# BOTANY

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## ABSTRACTS

**Effect of Abscisic Acid on Phospholipid Bilayers.** Blair Brengle, William Stillwell and Stephen Wassall, Departments of Biology and Physics, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——Our laboratory has been studying the effect of the plant hormone abscisic acid (ABA) on phospholipid bilayer membranes. We previously have demonstrated that ABA increases bilayer permeability to the non-electrolytes urea, erythritol and glucose, to the cation $Pr^{3+}$ and to water. The permeability enhancement for erythritol is much greater with mixed component bilayers than with membranes composed only of phosphatidylcholine. We have used several sophisticated biophysical techniques to study the nature of these ABA-membrane effects. The hormone adversely affects lipid vesicle stability by enhancing aggregation and/or fusion. ABA does not appear to behave as a general bilayer perturbing agent as no ABA dependent change in acyl chain motion can be detected by $^{13}C$-NMR of phospholipid vesicles or by ESR measurements of bilayers containing spin labelled fatty acids. The failure of ABA to alter $^{31}P$-NMR relaxation furthermore implies that the hormone is not strongly interacting with the phospholipid head groups and no significant ABA induced perturbations of phase behavior can be detected by differential scanning calorimetry or by the ESR-Tempo spin label technique. These results indicate that although ABA does have a profound effect on bilayer permeability, it must be altering very small regions of the bilayer, perhaps through channel formation.

**The Algae of an Acid Lake.** A.E. Brooks, W.N. Doemel and J.E. Miner, Department of Biology, Wabash College, Crawfordsville, Indiana 47933; and A.K. Konopka, Department of Biology, Purdue University, West Lafayette, Indiana 47907.——Reservoir 29, an acidic strip mine drainage lake in the Green-Sullivan State Forest, is populated chiefly by three algal species that include: *Chlamydomonas acidophila* (chlorophyta), *Euglena texta* (euglenophyta) and *Clorocloster pyreniger* (chrysophyta). A diatom, *Pinnularia* is also present but in relatively small numbers. In 1984, the algae exhibited a major bloom late in the spring and a secondary peak late in the summer. Discrete sampling methods have revealed that the algae exist mainly in a five centimeter metalimnetic layer that seems to extend over a large area of the lake. Observations also suggest that the algae exhibit a diurnal vertical migration. Isotopic carbon fixation experiments indicate that the algae are significant primary producers in this lake system. Preliminary experiments suggest that the algae may be carbon limited in the lake. High sulfide concentration in the hypolimnion also is thought to be important in regulating the vertical distribution of the algae. All three of the algae have been isolated and are being maintained in a simple salts medium supplemented with vitamin B and thiamine.

**Laticifer Differentiation in Embryoids Derived from Tissue Cultures of *Asclepias curassavica* (Asclepiadaceae).** KERRY B. DUNBAR AND KATHRYN J. WILSON, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223; BRUCE H. PETERSEN, Eli Lilly Company, Indianapolis, Indiana 46202; and DAVID D. BIESBOER, Department of Botany, University of Minnesota, St. Paul, Minnesota 55108.——Laticifers were detected in frozen sections of embryoids derived from callus cultures of *Asclepias curassavica* (blood flower) by an indirect fluorescent antibody technique. Sections were treated with IgG fraction rabbit anti-latex antiserum, produced with mature *Asclepias syriaca* latex as a source of antigens, and with fluorescein-conjugated IgG fraction goat anti-rabbit IgG. Laticifers were identified by their fluorescence in embryoids dissected from callus cultures. Stem explants from mature greenhouse plants were used to initiate callus on Murashige-Skoog (MS) medium supplemented with 1.0 mg/l benzyl adenine, 5.0 mg/l adenine, 2.0 mg/l 2,4 dichlorophenoxyacetic acid (2,4-D), 0.1 g/l myo-inositol, 0.4 g/l casein hydrolysate, 2.0% sucrose, and 0.9% agar, and adjusted to pH 5.8. Callus cultures were maintained for one year by transferring callus tissue to fresh media every 2 to 3 months. Embryoids were initiated by transferring tissue to the same MS medium minus 2,4-D. After 3 months callus subcultured to the 2,4-D-free medium contained embryoids similar to globular, heart, torpedo, and mature zygotic embryo stages. Embryoids similar to late heart stage zygotic embryos possess laticifers detectable by fluorescent microscopy in and at the edge of vascular tissue. Laticifers cannot be detected in paraffin or cryostat sections without the specific fluorescent marker. Sections on control slides, treated with whole serum of IgG fraction from whole serum, both from an uninjected rabbit, contained no fluorescent cells.

**The Natural Reduction of the Acidity of Acid Polluted Stripmine Lakes.** R.A. GYURE, W.N. DOEMEL, A.E. BROOKS, A.K. KONOPKA, AND J.E. MINER. Department of Biology, Purdue University, West Lafayette, Indiana; Department of Biology, Wabash College, Crawfordsville, Indiana, 47933.——Acidic strip-mine lakes polluted by the bacterial oxidation of exposed iron pyrite have been observed to become less acidic with time. In one of these lakes, Reservoir 29, a dimictic lake located in the Greene-Sullivan State Forest, the pH of the water column is 2.8 through the metalimnion, but in the hypolimnion the pH increases gradually through the summer from pH 3.0 to 3.5. This increase in pH is accompanied by a hypolimnetic increase in hydrogen sulfide to more than 1 mM. Cultural enrichments and isotope experiments with sulfur-35 indicate the presence of a metabolically active population of sulfate reducing bacteria. These observations support the hypothesis that the observed decrease in the hypolimnetic acidity is a direct result of the activity of sulfate reducing bacteria. With the relative abundance of sulfate, the factor(s) limiting the reduction of sulfate in this sytem is the electron donor or the carbon source.

**The Black Cherry Rust in the Americas.** R.M. LOPEZ-FRANCO AND J. F. HENNEN, Arthur Herbarium, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.——*Tranzschelia arthurii*, the black cherry rust, described in the Soviet Union by Tranzschel & Litvinov in 1938 and based on specimens from Iowa and Michigan, has been overlooked, ignored or questioned by North American authors and confused with *Tranzschelia pruni-spinosae* (Persoon) Dietel, sensu lato.

Using the name *Puccinia pruni-spinosae* Persoon, Arthur in 1905 reported experimental innoculations that demonstrated heteroecism in this rust with spermogonia and aecia on *Hepatica nobilis* P. Mill. var. *acuta* (Pursh) Steyemark, and uredinia

and telia on *Prunus serotina* Ehrh. var. *serotina*. The following year he established the genus *Tranzschelia* for this and related rusts based on the kind of spermogonia and fascicled teliospore pedicels.

Our preliminary study of the genus *Tranzschelia* revealed that *T. arthurii* is an easily identified taxon when teliospores are present. Its uredinia and telia occur on varieties of *Prunus serotina* and on *P. virginiana* L. in Canada, United States, Mexico, Guatemala, Colombia, Ecuador and Peru. The spermogonial and aecial stages are known only from Eastern North America.

Our inoculation experiments have confirmed the heteroecism of this rust and provided material for a developmental morphological study including light and scanning electron microscopy, which are included in this paper.

**Community Structure of an Indiana Gravel Hill Prairie with Special Reference to the State Endangered** Besseya bullii. ERIC S. MENGES AND KIMBERLY A. WADE, Holcomb Research Institute, Butler University, Indianapolis, Indiana 46223.——In Indiana xeric gravel hill prairies support a biota that includes several species rare in Indiana. Community composition at one such prairie, Wea Creek Nature Preserve, was determined in 1985 using 0.1 $m^2$ rectangular quadrats in four cover types: unburned prairie, spring-1985-burned prairie, edge thicket, and dry woods. Additional quadrats were centered on randomly chosen individuals of the state endangered perennial herb *Besseya bullii*.

Ordinations and classifications show that species composition of woods, edge thicket, and prairie are distinct. Prairie quadrats varied significantly in composition in relation to slope position, degree of disturbance to native turf, and edge effects from bordering woods. In particular, a shift in dominant grass species from *Bouteloua curtipendula* to *Andropogon scoparius* from upslope to downslope suggests Wea Creek Nature Preserve varies in soil moisture. The burning treatment has had very little effect. The relatively low prairie diversity (4-10 species per quadrat) may reflect the harsh conditions of the prairie and a history of disturbance to the native sod. Species richness was greatest in the edge thicket, and varied little between burned and unburned prairie.

*Besseya* individuals were found mainly in the edge thicket and in dry woods. Previously observed plants growing near an eroding cliff have disappeared. The lack of plants in the open, southwest-facing prairie is consistent with observations made by other investigators. We noted 66 individuals at Wea Creek N.P., and 24 of these were reproductive. Over 1/3 of all plants had less than three basal leaves. Reproductive individuals have significantly more and larger basal leaves than non-reproducing, but the best predictors of the quantity of reproductive output are measurements of flowering scape size. We have not yet noted *Besseya* seedlings in the field, but have initiated laboratory and field germination experiments aimed at understanding seed and seedling behavior.

**Analysis of DNA Methylation in the Growth and Development of the Early Alaska Pea *(Pisum sativum)*.** L.A. NEEB AND B.D. ALLAMONG, Department of Biology, Ball State University, Muncie, Indiana 47306.——The methylation of specific gene sites is thought to play a controlling role in gene expression in microorganisms, higher plants, and animals. The relationship between methylation of DNA and gene expression has been well documented in microorganisms and animals; however, the regulatory role of methylation in higher plants has remained relatively unresearched. The focus of this study was to investigate specific fluctuations in DNA methylation during the early development of the pea (*Pisum sativum*).

Pea seeds were grown for 12 days in vermiculite in a growth chamber. Duplicate samples of 30 seedlings were harvested daily. The samples were pulse-labeled with S-

Adenosly-L-methionine, (methyl-C3$H_3$) for 10 hours. The labeled methyl group was allowed to be incorporated into the DNA as the samples continued to grow and differentiate. The methyltransferase action was stopped by freezing. DNA was then extracted, quantitated, hydrolyzed, and separated on thin-layer chromatography into its bases. Each methylated product was quantitated in the scintillation counter.

Analysis of the location and fluctuation in the methylated DNA over the growth period of seedling differentiation was made. The percent of methylated base was quantitated and compared to the DNA extracted for each day of growth over the 12 day period. Fluctuations of methylated DNA bases correlates to growth patterns observed. The results lend supporting evidence to the above stated hypothesis. It was concluded that differentiation in pea plants may be a product of methylated DNA masking the expression of selective genes.

***Azolla caroliniana* and its Symbionts.** SOLOMON OYELEKE AND J.D. SCHOKNECHT, Indiana State University, Terre Haute, Indiana 47803.——The water fern *Azolla caroliniana* Willd. is known as an important agent for nitrogen fixation in water and is used extensively in wet cropping to provide nitrogen for plant growth. Azolla ferns were collected from cypress swamps in Southern Illinois. These ferns were examined with scanning electron microscopy and electron microscopy. The leaflets are formed in pairs along a rachis. The lower leaf of the pair appears to function primarily as a float. It is flat and is two cell layers thick. The upper leaflet has a layer of palisade cells that function in photosynthesis, below the upper epidermis. The cells of the upper epidermis are inflated on their upper surface. These act to prevent wetting of the plants surface and trap air between the inflations. The fern is able by this morphology to maintain the photosynthetic surface on the surface of the water even if mechanically submerged. The lower epidermis is separated from the palisade layer by a pouch which contains a species of the cyanophyte, *Anabaena* and an Actinomycete. The symbionts have been isolated and are being characterized and analyzed for nitrogenase activity.

**Induction of Embryogenesis in Embryo-derived Callus of *Ginkgo biloba* L.** WESLEY SHANKLIN AND WILLARD F. YATES, JR., Department of Botany, Butler University, Indianapolis, Indiana 46208.——Callus can be induced from explants of almost any tissue of *Ginkgo biloba* L. on a relatively simple medium such as Murashige and Skoog (MS), Nitsch and Nitsch, Gamborg's B-5, etc. We have obtained callus from leaf discs, meristems, root tips, megagametophyte and microgametophyte tissues. Although many of the callus cultures were vigorous and rapid growing, attempts to obtain embryo formation were unsuccessful.

In an effort to obtain callus with greater embryogenic potential, *Ginkgo* embryos were excised from mature seed and placed on a basal medium consisting of MS major and minor salts with minimal organics, 3% sucrose, 0.7% agar to which was added a range of kinetin (K) and napthalene acetic acid (NAA). Viability of the excised embryos approached 100%. All embryos developed callus at all concentrations of cytokinin and auxin. A 5 mg/l kinetin and 2 mg/l NAA concentration produced best results. Callus growth was produced on almost every embryo and appeared to be derived primarily from the base of the cotyledons. After 4 weeks, some callus cultures were transferred to reduced K and NAA concentrations. This pulse-transfer type treatment did not initially appear to effect embryogenesis, but may warrant further investigation.

After 4-6 weeks dark green globular bodies appeared distributed throughout a much lighter and more friable tissue. These were determined to be somatic embryos in varying stages of development. The most mature embryos to date have initiated cotyledons. The most striking difference noted between the induced embryos and those derived from gametes seems to be the intense green color of the former.

Attempts to obtain mature somatic embryos as well as a continuous culture tissue with high embryogenic potential are continuing.

**Micropropagation of Black Locust: A Controlled Method to Study the Rhizobial/Legume Symbiosis.** HENRY STELZER AND ROBERT J. REINSVOLD, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907.——The ability of *Robinia pseudoacacia*. L., black locust, to form a symbiotic association with the dinitrogen-fixing bacterium *Rhizobium* has been speculated as a primary factor contributing to its successful establishment on a diversity of sites. Due to the apparent ubiquitous nature of the rhizobia which can associate with black locust, it is often difficult to control against contaminating rhizobia in the non-inoculated controls. Another complicating factor in controlled experiments is the variability of the host genotype. The purpose of this study was to determine the feasibility of using micropropagated plantlets of black locust to more clearly understand the symbiotic association.

Genetically identical shoot propagules were produced by placing excised, disinfested shoot apices from a single one-year-old coppice sprout on Lloyd and McCown's woody plant medium (WPM) supplemented with 4 $\mu$M benzyladenine (BA) to stimulate axillary shoot production. A mean axillary shoot proliferation rate of six shoots per culture was achieved within six weeks. Rooting of the shoot propagules occurred within four weeks following the transfer back to WPM with no BA. *In vitro* plantlets were inoculated with selected strains of *Rhizobium*. Dinitrogen-fixing nodules were successfully formed on the root systems.