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ON THE BEGINNINGS OF MODERN STUDIES OF PLANT MORPHOGENESIS: A TRIBUTE TO RALPH H. WETMORE'S CONTRIBUTIONS[1]

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Early interests

Ralph Wetmore was awarded his Ph.D. degree at Harvard University in 1924, after an undergraduate career at Acadia University in Nova Scotia, and entered into a lifelong study of the form and differentiation of plants. When K. V. Thimann went to Harvard in the fall of 1935, Ralph Wetmore approached him immediately. Wetmore was working with two graduate students, R. Goodwin and A. Delisle, on the relative growth of leaves, using parent plants of two species and their hybrids. The approach had been based on Huxley's algebraic treatment of the relation between width and breadth, $y = ax^b$. Goodwin was using a *Solidago* hybrid and Delisle an *Aster*. Wetmore wanted these two men shown how to determine auxin production in leaves so that they could try to relate the size and shape of the leaves to auxin production. Thimann had just previously shown Avery these methods at Cal. Tech. for his study of auxin formation in *Nicotiana* leaves (AVERY 1935). The quantitative character of this approach demonstrated very well the direction of Wetmore's thinking at the time, that is, the quantitative expression of morphological characters and their analysis in causal terms. The results, which were published later (DELISLE 1937, 1938; GOODWIN 1937; WETMORE and DELISLE 1939) were useful beginnings in this direction. Goodwin's study of *Solidago* in the rosette stage was the first demonstration that the latest leaf in the rosette exerts an inhibiting effect upon the next in succession, comparable to the inhibiting effect exerted by a bud upon the next one below it.

Wetmore came to the experimental and analytical study of plant development via a natural ontogeny. In his own morphological and anatomical studies of the thirties and early forties, he combined a concern for internal structure and external form with the developmental origins of both, especially in the context of their comparative and evolutionary origins. Together with his close associate, I. W. Bailey, he had pursued teaching and research on anatomical matters, collaborated on the expansion of the Harvard Wood Collection, and developed a group of research students with strong comparative anatomical orientation with special concern for wood structure. These interests were evident in his published work and that of his students, as, for example, the comparative anatomical studies in the Moraceae by TIPPO (1938). The seminal review by ESAU (1943) on primary vascular tissues provoked interest in the primary plant body and served as a stimulus for more intensive studies. Wetmore encouraged V. Cheadle to study specialization of

[1] This essay is a rather personal review of the way things developed at Harvard around Ralph Wetmore; it was prepared collaboratively without any real attempt to be comprehensive. It is thought to be reasonably accurate, or as accurate as could be, short of consulting Ralph Wetmore himself. The authors bear the blame for omissions or commissions.

vessels in the monocots (CHEADLE 1944), H. Miller to work out the anatomy of the embryo and seedling of *Phlox* (MILLER and WETMORE 1945, 1946) and A. Spurr to study the organization of *Pinus* (SPURR 1949). HEIMSCH (1951) reflected Wetmore's influence in his studies on vascular tissues in barley roots as did GREENIDGE (1952) in his interests on vessel length and water movement in trees. His interest in the vascularization of the nonseed plants was evidenced by publications on *Lycopodium* (WETMORE 1943) and *Equisetum* (GOLUB and WETMORE 1948).

The beginnings of modern plant morphogenetic studies

World War II naturally brought about a dislocation of graduate work. Then in 1945 faculty and students alike returned to the universities and to the research problems whose resolutions had gone unheeded during the war years. New recruits joined the veterans in reexamining the classic problems, trying novel approaches, more drastic methods, and working with new techniques. In plant development, a new assault was made on the age-old problem of what the nature and origin of plant organization are. The word "morphogenesis" became a touchstone, and attention focused on the shoot apex and its well-concealed secrets of organogenesis.

The work of Wetmore and his students thus naturally shifted from the descriptive analyses of plant embryos or embryonic shoot apices of lower and higher vascular plants to experimental manipulation of these same structures. E. Ball, appointed to the staff at Harvard after a postdoctoral fellowship in E. Sinnott's laboratory at Yale, had audaciously cut living shoot apices of *Lupinus* and *Tropaeolum* transversely into 500-μ segments and attempted to grow them in sterile culture with some success. He had found that the apical dome together with the youngest leaf primordia would grow into a whole plant if explanted onto a relatively simple nutrient medium, whereas subjacent cubes cut from the apex formed only callus (BALL 1946).

In England C. W. Wardlaw had concluded his tropical studies on diseases of the banana and took the chair of cryptogamic botany in Manchester, where he mounted a major new attack on the shoot apex, using surgical approaches that forced the plants, mostly ferns, into new expressions of potentialities and new developmental patterns. Students in Wetmore's laboratory in the late forties joined in the searching analysis of the papers that began to pour from Wardlaw's laboratory and by 1950 had themselves begun surgical attacks on the shoot apices of a number of other vascular plants. In several talks, Wetmore had indicated his feeling that the problems of vascularization in lower vascular plants, as in flowering plants, could only be effectively attacked at the experimental level by tissue culture techniques. He was impressed by the observations of R. J. GAUTHERET (1942) and of G. CAMUS (1949) on the formation of xylem and phloem in tissue culture masses. Wetmore invited Georges Morel, who had studied tissue culture methods with Gautheret, to come to Harvard as a research associate to set up a plant tissue culture laboratory. Morel arrived in 1949, and no time was lost in organizing such a laboratory. By 1951 tissue cultures of fern (*Osmunda*) callus, *Lycopodium* prothalli, and *Selaginella* gametophytes had been established (WETMORE and MOREL 1951*a*, 1951*b*). At this time no monocotyledonous tissue had been cultured except for *Asparagus* tips, which had been cultured earlier by Loo (1945); Morel achieved true callus cultures of monocots at Harvard with the tuber tissue of *Amorphophallus* and *Sauromatum* (MOREL and WETMORE 1951*a*).

Meristem culture

Apical meristem culture became a continuing challenge. The interesting generalization was made that culture of very tiny pieces 200–300 μ in length, lacking visible leaf primordia and cut from the apical dome or promeristem of many of the lower vascular plants, including the ferns, led to successful cultivation of whole plants, whereas among the angiosperms tested, whole plants could be grown only from much larger apices (500 μ or greater) excised with several of the youngest leaf primordia attached. The nutrient requirements for apices of vascular cryptogams were quite simple, comprising only mineral elements and sucrose. Even with elaborate media, containing, in addition, organic nitrogen, vitamins, and complex components such as coconut milk, angiosperm apices proved much more difficult to cultivate successfully (WETMORE 1954).

Focus on the activities, potentialities, and properties of the apical meristem in vascular cryptogams and angiosperms, both dicots and monocots, in Wetmore's laboratory led to research on morphogenesis in a number of new and exciting directions. Most apparent was the need for more information about the distinctive chemical and biological properties of the apical meristem. In collaboration with J. P. Nitsch, previously a student at Cal. Tech. and successor to Morel for a few years as research associate at Harvard, Wetmore began an exploration

of the shoot apex of *Lupinus* and *Syringa* for comparison with those of the lower vascular plants, *Adiantum, Selaginella, Lycopodium,* and *Equisetum.* Nitsch's interests in auxin physiology and metabolism led to attempts to determine auxin precursor levels in the apex (NITSCH and WETMORE 1952) and the development of more sensitive assays for auxin determination (NITSCH and NITSCH 1956). With the collaboration of F. C. Steward at Cornell University, Wetmore and Nitsch pursued analyses of the alcohol-soluble free amino acids and the amino acid residues in hydrolyzed proteins extracted from thousands of shoot apical meristems and tiny leaf primordia (STEWARD et al. 1954). The results of this phase of the work on apex culture were summarized by Wetmore at the Brookhaven meeting in 1953, a meeting which served as one of the first postwar colloquia for the exchange of ideas about plant morphogenesis, tissue culture, and problems of developmental control in plants.

It is interesting to attempt to trace the lines of research at Harvard which followed (and preceded) these studies focusing on the shoot apical meristem and its morphogenetic capacities. Several clear directions of study are apparent in retrospect. A continuing preoccupation with auxin physiology in the shoot apex was inevitable due to the proximity and continuous interchange with Wetmore's colleague at Harvard, K. V. Thimann. Collaborative work on the auxin relations in shoot and leaf development had preceded the apical meristem culture work. Wetmore and Gunckel published an interesting comparative morphological study on the short and long shoots of *Ginkgo* (GUNCKEL and WETMORE 1946*a,* 1946*b*). Later, this was extended with Thimann into a physiological investigation of the auxin relations of these two kinds of shoots (GUNCKEL and THIMANN 1949; GUNCKEL, THIMANN, and WETMORE 1949). Here the analogy was developed between the short shoot of *Ginkgo,* which unfolds but does not elongate, and the inhibited lateral bud of a dicot, which does not even unfold. The latter system was explored from an anatomical point of view by Wetmore's student R. GARRISON (1949). Both axillary short shoots and axillary buds develop into long shoots after removal of the terminal buds. Some years afterward, Wetmore directed P. Titman in a comparable study of the long shoots and single-leaved short shoots of *Cercidiphyllum* (TITMAN and WETMORE 1955) in which a similar attempt was made to correlate the behavior with auxin relations. As in *Ginkgo,* it appears that the short shoots are kept short by auxin coming from the meristem; that is, in both these plants, apical dominance is exerted on bud elongation, but not on leaf unfolding. Also, as in *Ginkgo,* the yields of auxin diffusing from short and long shoots were the same when the buds first opened, but soon fell to zero in the short shoot while increasing in the long shoot during its whole elongation phase. A direct attack on the hormone content and synthesis in the apical meristem itself was never made. Closest to it were the determinations of diffusible auxin in developing leaves of *Coleus* made by WETMORE and JACOBS (1953) but carried out more in the context of leaf development and abscission than in the relationship of leaves to their origin at the apex.

The experimental work on xylem differentiation can be traced directly from the ideas about the apical meristem and young leaf primordia as possible sources of hormones, especially of the auxin type. As a junior fellow in Wetmore's laboratory at Harvard, W. P. Jacobs had begun the development of experimental evidence relating regeneration of xylem in wounded *Coleus* stems to the auxin diffusing from young leaves (JACOBS 1952), a line of work he was to pursue later with great industry at Princeton. Torrey's research on xylem differentiation and vascular tissue pattern formation in roots of *Pisum* (TORREY 1953, 1957) had its inception in experiments and discussions with Jacobs in Wetmore's laboratory while Torrey worked with K. V. Thimann on his Ph.D. thesis on lateral root initiation.

In culturing apical meristems, Wetmore found that excised apices could be maintained somewhat longer by placing them on unorganized callus tissue. Experiments after the fashion of those by CAMUS (1949) establishing grafts between excised apices and callus tissue allowed an analysis to be made of the chemical influences of apices on xylem differentiation (WETMORE and SOROKIN 1955). Replacement of grafted buds by auxin-sucrose solutions led to the concept that hormones control the vascular patterns in tissue culture explants (WETMORE and RIER 1963; WETMORE, DEMAGGIO, and RIER 1964) and the subsequent ideas of the multiple-factor control of cytodifferentiation (see the review by TORREY, FOSKET, and HEPLER 1971). Efforts were made also to analyze physical effects on vascular tissue formation (BROWN and SAX 1962).

A related research direction concerned the morphogenesis of leaves. Having excised and extracted thousands of shoot apices for chemical analyses, researchers in Wetmore's laboratory found it was a reasonable matter to place leaf primordia of different ages and sizes into culture in an effort to establish the ontogenetic difference between a

leaf and a bud initial. This line of research was pursued by Steeves and his students, initially in collaboration with Sussex, in the broader context of the question of how fern leaves develop (SUSSEX and STEEVES 1953; STEEVES 1962).

Focus on the vascular cryptogams

Meristems of the shoot and root have as immediate precursors the meristematic state, which develops in the embryo, which in turn has as its precursor the fertilized egg. Wetmore's long-term research orientation centered on the problems of the embryonic state, its origin, and its perpetuation. In the vascular cryptogams, the morphogenetic sequences of zygote to mature sporophyte and spore to fully developed gametophyte offered materials with ready access for experimental manipulation and analysis. Over a period of years, Wetmore directed much of his own energies and those of many of his students into investigations of these morphogenetic pathways and problems. In the ferns, Ward tackled fertilization, embryogeny, and experimental isolation and manipulation of embryogenesis in *Phlebodium* (WARD 1954*a*, 1954*b*; WARD and WETMORE 1954). DeMaggio dealt with similar problems in *Todea* (DEMAGGIO 1961; DEMAGGIO and WETMORE 1961), and later Steeves and Whittier explored the natural and experimental conditions leading to apogamy in *Pteridum* (WHITTIER and STEEVES 1960, 1962). Leaf ontogenesis itself was studied *in extenso,* in relation to cellular events, correlation phenomena, and hormonal control (STEEVES and WETMORE 1953; BRIGGS et al. 1955; BRIGGS and STEEVES 1958, 1959; STEEVES and BRIGGS 1958, 1960). Developmental problems in other groups among the vascular cryptogams were also studied, for example, in *Lycopodium* (FREEBERG and WETMORE 1957, 1967).

With the availability of and interest in organ and tissue culture techniques in Wetmore's laboratory, it was inevitable that efforts would be directed toward the establishment of true tissue cultures, that is, unorganized tissues subject to unlimited culture in vitro, of tissues of some of the vascular cryptogams. The major collaborative effort in this direction led to the early establishment of cultured callus tissue from gametophyte tissues of the fern *Osmunda* (MOREL and WETMORE 1951*b*) and *Pteridium* (STEEVES and SUSSEX 1952). Peculiarities, both cytological and morphogenetic, of these tissues were described (PARTANEN, SUSSEX, and STEEVES 1955; STEEVES, SUSSEX, and PARTANEN 1955) and a correlation established between abnormal nuclear condition and developmental behavior.

Although always preoccupied with the lower vascular plants and their plasticity in development, Wetmore and his associates over the years moved back and forth from the vascular cryptogams to the angiosperms, seeking similarities and differences and generalizations. They studied the anatomy and development of shoot apices in ferns, *Lycopodium, Selaginella,* and *Equisetum,* and the angiosperms, *Lupinus, Syringa, Helianthus,* and *Parthenocissus* (WETMORE 1954); later, they added the vegetative and flowering apices of *Xanthium, Chenopodium, Glycine, Hyoscyamus,* and *Papaver* (WETMORE, GIFFORD, and GREEN 1959). Thus, anatomy and physiology, descriptive and experimental, in both lower and higher vascular plants, were a part of the research in Wetmore's laboratory after the war years—the period of the ascendancy of plant morphogenesis.

Innovator and interpreter of experimental morphogenesis

During the period beginning about 1945, Wetmore played an important role beyond that of experimentalist and innovator in experimental plant morphogenesis. It is in character and one of his special attributes to act as mediator and interpreter. During the period when experimental manipulations, physiological and biochemical analyses, and cytochemical methods were beginning to be applied to problems of plant ontogenesis and cytodifferentiation, it was (and is) important to have experienced and soundly based anatomists and morphologists attempting to understand and interpret the experimental results in as unprejudiced a fashion as possible. In this role Wetmore played an important part. In the late forties and fifties, when distinguished anatomists such as Esau, Foster, and Gifford were squared off against the new surgical experimentalists like Ball, Wardlaw, and Cutter, as well as the unconventional anatomists and cytologists like Buvat, Nougarède, and Gautheret, it was essential to have an expert intermediary. Thus, in 1951, Wetmore collaborated with Wardlaw in writing a pioneering review on experimental morphogenesis for the *Annual Review of Plant Physiology* (WETMORE and WARDLAW 1951) interpreting the new experimental findings, especially from Wardlaw's group, for the plant anatomists, on the one hand, and pointing out the relevancies to the plant physiologists, on the other hand. To cover this subject without recourse to half-tone plates was a tour de force which occupied them both for many months. Further interpretation and unification came from later reviews (WETMORE 1956, 1959) which began to encompass the primarily physiologi-

cal information then becoming available from tissue culture studies, such as hormonal effects in morphogenesis (SKOOG and MILLER 1957) and the work from Steward's laboratory on carrot cell suspension culture (STEWARD, MAPES, and SMITH 1958). The most recent and perhaps most comprehensive review, which embodies many of the ideas discussed above, is that published with Steeves (WETMORE and STEEVES 1971) in the multiple-volume work edited by F. C. Steward.

Later, Wetmore shared anatomical researches with proponents on both sides. Thus, Gifford came as a postdoctoral fellow to Wetmore's laboratory at Harvard and later Wetmore spent a sabbatical year on the Davis campus of the University of California in continuous discussion and exchange of ideas—interpreting the new experimental work in ways compatible with more classically oriented anatomical research. To be sure, surgical experiments do introduce abnormalities and uncertainties into the developmental process, and the burden of proof of the significance of experimental data for normal development rests upon the experimenter (ESAU 1965). Happily, this battleground has now been left behind, but during the early skirmishes, Wetmore served a unique role in the mediation that was necessary for a continued rapprochement.

In another context, Wetmore served as an effective bridge between plant anatomist and plant physiologist and was able, in collaboration with Thimann and later with Steeves and then with Torrey, to create a research atmosphere in which students were encouraged to reach across the gap and bridge it. Through a succession of graduate students and postdoctoral fellows who became anatomically oriented physiologists or physiologically oriented anatomists, Wetmore and his associates encouraged a new breed of experimentalists of the variety needed to attack problems of plant morphogenesis. Their students and their students' students continue to expand horizons in this field.

Since retirement from teaching in 1962, Wetmore has continued active research, first, for a period of collaborative research in Versailles with Morel and DeMaggio (WETMORE et al. 1964) and then with DeMaggio at Hanover. Since his return to Cambridge, he has occupied a real place in the ongoing research in plant morphogenesis at Harvard. Students of Thimann, Torrey, Briggs, Bogorad, and others have found Wetmore ever responsive to the excitement of research and new discoveries and ideas. As personal friend and confidant as well as research mentor, he continues to influence students of morphogenesis. The dedication to Ralph Wetmore of the recent pictorial text *Plant Structure and Development* (O'BRIEN and MCCULLY 1969) by T. O'Brien, student of Thimann, and M. McCully, student of Torrey, is one more clear expression of his ongoing contribution to the field of plant morphogenesis.

Directions for future research

One can foresee at least four areas of importance for the immediate future, areas of morphogenetic research which evolve from these earlier studies. Still, what needs most to be understood is the problem of the distinctive nature and organization of apical meristems, of both roots and shoots. Since returning to Versailles, Morel has focused on the potentialities and activities of the apical meristem, establishing practical methods of propagating genetically stable, virus-free propagules from "mericlones" in orchids (MOREL 1962, 1964), in important crop plants such as *Solanum* and *Asparagus,* and in a wide variety of flowering plants of interest to commerce (MOREL and MARTIN 1955; MOREL and MULLER 1964; MOREL 1971). The practical applications of these techniques, which go back in origin to the early Wetmore-Morel collaboration in the early 1950s, have hardly begun to be exploited. Orchids are just a striking example of how a whole plant industry can be changed in a few years. Recently, SMITH and MURASHIGE (1970) have resolved some of the problems of culturing very tiny angiosperm apices, and new possibilities are opened by this achievement.

Another approach to the nature of the shoot apex is being pursued by those who would know the metabolic activities and changes in the cells of the apical region. Here, the techniques of histology, cytochemistry, chemical analysis, and biophysical manipulation can contribute, and all are being used (see the recent extensive review edited by BERNIER [1970] on the conversion of a vegetative apex to a floral apex). Given the present emphasis and state of the art, we are perhaps more likely to discover the macromolecular synthetic events determining the activities of the shoot apex than we are to learn about the control mechanisms which may reside at hormonal or perhaps more subtle levels. But surely here is an area for imaginative new research.

A second area with great promise is plant embryogenesis. By a unique coincidence, within the past few years achievements in researches on in vitro cultivation of embryos excised from the ovule (see the review by RAGHAVAN 1966), on induced embryo formation from cultured vegetative tissues grown in vitro (see the review by HALPERIN 1969), and on embryogenesis from pollen cells in excised

cultured anthers leading to haploid progeny (NITSCH and NITSCH 1969) have suddenly come together. As a result, the field of experimental plant embryology is now opened wide to new and exciting kinds of work.

A third and closely related type of research, early appreciated and used as a tool by Wetmore, involves exploitation of the increasingly well-defined methods of tissue and cell culture to simplify and control cellular events. As a result of improved techniques, one can ask more penetrating questions concerning cellular and cell-to-tissue events. Biochemical analyses of more uniform and synchronous events have become possible. As with all biochemical analysis, results from such studies must, of course, be related to the whole plant and to normal development. Particularly interesting and promising are the techniques now available for producing protoplasts (see the review by COCKING 1971). Isolation of naked protoplasts after plasmolysis and enzymatic treatment of a wide variety of tissues is now possible. Such isolated protoplasts can be made to reform new walls and to divide to form a tissue, and such tissue can be induced to form a new plant (TAKEBE, LABIB, and MELCHERS 1971). That protoplasts, while naked, can be made to fuse to form cell hybrids has been shown, and if new hybrid plants from such cell progeny can be made, a new era of somatic cell genetics will become a reality. The present rate of publication in the protoplast field is some measure of the expectations.

A fourth area for the immediate future is in extending our understanding of cytodifferentiation and its control. Here the botanists may in fact have available better tools and more clearly defined systems than are currently accessible to zoologists. Manpower to pursue these researches may be the greatest problem. Cytodifferentiation as part of the cell cycle or as an event stemming from the mitotic cycle is one of the most urgent and enticing problems facing biologists today. Experimental plant morphogeneticists are right there in the middle of it.

LITERATURE CITED

AVERY, G. S., JR. 1935. Distribution of phytohormone in leaf of *Nicotiana,* and its relation to polarized growth. Bull. Torrey Bot. Club **62:**313–330.

BALL, E. 1946. Development in sterile culture of stem tips and subjacent regions of *Tropaeolum majus* L. and of *Lupinus albus* L. Amer. J. Bot. **33:**301–318.

BERNIER, G. 1970. Cellular and molecular aspects of floral induction. Longman, London.

BRIGGS, W. R., G. MOREL, T. A. STEEVES, I. M. SUSSEX, and R. H. WETMORE. 1955. Enzymatic auxin inactivation by extracts of the fern *Osmunda cinnamomea* L. Amer. J. Bot. **30:**143–148.

BRIGGS, W. R., and T. A. STEEVES. 1958. Morphogenetic studies on *Osmunda cinnamomea* L. The expansion and maturation of vegetative fronds. Phytomorphology **8:** 234–248.

———, and ———. 1959. Morphogenetic studies on *Osmunda cinnamomea* L. The mechanism of crozier uncoiling. Phytomorphology **9:**134–147.

BROWN, C. L., and K. SAX. 1962. The influence of pressure on the differentiation of secondary tissues. Amer. J. Bot. **49:**683–691.

CAMUS, G. 1949. Recherches sur le rôle des bourgeons dans les phénomènes de morphogénèse. Rev. Cytol. Paris **11:** 1–199.

CHEADLE, V. I. 1944. Specialization of vessels within the xylem of each organ in the Monocotyledoneae. Amer. J. Bot. **31:**81–92.

COCKING, E. C. 1971. Fusion of isolated protoplasts: a first step towards the somatic hybridization of plants. Pp. 303–317 *in* Les cultures de tissus de plantes. Int. colloquium, Centre Nat. recherches sci.

DELISLE, A. L. 1937. The influence of auxin on secondary branching in two species of *Aster*. Amer. J. Bot. **24:**159–167.

———. 1938. Morphogenetical studies in the development of successive leaves in *Aster,* with respect to relative growth, cellular differentiation, and auxin relationships. Amer. J. Bot. **25:**420–430.

DEMAGGIO, A. E. 1961. Morphogenetic studies on the fern *Todea barbara* L. Moore. II. Development of the embryo. Phytomorphology **11:**64–79.

DEMAGGIO, A. E., and R. H. WETMORE. 1961. Growth of fern embryos in sterile culture. Nature **191:**94–95.

ESAU, K. 1943. Origin and development of primary vascular tissues in seed plants. Bot. Rev. **9:**125–206.

———. 1965. Vascular differentiation in plants. Holt, Rinehart & Winston, New York.

FREEBERG, J. A., and R. H. WETMORE. 1957. Gametophytes of *Lycopodium* as grown in vitro. Phytomorphology **7:**204–217.

———, and ———. 1967. The Lycopsida—a study in development. Phytomorphology **17:**78–91.

GARRISON, R. 1949. Origin and development of axillary buds: *Syringa vulgaris* L. Amer. J. Bot. **36:**205–213.

GAUTHERET, R. J. 1942. Manuel technique de culture des tissus végétaux. Masson, Paris.

GOLUB, S. J., and R. H. WETMORE. 1948. Studies of development in the vegetative shoot of *Equisetum arvense* L. II. The mature shoot. Amer. J. Bot. **35:**767–781.

GOODWIN, R. H. 1937. Cyto-genetics of two species of *Solidago* and its bearing on their polymorphy in nature. Amer. J. Bot. **24:**425–432.

GREENIDGE, K. N. H. 1952. An approach to the study of vessel length in hardwood species. Amer. J. Bot. **39:**570–574.

GUNCKEL, J. E., and K. V. THIMANN. 1949. Studies of development in long shoots and short shoots of *Ginkgo biloba* L. III. Auxin production in shoot growth. Amer. J. Bot. **36:**145–151.

GUNCKEL, J. E., K. V. THIMANN, and R. H. WETMORE. 1949. Studies of development in long shoots and short shoots of *Ginkgo biloba* L. IV. Growth habit, shoot expression and the mechanism of its control. Amer. J. Bot. **36:**309–316.

GUNCKEL, J. E., and R. H. WETMORE. 1946*a*. Studies on development in long shoots and short shoots of *Ginkgo biloba* L. I. The origin and pattern of development of

the cortex, pith and procambium. Amer. J. Bot. **33**:285–295.

———, and ———. 1946*b*. Studies of development in long shoots and short shoots of *Ginkgo biloba* L. II. Phyllotaxis and the organization of the primary vascular system, primary phloem and primary xylem. Amer. J. Bot. **33**:532–543.

HALPERIN, W. 1969. Morphogenesis in cell cultures. Annu. Rev. Plant Physiol. **20**:395–418.

HEIMSCH, C. 1951. Development of vascular tissues in barley roots. Amer. J. Bot. **38**:523–537.

JACOBS, W. P. 1952. The role of auxin in differentiation of xylem around a wound. Amer. J. Bot. **39**:301–309.

LOO, S. W. 1945. Cultivation of excised stem tips of asparagus *in vitro*. Amer. J. Bot. **32**:13–17.

MILLER, H. A., and R. H. WETMORE. 1945. Studies in the developmental anatomy of *Phlox drummondii* Hook. I. The embryo. Amer. J. Bot. **32**:588–599.

———, and ———. 1946. Studies in the developmental anatomy of *Phlox drummondii* Hook. III. The apices of the mature plant. Amer. J. Bot. **33**:1–10.

MOREL, G. 1962. Producing virus-free *Cymbidium*. Amer. Orchid Soc. Bull. **31**:495–497.

———. 1964. Tissue culture: a new means of clonal propagation of orchids. Amer. Orchid Soc. Bull. **33**:473–478.

———. 1971. Morphogenesis of stem apical meristem cultivated *in vitro:* application to clonal propagation. Symp. Plant Tissue Cult., Univ. Delhi.

MOREL, G., and C. MARTIN. 1955. Guérison de plantes atteintes de maladies à virus par cultures de méristèmes apicaux. 14th Int. Hort. Congr. Netherlands. Pp. 303–310.

MOREL, G., and J. F. MULLER. 1964. La culture in vitro du méristème apical de la pomme de terre. Acad. Sci. (Paris), Compt. Rend. **258**:5250–5252.

MOREL, G., and R. H. WETMORE. 1951*a*. Tissue culture of monocotyledons. Amer. J. Bot. **38**:138–140.

———, and ———. 1951*b*. Fern callus tissue culture. Amer. J. Bot. **38**:141–143.

NITSCH, J. P., and C. NITSCH. 1956. Studies on the growth of coleoptile and first internode sections: a new sensitive, straight-growth test for auxins. Plant Physiol. **31**:94–111.

———, and ———. 1969. Haploid plants from pollen grains. Science **163**:85–87.

NITSCH, J. P., and R. H. WETMORE. 1952. The microdetermination of free L-tryptophane in *Lupinus albus*. Science **116**:256–257.

O'BRIEN, T. P., and M. E. MCCULLY. 1969. Plant structure and development. Macmillan, New York.

PARTANEN, C. R., I. M. SUSSEX, and T. A. STEEVES. 1955. Nuclear behavior in relation to abnormal growth in fern prothalli. Amer. J. Bot. **42**:245–256.

RAGHAVAN, V. 1966. Nutrition, growth and morphogenesis of plant embryos. Biol. Rev. **41**:1–58.

SKOOG, F., and C. O. MILLER. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Soc. Exp. Biol. Symp. **11**:118–131.

SMITH, R. H., and T. MURASHIGE. 1970. In vitro development of the isolated shoot apical meristem of angiosperms. Amer. J. Bot. **57**:562–568.

SPURR, A. R. 1949. Histogenesis and organization of the embryo in *Pinus strobus* L. Amer. J. Bot. **36**:629–641.

STEEVES, T. A. 1962. Morphogenesis in isolated fern leaves. Pp. 117–151 *in* D. RUDNICK [ed.], Regeneration. Princeton Univ. Press, Princeton, N.J.

STEEVES, T. A., and W. R. BRIGGS. 1958. Morphogenetic studies on *Osmunda cinnamomea* L. The origin and early development of vegetative fronds. Phytomorphology **8**:60–72.

———, and ———. 1960. Morphogenetic studies on *Osmunda cinnamomea* L.: the auxin relationships of expanding fronds. J. Exp. Bot. **11**:45–67.

STEEVES, T. A., and I. M. SUSSEX. 1952. In vitro cultures of a fern callus. Nature **170**:672.

STEEVES, T. A., I. M. SUSSEX, and C. R. PARTANEN. 1955. In vitro studies on abnormal growth of prothalli of the bracken fern. Amer. J. Bot. **42**:232–245.

STEEVES, T. A., and R. H. WETMORE. 1953. Morphogenetic studies on *Osmunda cinnamomea* L.: some aspects of the general morphology. Phytomorphology **3**:339–354.

STEWARD, F. C., M. MAPES, and J. SMITH. 1958. Growth and organized development of cultured cells. I. Growth and division of freely suspended cells. Amer. J. Bot. **45**:693–704.

STEWARD, F. C., R. H. WETMORE, J. F. THOMPSON, and J. P. NITSCH. 1954. A quantitative chromatographic study of nitrogenous components of shoot apices. Amer. J. Bot. **41**:123–134.

SUSSEX, I. M., and T. A. STEEVES. 1953. Growth of excised fern leaves in sterile conditions. Nature **172**:624–627.

TAKEBE, J., G. LABIB, and G. MELCHERS. 1971. Regeneration of whole plants from isolated mesophyll protoplasts of tobacco. Naturwissenschaften **58**:318–320.

TIPPO, O. 1938. Comparative anatomy of the Moraceae and their presumed allies. BOT. GAZ. **100**:1–99.

TITMAN, P. W., and R. H. WETMORE. 1955. The growth of long and short shoots in *Cercidiphyllum*. Amer. J. Bot. **42**:364–372.

TORREY, J. G. 1953. The effect of certain metabolic inhibitors on vascular tissue differentiation in isolated pea roots. Amer. J. Bot. **40**:525–533.

———. 1957. Auxin control of vascular pattern formation in regenerating pea root meristems grown in vitro. Amer. J. Bot. **44**:859–870.

TORREY, J. G., D. E. FOSKET, and P. K. HEPLER. 1971. Xylem formation: a paradigm of cytodifferentiation in higher plants. Amer. Sci. **59**:338–352.

WARD, M. 1954*a*. Fertilization in *Phlebodium aureum* J. Sm. Phytomorphology **4**:1–17.

———. 1954*b*. The development of the embryo of *Phlebodium aureum* J. Sm. Phytomorphology **4**:18–26.

WARD, M., and R. H. WETMORE. 1954. Experimental control of development in the embryo of the fern, *Phlebodium aureum*. Amer. J. Bot. **41**:428–434.

WETMORE, R. H. 1943. Leaf-stem relationships in the vascular plants. Torreya **43**:16–28.

———. 1954. The use of in vitro cultures in the investigation of growth and differentiation in vascular plants. Pp. 22–38 *in* Abnormal and pathological plant growth. Brookhaven National Laboratory, Brookhaven, N.Y.

———. 1956. Growth and development in the shoot system of plants. Pp. 173–190 *in* D. RUDNICK [ed.], Cellular mechanisms in differentiation and growth. Princeton Univ. Press, Princeton, N.J.

———. 1959. Morphogenesis in plants—a new approach. Amer. Sci. **47**:326–340.

WETMORE, R. H., and A. L. DELISLE. 1939. Studies in the genetics and cytology of two species in the genus *Aster* and their polymorphy in nature. Amer. J. Bot. **26**:1–12.

WETMORE, R. H., A. E. DEMAGGIO, and G. MOREL. 1964. A morphogenetic look at the alternation of generations. J. Indian Bot. Soc. **42A**:306–320.

WETMORE, R. H., A. E. DEMAGGIO, and J. P. RIER. 1964. Contemporary outlook on the differentiation of vascular tissues. Phytomorphology **14**:203–217.

WETMORE, R. H., E. M. GIFFORD, JR., and M. C. GREEN. 1959. Development of vegetative and floral buds. Pp. 255–273 *in* Photoperiodism and related phenomena in plants and animals. American Association of Advancement of Science, Washington, D.C.
WETMORE, R. H., and W. P. JACOBS. 1953. Studies on abscission: the inhibiting effect of auxin. Amer. J. Bot. **40**:272–276.
WETMORE, R. H., and G. MOREL. 1951*a*. Sur la culture in vitro de prothalles de *Lycopodium cernuum*. Acad. Sci. (Paris), Compt. Rend. **233**:323–324.
———, and ———. 1951*b*. Sur la culture du gamétophyte de Sélaginelle. Acad. Sci. (Paris), Compt. Rend. **233**: 430–431.
WETMORE, R. H., and J. P. RIER. 1963. Experimental induction of vascular tissues in callus of angiosperms. Amer. J. Bot. **50**:418–430.
WETMORE, R. H., and S. SOROKIN. 1955. On the differentiation of xylem. J. Arnold Arboretum **36**:305–317.
WETMORE, R. H., and T. A. STEEVES. 1971. Morphological introduction to growth and development. Pp. 3–166 *in* F. C. STEWARD, Plant physiology: a treatise. Vol. **VIA.** Academic Press, New York.
WETMORE, R. H., and C. W. WARDLAW. 1951. Experimental morphogenesis in vascular plants. Annu. Rev. Plant Physiol. **2**:269–292.
WHITTIER, D. P., and T. A. STEEVES. 1960. The induction of apogamy in the bracken fern. Can. J. Bot. **38**:925–930.
———, and ———. 1962. Further studies on induced apogamy in ferns. Can. J. Bot. **40**:1525–1531.

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THE INFLORESCENCE AND FLOWER IN MALE MYRICA ESCULENTA VAR. FARQUHARIANA

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ABSTRACT

There are various ambiguities in the interpretations of the male floret and inflorescence in the Myricaceae. *Myrica esculenta* Buch.-Ham. var. *farquhariana* (Wall.) Chev., as a relatively primitive species in the family, was chosen for detailed study. The presence of tertiary bracts in one individual permits the interpretation of the male flower as consisting of only one or two stamens. The anatomy of the flower suggests that it is composed of but a single stamen whose single vascular bundle arises from a distinctive pedicellar vascular supply. A subtending bract may or may not be present. The so-called column has a complete vascular cylinder and is therefore to be interpreted as an ultimate branch of a compound inflorescence. There is no terminal floret in such ultimate axes, but rather, the vascular supply of that axis continues beyond the insertion of the floral supplies. The inflorescence is thus open or indeterminate, and is a panicle of spikes and spikelets. Phyllotactic considerations as well as vascular anatomy lead to the conclusion that the ultimate branch of the inflorescence may be reduced to a pseudocymule. This suggests that evidence for the "cymule" of the Betulaceae must be carefully reassessed.

Introduction

The organization of the male inflorescence of the Myricaceae has been variously interpreted. It is the object of the present study to examine carefully a member of the family which has a complex inflorescence (since the simple ones may be deceptively so!) in the hope that a logical reevaluation of the inflorescence and its components will emerge. *Myrica esculenta* Buch.-Ham. var. *farquhariana* (Wall.) Chev. has been chosen for this study on the basis of a preliminary survey of the floral organization and secondary xylem of the family (E. C. ABBE 1963; L. B. ABBE 1963). In this survey we found *M. esculenta* to be a relatively primitive species within the range of variation in the family.

The nature of the male floret needs review as part of the study of the inflorescence. But the identity of the individual male floret in this family is obscured by the absence of a perigon, by extreme reduction in size, and by its aggregation with many other florets. There is, as a result, a widely varying assessment of the number of stamens which constitute a floret. A range of two to 16 stamens per floret in the family was accepted by DE CANDOLLE (1864), EICHLER (1878), BENTHAM and HOOKER (1880), and WETTSTEIN (1924), while a range of two to 20 was advocated by BAILLON (1875), CHEVALIER (1901), RENDLE (1925), BACKER (1951), LAWRENCE (1951), MELCHIOR (1964), HUTCHINSON (1967), and ELIAS (1971). CLARKE (1858) reported from eight to 30 or even 40 stamens per floret. Many of these authors indicate that "often" or "usually" there are two to four, or four to eight stamens per floret. HJELMQVIST