

BOTANY

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Abstracts

Plant Cell and Tissue Culture for *In Vitro* Manipulation with Agronomic Species. N. P. MAXON, C. L. RHYKRD and C. L. RHYKRD JR., Departments of Agronomy and Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.——Application of novel techniques in plant cell and tissue culture with agronomic species is rapidly becoming a potent research tool for the plant scientist. There is great interest by plant breeders and geneticists to expand the genetic variability of existing gene pools. This objective is hampered by the conservative nature of the sexual cycle. Through the use of cell and tissue culture new genetic combinations are possible with such techniques as cell hybridization, exogenous DNA uptake or forced somatic cell association. Directed mutation induction has been limited by lack of selection screens for desirable mutants. *In Vitro* selection using suspension culture is a powerful tool for those interested in mutation breeding. Tissue and embryo culture can now be used to restore fertility to normally sterile F¹ plants. By allowing F¹ callus tissue to change its ploidy level, sexual recombination is possible with regenerated plants. Haploid plants can be recovered from anther or pollen culture and be induced to double forming a homozygous diploid. The factor limiting greater utilization of these research techniques is the difficulty of regeneration of plants from primary and secondary callus. It appears that the greatest success with regeneration comes from species that can be vegetatively propagated. By exploring various phytohormone combinations and pre-conditioning treatments, this impediment in application of these techniques will soon be removed.

Quantitative Experiments with Plant Catalase for the Beginning Botany Laboratory. WILLIAM W. BLOOM and GAYTON C. MARKS, Department of Biology, Valparaiso University, Valparaiso, Indiana, 46383.——An effective inexpensive manometer is described, together with a convenient method of extracting catalase from germinating cucumber seeds. A number of experiments are described for laboratory teams of four students. Quantitative data can be collected on the effects of variations in pH, temperatures, enzyme concentrations, and substrate concentrations. The inactivation of the enzyme by boiling and poisoning with heavy metals can also be demonstrated.

Use of Computers to Enhance Education in Plants and Human Affairs. THEODORE J. CROVELLO, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556.——Computers are used in an advanced undergraduate Plants and Human Affairs course which is open to all juniors and

seniors at the University. Computers were introduced to enhance both teaching and learning in the course. Major uses involved information retrieval (of literature, of 2 x 2 slides, etc.), multiple choice course review, and simulation of plant-related systems of relevance to society (the world model, and endangered species status). Reaction to all aspects of computer use in general has been good, but efforts must still be made to enhance understanding and clarity of the more difficult assignments, particularly those involving simulation. Many different types of people are required to make computer assisted education work, including biology professors, students in nonparasitic roles, and computer center personnel. We believe that students benefit from the use of computers in Plants and Human Affairs, and while computers will not replace good teachers, they can enhance their effectiveness.

The Trees of the Manchester College Campus after Sixty Years. PHILIP A. ORPURT, Biology Department, Manchester College, North Manchester, Indiana 46962.——Sixty years ago in 1917, E. E. FRANTZ, a Manchester College student chose as the topic for his required graduation thesis, "Trees of Manchester College." This paper is to acknowledge that earlier study and to report on the general status and growth of some of the trees which have survived to the present. The M. C. Campus was established in an "oak opening," consequently, *Quercus alba* L., the white oak comprised the most numerous species. Counts when compared with those of 1917 indicate that the stresses and hazards often associated with campus life have taken their toll. Of 441 white oaks in 1917 only 169 remain. From an average dbh of 10 inches and an average height of 45 feet in 1917 the white oaks remaining have grown to an average dbh of 21 inches and an average height of 75 feet with some attaining a height of 85 feet. The frequencies of oak trees ranging from 10 in. dbh to a maximum of 32 inches show a relatively even distribution.

Some Effects of Cadmium on Carbon Dioxide and Water Vapor Transfer in Leaves of *Acer saccharinum* L. ROBERT J. LAMOREAUX, WILLIAM R. CHANEY and RICHARD C. STRICKLAND, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47906.——Net photosynthesis, transpiration and dark respiration were measured in excised leaves of silver maple (*Acer saccharinum* L. exposed to 0, 5, 10 or 20 ppm Cd^{2+} added as $CdCl_2 \cdot 2\frac{1}{2} H_2O$. Rates of net photosynthesis and transpiration were reduced to 18 and 21%, respectively, of untreated controls after 64 hours, and reduced rates were highly correlated with solution concentration of Cd^{2+} . Dark respiration increased to 193% of untreated controls but was poorly correlated with solution concentration of Cd^{2+} . Diffusion resistances of leaves to carbon dioxide and water vapor transfer increased with increasing Cd^{2+} concentration. Cadmium treated leaves exhibited increased mesophyll resistance to carbon dioxide transfer which was probably an indirect effect of Cd^{2+} acting on diffusive and enzymatic factors associated with the mesophyll cells. These findings indicate that Cd^{2+} inhibited transpiration by interference with stomatal function, and that it inhibited net photosynthesis by increasing both stomatal and mesophyll resistance to carbon dioxide uptake.

Ultrastructural Changes of Chloroplasts in Attached and Detached, Aging, Primary Wheat Leaves. W. J. HURKMAN and G. S. KENNEDY, Department of

Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907 and Department of Botany, University of Wisconsin, Milwaukee, Wisconsin 53201.——In chloroplasts of mesophyll cells in attached, naturally senescing primary leaves of wheat, the first indications of aging are the appearance of osmiophilic globuli and reorientation of the thylakoidal system. Subsequently, the membranes of the grana and intergrana lamellae become distended and, later, dissociate into distinct vesicles. Concurrent with these membrane changes, osmiophilic globuli increase in size and number, the stroma becomes less dense, and the chloroplast envelope remains intact. In chloroplasts of mesophyll cells in detached, aging, primary leaves, initial changes also include appearance of osmiophilic globuli and lamellar reorientation. However, later stages of chloroplast degradation are strikingly different. The chloroplast envelope breaks down prior to lamellar breakdown. Osmiophilic globuli are fewer in number. Swelling of grana and intergrana lamellae is not pronounced and, additionally, the thylakoidal system degenerates without the formation of vesicles.

Storing Orchid Pollinia for Future Use in Hybridization: A Preliminary Report.

W. S. COURTIS, Assistant Professor of Biology, IUPUI, Indianapolis, Indiana 46205.——Data obtained to date suggest that optimal storage conditions may be different for different species. In general, this study confirms the previously reported detrimental effects of drying agents; and, that lower temperatures (4° and -6° C) are most effective. The data also suggest that stored pollen may germinate on artificial media and yet fail to produce seed when used for pollination.

Predicting Crop Yields by Use of Multiple Species Phenology Observations.

BYRON O. BLAIR*, V. L. ANDERSON, and C. L. RHYKERD.——Phenology observations on lilac (*Syringa persica*) have been in continued use since they were instigated in the mid 1960's over broad areas of the United States and more recently the Northeastern Provinces of Canada. These observations have been of limited value in the United States due to the very short period in the spring when phenological observations on lilac can be taken. However, these observations have been of greater value in predicting yields in Canada where agricultural production is limited by the shorter growing season. In 1964 a multiple garden system with several species was initiated in Indiana. Ten years of data have been collected from seven species with flowering patterns from late April to September. This paper is a correlation evaluation of species at several locations with yearly corn and soybean yields and demonstrates how phenological studies employing multiple species may be used in predicting crop yields.

Nutrient Capital and Substrate Quality of Logs in an Old-Growth Douglas-fir

Forest. PAUL C. MACMILLAN, Department of Biology, Hanover, College, Hanover, Indiana 47243. K. CROMACK, JR. and J. E. MEANS, School of Forestry, Oregon State University, Corvallis, Oregon 97330.——Visual criteria were used to classify Douglas-fir logs into 5 decay classes: 1 = recent input, to 5 = highly decayed log. These decay classes were used in our study of log nutrient capital and substrate quality in a 450 yr old stand in western Oregon. Mean residence time of logs by decay class was: 7, 16, 36, 82 & 159 yr. Over this time span wood

density decreased to less than one-half the original density. Percent of N, P, K, Ca, Mg & Na all increased with residence time; changes in absolute concentrations and total kg/ha of these elements will be discussed. Percent of lignin increased and % cellulose decreased with residence time; changes in absolute concentrations of carbon components and C/N ratios will be discussed.

Effects of Low Levels of Available Cadmium on Height Growth, Dry Matter Accumulation and Tissue Cadmium Levels in Soybeans. RICHARD C. STRICKLAND, WILLIAM R. CHANEY and ROBERT J. LAMOREAUX, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47906. —Soybeans (*Glycine max* L. var. Williams) were grown for seven weeks in a greenhouse in quartz sand amended with 0, 0.063, 0.125, 0.25, 0.50, 1.00, 2.00 or 4.00 ppm $\text{CdCl}_2 \cdot 2\frac{1}{2} \text{H}_2\text{O}$ (49.4% Cd). Heights were measured weekly and at harvest plants were separated into leaves, stems and roots for subsequent dry weight and Cd^{2+} determinations. Height growth was reduced over the entire study period in the 2.00 and 4.00 ppm treatments, while in all other treatments heights were not statistically different. Treatments above 0.25 ppm caused significant decreases in dry matter accumulation with leaves, stems and roots showing similar patterns of reduction. While Cd^{2+} levels in the various tissues increased with increasing treatment, the pattern of accumulation varied among tissues. For treatments up to 0.50 ppm, stems contained less Cd^{2+} than leaves, but in higher treatments the reverse was true. Cadmium levels in roots were always higher than in leaves and stems. Accumulation of Cd^{2+} by leaves, stems and roots ranged from 1.0 to 11.2, 0.2 to 61.3 and 1.5 to 582.7 ppm, respectively, and was highly correlated with treatment. This study showed: (1) substantial accumulation can occur at low levels of available cadmium, (2) accumulation of cadmium in roots is not necessarily reflected in dry matter reduction in roots or shoots, (3) height growth is less sensitive to cadmium than dry matter accumulation, (4) cadmium is not evenly distributed throughout the plant and (4) there is no growth stimulation at any level of cadmium used in this investigation.

Organic Matter Influences Availability, Uptake and Distribution of Cadmium in Soybeans. RICHARD C. STRICKLAND, WILLIAM R. CHANEY and ROBERT J. LAMOREAUX, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47906. —Soybeans (*Glycine max* L. var. Williams) were grown for six weeks in the greenhouse in quartz sand containing 0, 3, 6, 12, 24 or 48% (v/v) sterilized peat moss. The cation exchange capacities of the organic matter-sand (OM-S) mixtures ranged from 0 to 8.7 meg/100g dry weight. Imposed on each OM-S mixture was a $\text{CdCl}_2 \cdot 2\frac{1}{2} \text{H}_2\text{O}$ treatment of 0, 2.5, 5.0, 10.0, 20.0 or 40.0 ppm. Height growth was measured weekly and at harvest plants were separated into leaves, stems and roots for dry weight and tissue Cd^{2+} determinations. Height growth and dry matter accumulation in all tissues were reduced and Cd^{2+} content was increased for plants grown in sand alone. These effects were correlated with increasing Cd^{2+} concentration in the rooting medium. Inhibitions in growth by Cd^{2+} were reduced by addition of organic matter; the amount of alleviation was dependent on the level of organic matter and the Cd^{2+} treatment. In the 0, 3 and 6% OM-S mixtures, Cd^{2+} content

in the various tissues was correlated with metal treatments in the 12, 24 and 48% OM-S mixtures. The order of Cd^{2+} accumulation in the tissues was roots \ll stems $<$ leaves.

Greening in Albino Plants of a Green: Albino Strain of Tobacco without the Addition of an Amino Acid to the Culture Medium. SUSANNAH NELSON,* MARY JO DONOVAN and ANNE SUSALLA, Saint Mary's College, Notre Dame.——Albino plants of a green: albino strain of tobacco have consistently become variegated with green when grown on a specific culture medium that includes the amino acid, glycine. Because leucine produces greenness in other albino plant species and is relatively similar to glycine in its chemical configuration, it was used to substitute glycine in the culture medium. The leucine-grown plants became green to the same degree as the control glycine-grown plants. The plants were then grown on the culture medium without an amino acid. Greenness occurred and the plants could not be distinguished from control plants grown on the culture medium containing glycine. Thus, albino plants of this strain of tobacco do not need an amino acid in the culture medium for greenness to occur.

Possible Disadvantages of Isolating Plant Cuticles by the ZnCl_2 -HCl Method. JAY H. JONES, Department of Biology, Indiana University, Bloomington, Indiana 47401.——Isolated cuticles are used for a variety of purposes. These include studies of cuticular anatomy and morphology, ion exchange and permeability, water loss and chemical composition. Methods of isolating cuticles also vary. One of the most frequently used methods (Holloway and Baker, *Plant Physiol.* 43:1878-1879) involves digestion of leaf discs in a concentrated ZnCl_2 -HCl solution. Intuitively such a harsh treatment might be expected to cause molecular changes within the cuticle. To test this, the composition of cutin hydrogenolysates prepared from cuticles isolated by the ZnCl_2 -HCl and the less severe oxalate methods were compared. Results indicate that chemical changes do occur during the ZnCl_2 -HCl digestion. These changes involve unsaturated cutin components. The exact identity of the products is still under investigation. These changes are of little importance for anatomical and morphological work. However, when working with ion exchange, permeability and chemical composition, such changes should be avoided by using other methods or at least taken into consideration.

The Foliar Physiognomy of an Indiana Lake Bottom and its Paleoclimatic Implications. JOHN L. ROTH, Department of Biology, Indiana University, Bloomington, Indiana 47401.——The current method of leaf margin and leaf size analysis in paleoclimatology assumes that the foliar physiognomy of a fossil deposit accurately reflects the physiognomy of the living forest from which it was derived. However, a study of lake bottom sediments from a small lake near Bloomington, Indiana shows that depositional systems are selective and tend to distort the physiognomic composition of fossil deposits. Streamside vegetation and sun leaves tend to be overrepresented in fossil deposits, while large leaves tend to be fragmented during transport and underrepresented in fossil deposits. Seasonal variations in the rate of deposition and in the leaf fall of deciduous and evergreen species also distort the physiognomy of the deposit. Therefore, foliar physiognomic data compiled from a fossil deposit cannot be compared to foliar

physiognomic data compiled from herbarium collections and published regional floras as this would lead to a misinterpretation of the paleoclimate. It should be compared to new reference data that is compiled from modern sites of deposition and carefully correlated with local climatic conditions.