

## BOTANY

Chairman: KATHRYN WILSON

Department of Biology, Indiana University-Purdue University  
at Indianapolis, Indianapolis, Indiana 46223

Chairman—Elect: L. DAVID POLLEY

Department of Biology, Wabash College, Crawfordsville, Indiana 47933

### ABSTRACTS

**Growth of Green Ash Seedlings Influenced by Available Soil Phosphorus but not Mycorrhizal Inoculation.** D. S. EGEL and P. E. POPE, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907.—Vesicular arbuscular (VA) mycorrhizae are reported to improve the survival and enhance the growth of herbaceous and certain woody plant species on droughty, nutrient deficient sites. A high probability for survival and rapid early growth of outplanted tree seedlings are paramount for a successful reforestation program. This study reports the influence of the mycorrhizal fungus *Glomus macrocarpum* (Tul. and Tul.) on the growth, and nitrogen and phosphorus uptake of green ash (*Fraxinus pennsylvanica*, Marsh.) seedlings grown over a wide range of available soil P Levels.

Green ash seedlings, grown in a glasshouse for 21 weeks, were harvested at 3 week intervals. Seedlings were grown at 10, 30, 70, 110 and 125 ppm Bray I extractable soil phosphorus and were either inoculated or not with *Glomus macrocarpum* at the rate of 380 spores/kg of autoclaved loamy sand soil mix. At each harvest, seedling height, diameter, fresh and dry weight by component (root and stem plus foliage) were measured and 10-20% of roots (fresh weight basis) were taken for determination of the percentage of mycorrhizal colonization. Percentage foliar phosphorus and nitrogen were also measured for each seedling.

Neither seedling growth nor percentage foliar phosphorus was affected by mycorrhizal inoculation but both increased with increasing soil phosphorus levels. Percentage mycorrhizal colonization decreased with increasing soil phosphorus levels and tended to decrease with time. Shoot/root ratio of seedlings was unaffected by either mycorrhizal inoculation or soil phosphorus levels.

**Abnormal Dependence on Potassium in *Chlamydomonas*:** Evidence for Transport Deficient Mutants. C. JAMES FRANCOEUR and L. DAVID POLLEY, Wabash College, Crawfordsville, Indiana 47933—Mutants of *Chlamydomonas reinhardtii* which exhibit a dependence abnormally high concentrations of potassium for growth have been isolated. These have been characterized genetically and physiologically. Here we report the results of our studies in which the kinetics of potassium ( $^{86}\text{Rb}$ ) transport were examined.

Potassium transport in wild type strain R3+NM conforms to simple Michaelis-Menten kinetics. When these cells are grown on medium supplemented with 10mMK, the  $V_{\text{max}}$  of  $^{86}\text{Rb}$  uptake is 25 nmoles Rb/hr  $\times 10^6$  cells and the  $K_n$  is 0.75 mM. When these cells are stressed for potassium by a 24 hour starvation in medium lacking potassium, both the affinity for potassium and the capacity of transport increase. By contrast, two mutant strains which exhibit nearly identical kinetic parameters when grown in 10mMK can not absorb any potassium after 24 hours of starvation. The third mutant strain exhibits slightly increased

capacity when grown in 10mMK, but there is no change in kinetic parameters when stressed by starvation. The relationship between these observations and possible mechanisms which regulate potassium transport will be discussed.

**Genetic and Morphological Differences in Twelve Populations of *Collinsia verna* (Scrophulariaceae).** JUDITH K. GREENLEE, Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556. — *Collinsia verna* (Scrophulariaceae), a spring flowering annual, occurs in small isolated populations. The focus of the study was to determine population dynamics and genetics of *C. verna*, its relationship to other species in the genus, and the status of *C. verna*, in Indiana.

Populations were collected from Indiana, bordering Kentucky counties and southern Illinois. Plants were collected in the budding stage and crosses were made in all possible combinations between populations. Morphological and cytological data obtained show differences between populations based on gross external characters as well as chiasmata frequency and meiotic configurations.

Electrophoretic techniques being employed show polymorphisms which are diagnostic between *Collinsia* species. Between populations, within populations and F1 crosses are currently being analyzed. Microspectrophotometric techniques being employed show differences in the amount of DNA between and within species and populations.

*C. verna* populations were found to be decreasing and disappearing from sites disturbed by development of parks, mowing, home building, and trash dumping.

**Micromorphology and Chemistry of Fossil Laticifers from Brown Coal Deposits of the Eocene.** PAUL G. MAHLBERG and DONALD FIELDS, Department of Biology, Indiana University, Bloomington, Indiana 47405. — Cellular remains from brown coal deposits of the Geiseltal, near Halle, were determined to be a fossil form of the nonarticulated laticifer. A cellulosic wall was often present surrounding the cells which retained a tubular shape during their preservation. Small holes, possibly artifactual in nature, occurred at irregular intervals along the cells. The cells contained a dense, yet spong-like, content primarily polyisoprenoid in composition. Rapid polymerization of the rubber in these cells undoubtedly aided in the preservation of these cells. These contents, which filled the entire cell lumen, occasionally included structures that may have represented cellular organelles or nuclei in the cell. Branches were present along laticifer axes and, as for branched nonarticulated laticifers in extant plants, these branches projected in different directions from the principal axis of this cell. Gas-liquid chromatography of acetone extracts derived from cellular contents, demonstrated the presence of several sterols which formed a characteristic sterol profile for these remains. The cellular morphology and sterol profile of these laticifers can be employed as markers, in comparative studies with extant plants, to identify the species origin of these laticifers.

**The Effect of Organic Chelators and Divalent Cations on Nickel Toxicity to Two Species of Green Algae.** LORI H. NICHOLS and DAVID F. SPENCER, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223 — Cultures of two algal species (*Ankistrodesmus falcatus* var. *acicularis* and *Scenedesmus quadricauda*) were maintained in a chemically defined medium. Total nickel concentration was 10.2 micromolar, a concentration previously shown to be toxic to these species. Organic chelators, ethylenediamine tetraacetic acid (EDTA) and nitrilotriacetic acid (NTA), were used to adjust the concentration of free divalent nickel. Algal response was measured by growth

rate and cell size. These parameters were inversely related to the concentration of free divalent nickel and independent of the total nickel concentration in the medium. Equilibrium calculations for the medium suggest that changes in growth were due to changes in the concentration of free divalent nickel and not changes in the availability of other nutrients and trace metals. The effect of CA, Mg, and Mn on nickel toxicity was also examined. Results indicated that within the ranges tested, Ca, Mg, and Mn did not reverse nickel toxicity for *Ankistrodesmus falcatus* var. *acicularis*.

**A Fairy Ring of *Chlorophyllum molybdites* (Fr.) Sacc.** PHILIP A. ORPURT, Department of Biology, Manchester College, North Manchester, Indiana 46962. — The occurrence of a sizeable ring of large mushrooms in the yard of a nearby farm was called to the attention of the author in September 1981. In response to a request that the fungus be identified to determine its edibility, the site was visited where photographs and specimens were obtained.

*Chlorophyllum molybdites* (*Lepiota morgani* Peck) is known to form large fairy rings throughout our area, however, the particular occurrence of this very often impressive mushroom was the most spectacular I had ever observed. The ring of 78 large sporophores measured ten feet in diameter. The largest specimen was 10½ inches tall with a cap 8 inches broad. Many of the other individuals were not much smaller. While some of the sporophores possessed gills which were essentially white, the more mature specimens were beginning to exhibit an olivaceous coloration. Spore prints soon revealed that the spores were indeed an olive color which made possible a positive identification.

Specimen site: The Robert Bechtold farm, T29N, R7E, Section 2, Wabash Co., Indiana. Date of collection: September 7, 1981. Specimens are on deposit at Manchester College and at Southern Illinois University.

***Pisolithus tinctorius* Colonization and Site Conditions Influence Establishment of Red Oak Seedlings.** P. E. POPE, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907. — Planting oak seedlings as a method of reforestation has been only moderately successful. Once planted, the seedlings characteristically exhibit little above ground height or diameter growth of 3-5 years. The ectomycorrhizal forming fungus (*Pisolithus tinctorius*) which successfully colonizes and enhances the outplanting survival and growth of southern pines also colonizes red oak.

Seedling nursery beds of red oak (*Quercus rubra*) were inoculated (2.5 l/m<sup>2</sup>) with sterilized or viable vegetative fungal inoculum of *Pisolithus tinctorius* and seedlings grown for one year. Dormant barefoot seedlings were planted in replicated 25 tree plots in March 1978 on three major soil types to evaluate the influence of soil-site conditions and mycorrhizal inoculation on seedling survival, development and nutrient uptake. After 3 years, seedling survival ranged from 81 to 93% and was significantly influenced by soil-site and initial mycorrhizal condition. Annual and 3 year growth increments (cm<sup>3</sup>) were greater for inoculated seedlings but the interaction with the soil-site component was also significant. For all sites, foliar P concentrations were greater for seedlings inoculated with the test fungus but foliar P levels did not differ significantly among sites for the inoculated seedlings. For the inoculated seedlings, the percentage of feeder roots infected by the test fungus declined over time on all sites but after 3 years had not reached the natural infection levels of the non-inoculated seedlings.

**Dissolution and Chemical Composition of Laticifer Starch in *Euphorbia pulcherrima*.** STEVEN R. SPILATRO and PAUL G. MAHLBERG, Department of Biology, Indiana

University, Bloomington, Indiana 47405. — This investigation was undertaken in order to better understand the slow rate of starch degradation when laticifer starch granules are treated with hydrolytic enzymes. GLC of trimethylsilylated derivatives of acid hydrolyzed laticifer starch revealed glucose to be the only carbohydrate present. 'Blue value' and iodine binding capacity determinations showed laticifer starch to contain 30-35% amylose. Suspension of laticifer starch granules in DMSO at 100°C or lower resulted in partial (<65%) dissolution after 40 hours. Dispersion at 125°C resulted in 90% dissolution of the starch granules after 9 hours and complete dissolution within 16 hours. The refractory nature of laticifer starch granules to enzymatic hydrolysis appeared not to be related to the presence of any unusual carbohydrates within the granule. The amylose content of the granule, although higher than the typical 20-25% found in seed and tuber sources, also does not indicate a barrier to enzymic hydrolysis. The unusually slow rate of dissolution of the starch granules, however, does indicate that the molecular association of the starch molecules may be sufficiently strong to produce a structure inaccessible to hydrolytic enzymes.

**Kinetin and Absciscic Acid Increase the Rate of Water Permeability across Lipid Bilayers.** WILLIAM STILLWELL and PAUL HESTER, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223. — Kinetin and absciscic acid are shown to greatly enhance the rate of water permeability across lipid bilayer membranes. Rapid water permeability is followed by measuring spectrophotometrically the rate of liposome swelling in hypotonic solution or the rate of liposome shrinking in hypertonic solution as a function of kinetin or absciscic acid included in the bathing solution. The kinetin effect is measured from 16.3  $\mu$ M to 2.7 mM, from pH 2.0 to 11.0, at temperatures from 10°C to 50°C, with membranes made from both natural (egg lecithin and asolectin) and synthetic phosphatidylcholines. The effect is greatest for membranes in the more fluid, liquid crystalline state. Absciscic acid produces a much greater increase in water permeability with membranes composed of natural phospholipid mixtures as opposed to synthetic phosphatidylcholines. The absciscic acid effect is dependent on the presence of phosphatidylethanolamine in the membrane. These experiments indicate that these plant growth substances may exert their initial, rapid time effect on the lipid bilayer portion of membranes.

**Callus Growth and Embryogenesis in Tissue Cultures of *Asclepias tuberosa* L.** (Asclepiadaceae). KATHRYN J. WILSON, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223. — Tissue cultures of *Asclepias* species provide an experimental system suitable for specifically defining the biochemical and physical parameters of differentiation and growth of the cell type, the laticifer. Since laticifers typically present in *Asclepias* members differentiate in zygotic heart stage embryos, experiments were undertaken to determine culture media supporting rapid callus growth and ultimately promoting the most prolific asexual embryogenesis. Stem explants callused most rapidly when derived from seedlings no older than 25-30 days. To derive callus, explants were maintained on several combinations of 2,4-D (0-4.0 mg/l) and benzyladenine (BA) (0-2.5 mg/l) incorporated into a basic Murashige-Skoog (MS) medium. After 33 days greatest callus growth was obtained on media supplemented with 0.5 mg/l 2,4-D and 0.5 mg/l BA. Significantly greater numbers of embryoids were obtained on media supplemented with 0.5 mg/l 2,4-D combined with 1.0, 2.0, or 2.5 mg/l BA and their numbers were increased by transferring one month old callus to MS medium supplemented with 0.5 mg/l BA and either no or a relatively high concentration of (6.0 mg/l) of 2,4-D. High concentrations of auxin promote embryoid

development most similar to normal embryogeny. The effect on laticifer differentiation in embryoids proliferated on high auxin concentrations currently is being investigated.