonal lines of plants of potentially unlimited numbers would be of obvious value in many types of genetic and plant production work". Nevertheless, there is a big difference between the drawing of attention, almost as an aside, to *potential* by Curtis and Nichol and the *achievement* of a well-conceived goal by Rotor.

FOUNDATIONS OF STEM TIP CULTURE

Three separate lines of research led to the development of micropropagation methods for orchids: (1) *in vitro* culture of stem tips of other plants and regeneration of plantlets from them; (2) production of disease (mainly virus)-free clones of important crops; and (3) clonal propagation. The background of these will be explored separately and brought to the point where they converge. A short history of plant hormones will also be presented because these substances are often critical to the *in vitro* culture of plant cells, tissues and organs (see Krikorian, 1995 for a review).

Plant hormones

Gottlieb Haberlandt (1854–1945; Fig. 6), Professor of Plant Physiology in Berlin, was the first to suggest that hormones might play an important role in plant cell culture media. This is clear from his suggestion that "the effect (also reported by [Hans] Winkler) of the pollen tube on the development of the ovule in orchids, the swelling of ovaries etc., [occurs] probably [because] substances (*Wuchsenzyme*), are involved here which, released from the pollen tube, [act] as a chemical stimulus to the growth and division of the cells concerned … it would be worthwhile to culture together vegetative cells and pollen tubes; perhaps the latter would induce the former to divide" (Haberlandt, 1902; English translation by Krikorian & Berquam, 1969:

83). It does not seem that Haberlandt followed his own suggestion.[7] Had he done so, it is possible but not probable that his culture experiments would have been successful. Nevertheless, Haberlandt was the first to suggest clearly that exogenous hormones are part of the requirements for culturing cells and it is interesting to note that the first successes with animal cells by Ross G. Harrison (1870–1959) are said to have derived their inspiration from Haberlandt's researches (Oppenheimer, 1966: 533 et seq.).

Auxins

Pollen tubes release at least one substance which brings about post-pollination phenomena and ovule development in orchids (Avadhani *et al*., 1994). Hans Fitting (1877–1970; Fig. 7) was the first to show that it exists as a result of his work with *Phalaenopsis* pollinia and pollination at the Buitenzorg (now Bogor) Botanical Gardens in the then Netherlands Indies (now Indonesia) (Fitting, 1909a, b, 1910, 1911, 1921; several personal communications with J.A. shortly before his death; for reviews see Arditti, 1971, 1979, 1984, 1992; Avadhani *et al*., 1994). Fitting named the substance *Pollenhormon* and by doing so became the first to do more than speculate that plants have hormones. He was also "the first investigator to work with hormones and active extracts in plants" (Went & Thimann, 1937). Fitting claimed until his death that *Pollenhormon* was a specific substance, different from auxin. Current evidence suggests that his extracts contained several substances including auxin (for a review see Avadhani *et al*., 1994). In 1932 Friedrich Laibach reported that the active substance in Fitting's *Pollenhormon* could be extracted with diethylether (Laibach, 1932) and several years later Kenneth V. Thimann (b. 1904; Fig. 8) showed that the ether extract contained auxin. The story ends here as far as Fitting's *Pollenhormon* is concerned. Frits W. Went (1904–1990; Fig. 9) suggested once (in a conversation with J.A. at Irvine during the early 1970s) that Fitting could have discovered auxin, but did not because his interest shifted to other problems in plant physiology including *Reizung* (which is best translated as 'irritability' or to use the modern term, 'sensory physiology').

Went's discovery of auxin (Went 1926, 1928) and its subsequent identification as indole-3-acetic acid (IAA) in 1934 (Went & Thimann, 1937; Haagen-Smit, 1951) opened the road to early successes in tissue culture (Gautheret, 1983, 1985). Lyophilized leaf extract from leaves of dodder host plants (a preparation which probably contained auxin) and pure IAA were incorporated in culture media with mixed results starting about a decade after the discovery of this hormone (Fielder, 1936; Geiger-Huber & Burlet, 1936; for reviews see Gautheret, 1935, 1937; Loo, 1945a).

Coconut water and cytokinins

Inspiration and insight both seem to have played a part in discoveries associated with tissue culture. One such instance was responsible for the incorporation of coconut water (at that time commonly called coconut milk) by Johannes van Overbeek[8] (1908–1988; Fig. 10) into culture media for immature or recalcitrant embryos of *Datura stramonium* produced by Albert F. Blakeslee (1874–1954; Fig. 11).

When it was shown that the embryos grew (van Overbeek, Conklin & Blakeslee, 1941) a new and complex additive (see Arditti & Ernst, 1993 for composition)[9] became available for supplementing culture media.[10] Also, the growth of explants with requirements which could not then and still may not be satisfied with defined compounds can be fostered by addition of coconut water to the medium. Moreover, coconut water was shown to induce cell division in quiescent cells in carrot root phloem explants (Caplin & Steward, 1948; Krikorian, 1975; Gautheret, 1985) and to work synergistically with 2,4-dichlorophenoxyacetic acid (2,4-D) in potato tuber explants where neither coconut milk nor 2,4-D would alone bring about cell division (Steward & Caplin, 1951).

Tissue culture research broadened and accelerated in the late 1940s and early 1950s and investigators faced a number of problems, one of them being the recalcitrance of tobacco pith tissues (Gautheret, 1985; Skoog, 1994). In his attempts to culture these tissues Folke Skoog (b. 1908; Fig. 12) and his students and associates at the University of Wisconsin formulated media (for details see Skoog, 1944, 1951; Skoog & Tsui, 1948; Skoog & Miller, 1957) and evaluated the growth stimulating effects of a number of substances. These included a preparation of herring sperm DNA which had been stored for a long time (the time frame suggested that Skoog may have used nucleic acid preparations left over from research on orchid seed germination by Prof. John T. Curtis; however, Prof. Carlos O. Miller[12] (b. 1923; Fig. 13) recalls that the relations between Skoog and Curtis would have precluded this from happening). This research resulted in the discovery of plant kinins (later re-named cytokinins[11]) by Skoog, Miller and others in the Wisconsin Botany Department group (for historical details see Strong, 1958; Miller, 1961, 1977; Leopold, 1964; Gautheret, 1985; Skoog, 1994). With the need for certain vitamins (Gautheret, 1945) and auxins in culture media already established, Toshio Murashige (b. 1930; Fig. 14) refined an existing nutrient culture solution and formulated the now widely used Murashige and Skoog (MS) medium (Murashige & Skoog, 1962; for historical coverage of the development of MS medium see Smith & Gould, 1989; Skoog, 1994). Since then (as now) an appropriate medium (along with the explant source) was a very important factor in the establishment of a tissue culture (Krikorian, 1982, 1995), a large number of plants earlier viewed as recalcitrant became amenable to culture.

CULTURE OF STEM TIPS

The idea of using stem tips or buds for mass rapid clonal propagation is over 100 years old. In 1893 Carl Rechinger in Vienna attempted to culture stem sections and excised buds of *Populus nigra* and *Fraxinus ornus* as well as portions of roots on sand moistened with tap water (Rechinger, 1893; Krikorian, 1982; Gautheret, 1983). His attempts failed, but he concluded that to develop properly, sections must be thicker than 1.5 mm. Rechinger's procedures would not be called 'tissue culture' by today's standards, but they foreshadowed it because he used a medium (tap water), a support (sand) and explants. Orthodox tissue culture procedures now include: (1) a nutrient medium which contains several components, including organics like sucrose, that make it advisable to use aseptic techniques; (2) an explant; and (3) in some instances agar (or a related substance) as a solidifier or support. However, except for the explant, some of these factors are often not an absolute requirement and a matter of


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convenience or sometimes invoked for pedantic rather than functional reasons. Many media, especially those used for orchids (for a review see Arditti & Ernst, 1993) are very dilute and liquid, i.e. 'unsolidified'. Even sterility, which is certainly to be preferred, is not an absolute requirement as long as microbial contaminants can be prevented from over-running the explants (Thurston, Spencer & Arditti, 1978, 1979; Spencer *et al*., 1979/1980; Brown *et al*., 1982, 1984; Johnson, Perera & Arditti, 1982; Cvitanic & Arditti, 1984).

Nearly 20 years later the German experimental morphologist Karl [later von] Goebel[13] (1855–1932; Fig. 15) tried to grow excised buds of the water fern *Ceratopteris thalictroides* in peat moss, but obtained only abnormal plants (Goebel, 1902; Krikorian, 1982). This was not 'tissue culture' either as the term has been used during the last 30–40 years, but Goebel did use 'explants' and a medium. Studies on the effects of polarity, water and presence or absence of cork on root formation in *Salix* by the German botanist Herman Vöchting (1847–1917; Fig. 16) were more tangential (Vöchting, 1906), but nevertheless important steps on the road to tissue culture (Krikorian, 1982: 162).

The pace of research in the area was accelerating but some 15 years passed before the first stem- and root-tip cultures were attempted (Krikorian, 1982; Gautheret, 1983). William J. Robbins (1890–1978; Fig. 17) working at the University of Missouri germinated seeds of peas, maize and cotton under aseptic conditions, excised root and stem tips and attempted to grow them in the dark on sterile 'Pfeffer's Solution' with and without glucose or fructose (see Knop, 1884; Pfeffer, 1900; White, 1943, 1945; Krikorian, 1975, 1982; Murashige, 1978; Arditti, 1977, 1992; Arditti *et al*., 1982; Arditti & Ernst, 1993 for composition of media). The cotton explants did not grow normally, but those of maize and peas did (Robbins, 1922). They produced roots but were chlorotic and showed characteristics which were "typical of plants grown in the dark" (Robbins, 1922). The results obtained by Robbins are easy to explain from our present vantage point on culture requirements. He did not have plant hormones or vitamins at his disposal. Indeed, he did not then know that the latter may be required by explants.[14] Neither did he realize that the cultures would have benefited from illumination. Despite this, Robbins and his associates managed to maintain the root tip cultures for nearly 4.5 months (Robbins & Maneval, 1923, 1924).

Independently of Robbins, but at the same time, one of Haberlandt's students in the Pflanzenphysiologische Institut in Berlin-Dahlem, Walter Kotte (Fig. 18), cultured pea roots. He used a solution of Knop's salts (Knop, 1884) as his basic salt medium and added to them glucose, peptone, asparagine, alanine, glycine, a meat extract and a digest of pea seeds. Kotte's medium was more sophisticated than the

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Figures 12–25. Botanists who played major roles in the history of plant hormone, tissue culture and orchid research. Fig. 12. Professor Folke Skoog (Janick, 1989). Fig. 13. Professor Carlos O. Miller (photograph courtesy C. O. Miller; signature from a note to J.A.). Fig. 14. Professor Toshio Murashige (Janick, 1989). Fig. 15. Professor Karl von Goebel (Wittrock, 1897–1903). Fig. 16. Professor Hermann Vöchting (photograph and signature from portrait in Fitting, 1919). Fig. 17. Professor William J. Robbins (Gautheret, 1985). Fig. 18. Dr Walter Kotte (photograph and signature from White, 1943). Fig. 19. Dr Philip R. White (photograph from Gautheret, 1985; signature from White, 1943). Fig. 20. Professor Loo Shih-wei (from a transparency by Dr Franz Hoffmann taken in Beijing *c*. 1985; English and Chinese character signatures from a letter to J.A.). Fig. 21. Professor Roger J. Gautheret. Fig. 22. Professor Pierre Noubécort (Gautheret, 1985). Fig. 23. Professor Ernest A. Ball (photograph from a Kodachrome transparency by J.A.; signature from Ph. D. Dissertation by Dr Michael S. Strauss). Fig. 24. Mr Hans Thomale (photograph courtesy Hans Thomale; signature from a letter to J.A. both obtained with the help of E. Lucke and Dr N. Haas-von Schmude). Fig. 25. Professor Hans Burgeff (photograph from Haber, 1963; signature from a letter to Professor Robert Ernst).

one used by Robbins and probably contained vitamins, some plant hormones and inositol, all of which were probably components of the complex additives. The roots grew, but could not be subcultured (Kotte, 1922a, b; White, 1943).

Philip R. White (1901–1968; Fig. 19) of The Rockefeller Institute for Medical Research at Princeton, New Jersey reached the conclusion that apical and intercalary meristems "would be best to choose [as] materials for our first experiments" (White, 1931, 1933b). While on a visit to Haberlandt's plant physiology institute at the University of Berlin in the winter of 1930 and spring and summer of 1931 White attempted to culture root tips (White 1932a, 1933a) and "some 400 stem tips" of the "common weed" *Stellaria media* in hanging drops of the medium of Uspenski & Uspenkaja (1925), a formulation designed for pure cultures of *Volvox minor* and *V. globator*. White used this nutrient solution earlier for the culture of root tips, embryos and other explants (White, 1933b). He managed to keep the tips alive "for periods up to three weeks … [and] during this time there … occurred active cell division … growth … differentiation … into leaves, stems and floral organs" (White, 1933b). But his results were disappointing by today's standards. Accumulation of "excretory products, and the exhaustion of nutrient materials" were given as reasons for the limited success (White, 1933b). A more plausible explanation is the composition of the medium. It had no ammonium ion, and contained no vitamins or hormones because they were not yet discovered, new to science at the time, or not yet known to be required by organ and tissue explants.[15]

Still, White's medium was one of the best available at the time and maize shoot tips cultured on it produced plants (Segelitz, 1938). If the tips were shorter than 2 cm in length they had to be cultured under illumination. Longer shoots (2–4 cm) could grow in darkness (Segelitz, 1938). This is one of the earliest successes in culturing a monocotyledon *in vitro*. It was reported long before what has sometimes been claimed to have been the first real success with this group (Morel & Wetmore, 1951a). To be precise, however, it should be noted that Morel and Wetmore dealt with callus production in their cultures. That success was appropriately viewed as particularly significant since monocotyledons do not normally make wound tissue and hence many monocot cultures even today grow only with difficulty (see also Swamy & Sivaramakrishna, 1975; Hunault, 1979 on monocotyledonous recalcitrance).

The second monocotyledon to be propagated by what can in retrospect be described as a crude or 'prehistoric form' of tissue or explant culture was taro (*Colocasia esculenta*), an important and ancient crop in Hawaii and the Pacific region.[16] An attempt was made to accelerate taro propagation, though by culturing normally dormant buds " borne in the axils of the leaves on the surface of the taro corm" (Kikuta & Parris, 1941). Tuber slices, 2–5 cm thick and buds "together with approximately 1 cubic centimeter of corm tissue", planted in sterilized soil produced plants. Thus, excised buds and corm explants were cultured in a sterile medium, soil in this case, and produced plants. There is no valid reason why only a solution (semi-solid or liquid) should be defined as a culture medium. This method of taro multiplication (Kikuta & Parris, 1941) is analogous to present day tissue culture propagation even if the techniques are somewhat crude and the cultures are not *in vitro*. Unfortunately, this procedure and related ones are mentioned only in a few instances (Arditti & Ernst, 1993; Arditti & Strauss, 1979; Krikorian, 1994a) and are generally missing from historical reviews (Gautheret, 1980, 1982, 1983, 1985). Taro was cultured *in vitro* for the first time 30 years later (by Mapes & Cable in 1972; see also Krikorian, 1994a).

Rye was cultured early (de Ropp, 1945). Stem tips (the plumules) of excised embryos were cultured on White's medium containing 2% (w/v) sucrose. When "any isolated stem tip developed a root, the entire growing point was stimulated to meristematic activity, and leaves normal in form and size developed" (de Ropp, 1945). These explants were embryonic in nature and it may be suggested that they were not equivalent to shoot tips of mature plants. However, current evidence (at least that obtained from orchids, see Arditti & Ernst, 1993 for a review) suggests that embryonic stem tips from seedlings and mature plants do not differ greatly with respect to their requirements *in vitro*.

From the mid-1930s to the 1950s the California Institute of Technology in Pasadena was arguably the world centre for research in plant physiology. Its faculty (which included such major figures in plant physiology as Kenneth V. Thimann, James Bonner, Frits W. Went, Herman Dolk, Arie J. Haagen Smit, Johannes van Overbeek and others) attracted excellent graduate and post-doctoral students from all continents (Thimann, 1980). One of these was Shih-Wei Loo[17] (b. first decade of the 1900s; Fig. 20). For his doctoral dissertation Loo cultured excised stem tips, 5–10 mm long, of *Asparagus officinalis* on a medium utilized by James Bonner for the culture of tomato roots (Loo, 1945a). Some of Loo's explants developed buds, but none formed roots. He concluded that growth of the excised stem tips was "potentially unlimited" (Loo, 1945b). It seems reasonable to assume that the tips would have produced roots had IAA been added to the medium. Loo moved to Columbia University after his first paper on the subject (Loo, 1945b) and published yet another report on asparagus shoot tips (Loo, 1946a). He showed that a solution rendered semi-solid with agar was "as good, if not better, than liquid medium". In the process of ascertaining this fact, he devised a simple method for supporting stem tips (Loo, 1946a). Growth of the explants was normal and they were still alive after 22 months and 35 transfers (Loo, 1946a).

Loo also cultured stem tips of the parasite dodder (*Cuscuta campestris*). His cultures failed to produce leaves and roots but nevertheless fortuitously flowered *in vitro* (Loo, 1946b). This is probably the first case where "floral organs … developed on excised stem tips *in vitro*" (Loo, 1946b). Again, it is reasonable to speculate that dodder explants would have formed leaves and roots with hormones in the medium (Galston, 1948). Unfortunately Loo did not add any, but he did conclude that the explants required sugar for growth *in vitro*, a conclusion which was relatively new at the time [although, in some cases orchid explants develop in a more desirable fashion only on sugar-free medium (see Arditti & Ernst, 1993 for a review)]. Another contribution by Loo was the cultivation and flowering *in vitro* of the composite *Baeria chrysostoma*, a small annual sometimes grown in gardens and which belongs to a California genus of *c*. 20 species (Loo, 1946c).

Loo's papers suggest that tissue culture of angiosperms and micropropagation would have advanced more rapidly had he remained in the U.S.A. and/or if conditions in China had been different. His important contributions to stem tip culture and ultimately to micropropagation have thus far received only passing credit in a few reviews (Krikorian, 1982; Gautheret, 1983) and a few research papers (Steward & Mapes, 1971b; Koda & Okazawa, 1980). Loo's work is certainly not as well-known as it should be. It is worth mentioning here that Segelitz, de Ropp and Loo (independently of each other), and not subsequent workers (Morel & Wetmore, 1951a; Gautheret, 1983, 1985) were the first to have significant success in the culturing of monocotyledons *in vitro*.

Frits Went was also indirectly associated (through a gift of auxin) with the first successful culture by Carl D. LaRue (1888–1955) of an axillary bud meristem, that of water cress on White's mineral nutrients supplemented with 20 g (w/v) sucrose l^{-1} and "1 hetero-auxin added, 1 part to 20 millions" (LaRue, 1936).

That plant tissues can be cultured "for unlimited periods of time was announced independently" and almost at the same time but not "simultaneously" (for a review see Gautheret, 1985) by Phillip R. White (*c.* 31 Dec 1938), Roger J. Gautheret (b. 1910, Fig. 21; 9 January 1939) and Pierre Nobécourt (1895–1961, Fig. 22; on 20 February 1939). These findings on the potentially unlimited growth of callus cultures set the stage for the first successful culture of a stem tip.

Ernest A. Ball (b. 1909; Fig. 23) was interested in shoot tips and apical meristems (Ball & Boell, 1944), "the capacity for growth and development of vegetative plant cells", "polarity of the buds and subjacent cells, "the relation between respiration and development, independence of the tip from the rest of the plant, production of subjacent tissues by the apex", and the "totipotentiality of all living plant cells" (Ball, 1946). He excised shoot apices of nasturtium, *Tropaeolum majus* L. ("55 μ high and 140 μ thick") and lupin, *Lupinus albus* L. ("81 μ high and 250 μ thick"); the sections were 400–430 μ^3 in volume[18] (Ball, 1946). Ball made "no provisions to achieve and maintain asepsis", "inoculations were performed in the laboratory". He placed explants on Robbins' modification of 'Pfeffer's Solution' plus micro-elements and in some cases "unautoclaved coconut milk".[19] The medium was made semi-solid with agar which changed in colour from brown to white after being washed with thirty 24-hour changes of distilled water. His explants grew well (Ball, 1946; personal communication to J.A. in many conversations) and any insinuations to the contrary (Morel, 1974:177) are without foundation.[20]

THE SECOND ASEPTIC CULTURE OF AN ORCHID EXPLANT

Even before the availability of cytokinins and the formulation of MS, several culture media (Skoog, 1944; Skoog & Tsui, 1948, 1951; Miller & Skoog, 1955; White, 1951) were adapted for less demanding plants, especially with the addition of auxins, vitamins and coconut water. Four such media were used to culture geranium, *Pelargonium zonale* and cyclamen, *Cyclamen persicum* (Mayer, 1956), and this led a German horticulturist to the first reported culture of sections (*Teilstücken* or *Pflanzenteile*) and tissues (*Gewebe*) of orchids (Thomale, 1956, 1957:89–90, fig. 39).

Hans Thomale (Fig. 24) was born in Herne, Westphalia, Germany on 16 October 1919, raised in Cologne and now lives in Lemgo where he still grows orchids.[21] He started to study chemistry and medicine just before World War II broke out. When he "was half ready" Thomale was drafted and had to interrupt his studies. After World War II he "was forced to learn potato [cultivation] in a well-known nursery which had more orchids … than vegetables". The owner of the nursery, Mr H. Kuhlman, also had a "daughter [Lieselotte] who [earned] the title 'Doctor of Botany' [while] I was forced to be a soldier". They later married.

Thomale became interested in orchid seed germination and asked Prof. [Hans] Burgeff [1883–1976; Fig. 25] for his book *Samenkeimung der Orchideen* and used it to teach himself both symbiotic and asymbiotic seed germination. In 1946 he established a laboratory and utilized it to produce hybrids between the "many fine orchids [Mr Kuhlman] bought [in] England and Belgium before the war … after

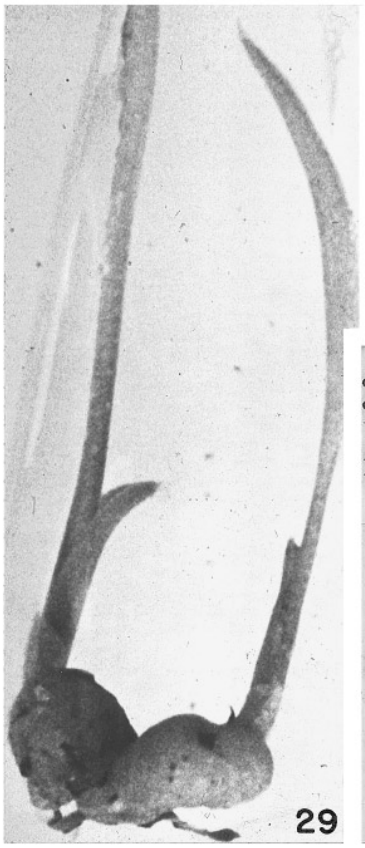
that I tried to raise … orchids [via] clonal propagation". The laboratory work brought him offers from Dorset Orchids Ltd., Plush, Dorset, U.K. in 1949 and Sanders Orchids, St Albans, Herts., U.K. in 1950 (neither exists any longer) to establish laboratories for them. Thomale wanted to propagate both tropical orchids and those which were native to Germany. It is clear from his writings that Thomale read widely and was familiar with the work of Gautheret, Mayer, Rotor, Skoog, Tsui and others.

Thomale based his own work on orchids on a paper by Dr Lucie Mayer (Mayer, 1956; Fig. 26) and on 23 September 1956 he was able to report to a meeting of the Deutsche Orchideen Gesellschaft [German Orchid Society] that explants of *Dactylorhiza (Orchis) maculata* (Fig. 27) and some tropical orchids *in vitro* produced shoots (Figs 28, 29) and subsequently plants. Thomale recollects, albeit with some uncertainty, that Mr Lecoufle of the French orchid firm Vacherot and Lecoufle (see below) was present at that meeting. A photograph of the *Orchid maculata* culture (Fig. 28) was published in the second edition of *Die Orchideen* (Thomale, 1957). The caption reads: "Section of *Orchis maculata* on agar medium (Mayer's method), which was induced to form roots and shoots" (Figs 28, 29). Thomale appreciated immediately the potential of his discovery. He wrote (Fig. 30, the translation below is from Arditti & Ernst, 1993):

> It should be noted that efforts to find a propagation method for European terrestrial orchids, based on the work by Dr L. Mayer [Mayer, 1956], through the culture of sterile explants on an agar medium were successful. It is well known that vegetative parts of orchids, for example, sterile sections of *Phalaenopsis* flower stalks [Rotor, 1949], which bear at least one adventitious bud [Note by J.A. and A.D.K.: these buds are lateral on the scape and not necessarily adventitious, at least not in the strict sense of the word], can produce shoots when cultured on an agar medium. Recently it has become possible to culture undifferentiated tissues on certain nutrient media to produce roots and shoots from them. Since sufficient details were not available by the time this book went to press [i.e., the second edition which appeared in 1957; the first edition was published in 1954], it is only possible to mention that whole plants can be produced from tissue explants one cubic centimeter in size. *This is a form of vegetative multiplication whose potential cannot be overlooked* [emphasis added]!

Thomale's work and his conviction about the value of explant culture as a means of mass rapid propagation was published (Thomale, 1957) before the first reports of *Cymbidium* 'meristem' cultures (Morel, 1960; Wimber, 1963), but it was overlooked. In this respect Thomale's work was similar to Rotor's. It is of some interest to note here that people were apparently aware of Rotor's work on *Phalaenopsis* propagation from stem sections, even if not, as Thomale states, "well known", but drawing attention to it in German language publications did little to publicize it. Another important point is that Thomale behaved professionally by calling attention to Rotor's work, first by mentioning his name (Thomale, 1956) and later by referring to *Phalaenopsis* (Thomale, 1957). Had Thomale not mentioned Rotor and *Phalaenopsis* he could have created the impression that he originated the entire idea of clonal propagation *in vitro*. Thomale did not describe his techniques in detail but credited Mayer's. In fact, Dr Mayer participated in Thomale's initial attempts (Haas-von Schmude, Lucke & Arditti, 1995; personal communication to J.A. by E. Lucke and Dr N. Haas-von Schmude, Wettenberg, Germany). Dr Mayer recalls that they also

Abb. 39. Teilstück von *Orchis maculata*, auf Agar-Nährboden (nach der Methode Mayer) zum Austreiben von Wurzeln und Trieb gebracht.



Nur der Vollständigkeit wegen sei darauf aufmerksam gemacht, daß der Wunsch nach einer Vermehrungsart europäischer Erdorchideen dazu führte, dies auf Grund einer Arbeit von Dr. L. Mayer*) durch

*) Wachstum und Organbildung an in vitro kultivierten Segmenten von *Pelargonium zonale* und *Cyclamen persicum*, „Planta", Bd. 47, 1956, Seite 401–446.

30 89

Kultur von steril gewonnenen Teilstücken auf Agar-Nährböden zu versuchen, was auch gelingt.

Bekannt war bisher, daß vegetative Teile von Orchideen, z. B. sterilisierte Stücke von *Phalaenopsis*-Blütenstielen, die über mindestens eine Adventivknospe verfügen, auf Agar-Nährböden zum Austreiben gebracht werden können. Neu ist hierbei, daß auch vollkommen indifferentes Gewebe einer Pflanze unter gewissen Nährbodenverhältnissen dazu gebracht werden kann, neue Wurzel- und Triebsprosse zu bilden.

Da zur Zeit der Drucklegung des Buches noch keine Veröffentlichung der Arbeitsweise vorliegt, muß es bei der Erwähnung der Tatsache bleiben, daß es bereits möglich ist, aus kubikzentimetergroßen Teilstücken irgendeines Pflanzengewebes neue Pflanzen zu erziehen. Eine Art der vegetativen Vermehrung von kaum zu übersehenden Möglichkeiten! 90

excised and cultured *Cymbidium* stem tips, but they never published that part of their work and therefore cannot be credited with it.

The following reasons may be responsible for the fact that Thomale's work did not become well known: (1) his findings were first published in German in an orchid hobbyist publication which at the time was not well-known outside Germany; (2) the second publication, also in German, was in a relatively obscure book aimed primarily at hobbyists and commercial orchid growers. As a result, few scientists read about Thomale's discovery. Practical growers who read it probably did not appreciate the technique and/or were bewildered by it (note this parallel between Rotor's and Thomale's publications).

Georges Morel (1916–1973; Fig. 31) is generally given exclusive credit for being the 'first' to culture an orchid explant *in vitro*. Clearly, he was familiar with Thomale's work at least as early as 1965 (Fig. 32). However, Morel cited it for the first time nearly 10 years later in a chapter written for Carl L. Withner's *The Orchids-Scientific Studies*. This was published some 14 years after his fame in the orchid world had been firmly established (Morel, 1974; Hass-von Schmude, Lucke & Arditti, 1995). Even then, Morel only cited Thomale's 1957 book and although he accurately reported that "pieces from the bulb of *Orchis maculata*, aseptically cultivated on nutrient medium, soon regenerated stems and roots …" He also added the qualifier "that [cases like this] are very exceptional." Morel included in his chapter a copy of a photograph provided by Thomale (Fig. 28) with the caption "Regeneration of roots and shoots occurring on a piece of tuber of *Orchis maculata*. (After Thomale.)" The wording ("stems and roots") tends to minimize Thomale's achievement by implying that what was produced were not 'whole' plants, and the context (included in a section entitled 'Regeneration from Inner Parenchyma') would seem to suggest that the new plants were produced from inner parenchyma rather than from buds, through bud formation, or via some other process commonly associated with tissue culture propagation (see Morel, 1974:170 ff.). Moreover, the photograph was not 'after Thomale'; it was provided by Thomale to Morel in response to his request (Fig. 32).

By the time Thomale was given any recognition (Arditti & Ernst, 1993; Haas-von Schmude, Lucke & Arditti, 1995), total credit for priority of discovery had essentially been established for and by Morel. This occurred, it may be argued, not only by virtue of the fact that Morel was already a well-known and established senior scientist in the world of plant physiology and plant pathology, but also due to his extensive travels and lectures. Orchid scientists who were unfamiliar with the historical details presented here, admiring hobbyists and grateful commercial growers, played a major role in elevating Morel to the position of being virtually the sole participant in the 'invention'. There was also, we submit, resistance to new knowledge (Gaffron, 1969) about which more will be said under our 'Concluding remarks'.

A note marking Thomale's 75th birthday (Lucke, 1994) makes no mention of his discovery because a statement to that effect was edited out by the editors of *Die*

Figures 26–30. The culture of *Orchis maculata* and Dr Lucie Mayer. Fig. 26. Dr Lucie Mayer (courtesy E. Lucke and Dr Norbert Haas-von Schmude). Fig. 27. Flowers and inflorescences of *Orchis maculata* (Landwehr, 1977). Fig 28. Explant of *Orchis maculata* in culture (courtesy of Mr Hans Thornale obtained with the help of E. Lucke and Dr N. Haas-von Schmude). Fig. 29. Plants of *Orchis maculata* produced from *in vitro* explants like the one in Fig. 28 (courtesy Dr Lucie Mayer obtained with the help of E. Lucke and Dr N. Haas-von Schmude). Fig. 30. The first description of the *Orchis maculata* cultures (from pages 89–90 in Thornale, 1957).

CENTRE NATIONAL DE RECHERCHES AGRONOMIQUES

STATION CENTRALE DE PHYSIOLOGIE VÉGÉTALE

Etoile de Choisy - Route de St-Cyr
VERSAILLES (S.-&-O.)
Tél. : 950 75-22

Références à rappeler :
N/Réf. : GM/JL
VERSA PHYSIO VE 52.211

V/Réf. :

Objet :

RÉPUBLIQUE FRANÇAISE
MINISTÈRE DE L'AGRICULTURE

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQ

G. MOREL , Directeur de Recherches
à
Monsieur Hans THOMALE
Kastanienwold 19
LEMGO
Lippe
(Allemagne)

Versailles, le 15 Décembre 1965

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Dear Sir ,

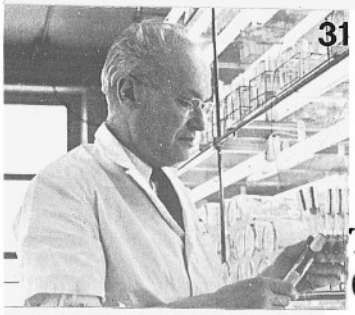
I have been asked by Dr. C. WITHNER to write a chapter on clonal propagation of Orchids for a new edition of his book .

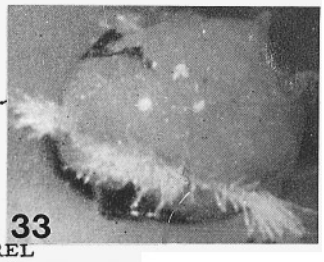
I would like to know if you did other experiments on propagation of Terrestrial Orchids since the one you mentionned in your book, p. 89 .

May I use the picture Ab 39 , of Orchis maculata, for this paper ? In that case, could you be kind enough to send me a print of it ?

Yours sincerely ,

G. MOREL


31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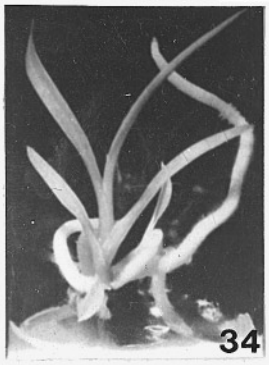
A Funny Thing Happened To The Orchid When They Operated On A Sick Potato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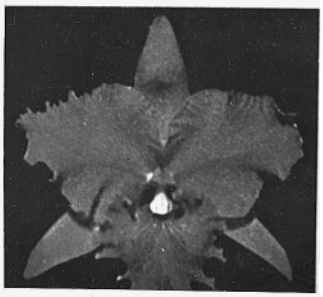
35

Orchidee (Dr Norbert Haas-von Schmude, Wettenberg, Germany, personal communication to J.A.). However his important contribution was eventually recognized in an American publication (Haas-von Schmude, Lucke & Arditti, 1995).

PLANT DISEASES AND MERISTEMS

The idea that healthy clones of horticultural plants can be obtained from stem tips, root cuttings and even leaves is at least half a century old (see Krikorian, 1982; North, 1953 for literature citations). A method for establishing *Verticillium*-free clones of chrysanthemums by making tip cuttings from 4–6 inch long shoots which were shown to be disease-free was reported by Arthur W. Dimock (1908–1972) during World War II (Dimock, 1943a, b) and subsequently refined and extended to other diseases (Brierly, 1952; Dimock, 1956). Similar methods were used for carnations (Dimock, 1943a, b, 1951; McFarland, 1948; Forsberg, 1950; Andreasen, 1951; Guba, 1952; Hellmers, 1955; Thammen, Baker & Foley, 1956).

That tips of virus-infected roots could be free of infection was reported over 60 years ago (White 1934a, b, 1943). Before that, viruses or 'abnormalities' could not be seen in stem tips of tobacco, tomato and *Solanum nodiflorum* (Clinch, 1932; Sheffield, 1933, 1942) but clearly virus infections were obtainable from them. For example, aucuba (*Aucuba*, Cornaceae is a genus of ornamental shrubs known as Japanese, greenleaf or sulphur leaf aucuba) and tobacco mosaic virus infections were obtained from isolated shoot- and root-tips (Sheffield, 1942). Thus, supposed absence of virus could have been due to the manner in which the tissues were excised, or to very low virus content (Samuel, 1934). Nevertheless, by 1948 stem tip cuttings could be used to eliminate the spotted wilt virus from *Dahlia* (Holmes, 1948, 1955). This method was extended to leaf spots associated with the internal-cork disease in sweet potato, *Ipomea batatas* (Holmes, 1956a) as well as aspermy virus (Holmes, 1956b) and other viruses (Brierley & Olson, 1956) in *Chrysanthemum*.

The use of stem tip cuttings to eliminate spotted wilt of *Dahlia* (Holmes, 1948) very clearly suggested that apical meristems might be virus-free. This was confirmed a year later in studies with tobacco mosaic infection of *Nicotiana tabacum* var. Samsun (Limasset & Cornuet, 1949). These findings were fortuitous. It is well-known today that apical meristems are not necessarily free of virus infection and this has led to considerable difficulties in freeing many clones and cultivars of viruses (Kassanis, 1967).

A problem facing French horticulture *c*. 1950 was viral infection of certain potato and *Dahlia* cultivars which would have caused them to be abandoned (Lecoufle, 1974a, b). Given the previous findings with *Dahlia* (Holmes, 1948) and tobacco (Limasset & Cornuet, 1949) the culture of stem tips provided a means of freeing these plants of viruses. And, indeed, Pierre Limasset and Pierre Cornuet "suggested to their colleagues Georges Morel and Claude Martin to cultivate shoot meristems of

Figures 31–35. Georges Morel: *in vitro* cultures, correspondence and folklore. Fig. 31. Dr Georges Morel (Orchids Orlando, n.d.). Fig. 32. Letter from Dr Morel requesting permission to use Thomale's photograph of *Orchis maculata* (copy of letter provided by Hans Thomale; photograph courtesy Hans Thomale obtained with the help of E. Lucke and Dr N. Haas-von Schmude). Fig. 33. Protocorm-like body (PLB) of *Cymbiium* (Morel, 1960). Fig. 34. *Cymbidium* plantlet produced from a PLB like the one in Fig. 33 (Morel, 1960). Fig. 35. Part of the cover of the Orchids Orlando catalog which listed orchids that were propagated clonally by the French firm of Vacherot and Lecoufle (Orchids Orlando, n.d.).

infected plants" (Gautheret, 1983, 1985). The suggestion was excellent, the attempts were successful and virus-free *Dahlia* (Morel & Martin, 1952) and potato (Morel & Martin, 1955a, b; Morel & Muller, 1964; Gautheret, 1983, 1985) plants were obtained from infected ones. The *Dahlia* and potato shoot obtained from stem tips *in vitro* by Georges Morel and his co-workers did not produce roots. Shoots produced *in vitro* by previous workers also failed to form roots. Therefore, following established laboratory practice, the shoots produced by Morel and his associates were grafted onto healthy seedlings (Gautheret, 1983). Later, other investigators were able to get rooting (Quak, 1961; Hollings & Stone, 1983). Attempts to free potatoes of virus through the culture of shoot tips were also undertaken by a number of others (Kassanis, 1957; Pirie, 1973; see Hirst & Harrison, 1988 for historical perspectives).

The success with *Dahlia*, potatoes and other plants (Morel & Martin, 1955b; Morel, 1964a) led Morel and his associates to the *in vitro* culture of *Cymbidium* shoot tips (Morel, 1960; Figs 33, 34). As already mentioned, this achievement has been heralded in a wide array of publications. A particularly adoring account in an advertisement-catalogue makes the claim on its cover that "a funny thing happened to the orchid when they operated on a sick potato" (Fig. 35) and in the text that "a beautiful thing happened to the orchids when they operated on a sick potato [because] Dr Georges Morel, distinguished French botanist, discovered the orchid meristem process while he was trying to figure out a way to prevent virus in potatoes" (Orchids Orlando, n.d; Fig. 36). Less maudlin but equally inaccurate statements asserting the same abound in the scientific and horticultural literature as well (for examples see Bertsch, 1966, 1967; Marston & Voraurai, 1967; Vacherot, 1967, 1977; Borriss & Hübel, 1968; Vanseveren & Freson, 1969; Hahn, 1970; Kukułczanka & Sarosiek, 1971; Lecoufle, 1971; Lucke, 1974; Allenberg, 1976; Champagnat, 1977; Rao, 1977; Loo, 1978; Murashige, 1978; Goh, 1983; Bouriquet, 1986; Griesbach, 1986; Hetherington, 1992). Much less frequently does one encounter attempts to be more precise about orchid micropropagation history (Arditti, 1977; Stewart, 1989; Arditti & Ernst, 1993).

Horticulture and plant agriculture are the major beneficiaries of stem tip culture in terms of the generation of plants free from specific pathogens as well as massive and rapid clonal propagation. The fact that both objectives can sometimes be accomplished simultaneously with one and the same explant has created "an apparent conception among horticulturists that tissue culturing and diseases-freedom (*sic*!) are synonymous. The same misconception was true of the so-called meristem cultured plants… A classic example of this misconception can be seen in the orchid industry… Before 'mericloning' orchid viruses were a minor problem … However [they] are now common, wide-spread and costly" (Langhans, Horst & Earle, 1977) because careless culturing spread rather than contained or eliminated viruses (see also Toussaint, Dekegel & Vanheule, 1984).

THE THIRD ASEPTIC CULTURE OF AN ORCHID EXPLANT

Most accounts and reviews of orchid micropropagation seem to start with a citation or at least a mention of Morel's 1960 paper on *Cymbidium* shoot tip culture.

Figures 36 & 37. Two accounts of Dr Georges Morel's work by firms which sold plants produced through his methods. Fig. 36. Page from the catalogue of Orchids Orlando (Orchids Orlando, n.d.). Fig. 37. Part of a letter to J.A. from the late Maurice Lecoufle, owner of the French orchid firm, Vacherot and Lecoufle.

The Meristem Story **36**

A Beautiful Thing Happened To The Orchids When They Operated On A Sick Potato

Columbus discovered America when he came up with the fantastic idea that the earth was round while toying with an egg.

Dr. Georges Morel, distinguished French botanist, discovered the orchid meristem process while he was trying to figure out a way to prevent virus in potatoes.

We never turned back to discover what happened to the sick potatoes, but we do know that the famous Frenchman hit upon a practical, realistic and successful way to put beautiful, exotic and expensive orchids within the reach of every pocketbook.

The story of meristem orchids is a beautiful one. Because it is the story of sharing.

Dr. Morel stumbled upon his great beneficial theory when the only method of reproducing a perfect orchid was by dividing the plant. But this took a long time, inasmuch as it takes about a year to produce divisions which will later on produce replica blossoms.

Thus, then, before Dr. Morel's discovery, you could recreate the image of your prize orchid only once a year. But, with the meristem system, you can produce 1,000 or 10,000 identical plants. Each one of the thousand or 10,000 plants will bloom exactly alike. Identical. You simply multiply your orchid as many times as you desire. This is meristem. (Continued)

Orchids Orlando 1717 EDGEWATER DRIVE
ORLANDO, FLORIDA 32804 / PHONE Area Code 305-424-5085 3

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ORCHIDÉES LES PETITS-FILS ET FILS DE VACHEROT & LECOUFLE

"LA TUILERIE"
30, RUE DE VALENTON
B. P. Nº 8
94470 BOISSY St-LEGER (France)

Dr. Joseph ARDITTI
Department of Development
and Cell Biology
UNIVERSITY of CALIFORNIA
IRVINE, Ca 92717
U. S. A.

BOISSY ST LEGER, April 1, 1985

Dear Dr. Arditti,

The first one who developed to me the theory of the excision and culture in vitro of the meristem has been Dr. Martin who was received at Vacherot & Lecoufle in 1956, explaining especially the great achievement made by Dr. Morel in the line of trying to have a virus-free orchid plant from a virus-infected plant, which corresponds to his article of 1960. This is the reason why I could in Sydney give these dates.

Sincerely yours,

ML/NF Maurice LECOUFLE

TEL. : 569-10-42 — CABLES VALSC - 94470 BOISSY ST LÉGER — C.C.P. PARIS 248-50 — SIREN 303 227 763 00015

A few examples are: "the potential of propagating orchids through tissue culture was observed first by Morel" (Murashige, 1974). Similarly, "… credit for the initiation of meristem culture technique goes to the late Dr G. Morel of INRA [Institut National de la Recherche Agronomique], Versailles, France" (Rao, 1977). Assertions that "the first application [of micropropagation] concerned the clonal propagation of orchids (Morel, 1960)" can be found in historical accounts by a 'founding father' of plant tissue culture (Gautheret, 1983, 1985). Since such reviews are often re-stated or quoted in other papers [for example, "the potentials of tissue culturing for plant propagation … have been … reviewed by Murashige …" (Langhans *et al*., 1977)] a historical 'factoid' has been elevated to truth and dogma. Once such a transformation happens, the forces which usually resist knowledge tend to maintain the status quo and thus strive to support dogma (Gaffron, 1969).

These factors seem to have come to bear on the history of orchid micropropagation. Attempts to question the accepted views have led to polemical exchanges in the literature (Arditti, 1985; Torrey, 1985a, b). Editorial demands for changes in manuscripts have also had to be agreed to (see e.g. Arditti & Arditti, 1985). The accepted history will be examined here for the sole purpose of placing historical facts in the most accurate perspective possible. Unfortunately, it may not be possible to do that "without apparent injustice to some other" (see opening quote). Indeed, this historical outline may appear, to some at least, to be somewhat 'unjust' only because many previous accounts have been imprecise enough to have done considerable violence to the truth.

Georges Morel (1916–1973) was born on 16 April 1916 in Béthune, France (Gautheret, 1977). In 1934 he entered l'Institut de Chemie in Paris where his interests led him to agriculture, plant pathology and I.N.R.A. (Gautheret, 1977).

Drafted into military service in 1939, Morel served with an artillery unit and was taken prisoner at the Belgian front in 1940. He escaped in 1941 (Gautheret, 1977). On returning to I.N.R.A. Morel was soon appointed "*chef de travaux*". In 1943 he joined Professor Gautheret's laboratory (Lecoufle, 1974a, b) and worked there towards his doctorate, which he received in 1948.

He went to the U.S.A. during the same year and worked until 1951 with Professor Ralph W. Wetmore (1892–1989) in the Biological Laboratories at Harvard University. They worked on tissue culture of monocotyledonous plants (Morel & Wetmore, 1951a) and ferns (Morel & Wetmore, 1951b).[22] Morel became friends and collaborated with Armin C. Braun (1912–1986) of the Rockefeller Institute in New York City on studies dealing with habituation and hormone autonomy in various plant tumours (Braun & Morel, 1950). Braun, a distinguished researcher on plant tumorigenesis, especially those induced by the crown-gall bacterium *Agrobacterium tumefaciens*, is appropriately regarded as one of the founding fathers of modern-day plant genetic engineering (Braun, 1982). Some gene transfer or transformation techniques rely heavily on the use of the Ti plasmid from that bacterium as a vector for inserting new genetic information (Bevan & Chilton, 1982). On Morel's return to France, he was appointed *Maître de recherches* (in 1951 or 1952) and in 1956 *Directeur de recherches* of the Station Centrale de Physiologie Végétale du Centre National des Recherches Agronomiques, Ministère de l'Agriculture (Lecoufle, 1974a, b).

It may be argued that Morel's first paper on shoot tip culture of *Cymbidium* (Morel, 1960) more closely resembled a news release or notice than a scientific paper. It sketchily reported what was done, minimally described the excision process and culture conditions, and referred to a nutrient medium, "Knudson III", which does

not last and may have been 'Knudson C'. The report concluded by stating "that it is relatively easy to free a *Cymbidium* from the mosaic virus … each bud will give several plants so the stock of a rare or expensive variety can be increased … [and that] experiments of the same kind are now being conducted with … Cattleya, Odontoglossum, and Miltonia, contaminated with different viruses" (Morel, 1960).

Morel's paper, did however, introduce a new phrase into orchid terminology and the English language. He used the term "protocorm-like body", (generally abbreviated as PLB)[23] to describe the "small flat bulblet looking exactly like [a] protocorm" (Fig. 33) which preceded the orchid plantlet formation (Fig. 34). Significantly, Morel's paper includes only two literature citations. One pertains to the mosaic disease (Jensen, 1951), the other deals with freeing plants from viruses through stem tip culture (Morel & Martin, 1955b). It would have been very difficult for anyone to repeat Morel's work because his article did not present sufficient details (competent plant scientists who took the trouble to study all of his previous work might have been able to reconstruct the procedures and medium or media; hobbyists or commercial growers would have had much more serious problems in doing that since many of them would have been looking for a detailed recipe, even a ready-made magic 'formula'). However, as the record shows, the orchid firm of Vacherot and Lecoufle 'La Tuilerie', Boissy-Saint Leget (Seine-et-Oise) had enough information to start commercial micropropagation of 'rare or expensive' orchids before any other establishment. They moved quickly enough to have a clonally propagated plant of *Vuylstekeara* Rutiland 'Colombia' bloom in December 1965 (Vacherot, 1966; Lecoufle, 1967).[24] This was 24 years before the publication of a specific method for this hybrid genus (Kukułczanka, Kromer & Roginska, 1989) and only two years after the reported excision date of the stem tips (Vacherot, 1966), and the development of culture methods (which were not published in detail at the time) for stem tips of the parent genera (Morel, 1963).

Two years from protocorm-like body to flowering appears to be very fast growth and development (but perhaps not impossibly so) for hybrids available at that time. In fact, according to one view "it will take just as long to grow the plants produced from meristem tissue as it takes to grow a new hybrid from seed" (Scully, 1964). As a rule, orchid plants grown from seed require at least 3 years to flower (excluding some recent *Phalaenopsis* hybrids which can be considerably faster) but there are also reports of hybrids which flowered only after 10 or more years (Goh, Strauss & Arditti, 1984; Goh & Arditti, 1985). However some "meristem-cultured plants may mature more quickly than plants raised from seeds" (Lecoufle, 1967). Plantlets of *Odontonia* Boussole 'Blanche' and *Odontonia* Moliere 'Lanni' removed from their flasks on 30 April 1965 "flowered ten to eleven months later and in blocks of hundreds, less than two years after being deflasked"[25] (Lecoufle, 1967). If the *Odontonia* plantlets were 'deflasked' on 30 April 1965, the cultures were probably started in 1964 or 1963 which is *before* the publication of culture procedures for this hybrid genus and its parent genera (*Odontoglossum* × *Miltonia*), but *after* Morel seems to have developed appropriate methods for them without publishing them (for a review see Arditti & Ernst, 1993).

In a subsequent paper, published in French, Morel added anatomical details regarding the protocorm-like body mentioned earlier and referred to attempts to extend the *Cymbidium* method to *Odontoglossum*, *Miltonia* and *Phajus* (Morel, 1963). However this paper did not provide additional details about excision or culture conditions.[26] It also tended to add to the confusion about a medium which those

seeking to duplicate his results might employ by reporting the use of 'Knop's Solution' supplemented with 2% glucose (Morel, 1963). The exact composition of the medium was not given. Morel used a modification of 'Knop's Solution' for potato stem tips but that paper (Morel & Martin, 1955a) was published in a journal not widely read outside France and is not cited in the orchid article. Therefore, it is safe to conclude that it would not have been easy for orchid scientists, and even more difficult for horticulturists, to gain access to the paper or the recipe.[27] Even if orchid scientists could find the composition of the potato stem tip medium there were no indications that it would be suitable for orchids. In fact, the potato stem tip medium is quite different from that subsequently used for orchids by Morel. It is also interesting to note that Georges Morel was very familiar with 'Knop's Solution' and the modified Berthelot trace elements formulation because he used them routinely in his doctoral dissertation work (Morel, 1948:137 *et seq*.). Those trying to learn more about the media used by Morel for orchids could have learned much from this paper (Morel, 1948), but the connection was not obvious, the published thesis was not well known, the journal was relatively obscure, and it was published in French.

Modifications of 'Knop's Solution' have been used for the culture of vegetative axis nodes of *Dendrobium* and *Bletilla* (Yam, 1989), and floral stem or scape nodes of *Phalaenopsis* (Ball, Reisinger & Arditti, 1974–1975; for a review see Arditti & Ernst, 1993) but there is no indication that these, or any, modifications of this solution would be suitable for shoot tips of other orchids. This is not surprising because the available evidence suggests that at present there is no single solution which is suitable for all orchids (see Arditti & Ernst, 1993 for a review).

A third paper (the second published in English) appeared a year later (Morel, 1964b). It was longer, had more illustrations, added the results of more work with three genera (*Cattleya, Miltonia, Phajus*) to those that were being cultured, and described the culture conditions. It has the potential to lead to confusion rather than clarification regarding the culture medium because it was listed as "Knudson III" again. This paper left no doubt that the culture of shoot tips could be used for mass rapid clonal multiplication but it still did not provide enough information for others to duplicate the technique. In retrospect, it is clear that even those who were familiar with all three papers (Morel, 1960, 1963, 1964b) would have had to guess which medium to use and how to modify it.[28] Guessing would not be conducive to success especially for commercial and hobby growers. Development of another suitable medium would have required time (i.e. caused delays for other investigators) and delayed knowledge of the 'right' formulation would have decisively secured for Vacherot and Lecoufle the lead they already enjoyed. This is an important point since the only published procedures in the literature even now for *Miltonia* and *Phajus* are the ones published (albeit unclearly) by Morel (see Arditti & Ernst, 1993 for details). It is not known, however, whether the medium is really pivotal, that is whether it is crucial to success. Several procedures and media are currently available for *Cattleya* and other orchids (Arditti & Ernst, 1993). The same may be true for *Miltonia* and *Phajus*.

A trio of additional papers appeared within the next three years (Morel, 1965a, b. 1966) and at this point the recipes were finally given. Some did but others did not resemble 'Knudson C' medium (more than likely Morel's 'Knudson III'), Knop's solution or the potato stem tip substrate (Morel & Martin, 1955a) enough to be called a modification of any of them. One medium for *Cymbidium* was actually described as "potato meristem medium" (Morel, 1966). Therefore, one is left wondering about

the listing of media (Morel, 1960, 1963, 1964b, 1965a, b) especially since the Knudson and Knop solutions were given as suitable for *Miltonia* and *Cymbidium* in a subsequent paper (Morel, 1970). That paper and an earlier one (Morel, 1966) also contained additional information about the micropropagation of *Cattleya*. Information about Vandaceous and European orchids and *Dendrobium* was published between 1966 and 1970 (Morel, 1966, 1970; see Arditti & Ernst, 1993 for details).

Two reviews (Morel, 1971a, 1974) were Morel's final contributions in English on the micropropagation of orchids. Both are excellent and contain a considerable amount of basic information. His last review (Morel, 1974), like some of the previous papers (Morel, 1965a, 1966), covers culture media and their components in some detail. The discussion is both interesting and enlightening. Media recipes and details about culture conditions are unambiguous. However, by that time the information was much less important and useful than it would have been in 1960. This is so because by 1965 a single firm in France (Vacherot and Lecoufle) had established a monopoly; also, as a result of research carried out throughout the world, several culture media and procedures for the micropropagation of orchids had been formulated and published. Publication of a suitable medium in 1960 would have made the technique available to all who wanted to use it even if (a) the medium used by Morel was not pivotal, and (b) several media were later shown to be suitable for some orchids (see Arditti, 1977 and Arditti & Ernst, 1993 for lists and media recipes).

With one exception (Morel, 1963), the initial orchid papers and several subsequent ones were published in periodicals aimed at hobbyists and commercial growers (Morel, 1960, 1964b, 1965a, b, 1966, 1970) and in proceedings of meetings (Morel & Champagnat, 1969; Morel, 1971a, b, c) rather than peer-reviewed scientific journals. One reason for this could have been the laudable intent to make the procedures available to growers. But if this was so, important information (e.g. culture media recipes, details about techniques etc.) would have had to have been included in each of them. It was not. Another conspicuous deficiency in these papers is the lack of literature citations. Previous papers by others which may have been the source of ideas, media and methods were not cited. This is not in line with the accepted standards of scientific publication. Lack of citations creates the erroneous impression that the ideas are original. Peer-reviewed scientific journals would have probably rejected most of these papers due to insufficient information about methods and media, and lack of citation of previous work. Yet there is no question whatever that the calibre of the early research was high enough to justify papers which could have been published in peer-reviewed scientific journals.

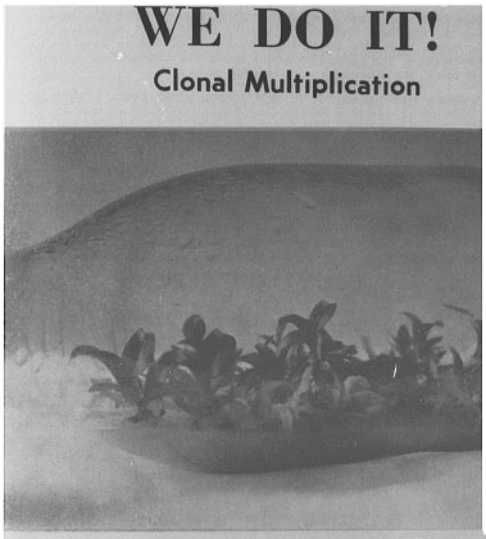
A key problem which cannot currently be solved is why there was only a single early, incomplete, paper in English, on *Cymbidium* (Morel, 1960) in a non-reviewed hobbyist journal. Subsequent papers on *Cymbidium* (Champagnat, 1965; Champagnat, Morel & Gambade, 1966; Champagnat *et al*., 1968), *Cattleya* (Champagnat & Morel, 1969; Champagnat, Morel & Mounetou, 1970), *Neottia nidus-avis* (Champagnat, 1971), and *Oprys* (Champagnat & Morel, 1972) were published in reviewed French journals. French is a language which has long since lost its scientific importance and is one with which most orchid growers in the world were, and are, not familiar. These papers did contain more details than the first one, but by this time the importance of the information was much reduced because a detailed procedure, complete with a medium recipe, had already been published by Wimber (1963).

"In 1955 Dr Morel performed the meristematic tissue culture of *Cymbidium, Cattleya, Miltonia* and *Phajus*" (Lecoufle, 1971). He "discovered in 1956 that it was possible to cultivate shoot apices" (Morel, 1964b). Also, "in 1956 [Morel] started to apply the techniques of meristem culture … previously developed to free potatoes, dahlias and carnations from viruses, to various Orchids" (Morel, 1965a). Additionally, in 1956, the year "meristem culture was achieved by Dr Morel", "Dr C. Martin [one of Morel's first students] … was received at Vacherot & Lecoufle in 1956 explaining especially … the great achievement made by Dr Morel" (Fig. 37; letter dated April 1 1985 to J.A. from the late Maurice Lecoufle). These statements raise yet another question about the first paper on *Cymbidium* which showed an 18-month old explant and included the statement that "some plants that are…10 cm high" (Morel, 1960). A *Cymbidium* plant *in vitro* or in a pot would certainly grow more than 10 cm in 4–5 years (from 1955 or 1956 to 1959 or 1960 when the time the paper was submitted and published). Therefore it is by no means clear whether the statements in the paper are accurate (Morel, 1960) or if the report is about plants which were produced specifically for that article.

"The possibility of producing unlimited numbers of plants from any single orchid clone" drew the attention of Dr Walter Bertsch, then living in Paris, who was involved with the breeding programme at Vacherot and Lecoufle (Bertsch, 1966). Bertsch suggested that Vacherot and Lecoufle enter the field. They did and were successful immediately (Bertsch, 1966, 1972). As a result "Vacherot and Lecoufle became the first nursery to develop, on an industrial basis, the meristemming of orchids. For ten years they held the monopoly" (Lecoufle, 1995). This monopoly started in the early 1960s or late 1950s (Orchid Digest Staff, 1995). Vacherot and Lecoufle published a full-page advertisement in the American Orchid Society Bulletin for June 1964 which included a photograph of a flask containing plantlets of *Laeliocattleya* Chine 'Bouton D'Or' and stated "we do it" (Fig. 38). The fact that this cross was registered in 1962 (Royal Horticultural Society, 1961–1963) and the size of the plantlets suggest that the cultures were started before publication of culture media for this hybrid genus or its parent genera (*Cattleya* and *Laelia*).

Vacherot and Lecoufle's "we do it" advertisement was followed in December of that year with a photograph of technicians performing aseptic manipulations in what appears to be a sophisticated laboratory (Fig. 39). The two advertisements appeared approximately one year before Morel first published extensive details about his procedure and the composition of some of his culture media. Georges Morel, the Vacherots and the Lecoufles were friends. One might speculate that this friendship prompted him and/or one of his associates to teach Vacherot and Lecoufle the technique and then to delay publication and withhold information for a while (Arditti, 1985). Morel was certainly willing to oblige an American orchid establishment which represented Vacherot and Lecoufle (Fig. 40a, b) with an endorsement (Fig. 41; Anonymous, *c*. 1965). At the time, this was not a common action for a research scientist. Another possibility, of course, is that the delay in divulging procedural details openly and generally was brought about by an unselfish and patriotic intent to allow a French firm to capture the market.

Figures 38–41. Commercialization of orchid 'meristem' culture. Fig. 38. The first advertisement by the French orchid firm, Vacherot and Lecoufle announcing the availability of in vitro-produced clonal divisions (*American Orchid Society Bulletin*, June 1964, p. 535). Fig. 39. Partial view of the Vacherot and Lecoufle laboratories *c*. 1964 (*American Orchid Society Bulletin*, page 1097). Fig. 40. Text (a) and photograph (b) in an advertisement by Orchids Orlando (*American Orchid Society Bulletin*, October 1964, pp. 898–899). Fig. 41. Commercial endorsement by Dr Georges Morel (Orchids Orlando, n.d.).

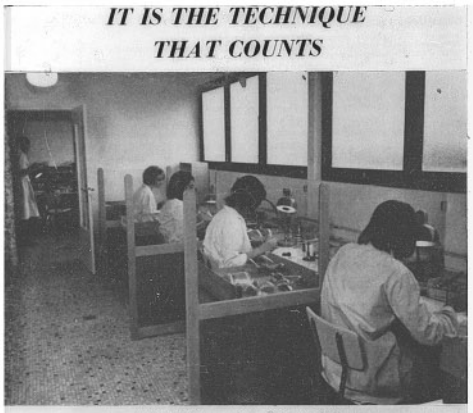


CLONAL DIVISIONS OF LC. CHINE 'BOUTON D'OR'

Our laboratories always use the latest techniques for progress in modern orchidology.

THE GRANDSONS & SONS OF

38 VACHEROT & LECOUFLE



(Partial View of our laboratories)

For Clonal Multiplication, Ask for the Advance Process of:

THE GRANDSONS AND SONS OF

VACHEROT & LECOUFLE

'La Tuilerie'
B.P. No. 8
BOISSY-SAINT-LEGER
(S. & O.), FRANCE

39

Kind Words to Orchids Orlando From Meristem System Discoverer

Republique Francaise
Ministere de L' Agriculture
Institut National De La Recherche Agronomique
Versailles, France 15 Fevrier 1968

It is a great satisfaction for a scientist to see the results of his discoveries widely applied.

That's why I am very happy to congratulate Mr. Martin Andersen of Orchids Orlando for his enterprise and courage in investing so much money in our new meristem theory.

These new techniques are going to bring a revolution in the orchid industry. I think he is on the right track because his processing program is sound and realistic.

G. MOREL

41

THE GRANDSONS & SONS OF

a

VACHEROT & LECOUFLE

are pleased to announce that

Orchids Orlando

has been appointed as our authorized agent for the U.S. and Canada, except for the state of California, where Frank Fordyce is our representative.

'LA TUILERIE'
BOISSY-SAINT-LEGER
(S. & O.), FRANCE

40

OCTOBER 1964

FRANCE COMES TO FLORIDA WITH NEW ORCHID BREAKTHROUGH - - -

Left to right: Larry Spencer, Mgr. Orchids Orlando, Maurice Lecoufle, owner of Vacherot & Lecoufle, pioneer French orchidologists, Tom Flynn, of McHutchison & Co., Ridgefield, N.J. and Martin Andersen, owner of Orchids Orlando. The plant Mr. Lecoufle holds is not a meristem product but will be shipped to him for clonal multiplication.

The questions posed here may be evaluated in connection with the facts presented above, and the rapid (approx. 2 years from the start of research to publication date) publication of the potato and *Dahlia* papers. Following that line of reasoning, it may be argued that the orchid papers might well have been published even more promptly because the stem tip cultures were well established by then.

Many consider Morel's orchid work to be highly original and innovative. However, a somewhat different picture emerges from our critical evaluation of the facts. None of what Morel did with potatoes, *Dahlia* and orchids was original. Media for plant tissue culture in general and stem tips of orchids in particular existed (Knudson, 1946; Rotor, 1949; Mayer, 1956, Thomale 1956, 1957) before Morel formulated his own by modifying existing ones. Several explant types (shoot tips, buds, nodes) from monocotyledonous plants in general and orchids in particular (Rotor, 1949; Thomale, 1956, 1957) were cultured before Morel did so. Further, a number of procedures were published following established scientific protocol prior to his. Shoot tips had been used to free plants of virus infection before Morel's work with dahlias, potatoes and orchids (see above). Even Morel's work on potatoes and dahlias was suggested by others, namely P. Limasset and P. Cornuet (Gautheret, 1983:402, 1985:42).

Morel's most significant achievement was to produce protocorm-like bodies (PLBs) which were sustainable via subculture and this made true mass and rapid clonal propagation possible. He did that by cleverly combining existing procedures and culture techniques into a very useful new application. Having done that, he was also able to generate publicity for an advance whose time had come. Clearly, he should be credited with imaginatively applying existing knowledge and technology to a new application. Indeed, in this he played a decisive role. However, he should not be given the accolades normally reserved for those who originate novel ideas, make basic discoveries and formulate new principles.

In the course of more recent reminiscences relating to the history of plant tissue culture, it has been claimed that "Ball is really the father of the so called micropropagation method" (Gautheret, 1985:16–17). Perhaps Gautheret felt justified in crediting Ernest Ball because he showed that stem tips can be cultured *in vitro*. But Ball does not seem to have appreciated and certainly did not express in print[20] the practical potential of his work. He was interested in the basic aspects of growth and development from meristems.[29] Therefore, he is perhaps better viewed as more of an 'uncle' than a 'father'. If we accept that Ball is not the father, then Morel could have been, except that (1) Gavino Rotor Jr. first thought of and implemented *in vitro* clonal propagation; (2) Hans Thomale was the first to culture orchid tuber explants; he also drew special attention to the mass propagation potential of his work, and (3) Donald Wimber was the first to publish a detailed shoot 'meristem' culture procedure.

Figures 42–48. Orchid tissue culture: people, plants, companies and a medium component (charcoal). Fig. 42. Samuel Mosher (Anonymous, n.d.). Fig. 43. Professor Donald E. Wimber (photograph from an Ektachrome transparency taken by J.A. in Hiroshima in 1987, signature from a letter to J.A.). Fig. 44. Protocorm-like bodies in liquid culture (Wimber, 1963). Fig. 45. Plantlets *in vitro* (Wimber, 1963). Fig. 46. Mr Everest McDade (photograph courtesy Everest McDade, signature from a letter to J.A.). Fig. 48. Rivermont Orchids from the air (back cover, *American Orchid Society Bulletin*, January 1952; this advertisement appeared over a number of years, several times and in more than one orchid magazine).


42
43
44
Don Dunlap
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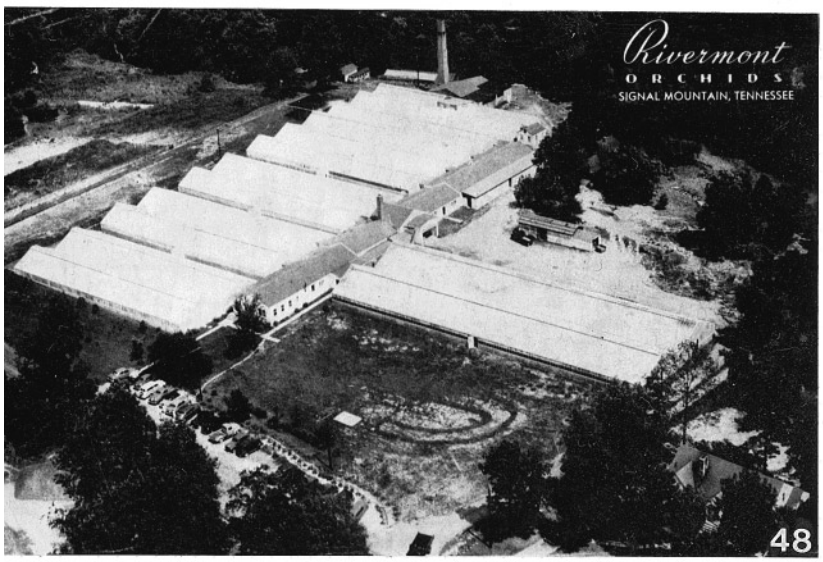
46
Everett McDade
47


Rivermont
ORCHIDS
SIGNAL MOUNTAIN, TENNESSEE
48

THE FOURTH ASEPTIC CULTURE OF AN ORCHID EXPLANT

Samuel Mosher (1893–1970; Fig. 42) grew orchids and eventually established the Dos Pueblos Orchid Company in Goleta, California. Mosher's enterprise included what was described as "the world's largest establishment for the breeding and growing of Cymbidium orchids" (Anonymous, n.d.). Mr Mosher was an enlightened and earnest grower and student of orchids, in many ways a throwback to the great British firms of yesteryear like Sanders, Veitch, Black and Flory McBean, Charlesworth and others (Arditti, 1990). He established a modern and well-equipped laboratory and hired a cytogeneticist, Dr Donald E. Wimber (b.1930; Fig. 43), to study orchid chromosomes and to manage a large, modern laboratory.

Wimber received his B.S. from San Diego State College in 1952 and M.S. and doctorate from Claremont College in 1954 and 1956 respectively. He became associated with the Dos Pueblos Orchid Company and worked there until 1957. In 1963 Wimber accepted an appointment at the Biology Department, University of Oregon, where he has remained and where he became a distinguished and honored (American Orchid Society Gold Medal) scientist (Ernst, 1992).

While associated with the Dos Pueblos Orchid Company, Wimber studied orchid cytology and engaged in seed germination. He had been introduced to the technique by Emil Vacin, co-formulator of the Vacin and Went medium (Ernst, 1992). Observing young plants and seedlings led Wimber to tissue culture of orchids. His first attempt was never published, but it pre-dated both Thomale's and Morel's work. The following account is based on a letter to J.A. from Dr Wimber dated 13 December 1976.

Research with embryonic leaves was carried out in the summer of 1955 while Wimber was still a graduate student. It involved several immature shoots from a *Cymbidium lowianum* clone. The shoots were 4–5 cm long. They were surface sterilized with a 10% dilution of the laundry bleach Clorox after a few of the outside scale leaves were removed. Several additional leaves were removed before the last 4–6 embryonic leaves were broken off and placed on semi-solid Vacin and Went nutrient medium. In addition, Wimber made several thin transverse sections through the shoot axis after removing many of the covering leaves. PLBs developed at the bases of the embryonic leaves and along the thin sections.

When some of the PLBs were quartered and spread on agar, the sections produced plantlets. Wimber showed his results to Sam Mosher and Kermit Hernlund, manager of Dos Pueblos at the time. They were not impressed because the tissues grew slowly. By Christmas of that year the plantlets were only 2–3 mm tall. In 1957 Wimber had a dozen plants in 10–15 cm (4–6 inch) pots. Wimber concluded his letter by stating "I knew I had something, but was rather fearful that some sort of chromosomal change might have occurred so that a faithful reproduction of the parent might not occur." If the cytogeneticist in Wimber had been less persuasive than the propagator he could have been the one credited with the discovery of mass rapid clonal propagation of orchids (see below under 'Concluding remarks').

In 1963, Wimber published his first paper on clonal propagation of *Cymbidium* (Wimber, 1963). Like Morel's first paper on shoot tip culture of *Cymbidium*, Wimber's report was published in the *American Orchid Society Bulletin* (Figs 44, 45), but the similarity ends there. Wimber followed standard scientific practice and provided full procedural details, gave the recipe of the medium (modified Tsuchiya) he used and carefully described the culture conditions (continuous illumination of 100 foot-

candles or less, constant temperature of 22°C, rotary shaker, 125 ml Erlenmeyer flasks sealed with rubber stoppers). Also, Wimber was very clear in calling attention to the propagation potential of shoot tip cultures. This wealth and clarity of details is especially remarkable in view of the fact that the procedure was developed while Wimber was employed by a commercial concern which had every right to keep the details secret. Morel, on the other hand, worked in a government laboratory and at one point received funding from the American Orchid Society. Anyone with the appropriate training or experience with orchid seed germination and the needed facilities could repeat Wimber's work immediately. (Incidentally, when Lewis Knudson established how orchid seedlings could be established asymbiotically, he too promptly published full details, see Arditti, 1990). A subsequent paper elaborated on the initial procedures (Wimber, 1965). Indeed, it could even be argued that Wimber was the first to publish on clonal propagation of orchids through stem tip culture because his was a scientific (albeit non-reviewed) paper (Wimber, 1963), rather than what can be called an announcement or news release (Morel, 1960).[30]

A PATENT

Following publication of the 'mericlone' process by Morel a claim was made by Mr Everest McDade (b. *c*. 1916; Fig. 46) that he developed the 'mericloning' process secretly as early as 1950 and kept it secret or at least did not publicize it at the time. This information (Bergman, 1972) as well as publications by Morel, Professor Harry C. Kohl (Kohl, 1962) and Wimber were used (Torrey, 1985b) to obtain eventually a patent (U.S. 3,514,900) for the process. According to McDade, an electronic engineer, science teacher and co-owner of Rivermont Orchids, Signal Mountain, Tennessee (Fig. 47) until *c*. 1949 (the firm no longer exists), the idea originated from a "photo of a Cymbidium bulb, with a cluster of buds at its base" (letter to J.A. from Everest McDade, Asheville, North Carolina). An article from *c*. 1946 or 1947 (which McDade does not have and claims he is still trying to find) that accompanied the photograph "was a very sudden clear message to us [McDade]. Just what we had been looking for: a renewable source of 'Ramets'…. We wanted to use the process for *Cattleya* types … I adapted the *Cymbidium* idea to cattleyas".

According to McDade, his secretary Dorothy Smith "made the first meristem cultures in 1950". He "wrote scores of letters … to authors … botany and genetics journals. [but] Only a few people took them seriously, or even guessed that we had discovered cloning and patents in [the] year 1950." McDade also claims that "in October, 1952 [he] actually gave the cloning process paper to the [American Orchid Society Bulletin] … for publication and *demonstrated a growth developing from a Cattleya flower stem, a flask, and community pot of a clone*" (emphasis by McDade).

However, McDade's claims are not borne out by the paper he published in the *American Orchid Society Bulletin* (McDade, 1952). Rivermont advertisements from that period did not offer for sale any orchids described as being clonally propagated. The granted patent itself 'relied on' work by Morel, Kohl and Wimber (U.S. patent 3,514,900 filed August 11, 1967 and awarded June 2, 1970; Torrey, 1985a, b). The chronology itself is suspect. It involves an idea which supposedly originated in 1946 or 1947, cultures that were presumably first made in 1950, work which was published between 1955 and 1963, and a patent not issued until 1970. An equally

good case, perhaps a better one, can be made for a suggestion that the patent was an attempt to benefit from the 'mericlone' process.

DARKENING OF MEDIA

Orchid seed germination and micropropagation media are often darkened with activated charcoal because the plants grow better in the presence of this additive. John T. Curtis was the first to darken a nutrient medium for orchid seedlings *in vitro*. He did it in an unsuccessful effort to simulate natural conditions and thereby bring about the germination of *Cypripedium reginae, C. pubescens, C. parviflorum, C. candidum* and *C. acaule* seeds (Curtis, 1943). Curtis used lampblack which has very little in common with activated charcoal except colour. Lampblack is soot produced by the burning of petroleum hydrocarbons. It does not have the large internal surface area, adsorptive properties and pore structure of charcoal. Lampblack has long been used in the production of black inks and paints.

Vegetable charcoal (Fig. 47) is made from wood, sawdust, peat and organic residues which are recovered during the production of pulp (West Virginia Pulp and Paper, n.d.), carbonized and activated to produce a large surface area. As a result for instance, one gram of Nuchar may contain up to 120 billion particles and have a total surface area of 500 to 2000 m^2. Pore distribution can range from $< 10\,\mu m$ to $> 500\,\mu m$ (Yam *et al*., 1990). The pore to volume ratio is 0.9 cc g^{-1}. Charcoals can contain many elements, some in very small amounts (Yam *et al*., 1990). Activation is carried out through treatment of the carbonized pyrolysis product with steam or carbon dioxide (Yam *et al*., 1990).

Charcoal was first used to darken an orchid culture medium by Prof. Peter Werkmeister in Germany (Werkmeister, 1970a, b, 1971). At the same time it was employed to germinate moss spores and grow filamentous algae (Proskauer & Berman, 1970; Krikorian, 1988). Werkmeister darkened the medium to study the growth of roots, gravitropism and proliferation of clonally propagated plantlets. He died not long after publishing the last of his orchid papers.

Robert Ernst (b. 1916; Fig. 49), a surfactant chemist and manufacturer, and Professor of Biology at the University of California, Irvine, was the first to add charcoal to practical seedling culture media and found that *Paphiopedilum* and *Phalaenopsis* seedlings grew well on it (Ernst, 1974, 1975, 1976). Ernst's findings resulted in the formulation and widespread use of charcoal-containing media for orchid seed germination, seedling culture and micropropagation (Ernst, 1974, 1975, 1976; Yam *et al*., 1990). The reasons for the beneficial effects of charcoal are still under discussion even if not the subject of intensive research (for reviews see Yam *et al*., 1990; Mohammed-Yasseen *et al*., 1995) and beyond the scope of this account.

TERMINOLOGY AND SEMANTICS

By 1964 clonal multiplication of orchids by means of aseptic culture had become an important part of orchid horticulture (Dillon, 1964; Scully, 1964). Discussions centered on techniques, growth, mutations during culture, costs and nomenclature. The question was what to call plants produced by this method which was inappropriately called "meristem culture" (Dillon, 1964; morphologically what was

being cultured were stem tips). Terms such as 'mass rapid clonal propagation', 'micropropagation', or 'tissue culture propagation' had not yet been invented. One suggestion by Dr Robert D. Patton of Galena, Ohio, namely, 'meristem division' was deemed "a good term" (Dillon, 1964), but 'mericlone', a term proposed by then Lt. and now orchid grower for Carter & Holmes Orchids in Newberry, South Carolina, Gene Crocker (Fig. 50) was judged to be better (Dillon, 1964). This term seems to have excellent marketing appeal. However, it is scientifically inaccurate and uninformative. Moreover, it is a linguistic abomination (Arditti & Ernst, 1993), whether used as a verb ("to mericlone a plant"), a noun ("this plant is a mericlone"), a description ("the mericlone industry", or "he/she is a mericloner") or part of the language ("this book is about mericloning"). The only potentially charitable statement that may be made for the term is that it upholds a long-standing 'tradition' in tissue culture in that it perpetuates the propensity to adopt misleading terminologies. I.W. Bailey (1884–1967), a pioneer and influential American plant anatomist and morphologist at Harvard University early complained that the term 'tissue culture' was a misnomer because only rarely are cultures derived from or are comprised of specific tissues (Bailey, 1943). His exhortations had little effect on the then relatively new field and the term 'tissue culture' seems to be with us forever.

SUBSEQUENT RESEARCH

The early history of orchid micropropagation ends around 1965. Many workers entered the field at that time. Scientists, hobbyists, commercial growers and laboratory operators attempted to culture stem tips and other explants from many genera. As a result, the number of papers on orchid micropropagation is now enormous (for reviews see Morel, 1974; Arditti, 1977; Arditti & Ernst, 1993). Numerous orchids have been cultured (Table 1; only the first publication regarding the aseptic culture or micropropagation of a specific genus or explant type is listed). Significantly, some genera of hobby or commercial interest like *Paphiopedilum* have not yet yielded to micropropagation despite being cultured experimentally in the laboratory (see Arditti & Ernst, 1993 for details). Many north temperate species including another 'slipper' genus, *Cypripedium*, have also been recalcitrant.

CELL AND PROTOPLAST CULTURE

The first attempts to culture free plant cells utilized mechanically isolated ones. Although fairly intensive efforts were made by Gottlieb Haberlandt in 1898 and 1902 (Krikorian & Berquam, 1969; Krikorian, 1975, 1982; Steward & Krikorian, 1975), he did not succeed. A suggestion that Haberlandt failed because he neglected the findings of a French naval architect and agronomist, Henri-Louis Duhamel du Monceau (1700–1782) who also studied wound healing in trees (Gautheret, 1985) probably has its roots in Gallic chauvinism rather than scientific reality. Haberlandt failed for the following reasons: (1) his ideas were more advanced than the plant science 'biotechnology' of his day; (2) his selection of cells to culture (mature, differentiated, specialized non-meristematic) was inappropriate; (3) his culture media did not include all necessary components — he used "tap water, one to five percent sucrose solutions, and Knop's solution with or without sucrose, dextrose, glycerine,

TABLE 1. Chronology of formulation of protoplast, cell, tissue and organ culture procedures for orchids[a,b]

Genus	Explant	Reference
Acampe praemorsa	Protoplasts	Seeni & Abraham, 1986
Acampe rigida	Leaf tips	Yam, 1989; Yam & Weatherhead, 1991a
Aëridachnis	Apical and axillary buds	Lim-Ho, 1981
Aerides	Protoplasts	Seeni & Abraham, 1986
Anacamptis pyramidalis	Shoot tips	Morel, 1970
Angraecum eburneum as *Angraecum giryamae*	Protoplasts	Price & Earle, 1984
Anoectochilus elatus	Protoplasts	Gopalakrishnan & Seeni, 1987
Anoectochilus formosanus (Anoectochilus roxburghii)	Lateral bud explants, cuttings, layering	Chow, Hsie & Chang, 1982
Arachnis hookeriana	Terminal and lateral buds	Vajrabhaya & Vajrabhaya, 1976a, b
Arachnostylis	Apical and axillary buds	Lim-Ho, 1981
Aranda	Shoot tips	Goh, 1973
	Protoplasts	Loh & Rao, 1985
Aranthera	Apical and axillary buds	Irawati *et al*., 1977
	Leaf bases	Lim-Ho, 1981
Arundina	Shoot tips	Mitra, 1971
Ascocenda	Young leaves	Fu, 1978
	Bud explants	Lim-Ho, 1981
(as *Schlechterara*)	Shoot meristems	Ichihashi, 1979
Ascocentrum	Protoplasts from flowers, leaves and roots	Oshiro & Steinhart, 1991
Ascofinetia	Inflorescences	Intuwong & Sagawa, 1973
Barlia	Protoplasts	Pais *et al*., 1982
Bletilla striata	Root tips, stem nodes	Yam, 1989
Brassavola	Protoplasts	Seeni & Abraham, 1986
Brassia	Protoplasts	Capesius & Meyer, 1977
Brassia brachiata	Protoplasts from root tip	Yasugi, 1989a, b
Brassocattleya	Buds	Scully, 1967
Bulbophyllum	Protoplasts	Seeni & Abraham, 1986
Burkillara	Apical and axillary buds	Lim-Ho, 1981
Calanthe	Protoplasts	Seeni & Abraham, 1986; Yasugi *et al*., 1986
Catasetum	Root tips	Kerbauy, 1984
Cattleya	Shoot tips	Morel, 1964a, 1970
	Lateral buds	Reinert & Mohr, 1967
	Dormant backbulb buds	Vajrabhaya, 1978
	Leaf bases	Champagnat *et al*., 1970
	Leaf tips	Arditti, Ball & Churchill, 1971
	Leaf tissues	Fu, 1978
	Protoplasts	Capesius & Meyer, 1977
	Protoplasts from flowers	Oshiro & Steinhart, 1991
Cattleya aurantiaca	Protoplasts from leaves and roots	Yasugi, 1989a, b
Cattleya skinneri	Protoplasts	Yasugi, 1900
Cymbidium	Cells, sloughed off from shoot tip cultures	Steward & Mapes, 1971a
Cymbidium	Protoplasts	Capesius & Meyer, 1977
Cymbidium	Floral organs	Kim & Kako, 1982
	Protoplasts	Oshiro & Steinhart, 1991
Cymbidium ensifolium	Shoot tips some cultures flowered in vitro	Wang *et al*., 1984, 1988a, b
Cymbidium faberi	Rhizomes	Hasegawa, Ohashi & Goi, 1985
	Various	Hasegawa, 1987
Cymbidium forestii	Various	Hasegawa, 1987

TABLE 1. *(continued)*

Genus	Explant	Reference
Cymbidium goeringii	Shoot tips	Wang *et al*., 1981
	Various	Hasegawa, 1987
Cymbidium hakuran	Various	Hasegawa, 1987
Cymbidium hybrids	Shoot tips	Morel, 1960; Wimber 1963
Cymbidium insigne	Shoots	Ueda & Torikata, 1972
	Various	Hasegawa, 1987
Cymbidium kanran	Various	Hasegawa, 1987
Cymbidium Kenny 'Wine Color'	Protoplasts	Yasugi, 1990
Cymbidium sinense	Various	Hasegawa, 1987
Cypripedium	Various	Morel, 1971b
Cyrtopodium	Root tips	Sanchez, 1988
Dactylorchis	Dormant shoot (not details)	Stokes, 1974
Darwinara	Embryonic callus	S. Ichihashi[b]
	Protoplasts	S. Ichihashi[b]
	Plants regenerated from protoplasts	S. Ichihashi[b]
Dendrobium	No details of any kind	Morel, 1965a, b
Dendrobium	Flower stalks	Singh & Sagawa, 1972
	Protoplasts	Price & Earle, 1984
	Protoplasts	Yasugi, 1990
	Protoplasts and cells were used to regenerate plants	Sajise *et al*., 1990
Dendrobium aggregatum	Protoplasts	Yasugi *et al*., 1986
Dendrobium antenatum	Axillary buds	Kukułczanka & Wojciechowska, 1983
Dendrobium aduncum	Stem nodes	Yam, 1989
Dendrobium crumenatum	Leaves	Manorama *et al*., 1986
Dendrobium chrysanthum	Pseudobulb segments	Vij & Pathak, 1989
Dendrobium hybrids	Axillary buds	Sagawa & Soji, 1967
	Nodes	Arditti, Mosich & Ball, 1973
	Protoplasts	Yasugi *et al*., 1986; Yasugi, 1989a, b
Dendrobium loddigesii	Stem nodes	Yam, 1989
Dendrobium nobile	Ovary	Ito, 1966
Dendrobium phalaenopsis	Shoot tips	Gandawijaja, 1980
Dendrobium transparens	Shoot tips	Yam, 1989
Disa	Shoot apices	Haas & Lückel, 1977
Doritaenopsis	Lateral buds from flower stalks	Lim-Ho, 1981
	Embryogenic callus	S. Ichihashi[b]
Doritis	Leaves	Sagawa & Kunisaki, 1982
Epidendrum	Node sections	Stewart & Button, 1976
	Root tips	Churchill, Ball & Arditti, 1972
	Leaf tips	Churchill, Ball & Arditti, 1973
	Protoplasts	Yasugi *et al*., 1986
	Protoplasts from flowers	Oshiro and Steinhart, 1991
Epidendrum radicans	Protoplasts	Yasugi, 1990
Epiphronitis	Shoot tips	Kusumoto, 1981
Eulophia hormusjii	Rhizome segments	Vij, Sood & Pathak, 1989
Geodorum densiflorum	Protoplasts	Seeni & Abraham, 1986
Grammatophyllum elegans	Protoplasts	Seeni & Abraham, 1986
Hetaeria	Creeping rhizome sections	Yam, 1989
Holttumara	Apical and axillary buds	Lim-Ho, 1981
Kagawara	Apical and axillary bds	Lim-Ho, 1981
Laelia	Shoots	Kako, 1973
Laeliocattleya	Shoot tips	Ishii, 1974
	Leaf tips	Arditti *et al*., 1971

TABLE 1. *(continued)*

Genus	Explant	Reference
Liparis nervosa	Stem sections	Yam, 1989
Liparis rigida	Leaf tips	Yam, 1989
Ludisia (Haemaria)	Single node sections	Teo, 1978
Luisia trichorhiza	Leaf segments	Vij & Pathak, 1988a
Luisia zeylanica	Protoplasts	Seeni & Abraham, 1986
Lycaste	No details of any kind	Morel, 1965a, b
Malaxis acuminata	Stem sections	Yam, 1989
Maxillaria tenuifolia	Protoplasts	Price & Earle, 1984
Miltonia	No details of any kind	Morel, 1965a, b
	Shoot tips	Kusumoto, 1981
Mokara	Flower buds	Lim-Ho, Teo-Lee & Phua, 1984
	Axillary buds	Lim-Ho, 1981
	Inflorescence tips	Abdul Ghani *c.*1988 as reported in Arditti & Ernst, 1983 (the following papers were just being written then: Abdul Ghani, Haris & bin Haji Ujang, 1992b; Abdul Ghani & Haris, 1992)
	Young leaves	Abdul Ghani *c.* 1988 as reported in Arditti & Ernst, 1993 (the following papers were just being written then: Abdul Ghani *et al*., 1992b; Abdul & Ghani Haris, 1992
Mormodes	Pseudobulb sections	Hölters & Zimmer, 1990a
Mormodes histrio	Root explants	Hölters & Zimmer, 1990b
Neofinetia falcata	Embryogenic callus	S. Ichihashi[b]
Neostylis	Various	Sagawa & Kunisaki, 1982
Neottia	Root	Champagnat, 1971
Nigritella	Tubers	Haas, 1977a, 1977b
Oberonia	Protoplasts	Seeni & Abraham, 1986
Odontoglossum	No details of any kind	Morel, 1965a, b
	Apical and axillary buds	Khaw, Ong & Nair, 1978a, b
	Protoplasts	Price & Earle, 1984
Oncidium	Flower stalks	Lim-Ho & Lee, 1987
	Apical shoot explants	Khaw *et al*., 1978a, b
	Apical buds	Lim-Ho, 1981
Oncidium papilio	Flower stalk tips	Fast, 1973
Ophrys apifera	Tuber sections	Champagnat & Morel, 1972
	Tuber and shoot base	Hoppe & Hoppe, 1987a, b, 1988
Ophrys bombylifera	Protoplasts	Pais *et al*., 1983
Ophrys fuciflora	Tuber sections	Champagnat & Morel, 1972
Ophrys lutea	Protoplasts	Pais *et al*., 1983
Orchis coriophora	Bud meristems	Allenberg, 1976
Orchis maculata	Tuber sections	Thomale, 1956 (first suggestion that mass rapid clonal propagation of orchids *in vitro* is possible), 1957 (first photograph and report of a regenerated culture *in vitro*)
Pachystoma senile	Tubers	Vij *et al*., 1983
Paphiopedilum	Protoplasts	Yasugi, 1989a, b
Paphiopedilum insigne	Protoplasts	Yasugi, 1990
Paphiopedilum species and hybrids	Shoot tips	Bubeck, 1973
	Leaf tips	Allenberg, 1976
	Protoplasts	Price & Earle, 1984
Phajus (misspelled as *Phaius*)	No details of any kind	Morel, 1965a, b

TABLE 1. *(continued)*

Genus	Explant	Reference
Phalaenopsis	Inflorescence nodes	Rotor, 1949; first time an orchid, or any plant, was propagated vegetatively *in vitro*
	Shoot tips	Intuwong & Sagawa, 1974
	Leaf tissue	Koch, 1974
	Root tips	Tanaka, Senda & Hasegawa, 1976
	Inflorescence internodes	Lin, 1986
	Protoplasts	Teo & Neumann, 1978a, b; Chen *et al*., 1990 Sajise *et al*., 1990
	Plants from protoplasts	Kobayashi *et al*., 1993
	Embryogenic callus	S. Ichihashi[b]
	Electrofusion of protoplasts	Chen *et al*., 1990, 1991, 1995
Phalaenopsis amabilis	Protoplasts	Yasugi, 1990
Pholidota cantonensis	Root tips	Yam, 1989
Pholidota chinensis	Leaf tips	Yam, 1989
Phragmipedium	Axillary bud explants	Stokes, Thomas & Holdgate, 1975; paper did not present any details and should be questioned
	No details of any kind	Morel, 1971b, c
	Flower buds	Fast, 1973
Pleione	Shoot tips	Weatherhead & Harberd, 1980
Renantanda	Leaf explants	Goh & Tan, 1982
	Shoot tips	Abdul Ghani as reported in Arditti & Ernst, 1993 (Abdul Ghani, Haris & bin Haji Ujang, 1992a was just being written)
	Protoplasts	Teo & Neumann, 1978b, c
Rhynchostylis gigantea	Buds and shoot tips	Vajrabhaya & Vajrabhaya, 1970
Rhynchostylis retusa	Leaf segments	Vij, Sood & Plaha, 1984
	Root segments	Sood & Vij, 1986
	Protoplasts	Seeni & Abraham, 1986
Rhynchostylis retusa	Root- and leaf-tips	Sharma & Chaturvedi, 1988
Schomburgkia superbiens	Shoot tips	Scully, 1967
Sophrolaeliocattleya	Shoot tips	Kako, 1969
Spathoglottis	Flower buds	Intuwong & Sagawa, 1974
Spathoglottis plicata	Rhizomes	Bapat & Narayanaswami, 1977
	Protoplasts	Seeni & Abraham, 1986
Thunia alba	Flower stalks	Singh & Prakash, 1984
Vanda	Leaf tips	Chaturvedi & Sharma, 1986
	Inflorescences	Valmayor, Pimentel & Martinez, 1986
	Leaf explants	Tanaka, Hasegawa & Goi, 1974
	Protoplasts from flowers	Oshiro & Steinhart, 1991
	Embryogenic callus	S. Ichihashi[b]
Vanda coerulea	Leaf bases	Anonymous, 1987, Seeni, 1988
Vanda Miss Joaquim	Shoot tips	Kunisaki, Kim & Sagawa, 1972
	Stems	Sagawa & Sehgal, 1967
	Axillary buds and root-tips	Goh, 1970
Vanda praemorsa	Protoplasts	Seeni & Abraham, 1986
Vanda sp.	Protoplasts	Sajise *et al*., 1990
Vanda testacea	Root tips	Vij & Pathak, 1988b
Vanda TMA X *V. teres*	Root- and leaf-tips	Sharma & Chaturvedi, 1988
Vandofinetia	Not mentioned	Intuwong & Sagawa, 1974
Vanilla planifolia	Nodal stem sections	Kononowicz & Janick, 1984
	Lateral buds	Gu, Arditti & Nyman, 1987
	Aerial root-tips	Philip & Nainar, 1986
	Axillary buds	Cervera & Madrigal, 1980

TABLE 1. *(continued)*

Genus	Explant	Reference
	Protoplasts	Seeni & Abraham, 1986
Vascostylis	Inflorescences	Intuwong & Sagawa, 1973
Vuylstekeara	Shoots	Kukułczanka *et al*., 1989

[a]Genera are listed alphabetically; the reference given is the first known for each genus and/or type of explant. Some of the genera listed here are hybrids. Their parentage and year of registration are listed below.

Hybrid genus	Parentage	Year of registration
Aëridachnis	*Aerides* X *Arachnis*	1954
Arachnostylis	*Arachnis* X *Rhynchostylis*	1966
Aranda	*Arachnis* X *Vanda*	1937
Aranthera	*Arachnis* X *Renanthera*	1936
Ascocenda	*Ascocentrum* X *Vanda* (some *Vanda* species were transferred to the genus *Euanthe*, see also *Schlechterara*)	1949
Ascofinetia	*Ascocentrum* X *Neofinetia*	1961
Brassocattleya	*Brassavola* X *Cattleya*	1889
Burkillara	*Aerides* X *Arachnis* X *Vanda*	1967
Darwinara	*Ascocentrum* X *Neofinetia* X *Rhynchostylis* X *Vanda*	1980
Doritaenopsis	*Doritis* X *Phalaenopsis*	1935
Epiphronitis	*Epidendrum* X *Sophronitis*	1890
Holttumara	*Arachnis* X *Renanthera* X *Vanda*	1958
Kagawara	*Ascocentrum* X *Renanthera* X *Vanda*	1968
Laeliocattleya	*Cattleya* X *Laelia*	1887
Mokara	*Arachnis* X *Ascocentrum* X *Vanda*	1969
Neostylis	*Neofinetia* X *Rhynchostylis*	1965
Renantanda	*Renantherra* X *Vanda*	1935
Schlechterara	*Ascocentrum* X *Euanthe* X *Vanda* (some *Vanda* species were transferred to the genus *Euanthe*, see also *Ascocenda*)	1966
Sophrolaeliocattleya	*Cattleya* X *Laelia* X *Sophronitis*	1897
Vandofinetia	*Neofinetia* X *Vanda*	1960
Vascostylis	*Ascocentrum* X *Rhynchostylis* X *Vanda*	1964
Vuylstekeara	*Cochlioda* X *Miltonia* X *Odontoglossum*	1911

[b]Personal communication from Dr. Syoichi Ichihashi, Department of Life Science, Aichi University of Education, Aichi, Japan.

asparagine and peptone in various combinations and concentrations" (Krikorian & Berquam, 1969);[31] (4) his choice of plants included monocotyledonous species which can be recalcitrant; (5) he had no previous information to guide him; and (6) his cultures, although sanitary, were not aseptic (Haberlandt, 1902 translated by Krikorian & Berquam, 1969).

Not as well known as his work with orchids, but well ahead of its time was Lewis Knudson's attempt to culture sloughed-off root cap cells of maize and Canada field-pea (Knudson, 1919). As a culture medium he employed water and, foreshadowing his work with orchids (for a review see Arditti, 1990), 'Pfeffer's Solution' modified through the use of monobasic potassium phosphate instead of the dibasic salt and with or without 0.5% sucrose. Some of the Canada pea cells survived for 50 days when roots were present in the culture medium. They lived for another 21 days after the roots were removed despite becoming contaminated. These experiments suggested the diffusion from roots of growth substances which the cells needed, but it needs to be borne in mind that this work was done: (1) 8 years before the discovery of auxins (Went, 1928, 1990; Thimann, 1980); (2) 20 years before it was shown that vitamin B_1, niacin and other factors enhance the growth of stem sections in general, and excised roots *in vitro* in particular (Bonner, 1937, 1938, 1940a, b; Addicott & Bonner, 1938; Bonner & Devirian, 1939); (3) 35 years before the discovery of cytokinins (Miller *et al*., 1955a, b; Miller, 1961, 1977; Skoog, 1994). Even if he surmised that his cells needed growth substances, few if any were available at the time and Knudson's attempts failed. However, it is surprising that he did not use aseptic techniques since the procedures were available in his laboratory by that time, even though this research was carried out 1–2 years before he started work on non-symbiotic germination of orchid seeds (for a review see Arditti, 1990).

The first isolated cells to be cultured successfully were those of tobacco, *Nicotiana tabacum* and marigold, *Tagetes erecta*. They were grown on filter paper platforms placed on top of proliferating callus masses (for reviews see Muir, Hildebrandt & Riker, 1954, 1958; Steward & Krikorian, 1975; Krikorian, 1975, 1982; Gautheret, 1983, 1985). Proof that the colonies on the platforms did not form from cells of callus origin that grew through the paper was obtained by culturing a single cell of *Tagetes erecta* on a platform placed on top of a sunflower callus (Muir *et al*., 1958). Other research followed (Bergmann, 1960) and convincing proof that a single isolated cell can divide and eventually yield a large callus mass was provided by tobacco cells which divided in drops of medium in microculture (Vasil & Hildebrandt, 1965a, b). Shortly after that, isolated mesophyll cells of *Arachis hypogea* were prompted to divide in culture and produced what can be described as protocorm-like bodies or structures which look like them (Joshi & Ball, 1968a, b).

Using an apparatus that slowly (1 r.p.m.) rotates 'nipple' culture flasks around a horizontal axis, F[rederick] C[ampion] Steward (1904–1994; Fig. 51) and his associates at Cornell University, Marion O. Mapes (1913–1981; Fig. 52) and Kathryn Mears (Fig. 53) obtained suspension cultures of carrot cells and eventually regenerated plants from them (for reviews see Krikorian, 1975, 1982, 1989b; Steward & Krikorian, 1975; Gautheret, 1983, 1985; Arditti & Ernst, 1993). The same technique was used to obtain the first orchid cell suspension cultures from which plants, those of *Cymbidium*, were regenerated (Steward & Mapes, 1971a). More recently *Phalaenopsis* plants have been regenerated from embryoids derived from a loose-celled callus (Sajise, Valmayor & Sagawa, 1990). See Krikorian (1996) for a discussion of 'free' cell culture and related terminology.

Protoplast preparations were first obtained in 1892 by surgically releasing them from plasmolysed cells of the water aloe *Stratiotes aloides*. Digestion of cell walls became the eventual method of choice and (for historical surveys see Steward & Krikorian, 1975; Krikorian, 1982). What is probably the first preparation of orchid protoplasts resulted from work with leaves (i.e. mesophyll cells) of *Cymbidium* Ceres and virus free protocorms of *Cymbidium pumilum*, *Brassia maculata* and *Cattleya schmobocattleya* (Capesius & Meyer, 1977).[32] The protoplasts were used for the isolation of nuclei but apparently no effort was made to produce callus masses or regenerate plants from them.

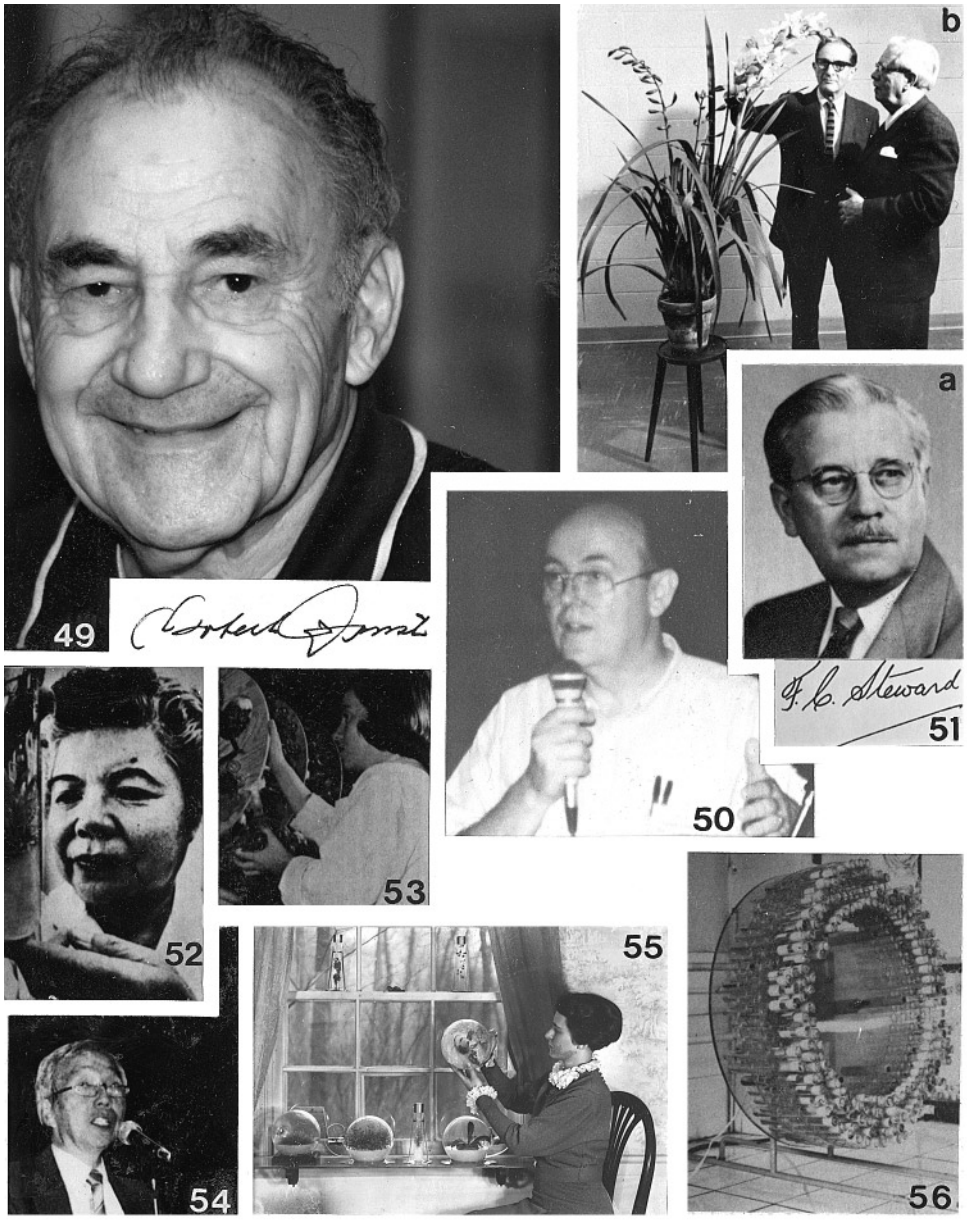
Production of orchid protoplasts and subsequent fusion between and within genera was first reported in 1978, but the ultimate fate of the fusion products has not been described in the literature (Teo & Neumann, 1978a, b, c). Subsequent isolations of orchid protoplasts have been reported from Portugal (Pais, Anjos, & Rangel de Lima, 1982, 1983); the U.S.A. (Price & Earle, 1984; Sajise *et al*., 1990), Singapore (Loh & Rao, 1985; Hew & Yip, 1986; Hew, 1987; Koh, Goh & Loh, 1988), Japan (Yasugi, Kagimiya & Katsura, 1986; Yasugi, 1986, 1989 a, b, 1990; Kobayashi, Kameya & Ichihashi, 1993), India (Seeni & Abraham, 1986; Gopalakrishnan & Seeni, 1987), and Taiwan (Chen *et al*., 1990). More recently, 4×10^6 protoplasts were obtained per gram of young leaf tissue of *Phalaenopsis* (Chen *et al*., 1995). Their average diameters were 31.2 μm. The diameters of protoplasts from root tips and petals were 36.4 μm and 31.1 μm respectively. Approximately 90% of the protoplasts were viable. Some of the protoplasts divided after 5 days. Only a few divided twice after 10 days. Very few clusters were formed after 21 days, and they eventually died (Chen *et al*., 1995). However, Yoneo Sagawa (b. 11 October 1926; Fig. 54) and his co-workers at the University of Hawaii have reported regeneration of *Phalaenopsis* plants from protoplasts (Sajise *et al*., 1990).

A direct electrical current pulse of 2500 V cm^{-1} of 2 milliseconds duration was sufficient to cause fusion in 10% of *Phalaenopsis* protoplasts to form hybrid cells. The fate of these fusion products was not described, but the context of the paper (Chen *et al*., 1995) suggests that they did not survive. Studies of transgenic *Phalaenopsis* are also in progress (Chen *et al*., 1995).

CONCLUDING REMARKS

Orchids were the first plants to be propagated *in vitro*, from seeds (symbiotically by Noël Bernard in France *c*. 1900 and asymbiotically by Lewis Knudson in the U.S.A. in 1921), or clonally (for reviews see Arditti, 1967, 1977, 1979, 1990, 1992; Arditti *et al*., 1982; Arditti & Arditti, 1985; Arditti & Ernst, 1993). The first papers on *in vitro* seed propagation were published in well-known scientific journals with complete details on all aspects of the procedures in line with accepted scientific standards. This was not the case with all of the early, and even several later papers on *in vitro* clonal propagation of orchids. Most of the papers were not peer-reviewed and were published in journals intended for orchid hobbyists. As a result, early publications often either remained unnoticed or did not meet all accepted scientific standards. They frequently lacked sufficient detail to enable duplication of results.

A person who could have gained substantial credit for her work in the early use of aseptic techniques to propagate plants but did not do so because none was published is Clara S. Hires (1897–1984; Fig. 55). She was encouraged by her family



Figures 49–56. Persons and equipment associated with *in vitro* culture of orchids and other plants. Fig. 49. Professor Robert Ernst (photograph by J.A., signature from J.A.'s files). Fig. 50. Mr Gene Crocker (photograph taken by J.A. in Brisbane, Australia in 1984). Fig. 51. Professor F.C. Steward, alone (a) and with Mr Russell C. Mott (b) admiring a *Cymbidium* plant derived from a cell suspension cultured by Mrs Marion O. Mapes (Fig. 52) under his direction. Mott was in charge of the Conservatory of the Liberty Hyde Bailey Hortorium at Cornell University and reared the 'free' cell-derived orchids to maturity. Fig. 53. Miss Kathryn Mears (now Mrs Trupin) placing 'nipple' culture flask of carrot cells on a rotating wheel or 'auxophyton' in F.C. Steward's laboratory in the Plant Sciences Building (Figs 51–53 from photographs and documents in the possession of A.D.K.; see also Krikorian, 1989). Fig. 54. Professor Yoneo Sagawa lecturing in Nagoya, Japan in 1990). Fig. 55. Miss Clara S. Hires examining a flask near a window (photograph supplied to A.D.K. by Clara Hires). Fig. 56. A rotating drum shaker for cultures (Morel, 1966).

to spend time in Knudson's laboratory after she had gotten her undergraduate degree at Cornell in the late 1920s so as to learn about asymbiotic orchid seed germination. The aim was to keep up supplies of vanilla for the family owned Hires Root Beer Company. Later Miss Hires operated a biological supply house under the title of 'Mistaire Laboratories' in Milburn, New Jersey. It provided various and sundry items like ferns in different stages of development to schools and colleges etc. These were aseptically propagated and/or multiplied *in vitro*. Pictures of the laboratory when it was in full operation are impressive and in many respects it was state-of-the-art. However, virtually none of the aseptic culture work was published (Hires, 1940). No doubt she was a pioneer in the industrialization of aseptic methods for propagating plants in the U.S.A. She not only grew lower plants (some of which increased vegetatively 'spontaneously' *in vitro*) but she was concerned with cinchona and, of course, orchids (Krikorian, 1979, 1982:171). Had Clara Hires published, one would perforce credit her work with lower vascular and non-vascular plants as pre-dating the higher plant 'micropropagation' work of Gavino Rotor Jr. with *Phalaeonopsis*.

We have already discussed the situation where workers suggest, peripherally or even directly, a process or a use but do not follow through on it (Curtis & Nichol, 1948 and p. 187 of this paper). In a similar vein, one may mention the carrying out of a process by an investigator not fully appreciating the potential of what he or she has accomplished. Finally, there are situations where valuable discoveries are not appreciated for a number of reasons.

From our more narrow perspective on orchid micropropagation, the problem caused by not strictly adhering to the rules of scientific publication was that the procedures did not become promptly available to all scientists and practical growers. As it turned out, only a single French commercial orchid establishment managed to obtain the information needed to produce plants from stem tips on a commercial scale. They held a monopoly for a long time. Growers wishing to have increased stocks of plants were obliged to send materials to France for 'mericloning'. These activities pre-dated substantially the development of 'new' technologies for germplasm exchange of *in vitro* materials (Roca, 1984; Krikorian, 1994a, b and references therein). Partial publication of orchid micropropagation research has made it difficult to unravel fully the 'muddied' historical track and adjudicate or resolve some largely unprovable or incorrect claims of priority in discovery. It is interesting that once perceptions of priority of discovery become established, they are indeed maintained by 'resistance to knowledge'.

'Resistance to Knowledge' is the title of a prefatory chapter by Hans Gaffron (1902–1979), a plant physiologist of note who worked on certain aspects of photosynthesis (Gaffron, 1969). While the chapter does not have much to do directly with photosynthesis or plant physiology, it points out eloquently that established institutions and dogma do not take kindly toward new ideas or challenges to 'proven' or generally accepted 'truths'. Two points in the chapter are of particular relevance to the question of how micropropagation came about. The first concerns 'Heisenberg's formulation', which states that 'science clears the fields on which technology can build'. In turn, technology delivers to science bigger and better bulldozers to clear the fields ever faster (Gaffron, 1969). This is relevant to orchid micropropagation history because most discussions of the subject by-pass early research and many pioneers. They ignore the clearing of the fields and start with, or

even showcase secondary developments and personalities that have attracted the most attention (i.e. louder and bigger bulldozers).

A second point made by Gaffron is that "those establishments whose power rests with God-given ignorance and thoughtlessness of their subjects rightly recognize free-science as an ever-present threat". He continues, "in the twentieth century … the refined modern way of minimizing the subversive power of research is to distract the minds of scientists from certain problems by offering them unheard-of-opportunities to run after … others …". In the context of orchid micropropagation the 'establishments' held generally accepted and widely held views which were, in large measure, derived from flawed information, unnoticed or ignored truths and/or constant repetition. The resistance to knowledge is indeed a fierce protector of these 'establishments'. Gaffron's "free-science" and the "subversive power of research" can be equated with attempts to seek and present facts which shake the foundations of the 'establishments' as well as the science and technology that preceded secondary discoveries. This, in turn, may eliminate the pedestals which support established but not always deserving idols. There is much in Gaffron's scholarly and entertaining essay to appeal to iconoclasts.

There is an extensive literature on what makes scientists 'tick', revealing how egos come into play in the scientific process (Schilling, 1958; Snow, 1961; Merton, 1973; Müller-Hill, 1993). Increasingly, attention has been given to how some scientists even come to 'betray the truth' (Broad & Wade, 1982), or go so far as to re-write history.[33,34]

In addition to recognizing the human aspects of the problem, it is instructive to consider the orchid micropropagation story in the context of the 'revolution' in plant biotechnology. From the outset, there was substantial confusion even among so-called experts about what was really achieved. F.C. Steward and A.D. Krikorian felt the need to denounce the increasingly common and disturbing trend in the reporting of plant 'biotechnology' research (Steward & Krikorian, 1975, 1979): "research, instead of an avocation quietly conducted by the few with limited means and little thought of priority or publicity [as in the so-called good old days], must now face the continued realities of funding. The temptation is to exploit ever-narrower objectives and, in the struggle for survival, minutiae are exaggerated, possibilities seen soon masquerade as realities, and, often aided by television and radio, the public or industry becomes involved so that patents and propaganda soon confuse the trail" (Steward & Krikorian, 1979:222; Krikorian, 1982, 1988, 1989a).

Plant scientists learned very early that it is virtually impossible to patent the processes of growth. It is easier to patent the products of plant breeding efforts and there are now many plant patents to protect 'breeders' rights'. It may be argued that perhaps the maximum commercial advantage for those interested in utilizing a new biotechnology process is derived from obtaining early access to information. This can be far more advantageous than attempting to defend a so-called process patent because it is, in the final analysis, relatively easy to achieve a given end in plant tissue culture using a variety of strategies. Sooner or later the competitors will have figured it all out. The 'sooner' is, however, what really matters from the commercialization perspective!

The technology of meristem culture was fairly simple to implement in a great many genera. However, it is very often a simple point of procedure that can make a major difference in the ease or efficiency with which a technique may be implemented. A nominally 'minor' procedural detail, overlooked or unappreciated

by one investigator but reported by another, can allow a person who has reached a 'bottle-neck' to proceed.

Morel's first orchid 'meristem' paper reports the use of a semi-solid nutrient medium. The use of such a medium limits the responsiveness of meristem explant, and a fair amount of time, ranging from days to weeks, is required to get the system going. A major procedural innovation was the adoption of a liquid nutrient medium and gentle shaking on a rotary 'shaker' (Fig. 44) which significantly speeded up the establishment of cultures. "Morel by 1960, was able, however, to grow *Cymbidium* meristems, and in 1963 Wimber grew clonal populations from rotating liquid meristem cultures of *Cymbidium* apices, using a mineral medium with sucrose and tryptone supplements" (Morel, 1974:177–178). This seems to indicate that Morel's group, at the outset at least, would have been severely limited in terms of the efficiency of the process for serious production purposes. Efficiency would have improved dramatically, however, upon adoption of liquid media and culture rotation.[35] As late as 1964 the indication was that 1.2% agar was being used in 'Knudson C' medium for *Cymbidium* etc. (Morel, 1964c:735). However, a photograph (Fig. 56) of a rotation aparatus for *Cattleya* was included by Morel in a 1965 article published in German (Morel, 1965b).

Again, it is significant to recognize that use of liquid medium for various tissue cultures was not 'invented out of the blue' and in fact, early in its history it encountered considerable criticism from the small, tightly 'networked' and sometimes arrogant 'tissue culture establishment'.[36]

Caplin and Steward developed an apparatus, the 'auxophyton', that rotated T-shaped tubes around a horizontal axis (Caplin & Steward, 1949). The tumbling action causes explants and cells to be alternately bathed in liquid nutrient and exposed to air (Steward, Caplin & Millar, 1952). Animal culture workers used roller tubers which bathed cells in liquid in their work whenever they could (the tubes apparently date back to a suggestion by Alexis Carrel (1873–1944) as early as 1913 (Gey, 1933)). Variations of the Steward & Caplin 'auxophyton' were adopted in several laboratories because liquid and appropriate aeration improved growth of carrot root plug cultures over what could be obtained on semi-solid media. For their early cell culture work, Albert J. Riker (1894–1982) and his co-workers at Wisconsin used a drum aerator that was a modification of the 'auxophyton' described by Caplin and Steward (Muir *et al*., 1958: fig. 2).

Phillip R. White (1953), mentioned above as one of the nominal 'inventors'[37] of plant tissue culture, took issue with the claims for increased growth. Roger J. Gautheret in France was no less displeased with the liquid culture work of Caplin and Steward, for his laboratory had concluded that liquid media presented nearly insurmountable problems. Gautheret and his student Heller devised a means of supporting cultures on a filter paper raft which drew nutrients by capillarity (Heller & Gautheret, 1949).

Further examination from a historical perspective of the particulars associated with the incorporation of liquid culture methods into the cloning protocols and the advantages that derived from this may well confirm one day that this was the pivotal development in orchid mericloning. Be that as it may, these points serve to underscore that it will not be possible to resolve all the issues relating to who made the most important contributions to the development of orchid micropropagation, and when they did it.

It is clear that Georges Morel was only one of the major participants in the

process. The work of many others, which received far less publicity, paved the way and provided seminal information. With most of the key participants now deceased, and crucial laboratory and business records not available, it will perhaps never be possible to fully re-construct the events. Nevertheless, if this account serves to provide a more historically precise record of the role of the many participants who were involved than has hitherto been attempted, then our efforts will have been justified.

ACKNOWLEDGMENTS

I dedicate my contribution to Professor Emeritus John L(uther, not Lucifer) Mohr, University of Southern California in Los Angeles for introducing me during my days as a graduate student to the pleasures and rewards of tracing the history of biology and to Professor Emeritus Arnold Samuel Dunn, also of U.S.C. for making it possible for me to study it 30 years later. — J.A.

I dedicate my contribution to the memory of the late George H. Pride. For many years he taught the only botany course at high school level in the U.S.A. His high academic standards and demand for precision and sustained critical analysis instilled a love for botanical and horticultural inquiry in several generations of students at South High School, Worcester, Massachusetts. — A.D.K.

APPENDIX

All comments and notes (superscript numerals) listed in the body of the paper can be found in this Appendix.

1. Micropropagation was originally defined as any aseptic procedure involving the manipulation of plant organs, tissues or cells that produces a population of plantlets thereby making it possible to by-pass conventional sexual or vegetative propagation. In some areas the term has largely been restricted to the use of stem tips and lateral buds for clonal multiplication *in vitro* (Krikorian, 1982:170). For orchids it describes all *in vitro* clonal propagation (Arditti & Ernst, 1993).

2. Many of the photographs used in this paper are re-photographed copies of old, grainy, faded (with age), poorly printed (due to the technology of the time) sometimes on acid paper, small and/or occasionally scratched originals. Readers are asked to overlook the poor quality of some of the illustrations.

3. Dr Gavino Rotor Jr. provided biographical information about himself in a letter to J.A.

4. Clara S. Hires (1897–1984) pioneered in the U.S.A. the commercialization of propagation methods via aseptic seed and spore germination, especially of ferns but also a number of higher plants. She was able to apply and perfect a number of available laboratory techniques for producing aseptic plants.

5. Post acknowledged Rotor for his help in making drawings for his book on floriculture. Knudson was a consultant for the orchid section. However, Rotor's work is not mentioned in the book. The reason for this may be that both Post's book and Rotor's paper were published in 1949 allowing no lag time for inclusion. Also there were no new editions (only reprintings) of Post's book after 1949 (Post, 1959).

6. While working for the late Roy J. Scott in Bel Air, California 1957/1958–1960 J.A. tried Rotor's method. The first cultures failed and a second attempt was not made.

7. Recognizing that he had to understand more about factors controlling cell division, Haberlandt diverted his attention to that and what came to be called 'wound hormones' (Krikorian, 1975:71 ff.).

8. Like many other Dutch (German and other European) botanists before World War II, van Overbeek spent time at Buitenzorg. No doubt coconuts and their liquid endosperm, a popular beverage in the then Netherlands Indies, came to his attention.

The terminology of coconut 'milk' versus 'water' often presents a problem to those who live or have spent time in the tropics and/or are familiar with coconuts other than the mature, brown ones purchased in food stores (i.e. unripe, fresh green fruits with jelly-like solid endosperm and a clear liquid one). The colourless liquid endosperm of *Cocos nucifera* is usually referred to as 'coconut water'. 'Coconut milk', on the other hand, is the white liquid obtained by squeezing, grating or extracting the solid white 'meat' (i.e. the solid endosperm which is dried to make copra) of the coconut.

9. The story of the introduction of coconut 'milk' to aseptic 'tissue culture' media formulations is of considerable historical interest and complexity (for reviews see Steward & Shantz, 1955; Tulecke *et al*., 1961; Krikorian, 1975).

While much of the detail is beyond the scope of this paper, it is interesting to note that van Overbeek and co-workers initially concluded that at least three factors or complexes that affected *Datura* embryo growth were present in coconut milk: (1) a thermolabile factor that caused both growth and differentiation; (2) a thermostable principle that leads in some cases to callus-like growth but no differentiation; and (3) a heat-stable substance which inhibited root growth and was probably related to auxin. Van Overbeek found that non-autoclaved coconut water contained an embryo growth factor, and that there was a toxicity associated with the raw coconut milk. This toxicity could be largely overcome by treatment with 80% ethyl alcohol. The so-called embryo factor had to be of relatively low molecular weight since it was dialysable through a cellophane membrane. They also learned that the auxin content of coconut water could be increased by autoclaving or by treatment with diethyl ether. Autoclaved coconut milk added to the basal medium yielded an unorganized, callus-like growth of the *Datura* embryos but this was for all practical purposes ignored. They focused on the discovery that undifferentiated proembryos grew and became differentiated embryos in the presence of 'embryo factor'. Smaller embryos required a higher concentration than larger ones. It was suggested that the threshold value depended on the embryo's ability to synthesize its own growth factor.

The only attempt to identify the growth-promoting substances of coconut milk at that time (van Overbeek, Siu & Haagen-Smith, 1944) did not proceed to the point of identifying the compounds responsible for the effects but some concentration of activity was achieved through ethanol extraction. Eventually the effects of coconut water were attributed largely to the balance of ordinary and well-known, inorganic and organic nutrients rather than to the presence of unidentified substances.

10. Considerable chemical work has been carried out on coconut water since its first use in plant culture media but the task of identifying all the components became recognized as gargantuan. A number of sugar alcohols were isolated from coconut water more than three decades ago (Pollard, Shantz & Pollard, 1961). Several years later the cytokinin zeatin was isolated from it (Letham, 1968). Some concluded (Galston, 1969) that the identity 'problem' of the components was solved with the identification of zeatin (Letham, 1968; Skoog, 1994; for an alternative view and summary of what are believed to be still outstanding problems see Steward & Krikorian, 1971). Attempts have been made from time to time provide summary compilations of components. One such attempt (Tulecke *et al*., 1961) lists levels of sorbitol, *meso*-inositol and *scyllo*-inositol as being in the range of 15, 0.01 and 0.05 ppm respectively rather than 15.0, 0.10 and 0.50 gm/l. This error, recognized and acknowledged by Tulecke (gracious letter of apology to Pollard, Shantz and Steward is in possession of A.D.K.) has unfortunately crept into the literature (Raghavan, 1966:10 and Arditti & Ernst, 1993:47).

11. Since animal physiologists had already adopted the term kinins for unrelated, physiologically active molecules, cytokinin was selected as a term which meets the needs of plant physiologists (Skoog, Strong & Miller, 1965).

12. Carlos Miller also showed that some mycorrhizal fungi produce cytokinins (Miller, 1967, 1969; Crafts & Miller, 1974). This could have a bearing on our understanding of the physiology of orchid mycorrhizae and symbiotic seed germination.

13. Like Went, van Overbeek and many other European botanists, Goebel spent some time at the Buitenzorg Botanical Gardens (Dammerman, 1945).

14. Robbins eventually made many significant contributions to our understanding of the activity of vitamins in plant tissue cultures (Kavanaugh & Hervey, 1981).

15. Ascorbic acid (vitamin C), first isolated in 1928, was studied more intensively in 1933. Biotin was identified in egg yolks in 1936. Folic acid was crystallized from liver in 1947, yeast in 1947 and identified in 1948. Although not really a vitamin, *myo*-inositol was isolated from muscles in 1850, but was not used in plant tissue culture media until much later. Niacin (nicotinic acid) was first made by oxidizing nicotine in 1925. Pantothenic acid was isolated from liver and its structure was first elucidated *c*. 1940. The structure of riboflavine, originally isolated from eggs, was described in 1935. Thiamine was isolated from rice bran in 1910–1911, but its structure was elucidated only in 1926 (for a review of vitamins and orchids see Arditti & Harrison, 1977). Of the plant hormones used in tissue culture, auxins were discovered in 1928 (Went, 1928, 1990) and [cyto]kinins in 1955 (Miller, 1961). Information that vitamins and hormones are required by explants in culture started to accumulate in 1936–1938 (for a review see White, 1943; see also Schopfer, 1949 and Åberg, 1961).

One of the earliest records on the deliberate inclusion of inositol in a modern plant tissue culture medium is by Jacquiot (1951). Interestingly, results from use of inositol have generally been inconclusive (Åberg, 1961:423). It was not until the sugar alcohols sorbitol, *meso*- or *myo*-inositol and *scyllo*-inositol were isolated and identified as components of coconut water (Pollard *et al*., 1961) that inositol was rationalized as a potentially useful addition to culture media. Although from time to time it has been implicated in signal perception as part of the phosphoinositide system, it remains to be shown that it plays a major positive role in the growth of plant tissues *in vitro*. Nevertheless, routine addition of *myo*-inositol as part of the MS medium allows one to 'play it safe'. At least the addition seems to do no harm.

16. Taro corms were used to make *poi*, a paste which was a very important part of the diet of the Hawaiian people when Cook arrived in the islands. The leaves of taro, *Colocasia esculenta*, called *luau*, were used as a vegetable during cannibalistic feasts. Nowadays, 'luau' refers to a Hawaiian barbeque, more often than not, staged for tourists.

17. Loo Shih-wei (Chinese style; the western form, Shih-wei Loo appears on papers he published in the U.S.A.) returned to China not long after the completion of his studies. This coincided with the communist takeover. He became a well-known plant scientist there. During the Cultural Revolution Prof. Loo suffered considerably. He became active again after the 'Gang-of-Four' was overthrown and returned to the Shanghai Institute of Plant

Physiology. S.W. Loo and J.A. started to correspond in 1983. Despite advanced age and failing health, Loo is still active scientifically.

18. Ernest A. Ball came from NC State University to UC Irvine in 1968 and left after about 10 years. J.A. collaborated with him for a while on tissue culture of orchids, taro and redwoods. His manual dexterity, ability to excise minute tissue sections (see for example Ball, 1972), and capacity for work were phenomenal.

19. While at UCI Ball used coconut water for several culture media. There is no doubt that by 'coconut milk' he means 'coconut water'. In Britain, continental Europe and the U.S.A. the term was, and still is, normally used for what ought to be termed 'coconut water'.

Ball, best described as an experimental plant morphologist, was one of the first to use coconut 'milk'. He did this in the attempt to culture stem segments of certain dicotyledons. Ball found that tissues subjacent to the stem tip of garden nasturtium *Tropaeolum* and lupin, *Lupinus* grew best in liquid media to which coconut milk was added. One of us (A.D.K.), while seated next to Professor Ball at a banquet in 1964 learned that he believed his role in establishing the value of coconut milk in plant tissue culture was pivotal. It is noteworthy therefore that Ball wrote in 1946 that [his findings] "… argue against the possibility that coconut milk constituted a causative agent in that growth. This liquid endosperm appears merely to have brought certain organic substances to the culture medium, without which these small bits of tissue would not have displayed cell division and growth. Its utilization here may be compared to that in growth of small *Datura* embryos in vitro by van Overbeek, *et al.* …"

20. An examination of Ball's publications indicates that he appears not to have appreciated (or perhaps cared about?) the significance of his own observations from an applied or practical perspective. This view is justified because Ball could have at least mentioned in passing the potential for mass multiplication of plants for horticultural purposes when he wrote an article on regeneration of seedlings after surgically manipulating the central initials of the meristems (Ball, 1950). He discussed only the theoretical aspects.

21. Thomale's letter, his photograph and some information were provided by himself, Mr E. Lucke and Dr Norbert Haas-von Schmude (Lucke, 1974, 1994; Haas-von Schmude, Lucke & Arditti, 1995). They also provided information about Dr Lucie Mayer and her photograph. Thomale provided a copy of Dr Morel's letter to him of 15 December 1965. This letter shows that Morel was familiar with Thomale's work long before crediting it in print (Haas-von Schmude, Lucke & Arditti, 1995).

22. In a paper that summarized research during a number of years in his laboratory at Harvard, Wetmore (1954) stated in a footnote on page 22: "The author wishes to acknowledge his indebtedness to Dr Georges Morel whose cooperation made the *in vitro* culture techniques a reality in these investigations" (see also Wetmore & Wardlaw, 1951; Torey & Thimann, 1972). In fact, Morel had been invited to Harvard University by Wetmore to help set up a plant tissue culture laboratory (Torrey & Thimann, 1972:200).

23. The term protocorm was coined by the noted and long-time director of the Buitenzorg Botanic Gardens, Melchior Treub (1851–1910; for a photograph see Arditti, 1992) to describe a stage in lycopod development. Noël Bernard (1874–1911; for photographs see Arditti, 1990, 1992) applied it to orchids between 1899 and 1910 using it to describe the early corm-like stage of seed germination. Morel modified the term to describe the corm-like structures which were formed by the *Cymbidium* stem tips he cultured. By definition 'protocorm' is used only for the structures formed by seeds; 'protocorm-like body' (often abbreviated as PLB or plb) is reserved at present for the bodies which resemble protocorms but develop from explants (see Arditti, 1990, 1992; Arditti & Ernst, 1993 for more extensive discussions).

24. *Vuylstekeara* is a trigeneric hybrid between *Odontioda* (bigeneric hybrid: *Cochlioda* X Odontoglossum) and *Odontonia (Miltonia* X *Odontoglossum)*. A specific procedure for it was first published in 1989 in Poland (Kukułczanka *et al*., 1989). Until then the procedures used for *Cymbidium* (Morel, 1960, 1963, 1964a, b, 1970, 1974) were described as being suitable for it (Arditti & Ernst, 1993). Morel did develop procedures for *Miltonia* and *Odontoglossum* but did not provide details until 1974 (Morel, 1974).

25. In orchid parlance, 'flasking' (a noun incorrectly made into a verb) was initially used to describe the process of placing orchid seeds in culture (i.e. germinating them in flasks). In recent years its use has extended to the placing of explants in culture. 'Deflasking' (an even worse linguistic abomination) refers to the removal of seedlings or plantlets from a flask and planting them in a potting mix. 'Transflasking' (another linguistic horror) means transferring protocorms, protocorm-like bodies, seedlings or plantlets from flask to flask.

26. In 1958 Frederika Quak from the Institute of Plant Virology in Wageningen, Netherlands presented a paper in Colloque-Symposium 4, Diagnostic et Cure des Maladies à Virus (diagnosis and control of virus diseases) at the International Horticultural Congress in Nice organized by P[ierre] Cornuet (one of the plant pathologists who suggested shoot tip cultures to Morel) and C[laude] Martin (one of Morel's collaborators), of I.N.R.A., Versailles. The secretary of that symposium was Jean-Paul Marrou, Ing. Hort., Chargé de Recherches (I.N.R.A.), Station Centrale de Pathologie Végétale, Route de Saint-Cyr, Versailles (Seine-et-Oise, France). Georges Morel Dir. de Recherches à la Station de Physiologie Végétale (I.N.R.A.), 28 rue du Plateau Saint-Antoine, Le Chesnay (Seine-et-Oise, France) and M^{me} Morel were listed as attendees at the conference. In that presentation, which did not get published until 1961, Dr Quak focused on her work with potato and the use of White's medium [White, 1954] supplemented with "10 p.p.m. thiouracil, 0.1 p.p.m. 2,4-D or 0.1 p.p.m. IAA". (Quak, 1961:146). Nothing was said in the 1961 paper about orchids. There is no evidence that Morel presented anything at that meeting.

27. Quak and a colleague (Baruch & Quak, 1966) do not cite Morel's paper on *Cymbidium* as an example of an apical meristem culture that could yield a virus-free plant. In connection with their work on iris meristems they state that "best results were obtained with media based on that of Morel. Therefore the formula of that medium only is presented here: $\frac{1}{2}$ concentration Knop solution 1000 ml; Berthelot solution 0.5 ml; cystein 1 mg; adenine 5 mg;

hydrolysate of casein 200 mg; saccharose [sucrose] 20 g; agar 6 g; vitamin solution (containing calcium pantothenate 1 mg, inositol 100 mg, biotin 10 mg, nicotinic acid 1 mg, pyridoxin 1 mg, distilled water 100 ml. The media were adjusted to pH 6" (Baruch & Quak, 1966:271). Baruch and Quak started their experiment in January 1963 and used about 700 meristems of 'Wedgewood' iris. The abstract of the paper (in English) draws attention to the fact that the "medium of Morel (personal communication) gave the best results". The Dutch summary repeats the statement. So at least by January 1963 Morel was willing to divulge his nutrient medium recipe and it was published in full by Quak. This was three years after Morel's initial publication on orchids and one year before the French orchid firm of Vacherot and Lecoufle announced that they could propagate orchids via shoot tip cultures. Whether anyone could or did make a connection between Quak's paper and orchids is open to speculation.

28. It would be interesting to know whether a written request for the recipe of the nutrient medium would have elicited a positive response. We do not know if such requests were made regarding any of Morel's orchid media. No such requests would have been necessary for the potato stem tip medium because it was published. In his important work with potato meristems Kassanis (1957) states that … "the apical meristems were excised as described by Morel and Martin (1955). The medium in which the meristems were cultured was suggested by Dr G. Morel, but differs from the one which was described by him (Morel and Martin, 1955). It consists of $\frac{1}{2}$ concentration of Knop solution, 10 drops of Berthelot solution (Morel, 1948)…" Basil Kassanis spent a few months with Morel at Versailles in 1954 (Hirst & Harrison, 1988). At least one British grower made "arrangements … to visit Prof. Morel's laboratory in May of 1964 [and] found Prof. Morel and his staff extremely helpful and they taught [him] the technique, giving [him] details of the formula used to produce plants from meristematic tissue. Late in 1964 Morel also visited McBean [McBeans Orchids Ltd., Cooksbridge, Lewes, Sussex, U.K.] and [the grower] was privileged to work with him …" (Bilton, 1985). It is not clear whether these reciprocal visits with the British growers were made on a voluntary basis or as a consulting arrangement. Nevertheless, by May 1964 the French orchid firm of Vacherot and Lecoufle had in effect firmly established its monopoly.

29. An example of having achieved something without having fully recognizing its full significance may be cited from the commentary by Carl D. LaRue, an early pioneer of tissue culture (Krikorian, 1982). LaRue pointed out on page 39 of the discussion after Ralph Wetmore's Brookhaven presentation "that years ago (1936) I did succeed in getting growth of a meristem of *Radicula aquatica*, which is I now believe, *Nasturtium officinale*. I did succeed in growing just a short typical meristem into a whole plant. Now that seems much more remarkable to me in view of what Dr Wetmore has said, than it did before" (Wetmore, 1954).

30. Ironically, publications now bear the statement that they are 'advertisements' to conform with tax regulations. For example, in the prestigious *Proceedings of the National Academy of Sciences* of the U.S.A., one may read footnotes to the effect that "The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked 'advertisement' in accordance with 18 U.S.C. § 1734 solely to indicate this fact."

31. Haberlandt spent time in Buitenzorg (November 1891 to February 1892, see Dammerman, 1945:64) and several locations in tropical Asia (Haberlandt, 1910). Krikorian & Berquam (1969) raise the question of what might have happened "had coconuts been generally available in Berlin", but coconuts may not have caught his fancy.

32. There is no '*Cattleya schombocattleya*'. It is not clear if this was meant to be '*Cattleya, Schombocattleya*'. It could be '*Cattleya* or Schombocattleya', '*Cattleya* and *Schombocattleya*', or '*Cattleya* X *Schombocattleya*'. *Cattleya* is a naturally occurring genus. *Schomboccatleya* is a hybrid genus between *Schomburgkia* and *Cattleya*. The first hybrid, *Schombocattleya* Spiralis was produced in 1905 from the cross *Cattleya mossiae* X *Schomburgkia tibicinis* (Garay & Sweet, 1974).

33. In some institutions, attendance at lectures on 'ethical issues in science' are mandatory for graduate students and post-doctoral fellows. At one extreme is the scientist who unwittingly biases the gathering and interpretation of data, at the other extreme is outright fraud and data fabrication.

34. In 1923 the Nobel Prize in Physiology or Medicine was awarded to Frederick Banting and James J.R. Macleod for the discovery of insulin. Banting shared his prize money equally with Charles Best, and Macleod shared his with James B. Collip. It has been pointed out that after Banting's death in 1941 Best started to re-write history about the details of the discovery of insulin and worked diligently to promulgate the view that Banting had discovered insulin with his help only. It has been suggested that claims were made that "were unprovable, misleading, or inaccurate" Bliss, 1993:257).

35. This also holds in the case of excised apices of bananas and plantains. The system is much more quickly established in liquid than on semi-solid (Cronauer & Krikorian, 1984). Some orchid explants are also considerably easier to start or made to proliferate in liquid media (Arditti & Ernst, 1993).

36. For many years Phillip R. White and Roger J. Gautheret saw themselves as the 'arch-priests' of the subject, and generally acted publicly and in print as though they were the ultimate arbiters of all 'real' progress in the field (Krikorian, 1975). Gautheret refused to mention Rotor's work in one of his historical accounts even after he was sent a copy of the paper by J.A.

37. It has been stated that Phillip White was taught aseptic procedures by W.J. Robbins at the University of Missouri (Kavanaugh & Hervey, 1981:108).

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