

BOTANY

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ABSTRACTS

Cytofluorometric Measurements and Nuclear and Nucleolar Morphological Changes in Successive Epidermal Cells in Response to *Botrytis allii* Infection. PRADEEP K. BHATTACHARYA, Department of Biology, Indiana University Northwest, Gary, Indiana 46408, and ARISTOTEL J. PAPPELIS, Department of Botany, Southern Illinois University, Carbondale, Illinois 62901.—Six nuclear indices were cytofluorometrically measured across 40 consecutive cell-rows in *Botrytis allii* infected onion bulb leaf base outer epidermis. Mycelium had penetrated 10 to 15 rows of cells adjacent to the inoculation site. Host cells in the infected region contained the least amount of total nucleic acids, DNA, RNA, total nuclear protein, histone, and non-histone protein while those 20 to 30 cells beyond the infected area contained the great amount of these. Nuclei in the infected area were pycnotic and the nucleoli in these were small, round, and generally without nucleolar vacuoles (indicating physiological inactivity). Cells in 20 to 30 cell-rows beyond the infected area contained normal nuclei and the nucleoli in these tended to be oval or round, twice the diameter of inactive round nucleoli, and, usually contain many nucleolar vacuoles (indicating physiological activity). Nuclei and nucleoli in the cells between those in infected areas and 20 to 30 cell-rows beyond were intermediate in nuclear and nucleolar traits. We conclude that *B. allii* kills host cells, greatly reduces the macromolecular content of the host nucleus, and inactivates nuclei and nucleoli in advance of the spread of mycelium in host tissue.

Viability of Sixty-one Year Old Spores of *Marsilea quadrifolia*. WILLIAM W. BLOOM, Valparaiso University, Valparaiso, Indiana 46383.—In 1952 the author reported on the viability of spores of *Marsilea quadrifolia* that were thirty years old. At that time seven of the ten sporocarps tested contained viable megaspores and produced embryo sporophytes. This year three remainig sporocarps (now 61 years old) from that collection were tested with the following results: one released spores from the sori but no spores were viable; one retained the spores in the sori and no spores were viable; one released all spores but only sixty-two of seventy-eight megaspores were viable and produced normal gamteophytes. Only a few microspores showed evidence of viability and no functional sperm were observed. No embryo sporophytes were produced.

Abscisic Acid-Kinetin Interactions on Lipid Bilayers. PAUL HESTER and WILLIAM STILLWELL, Department of Biology, Indiana University-Purdue University at Indianapolis, Indiana 46223.—Although plant hormones are known to alter many physiological processes, a precise mode of action has not been described for any of them. The hormones kinetin and abscisic acid (ABA) have been shown to affect the permeability of several plant membranes, often in an antagonistic manner. Because of the complex nature of biological membranes it has not been possible to deduce the nature of these

hormone-membrane interactions. We have, therefore, used the well-defined, protein-free artificial bilayer systems (liposomes) in place of biological membranes to more accurately assess the effects of kinetin and ABA on membrane permeability.

ABA is shown to enhance the permeability of crude egg lecithin bilayers to urea, erythritol and water. The effect is pH dependent (undissociated ABA is much more effective) and requires the presence of phosphatidyl ethanolamine (PE) in the bilayers (no effect is noticed with PE-free, dimyristoylphosphatidylcholine bilayers). Kinetin also enhances permeability of similar bilayers. When the membranes containing PE are exposed to the two hormones simultaneously, however, a clearly antagonistic effect is seen with kinetin reversing the ABA-induced permeabilities. On the basis of these experiments we propose that undissociated ABA enhances membrane permeability by interacting with PE, possibly forming channels which in turn are blocked by kinetin. This model can explain many of the rapid-time physiological effects of these two plant hormones.

Evidence for Increased Primary Productivity Under Dynamic Conditions. JEFFREY KING and AUSTIN BROOKS, Wabash College, Crawfordsville, Indiana 47933.—The estimation of the primary productivity in an aquatic habitat has long been accomplished by the light and dark bottle method. The accuracy of this method was questioned by John H. Rodgers Jr. (1976, 1978). In 1978 our laboratory began to investigate primary productivity estimations under various conditions. We propose that the current light and dark bottle method may underestimate the aquatic primary productivity.

Cultures of *Oscillatoria* were grown in a sterile, synthetic media (Alga-grow Freshwater, Carolina Biological Supply) for seven to ten days, at 23°C and a light intensity of about 300 ft.c. Through agitation, the cultures were made homogeneous. The homogeneity of the culture was confirmed by correlating the optical density, measured at 525 nm, with the total protein, and chlorophyll *a* content. Chlorophyll was extracted using the acetone/DMSO technique, while total protein was determined using the Lowry method. The cultures were then placed under various physical parameters; both static, and dynamic, and incubated with $\text{NaH}^{14}\text{CO}_3$ for 30 minutes at 23°C, with a light intensity of about 300 ft.c.

We observed an increase in the primary productivity under dynamic conditions of nearly 20 percent over that observed in the static environment. However, after the current speed exceeded 110 cms^{-1} , productivity decreased to a level lower than the static productivity. This was most pronounced at a speed of 145 cms^{-1} . These current speeds are similar to those that *Oscillatoria* may encounter in nature.

While the reduction in productivity at fast current speeds is reproducible, we are unsure of its cause. Through a dilution and enrichment scheme we feel that the nutrient concentration is not the controlling factor in this process. We have observed that by first stressing the cultures (prestirring them at 145 cms^{-1}) for various periods of time, then carrying out our normal dynamic incorporation procedure, that *Oscillatoria* appears to show an ability to adapt to the turbulent environment. After approximately 25 minutes of high speed prestirring, the cultures exhibit an estimated primary productivity that approaches that obtained under static conditions.

A Comparative Study of Photosynthetic Rates in Deep (30m) Shallow (1-2m) Water Species of Algae. ROSALIE KRAMER, Department of Biology, Indiana University East, Richmond, Indiana 47374.—Preliminary studies of the comparative photosynthetic rates of deep (30m) and shallow (1-2m) water species of tropical marine algae indicate significant differences in the plant's photosynthetic mechanisms. As plants growing at different depths utilize different wave lengths and different pigment systems, this was

to be expected. Algae were selected from the families chlorophyta, rhodophyta, and phaeophyta. The study was carried out at Glover's Reef Atoll, Belize, Central America. This study attempts to demonstrate possible adaptability of algae to differing photosynthetic conditions by "transplanting" deep water specimens into shallow water and shallow species into deep water. The algae were maintained in the new environment for one month and comparative photosynthetic rates were taken on a weekly basis. Readings were based on the amount of O₂ evolved in one hour in a standard 300 ml B.O.D. bottle as read with a dissolved oxygen probe attached to a portable Orion pH meter.

Sequential readings over the month-long period indicated differences in the ability of different species of algae to acclimate to the new habitat.

Further work in terms of a long-term study is needed to validate results and to collect data on additional species.

The Effects of Hormone Treatment on Superoxide Dismutase Activity in Etiolated Decapitated Pea Seedlings. JULIE A. OLSEN and CURTIS R. COOK, Wabash College, Department of Chemistry, Crawfordsville, Indiana 47933.—Tissue taken from the cut surface of etiolated decapitated pea seedlings shows rapid decline (within 30 minutes) in superoxide dismutase activity to approximately 40% of the original level. Protein concentrations also fall within similar time span to approximately 70% of the initial concentration. The tissue recovers about 90% of the protein and 50% of the superoxide dismutase activity within 2.5 hours of decapitation. Treatment of the cut surfaces with solutions of indole acetic acid, kinetin gibberellic acid or ethylene gas indicate that both indole acetic acid and kinetin provide some selective protection for superoxide dismutase from general proteolysis. Indole acetic acid, kinetin and gibberellic acid also appear to enhance the rate of protein synthesis following wounding.

Potassium Transport Defective Mutant Strains of *Chlamydomonas reinhardtii* L. DAVID POLLEY, Wabash College, Crawfordsville, Indiana 47933.—Mutants of *Chlamydomonas reinhardtii* which exhibit a dependence on abnormally high concentrations of potassium for growth have been isolated. These have been characterized genetically and physiologically. Results of genetic analysis show that the potassium dependent phenotype is inherited as a nuclear gene. Characterizations of potassium transport show that wild type cells have two potassium transport systems and that the mutant strains lack one of the transport activities (a potassium "scavenging" activity).

Potassium transport defective strains also were examined for possible pleiotrophic effects of the lesion in the transport gene. Mutant strains were observed to stop swimming after four hours of potassium starvation. The effects of potassium starvation on the cell's ability to mate is also being explored. The potential these other pleiotrophic effects may have in developing enrichment schemes for future mutant hunts will be discussed.

***Pisolithus tinctorius* Inoculation Enhances the Nursery Production of *Quercus rubra* L. Seedlings.**—P. E. POPE, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907 and J. D. RHODES, Research Associate, Texas A & I University, Kingsville, Texas 78363.—Nursery beds planted with *Quercus rubra* L. were inoculated with vegetative mycelium of the mycorrhizal fungus *Pisolithus tinctorius* (Pt) or autoclaved inoculum at a rate of 200 ml/0.1 m² in early May of 1980, 1981 and 1982. Both treatments received the same amounts of irrigation and fertilization during the growing season. All seedlings from the four 1.2 x 1.9 m replicates were lifted in mid to late November of each year and graded by nursery standards for height and diameter for marketability. In addition, the seedlings were separated into four size classes and subsamples taken to determine total dry weight, root/shoot

ratio, methanol soluble carbohydrates and extent of mycorrhizal infection. Inoculation of red oak nursery beds significantly increased the number of salable seedlings and the average total dry weight/seedling each of the three years. Root to shoot ratio and methanol soluble carbohydrates of inoculated seedlings were significantly greater than the control in 1980 and 1982. Enhanced seedling growth is influenced by the mycorrhizal inoculation treatment but percent root colonization by the mycorrhizal fungus is poorly correlated with many of the seedling growth parameters. Mycorrhizal development on seedling roots inoculated with Pt was significantly greater than the control for all years.

Cellar Storage Shows Promise for Preserving Viability of Black Walnut Seed. ROBERT D. WILLIAMS, U.S. Forest Service, Bedford, Indiana 47421.—Black walnut (*Juglans nigra* L.) seeds from nine southern Indiana seed trees were collected in the fall of 1978 and either sown into Vallonia Nursery seedbeds soon after collection or stored one full year in soil pits or in root cellars, then sown at the nursery in the fall of 1979.

Differences in germination attributable to both seed source and storage facility were statistically significant at the 1-percent level. Also, the interaction between seed source and storage facility was significant at the 10-percent level. Some seed sources germinated best after pit storage while others germinated best after being stored in one of the root cellars.

Germination of some seed sources was more than 50-percent after a full year in cellar storage. So cellar storage has the potential for cheap, effective storage of black walnut seed for one year.

Seed Source Affects Black Walnut Seedling Growth More Than Mycorrhizal Inoculation. ROBERT D. WILLIAMS, U. S. Forest Service, Bedford, Indiana 47421.—In 1980 containerized black walnut (*Juglans nigra* L.) seedlings from four local families and one mixed seed source, both inoculated and non-inoculated with vesicular-arbuscular mycorrhizae, were outplanted into an old field in southern Indiana.

Fourth year results from the study show that seed source, even though all were local families, has had more effect on seedling growth than mycorrhizal inoculation.

Fourth year survival was not affected by seed source or inoculation. Although small, height differences attributable to seed source are statistically significant at the 1-percent level and diameter differences attributable to seed source are significant at the 5-percent level. Neither height nor diameter differences attributable to mycorrhizal inoculation are statistically significant.

Immunocytochemical Identification of Laticifers. KATHRYN J. WILSON, and BRUCE H. PETERSEN, Department of Biology, Purdue University School of Science, Indianapolis, Indiana 46223, and DAVID D. BIESBOER, Department of Botany, University of Minnesota, St. Paul, Minnesota 55108.—A sensitive immunocytochemical method for the identification of laticifers has been developed. To prepare latex antiserum latex serum was prepared from whole latex of wild *Asclepias syriaca* plants and injected into rabbits. The IgG fraction of the rabbit serum was separated by ion exchange chromatography and antibodies not specific to laticifer cells were removed by absorption of the IgG fraction with cells derived from liquid cell cultures of *A. syriaca* determined not to contain laticifers. Frozen sections of various laticifer-bearing plant material were mounted on slides. Sections were flooded with anti-latex antiserum followed by fluorescein-conjugated IgG fraction goat anti-rabbit IgG to visualize laticifers. Positive fluorescence was observed for laticifers in shoots and embryos of *A. syriaca* and *Stapelia bella*, and embryos of *A. tuberosa*. Laticifers did not fluoresce in shoots of *A. tuberosa*

and *Euphorbia tirucalli*, in embryos of *E. marginata*, or in petioles of *Musa pardisiaca* and *Cichorium intybus*. Controls prepared with uninjected rabbit serum were negative (no fluorescence).

A Survey of Tree Growth in the Merry Lea State Nature Preserve, Noble County, Indiana. STEPHEN C. YODER and LARRY R. YODER, Merry Lea Environmental Learning Center of Goshen College, Goshen, Indiana 46526.—Line strip surveys in a 56 acre portion of the Merry Lea State Nature Preserve were conducted by Alton A. Lindsey in 1972. The site is well suited as a permanently protected example of an oak-hickory association that experienced earlier selective harvests but is now growing without further human disturbance. A re-survey of the same line strips in 1982 revealed an 8.6% reduction in stand density which represents a loss of 1.2 trees per acre per year. During the same period the basal area increased by 10.6% or 1.1 ft² per acre per year. Among 15 species present, white oak (*Quercus alba*) and pignut hickory (*Carya glabra*) showed the greatest decrease in stand density. All species except for pignut hickory increased their basal area at a rate that more than off set density declines. The presence of shade intolerant species and a disjunct distribution of sizes indicates past disturbance from harvests. In both surveys, shagbark hickory (*Carya ovata*) was the most frequent species followed by black oak (*Quercus velutina*), wild black cherry (*Prunus serotina*), white oak (*Quercus alba*), pignut hickory (*Carya glabra*), and sassafras (*Sassafras albidum*). Future studies at 10 year intervals will provide an important base for assessing species composition and growth patterns in similar ecosystems. The 1982 survey was supported by an IAS research grant.

