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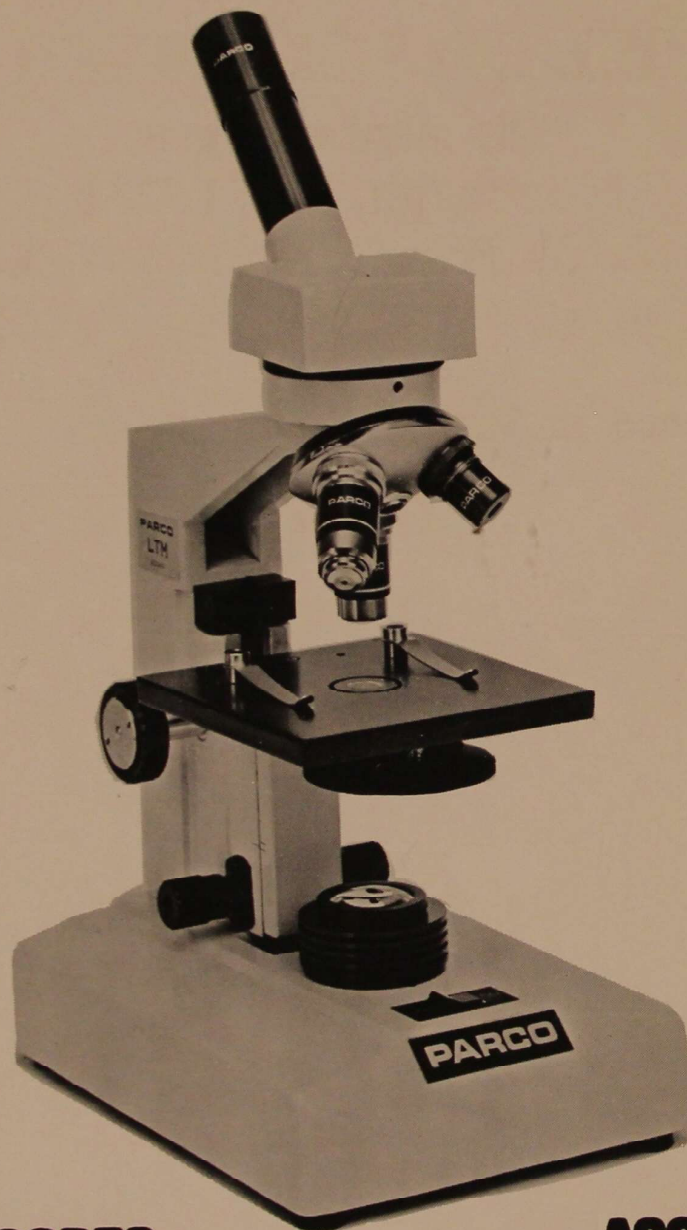
Proceedings of the West Virginia Academy of Science 1993



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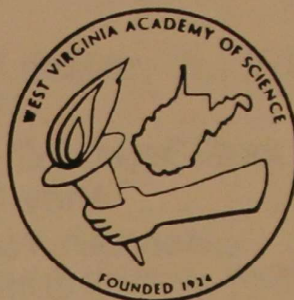


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Abstracts of the 1993 Annual Meeting

Acid Rain Symposium

A.T. HERLIHY, P.R. KAUFMANN (Oregon State Univ., c/o EPA, 200 SW 35th St., Corvallis, OR 97333), M.R. CHURCH, P.J. WIGINGTON, JR. (EPA Env. Research Laboratory-Corvallis), and J.R. WEBB (Univ. of Virginia).

The Effects of Acidic Deposition on Streams in the Mid-Appalachian Mountains - Summary of NAPAP Findings

Streams in the Appalachian Mountain area of the Mid-Atlantic receive some of the largest acidic deposition loadings of any region of the United States. A compilation of survey data from the Mid-Appalachians yields a consistent picture of the acid-base status of streams. Acidic streams, and streams with very low acid neutralizing capacity (ANC), are almost all located in small ($< 20 \text{ km}^2$), upland, forested catchments in areas of base-poor bedrock. In the subpopulation of upland forested systems, which comprises about half the total stream population in the Mid-Appalachian area, data from various local surveys show that 5–20% of the streams are acidic ($\text{ANC} < 0$), and about 25–50% have $\text{ANC} < 50 \text{ } \mu\text{eq/L}$. National Stream Survey estimates for the whole region show that there are 2,330 km of acidic streams and 7,500 km of streams with $\text{ANC} < 50 \text{ } \mu\text{eq/L}$ that have strong acid anion concentrations dominated by sulfate from atmospheric deposition. Many of the streams with baseflow $\text{ANC} < 50 \text{ } \mu\text{eq/L}$ become acidic during storm or snowmelt episodes. In these acidic streams, the low pH (median=4.9) and high levels of inorganic monomeric aluminum (median=129 $\mu\text{g/L}$) leached through soils by acidic deposition are causing damage to aquatic biota. Quantification of the extent of biological effects, however, is not possible with available data. Localized studies have clearly shown that streamwater ANC is closely related to bedrock mineralogy. Attempts to quantify this relationship across the Mid-Appalachians, however, were frustrated by the lack of adequate scale geologic mapping throughout the region. Sulfate mass balance analyses indicate that soils and surface waters of the region have not yet realized the full effects of elevated sulfur deposition due to watershed sulfate retention. Sulfur retention will likely continue to decrease in the future, resulting in further losses of stream ANC.

ADAMS, MARY BETH, JAMES N. KOCHENDERFER
PAMELA J. EDWARDS, and THEODORE ANGRADI,
USDA Forest Service, Northeastern Forest Experiment
Station, Timber and Watershed Laboratory, Parsons, WV
26287. Research on Acidic Deposition and Other Air
Pollutants at the Fernow Experimental Forest.

For a number of years, scientists at the Fernow Experimental Forest have been involved in research evaluating the effects of acidic deposition on forest ecosystems. Precipitation and other air pollutants have been monitored since 1978 on and near the Fernow. In 1989 researchers began a unique study on an 85 acres gaged experimental watershed. This study was designed to evaluate the effects of artificial acidification on forest ecosystems. This research project involves a large number of cooperators from West Virginia

and elsewhere in the U.S., and is one of only two artificial acidification projects in the U.S. Ammonium sulfate fertilizer treatments are being applied by helicopter three times a year, at rates that approximate twice-ambient levels of sulfur and nitrogen deposition. The effects on stream water chemistry, soil water chemistry, nutrient cycling, and various biota are being examined. Four years of treatment have been completed; we expect to continue this study at least one more year. Other research related to acidic deposition in West Virginia will also be discussed.

RAYMOND P. MORGAN II, University of Maryland,
CEES-AEL, Gunter Hall, Frostburg, MD 21532.
Impact of episodic events on stream water chemistry
in the Allegheny Plateau.

A three-year (1989-1992) study on the Big Run watershed of Garrett County, MD was designed to observe impacts of acidic deposition on stream chemistry. The fundamental protocol was for weekly sampling of analytes associated with acid rain studies at a number of set stations within the watershed, followed by more intensive sampling during storm events. Three basic types of episodes were examined: major storm fronts moving across the Allegheny Plateau during fall and spring, snowmelt, and thunderstorms.

Depending on bedrock composition, episodic depressions of acid neutralizing capacity (ANC) were observed to drop to $0 \mu\text{eq/L}$, with pH reaching 5.0 -- an indication of an acidic event. Large depressions ($> 250 \mu\text{eq/L}$) of ANC were frequently observed throughout the watershed. However, pH depressions into the 4.2-4.6 range were not observed at any sampling site. Elevated aluminum concentrations were noticed at a number of stations during episodic events, with levels approaching toxic concentrations to biota. Dilution of base cations was also observed, but was highly dependent on stream source geology. The degree of acidic impacts in the watershed is highly dependent on bedrock composition.

THOMAS K. PAULEY, Department of Biological
Sciences, Marshall University, Huntington,
WV and JAMES KOCKENDERFER, Fernow Experi-
mental Forest, Parsons, WV. Potential impacts
of an artificially acidified watershed on
forest salamanders.

Three watersheds (two controls and one treatment) at the Fernow

Experimental Forest in Parsons, West Virginia have been monitored annually since 1988 to determine potential impacts on terrestrial and aquatic salamanders. Ammonium sulfate fertilizer has been applied to the treatment watershed each year in March, July, and November. To date, 12 applications have been made. Soil water chemistry is monitored by lysimeters and stream flow chemistry is monitored at watershed outlets. Three species, the redback salamander (Plethodon cinereus) and the mountain dusky salamander (Desmognathus ochrophaeus) in terrestrial habitats and the seal salamander (Desmognathus monticola) in streams, have been monitored in the field for potential impacts on surface density and sex and age ratios. Laboratory studies included potential effects on food items, percentages of fat in the tails and carcasses, and number, size, and volume of ovarian eggs. To date, no significant differences have been found in any of the parameters in relation to salamander ecology.

J.R. Webb, B.J. Cosby, F.A. Deviney, J.N. Galloway, and M.E. Mitch, Dept. of Environmental Sciences, Univ. of Virginia, Charlottesville, Va. 22903 and D.M. Downey, Dept. of Chemistry, James Madison Univ., Harrisonburg, Va. 22807. Nitrogen release and stream acidification after forest defoliation by the gypsy moth.

During the period 1984 to 1991, the range of the gypsy moth (Lymantria dispar) expanded southward about 30 km/yr along the mountain ridges of western Virginia. This infestation resulted in late-spring defoliation of the deciduous forest of these ridges. An association between this defoliation and both nitrogen release and streamwater acidification is indicated by examination of geographic defoliation data in relation to ion concentration and flux measurements obtained for streamwaters included in a regional watershed research program. Virginia mountain streams are highly susceptible to acidification because of inherent geologic sensitivity and an elevated flux of acidic sulfate from atmospheric deposition. An assessment based on the magnitude of the initial response and the extent of sensitive systems indicates that the gypsy moth contribution to regional streamwater acidification may be substantial and that impacts to aquatic biota should be anticipated.

GILLIAM, FRANK S. Dept. of Biol. Sci., Marshall Univ.,
Huntington, WV 25755 and MARY BETH ADAMS, NE For.
Exp. Sta., Parsons, WV 26287. Precipitation chemistry for
West Virginia Appalachian forest sites: temporal and spatial
variation.

Acidic deposition continues to be an environmental concern in the forested Appalachian mountain region. Although extensive research has been directed at spruce-fir forests (in response to dramatic declines), far less research has investigated the impacts of air pollution on eastern high-elevation hardwood forests. The purpose of this study is to describe precipitation chemistry for several Appalachian hardwood forest sites within the Fernow Experimental Forest (FEF) to assess the potential for problems associated with acid deposition in these forests. Emphasis is placed on 1) seasonal and long-term (8 yr) patterns of ionic concentrations and deposition (H^+ , Ca^{++} , NH_4^+ , NO_3^- , SO_4^{--}), and 2) spatial variability of these ions among sites. Precipitation chemistry for FEF was predictably similar to data summarized for sites throughout the eastern United States, with the notable exception that H^+ represented approximately 70% of total cations for FEF and only about 50% for the eastern U.S. Seasonal patterns of most ions showed highest concentrations and deposition during the summer months. There were no real long-term trends for any ions, although mean $[H^+]$ exhibited a slight pattern of increase during the study period. Deposition of all ions was consistently higher (related to higher precipitation) at the three high-elevation sample sites compared to the one low-elevation site. These data suggest the potential for problems with acid deposition at these remote deciduous forest sites. Precipitation is chronically quite acidic, more so during the growing season and highest at higher elevations where environmental stresses can be most severe.

HAROLD S. ADAMS, Div. of Arts and Sciences, Dabney S.
Lancaster Community College, Clifton Forge, Virginia 24422,
STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State
College, Fairmont, West Virginia 26554, DAVID M. LAWRENCE,
Dept. of Environmental Sciences, University of Virginia,
Charlottesville, Virginia 22903, and MARY BETH ADAMS, USDA
Forest Service, Timber and Watershed Laboratory, Parsons, West
Virginia 26287. An ecological analysis of the red spruce/northern
hardwood ecotone in the Allegheny Mountains of West Virginia

The ecotonal boundaries that exist between mid-Appalachian red spruce (*Picea rubens* Sarg.) communities and the northern hardwood communities that surround them are usually quite narrow and abrupt. This is in marked contrast to hardwood/hardwood ecotones in the same region, where communities tend to intergrade almost imperceptibly and form a mosaic of vegetational continua. Recent evidence from a number of studies suggests that high-elevation red spruce forests throughout the eastern United States are declining, possibly in response to airborne chemical pollutants. Using techniques of direct gradient analysis, we are currently investigating patterns of species composition and distribution, ecologically important population

processes, and microenvironmental gradients in permanent transects (each consisting of a series of contiguous 100 m² quadrats) established across spruce/hardwood ecotones at two localities (McGowan Mountain in Tucker County and Cheat Mountain in Randolph County) in the Allegheny Mountains of West Virginia. Primary emphasis of our research is directed toward testing three basic hypotheses: (1) red spruce communities are decreasing in areal extent due to encroachment of surrounding hardwood communities, (2) stress-induced growth decline in red spruce is a factor in this decrease, and (3) the direction and rate of successional change can be predicted from models developed from quantitative data obtained from field studies of spruce/hardwood ecotones. Preliminary data obtained during the 1992 field season suggest that mid-Appalachian red spruce communities presently exist in static equilibrium with respect to surrounding hardwoods. (Supported in part by funds provided by the USDA Forest Service.)

Biology

MICHAEL B. GRIFFITH, SUE A. PERRY, and WILLIAM B. PERRY, WV Coop. Fish & Wildlife Res. Unit, West Virginia University, West Virginia 26506. Macroinvertebrate communities in headwater streams of different baseflow alkalinity in the central Appalachians.

We compared the species and functional group composition of the benthic macroinvertebrate community in four streams located on the Allegheny Plateau of West Virginia; these streams were characterized by different bedrock geology, streamwater alkalinity, and pH. Mean alkalinity was 0.0, 0.9, 0.7, and 40.8 mg/L, and mean pH was 4.26, 6.07, 5.99, and 7.48 in the four streams. Macroinvertebrate samples were collected monthly from each stream from September 1990 to October 1991. To collect the samples, we used a pump depletion sampler.

Densities of shredders were higher in the acidic stream, but the higher densities were accompanied by differences in the dominant shredder species. The dominant shredder species in the acidic stream were small stoneflies Leuctra sp. and Amphinemura sp. The dominant shredders in the neutral streams were Peltoperla arcuata and Pycnopsyche sp. In the more alkaline stream, the dominant shredder was Gammarus minus. Densities of collector-gatherers were less in the acidic stream, and these differences were accompanied by fewer species of Ephemeroptera. Several mayflies were tolerant of low pH, though, including Ameletus sp. and Epeorus sp. Densities of other functional groups including grazers and predators did not seem to differ among streams.

ADAMS, MARY BETH, USDA Forest Service, Northeastern Forest Experiment Station, Timber and Watershed Laboratory, Parsons, WV 26287. Acidic deposition effects on high-elevation red spruce forests.

In the northeastern U.S., spruce-fir forests are experiencing visible decline, and many hypotheses have been developed to explain the decline. The Spruce-Fir Research Cooperative, a regional program of the Forest Response Program of NAPAP (National Acid Precipitation Assessment Program), was charged with evaluating a number of hypothesized effects of acidic deposition on these spruce-fir forests: (1) soil-mediated effects; (2) altered physiological processes; (3) increased foliar injury; (4) increased susceptibility to winter injury. After six years of research by more than 120 scientists from different institutions, a significant body of research has accumulated, and some conclusions can be drawn. Based on extensive field and laboratory data,

regional air pollution has played a significant role in the decline of red spruce in the eastern U.S. Winter injury is associated with decreased growth and increased mortality in the northern Appalachians, while aluminum interference with cation uptake, carbon metabolism and growth has been documented in the southern Appalachians. The sensitivity of red spruce to winter injury, aluminum mobilization and cation loss is increased by atmospheric deposition of acids in amounts currently occurring in the eastern United States.

STEVEN D. KOENIG (PRESENTER), SUE A. PERRY, AND WILLIAM B. PERRY, WEST VIRGINIA COOPERATIVE FISH AND WILDLIFE RESEARCH UNIT, WEST VIRGINIA UNIVERSITY, P.O. BOX 6125, MORGANTOWN, WV 26506-6125. PHONE: (304) 293-3794. THE EFFECT OF TEMPERATURE AND PH ON THE USE OF ENDOGENOUS ENERGY SOURCES BY LARVAL BROOK TROUT (SALVELINUS FONTINALIS).

Recruitment failure has been identified as a major mechanism responsible for the disappearance of fish populations in acid-stressed ecosystems. Chronic exposure to sublethal levels of pH may alter partitioning of endogenous energy resources during egg development. The primary objective of this study was to measure the metabolic cost of chronic exposure of developing brook trout to low pH. Brook trout eggs were incubated under static-renewal conditions at 9°C or 12°C from fertilization to hatch and exposed to a series of buffered pH solutions (pH 5 to 7) of reconstituted soft water. Size, dry weight, calories, caloric density, protein, lipid, and carbohydrate of unfertilized, fertilized, eyed eggs, and larvae at day one post-hatch were measured.

Total calories decreased from 189.99 calories for the unfertilized egg to 142.98 calories at hatch. Total calories of the yolk and larval somatic tissue at hatch were 133.00 and 9.98, respectively. Caloric density increased significantly throughout development. Neither temperature nor pH had a significant effect on total calories remaining at the eyed stage. Caloric density at the eyed stage was correlated with temperature (p-value = 0.0001) and pH (p-value = 0.0600). Total carbohydrate decreased 88.6% by the eyed stage.

VINCENT GEORGE and THOMAS E. WEAKS. Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755. Periphyton communities of dominant bryophytes of the Greenbottom wetlands area.

Comparisons of periphyton communities from two distinct plant communities were made at the Greenbottom wetlands area located along

the floodplains of the Ohio River in Cabell and Mason Counties, West Virginia. Bryophytes were sampled from submerged substrates and analyzed for periphyton species within each plant community. Two mosses, Amblystegium serpens (Hedw.) B.S.G. and Brachythecium acutum (Mitt.) Sull., were dominants in the Acer saccharinum-Plantanus occidentalis plant community. In the second plant community, Typha latifolia-Saururus cernuus, the dominant bryophyte was Leptodictyum riparium (Hedw.) Warnst. Blue-green periphyton found to occur on each of these three bryophytes were Oscillatoria, Johannesbaptista, and Anabaena. The green algae, Oedogonium, Ulothrix, Zygnema, and Closterium were also present on all three of these bryophytes. Species richness and species diversity were highest for L. riparium. The dominant periphyton species were the pennate diatoms, Navicula radiosa Kutz., Tabellaria fenestrata Kutz. and Eunotia pectinalis Kutz.

KIMBERLEY D. WAYBRIGHT and THOMAS E. WEAKS,
Department of Biological Sciences, Marshall
University, Huntington, West Virginia 25755.
Bryophytes of eight plant communities of Green-
bottom Swamp in Cabell and Mason Counties, West
Virginia.

Studies of bryophytes were conducted in eight distinct plant communities in Mason and Cabell Counties, West Virginia. Two or more 50 m transects were sampled at 25 m intervals from the center of each plant community. Epiphytic bryophytes, within 1 m of the transect, were sampled along tree bases and at 1 m heights at cardinal points. Thirty-one species of bryophytes (27 mosses and 4 liverworts) were observed. Three aquatic species, Leptodictyum brevipes (Card. & Ther. ex Holz.) Broth., Leptodictyum riparium (Hedw.) Warnst. and Amblystegium serpens (Hedw.) B.S.G. were the dominant mosses. The dominant liverwort was Riccia fluitans L. Highest species diversity was for the Salix nigra-Acer saccharinum dominated community. Lowest species diversity was observed for the Typha latifolia-Saururus cernuus and Cephalanthus occidentalis dominated communities.

TOM WEAKS, Dept. of Biological Sciences,
Marshall University, Huntington, West Virginia
25755. Periphyton community structure of
wetlands associated with the Ohio River.

The community structure of periphyton assemblages of representative wetland types associated with the Ohio River was examined along with

selected aspects of the physiochemical environment. An attempt was made to identify major causes of differences in periphyton communities in seven wetland types. Of the physical factors evaluated, duration of flooding and depth of water were found to exert the greatest influence on species diversity. Mean species diversity (d) ranged from 0 to 4.0 and was generally higher for the deep open water sites than for shallow wetlands. Members of the Bacillariophyceae were the predominant algal type with the majority of those being of the genus Navicula. Results of the study suggest that periphyton community structure integrity is correlated with secondary succession of wetlands and as succession progresses a decrease in species diversity plus supplanting of dominant species can be expected. In addition, comparisons of community diversity among wetland types indicate that the habitats are quite different with respect to periphyton present and their apportionments.

S. CRAIG STAMM, BAO-ZHEN ZHONG, TONG-MAN ONG, WEN-ZONG WHONG, National Institute for Occupational Safety and Health, Morgantown, West Virginia 26505 and E.C. KELLER JR., Department of Biology, West Virginia University, Morgantown, West Virginia 25506. Mutagenicity of two smokeless tobacco extracts in *Salmonella typhimurium* strains with enhanced sensitivities to aromatic amines/nitroarenes.

Epidemiological studies of coal miners have indicated a positive relationship between this occupation and the incidence of gastric cancers occurring in this industry. It has been suggested that coal dust, inhaled by miners and cleared by mucociliary functions, undergoes nitrosation and/or other chemical interactions at low pH in the stomach resulting in the formation of carcinogenic compounds. The source of the nitrites/nitrates responsible for the nitrosation reactions is thought to be dietary components such as preserved meats, vegetables, or lifestyle factors such as the use of smokeless tobaccos. Since there is a high incidence in the use of smokeless tobacco products in the mining business, especially for underground miners, and because synergistic interactions have been shown to occur between coal and tobacco extracts, the role of tobacco-related nitrites and other compounds are of particular interest in this problem. Aromatic amines and nitroarenes are genotoxic agents that have been found to be present in tobacco in a limited number of studies. The chemical detection of these compounds in tobacco is very difficult; due to both the complexity of tobacco and the generally low concentrations of these volatile substances therein. However, biological assays in the form of *Salmonella* microbial mutagenicity assays with greatly enhanced sensitivities to these agents now exist. These test systems utilize bacteria with multiple copies of the O-acetyltransferase (OAT) or nitroreductase (NR) genes which are necessary for the conversion of aromatic amines and/or nitroarenes to their ultimately mutagenic nitrenium species. This study examines both nitrosated and non-nitrosated extracts of a chewing tobacco and a snuff for these compounds by contrasting the activities of a conventional versus an OAT tester strain in the Ames assay. Additionally, the non-nitrosated snuff extract was examined on five tester strains with varying sensitivities to aromatic amines/nitroarenes. The results of this study suggested the presence of nitroarenes in both of the nitrosated smokeless tobacco extracts, and the presence of aromatic amines in the

non-nitrosated snuff extract. These findings may have implications in cancers of both oral and gastric sources for the users of these products, and may be of particular importance in the etiology of gastric cancer in coal miners.

JOHN C. LANDOLT, Div. of Science and Mathematics, Shepherd College, Shepherdstown, West Virginia 25443 and STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554. Temporal changes in the structure and composition of cellular slime mold communities.

A study of the occurrence and distribution of dictyostelid cellular slime molds (CSM) in four watersheds of the Fernow Experimental Forest in Tucker County, West Virginia, was carried out during the period of 1989-92. Soil/litter samples for isolation of CSM were collected from each watershed in late spring and early fall during each of the four years. In 1991 and 1992, data were obtained for numbers of heterotrophic, aerobic soil/litter-inhabiting bacteria, which represent the primary food resource for CSM, in the same four watersheds. At least twelve different species of CSM were isolated in the 4-year study, with the most common species generally represented in all watersheds on each sampling date. Both species richness and density of CSM were higher in the fall than in the spring, and one species, Polysphondylium violaceum, was noticeably more abundant in the fall than in the spring. Numbers of soil/litter bacteria were quite variable within a single watershed on a given sampling date. However, averages for the four watersheds were generally similar for a particular sampling date and tended to show an increase from spring to fall. (Supported in part by funds provided by the USDA Forest Service.)

JOHN C. LANDOLT, Dept. of Biology, Shepherd College, Shepherdstown, West Virginia 25443, STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, GARY A. LAURSEN, Dept. of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775, and ROSEANN DENSMORE, National Park Service, P. O. Box 9, Denali National Park, Alaska 99775. Notes on the occurrence and distribution of dictyostelid cellular slime molds in Denali National Park, Alaska.

The occurrence and distribution of dictyostelid cellular slime molds (CSM) and the heterotrophic, aerobic bacteria upon which these organisms feed were studied in the 1992 field season. Soil/litter samples for isolation of CSM and for determining bacterial densities were collected from a number of different study sites in Denali National Park and Preserve in central Alaska. Among these were four permanent plots (alpine tundra, white spruce treeline, white spruce forest and riparian shrub) established as part of a long-term vegetation monitoring project. In general, numbers of bacteria (colony forming units/g wet soil) were comparable to those obtained for forest study sites in West Virginia. Numbers of CSM (clones/g wet soil) were often much lower than those reported for most forest study sites at lower latitudes. CSM isolated from soil/litter samples in the present study included all of the species reported previously from central Alaska as well as several forms which may represent new records for high-latitude North America or perhaps even new taxa. (Supported in part by funds provided by the National Park Service.)

Botany

MICHAEL A. FOX AND RONALD H. FORTNEY, School of Engineering and Science, West Virginia Graduate College, Institute, West Virginia 25112. A Survey of Common Plant Species of Wetland Habitats in West Virginia.

Advances in computer technology during the past decade has made it possible to easily compile large quantities of information into easily useable formats. As a result, the key objective of this study was to assemble ecological information on wetlands into an easily accessible format for use by educators, government agencies and wetland delineators, as well as to provide information for the general public.

It was the intent of this study to collect and document information on hydrologic, geologic and biologic parameters important to approximately three hundred wetland vascular plant species occurring in West Virginia. The data were then arranged in an original format to facilitate transference into a computerized data base.

The survey included reviewing approximately 24 sources including, technical articles, books and technical reports on wetland habitats and species in the Central Appalachian Mountain Region. This paper demonstrated that (1) plant descriptions are generally not multi-disciplinary in scope and (2) there is no standardization of terms among authors in describing habitat parameters. The work may be considered a first attempt to fill the gap that exists between users who, because of their vocational or educational work, need information on wetlands for which there are no readily available sources.

Stark, T.J., D.K. Evans, and F.S. Gilliam, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25755 and P.A. Robertson, Dept. of Plant Biology, Southern Illinois University at Carbondale, Carbondale, Illinois 62901. Characterization of the vascular plant communities of Green Bottom Wildlife Management Area, a middle Ohio River floodplain.

Plant communities of the 338 hectare Green Bottom Wildlife Management Area were described by several multivariate and classical methods, including detrended correspondence analysis (DECORANA), and canonical community ordination

(CANOCO). Overstory and shrub layer species from 147 100m² plots were treated separately, as were herb layer cover data from 588 1m² subplots along the same overstory transects. Gross community types include high and low bottomland hardwood forest, river beach, river mud flat, river berm, marsh, wet meadow, full canopy swamp, and open canopy shrub swamp. The defined plant communities are arranged along hydrologic and soil texture gradients. Classification of the sample points, and their correlation to these environmental variables, provide insight into temporal community succession in the rapidly expanding wetland. This information is necessary for making management decisions concerning wetland conservation, restoration, and creation projects.

Stark, T.J., and D.K. Evans, Dept. of Biological Sciences, Marshall University, Huntington, West Virginia 25755. The floristic value of Green Bottom Wildlife Management Area, a middle Ohio River wetland floodplain.

Green Bottom Wildlife Management Area (Cabell county, West Virginia) contains several hydrologically diverse habitats recognized by their plant species composition and gross physiography. Both forested and open canopy wetlands, old fields, and riparian sand and mud flats are observed in several stages of succession. Species richness in these habitats often surpasses 20 species per 1m². The objective of this study was to produce a vouchered flora of the 338 hectare site, ignoring fields under cultivation. Over 20 scholarly papers are based on field sampling at Green Bottom. This work is intended as a comprehensive baseline for future conservation, management, and and research decisions. A single season taxonomic survey documented 330 species of vascular plants, including 63 obligate, and 217 facultative wetland species. Of these, 238 are native to West Virginia, 71 are exotic to North America, and the remainder are considered North American adventive. Green Bottom is the first described location of several of West Virginia's vascular plant species. In all, six state endangered species (found in five or fewer locations statewide), and four state threatened species, were recorded. The flora are examined for geographical affinity, wetland status, and obligation to habitat. Pertinent literature, concerned herbaria, and West Virginia State Natural Heritage Program records for Cabell county are being searched and verified in order to construct as complete a vouchered flora as possible.

D. W. CHAFFIN, D. K EVANS, and M.A HARRISON, Dept. of Biological Sciences, Marshall University and R. DEAL, Dept. of Science, Shawnee State University. Secretary reservoir anatomy in leaves and involucral bracts of Amazonian Clibadium asperum (Aubl.) DC (Asteraceae).

Epithelium lined secretory reservoirs occur concomitant with the vasculature in stems, leaves and involucral bracts of Clibadium asperum. Upper leaves and infructescences of this shrub are utilized as a source of piscicide by native people of Ecuador. The agents responsible for the fish poisoning activity of the plant are ichthyothereol and its acetate. Presence of polyacetylenes, produced and stored in reservoirs, is suggested by fluorescent action and methods of colorimetric detection on nitrocellulose tissue prints. In general, reservoirs of varying length are located abaxially in relation to lower order veins and variable to higher vein orders. Involucral bracts possess reservoirs of relatively larger diameter compared to those of leaves. Certain developmental aspects, as well as possible function of leaf and bract reservoirs, will also be discussed.

D. K. EVANS and D. W. CHAFFIN, Department of Biological Sciences, Marshall University, Huntington, WV 25755. Ethnobotany of Clibadium asperum (Aubl.) DC (Asteraceae) of southeastern Ecuador.

The Shuar and Achuar, formerly the Jivaro, of southeastern Ecuador, employ several plants that kill or stun fish. The most important are Lonchocarpus nicou (Aubl.) DC and varieties (Fabaceae), Tephrosia sinapou (Bucholz) A. Chev. (Fabaceae) and Clibadium asperum (Aubl.) DC (Asteraceae). All are woody plants, cultivated in gardens in or near villages and are harvested nondestructively. In the former two taxa roots sections are dug, pounded into shreds and introduced in the stream or pool with a vine basket or simply thrown into the water. In the latter, upper leaves and infructescences are collected, placed into a prepared hole in the ground and crushed into a wet pulp with blunt-ended poles. The material is then introduced into the water with a vine basket passed back and forth in the stream or impounded pool. In all cases fish become disoriented in a matter of minutes and are easily caught and eaten without ill effects. In both Lonchocarpus and Tephrosia the active compound is rotenone the action of which is well established. In Clibadium asperum the poisonous principle

is ichthyothereol, a polyacetylenic compound which apparently acts as a neurotoxin. Laboratory studies of fish killing potency and of ichthyothereol reservoirs in plant tissue are currently in progress.

TARA DUBEY, Dept. of Biology, West Virginia Institute of Technology, Montgomery, West Virginia 25136, STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, and PAMELA J. EDWARDS, USDA Forest Service, Timber and Watershed Laboratory, Parsons, West Virginia 26287. The effects of acidification on the aquatic fungi associated with West Virginia mountain streams

The distribution patterns of the aquatic fungi occurring in six streams located on or near the Fernow Experimental Forest in Tucker County, West Virginia, were studied during the 1991 and 1992 field seasons. Water pH averaged >5.5 in three of the streams, whereas the others were relatively more acidic (average pH = 4.2, 3.9 and 3.2, respectively). Sampling methods used included (1) isolating conidia from stream water by means of a membrane filtration technique, (2) placing litter bags prepared with leaves from four different tree species in the streams for periods ranging from 14 to 112 days, and (3) "baiting" the streams with various types of organic material. The mycoflora of the streams we sampled includes at least 142 taxa (44 Phycomycetes, 49 non-Ingoldian Hyphomycetes, and 49 Ingoldian Hyphomycetes). The total number (27 taxa) of Phycomycetes [chytridiaceous fungi and water molds] present in the stream with the highest pH (7.9) was appreciably higher than the numbers (15-18 taxa) recorded for the other streams. The general pattern for aquatic Hyphomycetes, based on the presence of conidia filtered from water samples, was for the number of taxa to decrease with decreasing pH, whereas the lowest numbers of conidia per 1000 ml of water were recorded at the two extremes of the pH gradient. Red oak (Quercus rubra) litter was colonized by an average of 14.6 taxa of aquatic Hyphomycetes in the six streams, sugar maple (Acer saccharum) litter by 12.6 taxa, and mixed red maple (A. rubrum) and beech (Fagus grandifolia) litter by 10.6 taxa. For all three types of litter, numbers were generally higher for the less acidic streams and lower for the more acidic streams. (Supported in part by funds provided by the USDA Forest Service.)

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, TARA DUBEY, Dept. of Biology, West Virginia Institute of Technology, Montgomery, West Virginia 25136, GARY A. LAURSEN, Dept. of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775, and ROSEANN DENSMORE, National Park Service, P.O. Box 9, Denali National Park, Alaska 99755. Aquatic fungi in streams of Denali National Park and Preserve, Alaska

The aquatic fungi occurring in six streams located within Denali National Park and Preserve in central Alaska were studied during the 1991 and 1992 field seasons. Sampling methods used included (1) isolating conidia from stream water by means of a membrane filtration technique, (2) placing litter bags prepared with leaves from three species of shrubs characteristically present as important components of riparian communities directly in the streams and retrieving these bags after a period of approximately two weeks, and (3) "baiting" with various types of organic materials (e.g., pine pollen) for the same period of time. In addition

to the stream survey, preliminary data were obtained for the aquatic fungi associated with decaying plant material in seepage pools located in areas of subarctic alpine tundra. The total number of taxa of aquatic Hyphomycetes recorded from a single stream, based on the presence of conidia filtered from water samples, ranged from 12 to 45. The majority of these were Ingoldian Hyphomycetes; non-Ingoldian Hyphomycetes were much less numerous. For Phycomyces (chytridiaceous fungi and water molds), the total number of taxa present (15 to 19) varied little among the three streams sampled for these organisms. Green alder (Alnus crispa [Ait.] Pursh) litter bags were colonized by an average of 10.0 taxa of aquatic Hyphomycetes, whereas the corresponding numbers for thinleaf alder (Alnus tenuifolia Nutt.) and feltleaf willow (Salix alaxensis [Anderss.] Cov.) litter bags were 12.3 and 13.7 taxa, respectively. Thirteen taxa were recorded from all three types of litter; Tetracladium marchalianum de Wildeman was particularly abundant. At least 24 taxa were recorded from decaying plant material collected from seepage pools located in areas of subarctic alpine tundra. (Supported in part by funds provided by the National Park Service).

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, SUSAN M. STUCLAR, Dept. of Botany and Microbiology, Oklahoma State University, Stillwater, Oklahoma 74078, CAROLYN J. MCQUATTIE, USDA Forest Service, 359 Main Road, Delaware, Ohio 43015,, and PAMELA J. EDWARDS, USDA Forest Service, Timber and Watershed Laboratory, Parsons, West Virginia 26287. The effects of acidification on the bryophytes associated with West Virginia mountain streams.

Various species of bryophytes (mosses and liverworts) are the major primary producers in many mountain streams, which are often virtually free of higher plants. In the present study, six streams located on or near the Fernow Experimental Forest in Tucker County, West Virginia, were sampled to obtain data on the structure and composition of the bryophyte communities present. Water pH averaged >5.5 in three of the streams, whereas the others were relatively more acidic (average pH = 4.2, 3.9, and 3.2). A number of other studies have demonstrated that species distribution patterns for stream-associated bryophytes are strongly influenced by water pH. The general pattern for the six streams we sampled was for species richness to decrease with decreasing pH, and no bryophytes were recorded from Finley Run, the most acidic of the six streams. Our data indicate that Scapania undulata, a leafy liverwort, is commonly the dominant bryophyte in most West Virginia mountain streams. However, S. undulata is absent from alkaline streams (pH >7.0) and from streams that are exceedingly acidic (pH <3.5). Transplant experiments carried out during the 1991 and 1992 field seasons indicate that this species can survive under these low pH conditions, but a number of changes in cell ultrastructure (as revealed by examination with TEM) are evident. Cell walls of S. undulata collected from a highly acidic stream (pH = 3.9) were thin, mottled, and densely stained compared with cell walls of material from a less acidic stream (pH = 5.5). A deterioration in chloroplast structure and other cellular organelles was also observed in material from the highly acidic stream. Scapania undulata transplanted from the less acidic stream to an extremely acidic stream (pH = 3.2) showed a similar (or less severe) breakdown in cell wall structure and disruption of cellular contents after a period of approximately three months. However, since the most acidic streams included in our study are impacted by acid mine drainage and have high sulfate and/or aluminum levels, low pH may not be the only factor responsible for the observed changes. (Supported in part by funds provided by the USDA Forest Service.)

TARA DUBEY, Dept. of Biology, West Virginia Institute of Technology, Montgomery, West Virginia 25136, STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, and PAMELA J. EDWARDS, USDA Forest Service, Timber and Watershed Laboratory, Parsons, West Virginia 26287. Effect of DIMILIN on the growth, sporulation, and enzymatic activities of the aquatic Hyphomycetes in streams of the Fernow Experimental Forest under natural conditions and in laboratory culture.

The effect of DIMILIN on the growth, sporulation, and leaf litter colonization of aquatic Hyphomycetes under natural conditions was studied by (1) isolating conidia from samples of stream water by means of a membrane filtration technique and (2) direct microscopic examination of the fungi colonizing litter bags containing leaves of red oak (Quercus rubra) and sugar maple (Acer saccharum) placed in two treated and two control watersheds of the Fernow Experimental Forest prior to the application of this chemical insecticide. In addition, mycelial growth and cellulase enzyme activity of six species of Hyphomycetes isolated from the watersheds were studied in the laboratory. Cellulase enzyme activity was assessed using a depth of clearance (DC) test and spectrophotometric analysis of the reducing sugars produced as a result of exoglucanase and endoglucanase activities. Appreciable increases in (1) the numbers of conidia filtered from water samples and (2) the extent of colonization of litter bags were noted two days after spraying of DIMILIN to the treated watersheds. The tolerance of aquatic Hyphomycetes to different concentrations of DIMILIN varied among the species tested, but DC was maximum at 5 ppm for five of the six species tested. In liquid culture an increase in endoglucanase activity at 20 ppm of DIMILIN was noted for four of the six species, whereas three species displayed a decrease in exoglucanase activity in the presence of DIMILIN. (Supported in part by funds provided by the USDA Forest Service.)

HAROLD S. ADAMS, Div. of Arts and Sciences, Dabney S. Lancaster Community College, Clifton Forge, Virginia 24422 and STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554. A comparative study of growth-trend patterns of red spruce in the southern Appalachians during the past two centuries.

Growth-trend patterns exhibited by red spruce (Picea rubens Sarg.) during the 20th century were analyzed using data obtained from increment cores collected at two sites in western Virginia (mean elevation = 1125 m), two sites in southwestern Virginia (mean elevation = 1698 m), and three sites in eastern Tennessee/western North Carolina (mean elevation = 1833 m). The set of cores obtained from each region and in each of three different age classes (<99 yr, 100-149 yr, and >150 yr) were analyzed separately. Based on average radial growth during each ten-year period (i.e., 1901-1910 through 1981-1990), trees at all three localities and in all three age classes have exhibited declining growth since 1960. This pattern is most apparent for the oldest trees and for trees at the two sites in southwestern Virginia. The percentage of trees showing reduced growth from the previous decade ranged from 18.8 to 93.3 for all sites in all decades. More trees showed reduced growth for sites in southwestern Virginia (64.7%) than for either western Virginia (53.3%) or Tennessee/North Carolina (53.1%). Our data indicate that the growth-trend pattern of red spruce has remained essentially the same during the two most recent decades. Growth-trend patterns of red spruce during the past two centuries were compared using data from the present study and comparable data obtained for cores collected from 21 old growth (>194 rings) trees (11 from western Virginia and 10 from the Great Smoky

Mountains National Park) during an earlier study. During the 19th century, these trees would have been comparable in age to most of those analyzed in the present study. The pattern of growth of these trees during the first nine decades of the 19th century was similar to that of the cores analyzed for the first nine decades of the 20th century. A similar group of 23 cores collected from old-growth (>200 rings) red spruce trees from West Virginia did not exhibit a comparable pattern. Instead, these trees were characterized by a period of accelerated growth during the 1850s, followed by a period of generally declining growth during the 1870s and 1880s. The very end of the 19th century was marked by a growth-trend increase which continued on into the early part of the 20th century.

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Division of Natural Resources, P. O. Box 67, Elkins, WV 26241,
BILL TOLIN and LINDA SMITH, US Fish & Wildlife Service,
Ecological Services, P. O. Box 1278, Elkins, WV 26241. New
Occurrences in West Virginia For Running Buffalo Clover (*Trifolium
stoloniferum*): Is It Really Endangered?

Running Buffalo Clover (*Trifolium stoloniferum*), once thought to be extinct but rediscovered in WV in 1983, was declared a federally endangered species in 1987. Since that time, Natural Heritage programs and cooperators in Indiana, Kentucky, Missouri, Ohio, and West Virginia have slowly discovered a number of new populations. The most recently discovered sites in West Virginia may prove to be the most significant in understanding some of the autecology and management implications pertinent to this species. New populations of Running Buffalo Clover were discovered at several sites within the Monongahela National Forest in 1991 and 1992 by cooperators and staff of the West Virginia Natural Heritage Program. These populations are significant because of their habitats, namely old forest service roads that are annually mowed, recently developed dirt roads, and logging skidder trails and haul roads that are dramatically disturbed at least once every 10 years. Populations range in size from 1 plant to thousands of plants. Information gathered at a recent regional meeting of conservation biologists working on the recovery of this endangered species provided an opportunity to summarize all of the known data about it and to review its status in each state and globally. In this paper, the new population sites and the data from the regional meeting are reviewed and summarized, and the implications for conserving Running Buffalo Clover and other rare plant species in West Virginia are discussed.

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**REGULATION OF ETHYLENE BIOSYNTHESIS IN
PLANTS GROWN IN CLOSED ENVIRONMENTAL
CHAMBERS**

Growth space is a limiting factor in NASA's Space Shuttle experiments and will be critical for the cultivation of plants in space. Small closed environmental chambers are

currently used for plant studies aboard the Space Shuttle's mid-deck locker facility. The plant hormone, ethylene, is produced by all plant tissues and can accumulate in these chambers. In ground-based experiments, ethylene accumulation alters plants' perception of gravity, growth, and productivity. Understanding the role ethylene biosynthesis in plants grown in small chambers may provide basic information necessary for the design of environmental chambers for space biology studies. In this investigation, tissue levels of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), and its malonyl-conjugate (MACC) were used as an index of ethylene biosynthesis in plants grown in closed environmental chambers. Pea seeds were germinated and grown in open or closed chambers at 23°C in darkness. Chambers were made to the dimensions of those currently used for Space Shuttle experiments. Root and stem tissue was analyzed for ACC and MACC content after 5 days incubation in chambers. Our results showed an increase in ACC levels in peas grown in closed chambers above those in open chambers. MACC levels were greater than ACC levels in all plant samples indicating active conjugation of ACC to MACC. Since, ACC accumulation may be due to inhibition of ACC oxidation to ethylene, ethylene accumulation and measurements of ACC oxidase activity will be investigated. Thus, this investigation will provide information vital for the design of chambers with optimum growth conditions under reduced air volume to plant ratio.

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TISSUE LOCALIZATION METHODS IN PLANTS:
NEW APPLICATIONS

Methods for cytochemical localization of plant products or enzymes can be complicated or impossible when dealing with living or fixed material which many substances cannot penetrate. The focus of these experiments was to explore techniques which allow for relatively simple tissue localization of plant products or enzymes, using polyacetylene and peroxidase as examples. Plant tissues left in 10% NaOH for short time periods become partially cleared with respect to tissue surface layers. When leaves and involucre bracts of *Cleistanthus asperum* (Aubl.) DC. are partially cleared, polyacetylenic constituents of secretory reservoirs appear either as clear oily pools or become dark brown, depending on drying time length prior to clearing. This method provides good resolution of secretory reservoirs and aids in three dimensional analysis of reservoir organization. Tissue printing is accomplished by pressing a tissue section on nitrocellulose paper. This procedure results in the transfer or printing of cell images and contents onto the nitrocellulose. Imprints allow the visualization of cells in the tissue with a resolution of approximately 5 μ m. Most colorimetric analyses for plant products are

appropriate for tissue prints. Peroxidase activity localization was achieved by reacting prints with substrates, guaiacol and peroxide, to produce an orange product. Peroxidase activity in stem prints was greatest in the epidermal layer and vascular tissue. Polyacetylenes were detected on tissue prints using two different colorimetric methods adapted from indicators used in thin layer chromatography. Polyacetylenes appear as orange spots on tissue prints reacted with 0.4% isatin in conc. H_2SO_4 and heated for 5 minutes. Polyacetylenes also appear dark when reacted with 1% $KMnO_4$ in 2% aqueous Na_2CO_3 . In addition, since polyacetylenes are autofluorescent, they can be seen in untreated tissue prints exposed to uv light. Polyacetylene accumulation was observed consistently in reservoir areas on tissue prints of leaf and stem. We used colorimetric localization of polyacetylene and peroxidase to demonstrate the versatility of tissue printing in studying living organisms at the tissue level.

Chemistry/Bio-Chemistry

ULRIKE KLÖTZ, RANDI B. WEISS, and PATRICIA E. GALLAGHER, Department of Biology, West Virginia University, Morgantown, WV 26506.
Substrate Specificity of a Human 3-methyladenine DNA Glycosylase.

Dimethyl sulfate and methylmethane sulfonate are methylating agents which belong to the largest group of chemicals that modify DNA bases. Methylating agents have numerous reaction sites in DNA each with potential biological significance. The most predominant lesions formed are the N-methylpurines, 7-methylguanine and 3-methyladenine, while the N-methylpyrimidines are produced in lesser quantities.

In *Escherichia coli*, an excision repair pathway that removes methylpurines as well as methylpyrimidines from damaged DNA is initiated by specific DNA glycosylases. However, it has been shown that the specificity of repair enzymes isolated from *E. coli* differs greatly from repair enzymes isolated from mammalian cells. Attempts to isolate enzymes from eukaryotic sources that are able to measurably remove methylpyrimidines have failed in the past. Methylpyrimidines, however, if not excised from damaged cellular DNA, may mispair upon DNA replication and therefore contribute to mutagenesis and carcinogenesis.

Using a polynucleotide release assay, we have investigated the mechanism by which mammalian repair enzymes remove methylpyrimidines from damaged DNA. Poly(dG-dC)-poly(dG-dC), a synthetic polymer, radiolabeled with [³H]-dCTP, and poly(dA)-poly(dT), radiolabeled with [³H]-TTP by nick-translation, was subjected to methylation by dimethyl sulfate and used as a substrate. A methylpurine-DNA glycosylase, partially purified from human lymphoblasts, was incubated with methylated and unmethylated polymer. Release of TCA-soluble reaction products was linear with respect to protein concentration and time of incubation when methylated poly(dG-dC)-poly(dG-dC) was used as a substrate. Heat inactivation experiments confirmed the enzymatic nature of the reaction. HPLC analysis of the reaction products confirmed the release of methylcytosines from the damaged substrate by the human DNA glycosylase. There was no release of TCA-soluble material from methylated poly(dA)-poly(dT) or unmethylated polymer. These results suggest that mammalian cells contain a DNA glycosylase that releases methylcytosines but not methylthymines from synthetic methylated polymers. (Supported by NIH grant CA-47457)

DEEPIKA WALPITA, RANDI B. WEISS, and PATRICIA E. GALLAGHER, Department of Biology, West Virginia University, Morgantown, WV 26506. Enzymatic Repair of Oxidative DNA Damage in Actively Growing and Quiescent Human Neuroblastoma Cells.

In neuronal cells, oxygen free radicals may arise as a result of ischemic and reperfusion events, which is characterized by a sudden decrease or total loss of oxygen to the brain followed by restoration of blood flow. Reoxygenation triggers a series of biochemical reactions which release oxygen free radicals as byproducts. These free radical species can react with cellular macromolecules such as DNA resulting in the formation of a number of base damages including ring-saturated pyrimidines. Mechanisms for the repair of many of these lesions have been well defined in both bacterial and mammalian systems. Ring-saturated pyrimidines are removed from mammalian DNA by the action of the redoxyendonuclease, the counterpart to *E. coli* endonuclease III. We have investigated the levels of this enzyme in actively growing and quiescent human neuroblastoma cells using an oxidized, radiolabeled polynucleotide as substrate. Extracts from logarithmically growing cells released oxidized pyrimidines into the TCA-soluble fraction, showing linear kinetics in response to protein concentration. Little release of modified pyrimidines was observed with extracts from quiescent cells. These data suggest that the redoxyendonuclease activity in actively dividing cells enables these cells to repair oxidative DNA base damages while nondividing cells, such as neurons, may be more susceptible to DNA damages caused by oxygen radicals. These DNA damaging species are produced during ischemia and reperfusion, events which are characteristic of stroke. (Supported by NIH grant CA-47457 and ACS Institutional Research Grant #IRG-181A).

THEODORE Y. KIM, RANDI B. WEISS, and PATRICIA E. GALLAGHER, Department of Biology, West Virginia University, Morgantown, WV 26506. Analysis of the Action Spectrum for the Formation of Adenine-Containing Photoproducts.

Ultraviolet irradiation produces a number of base damages in DNA. The best characterized of these lesions are the bipyrimidine adducts,

cyclobutane pyrimidine dimers and 6-4'-(pyrimidin-2'one)-pyrimidines. Little information is available concerning the formation of purine-containing photoproducts. To date, two distinct adenine photodimers have been reported. An endonuclease activity which recognizes adenine-containing photoproducts has recently been purified from *Escherichia coli*, using UV-irradiated poly(dA)-poly(dT) radiolabeled with [8-³H dATP], as a substrate. The enzyme releases TCA-soluble, radiolabeled material from irradiated polynucleotides. DNA sequence analysis was used to confirm the site of enzymatic incision. An action spectrum for the formation of the adenine-containing photoproduct was performed using the purified endonuclease as a probe. The results demonstrated that the purine photoproduct which is substrate for the *E. coli* endonuclease is formed maximally by irradiation with 270 nm monochromatic light. These results suggest that the adenine photoproduct may be important in the induction of skin cancer. (Supported by NIH grant CA-47457 and WVU Senate Grant).

JOHN R. LENHART, RANDI B. WEISS, and
PATRICIA E. GALLAGHER, Department of
Biology, West Virginia University, Morgantown,
WV 26506. An Endonuclease Activity From Plant
Tissue Which Incises Oxidized DNA.

Oxidation of the DNA bases is caused by a variety of physical and chemical agents in the environment as well as intermediates of normal cellular metabolism. Organisms have evolved mechanisms to repair numerous types of DNA damages; while these DNA repair systems have been well characterized in bacteria and mammalian cells, surprisingly little is known about repair of potentially harmful DNA lesions in plants. An enzyme that recognizes and incises chemically oxidized DNA has been partially purified from plant tissue. Glycosylase-produced base loss sites were detected by a nitrocellulose filter-binding assay using oxidized PM2 viral DNA as the substrate. Linear kinetics have been observed with respect to both enzyme concentration and time of incubation. Using a DNA sequencing assay, we have shown that the enzyme incises oxidized DNA at sites of thymine damage and UV-irradiated DNA at altered cytosines. These data suggest that the partially purified endonuclease is the plant counterpart to *E. coli* endonuclease III and the mammalian redoxendonuclease. (Supported by NSF Grant DMB-9005762)

JEFFREY A. ANDERSON, AND DEBORAH A. LEONARD, Dept. of Biology, West Virginia University, Morgantown, WV 26506 and LEAH L. FRYE, AND KEVIN P. CUSACK, Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180 15-SUBSTITUTED LANOSTEROLS: DUAL-ACTION INHIBITORS OF CHOLESTEROL BIOSYNTHESIS.

To reduce the risk of death from coronary heart disease, the nation's leading killer, diet modification and/or drug therapy may be used to lower serum cholesterol levels. Compounds which are inhibitors of cholesterol biosynthesis at more than one enzymatic step may be useful as anti-hypercholesterolemic agents. 4,4,14 α -trimethyl-substituted 15-oxygenated sterols are known dual-action inhibitors of the cholesterol pathway, inhibiting both 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), the rate-determining enzyme of cholesterol biosynthesis, and lanosterol 14 α -methyl demethylase (P450_{DM}); however, little is known about their mechanism of action. These compounds are of particular interest because the presence of a functional group at C-15 should enhance their metabolic stability; the proton at this position is essential for the removal of the 32-methyl group by P450_{DM}. We have synthesized a novel compound, 3 β -hydroxy-lanost-7en-15-one 15-oxime, which is structurally similar to the known inhibitor 3 β -hydroxy-lanost-7en-15-one. In analyzing how these compounds affect cholesterol biosynthesis, crucial information on the regulation of this pathway will be gained which may aid in subsequent drug discovery efforts. We have studied the effects of these oxylanosterols as well as the 15 α - and 15 β -hydroxylanosterols on cholesterol biosynthesis in Chinese hamster ovary (CHO) cells and a P450_{DM}-deficient cell line. All compounds are potent inhibitors of HMGR *in vivo*, but not *in vitro*. In contrast, all four compounds inhibit P450_{DM} directly. These sterols inhibit HMGR by regulating gene expression as evidenced by parallel decreases in enzyme activity and immunoreactive protein. Northern blot analysis was used to measure HMGR mRNA levels. Preliminary results indicate that these oxylanosterols are all post-transcriptional regulators of HMGR since enzyme protein decreased in the absence of any change in mRNA levels in treated cells. Further characterization of the 15-ketolanosterol and lanosterol 15-oxime indicates that these analogs affect both HMGR translation and degradation as shown by immunoprecipitation of [³⁵S]methionine-labelled proteins. Supported by NIH grant HL45287-01 (LLF)

KIMBERLY S. CROSS, AND DEBORAH A. LEONARD, Dept. of Biology, West Virginia University, Morgantown, West Virginia 26506 and LEAH L. FRYE, AND KEVIN S. CUSACK, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12180. 7-Substituted Oxysterol Inhibitors of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase.

Coronary heart disease, which is associated with elevated serum levels of cholesterol, is the leading cause of death in the United States. Cholesterol, an amphipathic lipid, plays an essential role in mammalian cell membrane function. A negative feedback mechanism regulates 3-hydroxy-3-methylglutaryl-coenzyme A

reductase (HMGR), the rate-limiting enzyme in the biosynthesis of cholesterol. Oxysterols, oxygenated precursors and products of cholesterol, inhibit the activity of HMGR by both transcriptional and post-transcriptional mechanisms. Metabolites of cholesterol with oxygen substitutions on the 7-carbon are known to be potent inhibitors of HMGR activity. To gain a better understanding of the relationship between sterol structure and the mechanisms by which these compounds regulate HMGR, oxygenated derivatives of lanosterol, a precursor of cholesterol, were synthesized and tested for inhibition of HMGR activity in Chinese hamster ovary (CHO) cells. 7 β -hydroxylanosterol and 7-ketolanosterol are potent inhibitors of HMGR and reduce the level of immunoreactive protein in both wildtype CHO and mutant demethylase-deficient cell lines. Northern blot analysis of HMGR mRNA and measurements of enzyme synthesis and degradation rates were used to determine the mechanism of HMGR inhibition by these oxylanosterols. Preliminary data suggest that these compounds act by post-transcriptional mechanisms. In oxylanosterol-treated cells, the rate of enzyme synthesis declined in the absence of any change in HMGR mRNA levels. The half-life of HMGR also decreased by more than 50%. In contrast, 7-ketocholesterol causes a decrease in HMGR mRNA, suggesting that its mechanism of action is similar to 25-hydroxycholesterol, a known inhibitor of HMGR transcription. In most cases, elevated serum cholesterol levels are controlled by modifications in the diet and with exercise. However, many individuals will benefit from drug therapy. An understanding of the mechanisms of oxysterol regulation of cholesterol biosynthesis will aid in the development of effective therapeutic agents. Supported by NIH grant HL45287-01 (LLF).

JOHN M. SQUIER, DEBORAH A. LEONARD, Dept. of Biology, West Virginia University, Morgantown, West Virginia 26506 and LEAH L. FREY, KEVIN P. CUSAK, Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12108. 32-Substituted Oxylanosterols Inhibit 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase by Two Different Mechanisms.

The incidence of coronary heart disease, the leading cause of death in the U.S., has been directly correlated with high cholesterol levels in blood plasma. Both diet modification and drug therapy have been used with hypercholesterolemic patients, but not all individuals are diet sensitive, and there is currently only one F.D.A. approved drug which effectively inhibits cholesterol biosynthesis. Clearly, further efforts to identify additional hypocholesterolemic drugs are in order. The rate-limiting enzyme of cholesterol biosynthesis is 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), an enzyme which is regulated both transcriptionally and post-transcriptionally. Oxygenated derivatives of lanosterol, which are intermediates in the cholesterol biosynthetic pathway have been proposed as negative feedback regulators of HMGR. However, because these compounds are rapidly metabolized, observing their effects upon HMGR activity is very difficult. We have synthesized 32-oxylanosterol analogues which are predicted to undergo only limited metabolism by lanosterol 14 α -methyl demethylase (P450_{DM}.) The effects of these compounds upon HMGR and P450_{DM} in cultured Chinese hamster ovary (CHO) cells has been measured. To determine if these compounds inhibited HMGR independent of their effects on P450_{DM}, a CHO cell line deficient in P450_{DM} was used. HMGR activity was determined in cells treated with increasing concentrations of oxylanosterol and quantitative immunoblot analysis was used to measure enzyme protein levels.

Incorporation of [^{14}C] acetate into nonsaponifiable lipids was used to detect inhibition of P450_{DM} by these oxysterols. Our data suggest there are two classes of 32-substituted compounds; those which act to inhibit HMGR directly, and those which inhibit HMGR by the generation of endogenous oxysterols. The effects of a direct inhibitor, 3 β -hydroxy-32-oxime, on HMGR mRNA levels were determined by northern blot analysis. Preliminary data suggest that this compound is a post transcriptional regulator which decreases enzyme activity and immunoreactive protein levels while doing nothing to lower HMGR mRNA levels. From these data, we propose there are two mechanisms of action for the inhibition of HMGR activity. Supported by NIH grant HL45287-01 (LLF).

DAVID W. MILLER and MARK CHATFIELD,
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cloning of a cDNA and characterization of
the gene for soybean ascorbate peroxidase.

Ascorbate peroxidase (AP) is involved in the destruction of harmful H₂O₂. In soybean (*Glycine max* [L.] Merr.) root nodules ascorbate peroxidase initiates a sequence of coupled redox reactions (ascorbate-glutathione pathway) which result in peroxide scavenging.

We report the isolation of a full length cDNA clone that encodes an ascorbate peroxidase subunit. The isolation strategy was to screen a nodule cDNA expression library with a polyclonal antibody prepared from purified root nodule AP. Putative AP clones were isolated at a frequency of 1 in 800 and 80% of the isolates contained a 1.1 kb insert. Double-stranded dideoxysequencing of one clone resulted in a 1054 nucleotide sequence with a 3'-poly A tail and an open reading frame encoding 249 amino acids. Comparison of the N-terminal amino acid sequence obtained by automated Edman sequencing (20 residues) confirmed that the clone was authentic ascorbate peroxidase. A comparison of deduced protein sequences [249 a.a.] revealed a sequence identity of 90% to pea and 78% to *Arabidopsis*. Southern blot analysis (used to evaluate gene copy number) and isolation of genomic clones are in progress.

The high activity of AP in nodules suggests that it may provide an essential protective action in processes related to nitrogen fixation. This analysis was a first step to studies addressing the genetic regulation of the ascorbate-glutathione pathway in root nodules.

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Reaction of α -(p-nitrophenyl)-4-pyridinemethanol
with aqueous NaOH.

The principal product of this reaction is 4-4'-di(p-pyridoyl)-
azoxybenzene. Efforts are continuing to identify the phenolic
component of this reaction. These results will be compared with
results of the reaction of α -phenyl-4-nitro-benzenemethanol and of α -
(p-nitrophenyl)-4-pyridinemethanol N-oxide with aqueous NaOH.

Ecology

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, ASHOK KUMAR, Oilseeds Research Station, Kangra (H.P.), India, RAJENDRA P. BHATT, Dept. of Botany, H. N. B. Garhwal University, Garhwal (U.P.), India, and MARY BETH ADAMS, USDA Forest Service, Timber and Watershed Laboratory, Parsons, West Virginia 26287. A comparative ecological and taxonomic study of the ectomycorrhizal fungi associated with the upland forest communities of northwestern India and the mid-Appalachians of the eastern United States.

The dependence of certain forest trees on ectomycorrhizal fungi has long been known, but only recently has the biological nature of the fungus-tree association been examined in some detail. On the basis of studies that have been carried out, it would appear that ectomycorrhizal fungi are usually unable to enzymatically degrade the complex carbohydrates of most organic detritus and, instead, rely on their hosts (i.e., forest trees) for their energy needs. In return, they are able to take up such metabolites as phosphorus, nitrogen, sulfur, and zinc from the soil and translocate these to their host. In this way, they greatly extend the functional root system of the host. Most ectomycorrhizal fungi are Basidiomycetes, with Amanita, Cortinarius, Lactarius, Russula, and Suillus among the best known ectomycorrhizal genera. Ectomycorrhizal associations are widespread, particularly in temperate regions, and involve many of the ecologically important trees making up the forests characteristic of these regions. Prominent examples include hemlock (Tsuga), spruce (Picea), pine (Pinus), fir (Abies), willow (Salix), oak (Quercus), birch (Betula), and beech (Fagus). Although geographically separated, the upland forests of the eastern United States and northwestern India share a number of ecologically important ectomycorrhiza-forming tree genera in common. In both regions, Picea and Abies are the usual dominants in forests found at higher elevations (generally >1200 m in the mid-Appalachians of the eastern United States and >2500 m in northwestern India), whereas Pinus and especially Quercus are often major components of forests located at somewhat lower elevations. Relatively few studies have considered the ectomycorrhizal associations represented in these forests, and most such studies have been rather limited in scope. The objectives of our ongoing research are (1) to compare the species composition, species diversity, taxonomic diversity, fruiting phenology, and sporocarp productivity of the ectomycorrhizal fungi associated with upland coniferous and oak-dominated forest communities in northwestern India and the mid-Appalachians of the eastern United States and (2) to elucidate, using data from sexual incompatibility tests, cultural studies, and isozyme analysis, the biological and taxonomic relationships that exist between populations of fungi that appear to represent the same taxonomic entity (i.e., morphospecies) in these two different regions of the world. The primary study sites being used are the Narkanda and Dharamshala areas of Himachal Pradesh and the Garhwal area of Uttar Pradesh in northwestern India and the USDA Fernow Experimental Forest near Parsons in Tucker County, West Virginia, in the United States. (Supported in part by funds provided by the USDA Forest Service.)

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554, GARY A. LAURSEN, Dept. of Biology and Wildlife, University of Alaska-Fairbanks, Fairbanks, Alaska 99775, ROLAND TREU, Dept. of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, JOHN C. LANDOLT, Div. of Science and Mathematics, Shepherd College, Shepherdstown, West Virginia 25443, ERNEST C. BERNARD, Dept. of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tennessee 37901, LONI J. ROSSOW, Dept. of Biology and Wildlife, University of Alaska-Fairbanks, Fairbanks, Alaska 99775, and ROSEANN DENSMORE, National Park Service, P.O. Box 9, Denali National Park, Alaska 99755. A preliminary report on mycorrhizal studies in Denali National Park and Preserve, Alaska.

The patterns and rates of succession in many terrestrial habitats are influenced by factors related to the successful establishment of mycorrhizal associations. These fungus-root partnerships play an important role in plant growth and nutrition, especially in stressful environments such as those found in high-latitude regions of the world. In the present study, which is still ongoing, an effort is being made to characterize vesicular-arbuscular mycorrhizal (VAM) fungi and ectomycorrhizal fungi from soil and root samples collected from seven permanent revegetation plots established along Glen Creek in the Kantishna Hills of Denali National Park and Preserve, Alaska. In addition, roots of 39 herbaceous and woody plant species from alpine habitats have been examined for mycorrhizae. Root samples from feltleaf willow (Salix alaxensis [Anderss.] Cov.) field-planted as cuttings and green alder (Alnus crispa [Ait.] Pursh) field-planted as seedlings have been examined for three and four field seasons, respectively. Ectomycorrhizae were not detected in willow roots in the first year but were present in the second year. Ectomycorrhizal infection in alder increased from 24.5% in the first year to 72.5% in the fourth year. Soil samples were assayed for VAM using sorghum as the trap crop. After three months, roots were stained with a trypan blue method. Sorghum roots in all samples were colonized by VAM fungi, as indicated by the presence of arbuscules and vesicles. Based on arbuscule and vesicle size and shape, at least two VAM fungal taxa are represented in our samples. Chlamydospores have not been observed in culture. Mycorrhizal types present in roots of plants from alpine habitats included ectomycorrhizae (six of the species examined), ericoid mycorrhizae (six species), arbutoid mycorrhizae (one species), and VAM (two species). A variety of intracellular infections not attributable to any known mycorrhizal type occurred in 11 species. (Supported in part by funds provided by the National Park Service.)

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, West Virginia 26554. Forest vegetation of New Zealand.

New Zealand is the most isolated land mass of its size in the world. The native vegetation, which had its origin in Gondwanaland, became isolated from the rest of the world about 80 million years ago. About 80 percent of all native vascular plants are endemic, including all of the conifers and 83% of the angiosperms. Although New Zealand spans 13 degrees of latitude, the climate throughout is temperate, with warm summers and relatively mild winters. Two major forests types are generally recognized--podocarp-broadleaf (or podocarp-hardwood) forests and southern beech (Nothofagus) forests. A podocarp-broadleaf forest typically consists of a mixture of several podocarps (members of the Podocarpaceae) and various broadleaf evergreen

angiosperms, tree ferns, and other plants. Southern beech forests are made up of one or more species of Nothofagus, with or without various podocarps and hardwoods also present. In general, podocarp-broadleaf is (or was) the main forest type on North Island, with southern beech generally confined to higher elevations in the main mountain ranges. On South Island, the predominant forest type is southern beech, and almost pure Nothofagus forests occur in subalpine areas of the Southern Alps. As is the case for other regions of the world, past human activities (including the introduction of exotic animals) have had a profound effect upon New Zealand forests. (Supported in part by funds provided by the National Geographic Society.)

AULICK, STACI D., GILLIAM, FRANK S., EVANS, DAN K., Dept. of Biol. Sci., Marshall Univ., Huntington, WV 25755 and MARY BETH ADAMS, NE For. Exp. Sta., Parsons, WV 26287. A study of herbaceous layer communities in a central Appalachian mixed hardwood forest.

Herb layer communities respond to a variety of environmental variables in forest ecosystems. The objectives of this study were to (1) observe floristic trends within three watersheds of the Fernow Experimental Forest, Parsons, WV, and (2) examine stand and soil variables and their influence on the herbaceous communities of the forest. Watersheds were selected on the basis of stand age/history (WS3 ~20 yr, select cut; WS7 ~20 yr, clearcut and herbicide; WS4 ~80 yr, control). Each watershed was sampled floristically by walking its entirety four times from May through August. The voucher specimens collected from this sampling method were used to create a species list for all three watersheds. In addition to the floristic survey, the herb layer was sampled within 15 0.04-ha circular sample plots per watershed. All vascular plants ≤ 1 -m in height within 10 1-m² subplots per plot were identified to species and estimated for cover (%). The floristic study identified several general communities, including ridgetop, stream bank, stream bed, slope, and disturbed areas. Within these communities, a total of 231 species were identified. The largest family represented (in terms of numbers of species) was Cyperaceae. Plot data revealed relationships between herb layer species diversity and soil variables for neither WS3 nor WS4. However, on WS7, there was a significant negative correlation between species diversity and soil variables, including clay content and cation exchange capacity. No correlations were found for any of the watersheds for stand variables (stem density and basal area) and herb layer species diversity or richness. Principle component analysis identified several potential herb communities. These communities appeared to be determined in part by similarities among herb layer species for resource requirements.

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44115. Annual variation in nest-site selection and reproductive success in the Carolina Wren.

Carolina Wrens (*Thryothorus ludovicianus*) nesting in farm and forest habits were found to differ considerably in annual variation of nest-site selection and reproductive success. Birds nesting in and around farms were more successful and fledged more young per year than those nesting in forests. The mean clutch size, mean number of fledglings per initiated brood and per successful brood, percentage of initiated clutches, and percentage of eggs producing fledged young in initiated clutches were significantly higher for farm nesting pairs. In addition, forest nesting birds had a marginally shorter renesting interval than did farm nesting pairs. This would support the hypothesis that pair density, nest-site selection, and reproductive success are positively correlated to habitat selection. Moreover, birds nesting at farms may be at a selective advantage and this may be why Carolina Wrens prefer nest-sites located around human occupation.

D. R. NASH, M. K. NOWLIN, C. VANNATTER, T. E. WEAKS, and F. S. GILLIAM, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Bryophyte response to wildfire in a southwestern West Virginia mixed hardwood forest.

Bryophytes play a significant part in recovery from disturbance in many terrestrial ecosystems, having particularly critical roles in nutrient cycling and water relations of plant communities. The purpose of this study was to quantify bryophyte response to fire in a hardwood forest. A section of upland mixed hardwood forest in Wayne County, West Virginia, which burned from a wildfire in November 1991, was divided into four fire intensity types based on the amount of overstory stem charring, survival of understory stems, and removal of litter layer. These types were high intensity burn (HB), medium intensity burn (MB), low intensity burn (LB), and unburned (UB). Three plots were randomly located in each of the four burn types and five 1-m² subplots were established in each plot by stratified random placement. All 60 subplots were measured in May, July and September for amount of bryophyte cover (%), herb layer cover (%) and overstory canopy closure. Bryophyte cover was greatest at the HB site (24.7%) and lowest at the UB site (3.7%) but did not correlate significantly with canopy closure at any site. Patterns of bryophyte recovery over time varied substantially with burn type. Bryophyte cover appeared to be more sensitive to herb layer development (closure) than to overstory canopy closure.

These results suggest that bryophyte response to wildfire in this hardwood forest is greatly mediated by an interaction of substrate availability (e.g., exposed soil) and the microclimatic conditions created by herbaceous layer cover.

NICOLE L. TURRILL, FRANK S. GILLIAM, Dept. of Biol. Sci., Marshall Univ., Huntington, WV 25755, and MARY BETH ADAMS, NE For. Exp. Sta., Parsons, WV 26287. Variations of herb layer-soil relationships in young vs mature stands of northeastern West Virginia forests.

Herb layer vegetation (vascular plants ≤ 1 m in height) responds to a variety of environmental factors and is often indicative of soil fertility. The purpose of this study was to compare herb layer-soil relationships of a West Virginia montane forest in relation to stand age. Herb layer, soil, and woody stem sampling was conducted in two young (~20 yr) and two mature (>~70 yr) watersheds of the Fernow Experimental Forest, Parsons, WV. Despite their differences in stand age, the watersheds were very similar in summer herb layer species composition, richness (3.6-5.0 species/m²), and cover (17.9-37.5%). Soils of all watersheds were acidic sandy loams of similar fertility. Correlations of herb tissue N:P and N:base cations suggest N-limitation for the herb layer in these soils. Herb tissue and soil nutrient correlations were significant on watersheds dominated by *Laportea canadensis* but not on those dominated by *Dryopteris marginalis*. Principal component analysis of individual watershed soil and canopy data (correlating herb layer cover to each axis) revealed that herb layer cover was negatively correlated to canopy characteristics on the mature watersheds and positively correlated with soil nutrients on the young watersheds. These data suggest that although soil and herb nutrient concentrations do not differ with stand age, herb-soil interactions vary such that the herb layer is nutrient-limited early in succession and light-limited (via canopy closure) in later successional stages.

M. K. NOWLIN and F. S. GILLIAM, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Effects of wildfire on the herbaceous layer of a southwestern West Virginia mixed hardwood forest.

The effects of fire on the mixed hardwood forests in West Virginia have long been of interest both biologically and economically. This study examined the effects of a Fall, 1991 wildfire in Wayne County, West Virginia on soil fertility and herbaceous layer response. Study plots were established in areas representing fires of high, moderate and low intensities with a control established in an

adjacent unburned area of similar aspect and elevation. Composite soil samples were taken from each site. Soil fertility response was evaluated with a plant bioassay experiment, using Poa pratensis (Kentucky bluegrass) grown in soil from each burn type and amended with a variety of nutrient solutions. Herbaceous layer and canopy cover measurements were made at each site during the spring, summer and fall of 1992. A seedbank study was performed to further assess potential herb layer recovery. Replicate sections of forest floor and surface soil were taken from each burn type and allowed to develop in the greenhouse with only periodic watering. The plant bioassay suggested that soil fertility was increased by fire at all three burn intensities. There was a generally negative correlation exist between herbaceous cover and canopy opening. This was significant on the high and moderate burned plots. Herbaceous cover was highest on high burned plots and lowest on unburned plots. Species which developed in the seedbank study were generally well-represented in the field plots during the course of this study.

LEE ANN MULLINS and DONALD C. TARTER, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Low pH tolerance, under static bioassay conditions, of naiadal green jacket skimmers, *Erythemis simplicicollis* Say (Odonata: Libellulidae), from the Green Bottom Wildlife Management Area in southwestern West Virginia.

Although adult odonates are highly vagile and thus of limited value as pollution indicators, the naiads as a group are highly sensitive to habitat degradation. Naiads of the green jacket skimmer, *Erythemis simplicicollis*, were experimentally tested under static bioassay conditions to determine their tolerance to low pH. Naiads were exposed to four pH values (1.5, 3.0, 5.0, and 7.0) in the laboratory investigation. The 96-hour TLM (median tolerance limit) test was used as the measure of acute toxicity to low pH. The straight-line graphical method was employed to determine the pH value at which 50 percent of the naiads survived after 96 hours. Tests were duplicated and the mean was plotted as the final TLM value. Results from this investigation will be compared with other pH tolerance studies of naiadal odonates.

GILLIAM, FRANK S., TURRILL, NICOLE L., Dept. of Biol. Sci., Marshall Univ., Huntington, WV 25755 and MARY BETH ADAMS, NE For. Exp. Sta., Parsons, WV 26287.
Tree species composition of young vs. mature montane hardwood forests of West Virginia.

Secondary succession in high-elevation hardwood forest ecosystems can be very site-specific. We studied hardwood forest successional change by comparing tree species composition among four watersheds of the Fernow Experimental Forest, Parsons, WV: two young (~20 yr) and two mature (>~70 yr) watersheds. All trees ≥ 2.5 cm diameter at breast height (DBH) were tallied and measured for DBH within 15 0.04-ha circular plots per watershed. Multivariate statistical analyses [canonical discriminant analysis (CDA) and principle components analysis (PCA)] were performed on ln-transformed importance values based on the sum of relative density and relative basal area per plot. For both CDA and PCA, axis scores were compared to ln-transformed environmental variables (e.g., elevation, soil nutrients, soil organic matter) to determine possible relationships of species composition and successional change to these variables. Watersheds varied greatly with respect to stand age (young vs. mature, respectively) for density (1278 vs. 465 stems/ha), basal area (22.5 vs. 42.8 m²/ha), and species richness (17 vs. 20 species/watershed). Several important tree species were common to all four watersheds, including *Acer saccharum*, *Quercus rubra*, *Prunus serotina*, and *Liriodendron tulipifera*. Species composition and dominance, however, varied greatly between watersheds. CDA separated the watersheds along a stand age gradient from mature to young stands, with *A. saccharum* and *Q. rubra* loading negatively and *P. serotina*, and *L. tulipifera* loading positively on the first axis. PCA indicated that tree species composition within each watershed responded substantially to a variety of soil variables (including nutrients and texture) and elevation. These data suggest a species sequence for this central Appalachian hardwood forest from *P. serotina* and *L. tulipifera* dominating early in succession and being replaced by *A. saccharum* and *Q. rubra* later in succession.

RONALD A. CANTERBURY, Department of Biology, Cleveland State University, Cleveland, Ohio 44115 and BEVERLY D. CANTERBURY, Veterans Administration Medical Center, Cleveland, Ohio. Nesting ecology of the Eastern Phoebe in southern West Virginia.

During the 1988-1991 nesting seasons, we studied several aspects of the Eastern Phoebe (*Sayornis phoebe*) nesting ecology in southern West Virginia. The main objective was to ascertain the relationship among clutch-size, hatching success, nestling success, fledging success, overall reproductive success and nest-site selection. We

define clutch size as the number of eggs laid, hatching success as number of eggs hatched/number of eggs laid, nestling success as number of young fledged/number of eggs hatched, fledging success as number of young fledged/number of eggs laid, reproductive success as number of nests that fledged at least one young, and nest-site selection as habitat type (natural or man-made) and nest placement (horizontally or vertically against the substrate). In West Virginia, Eastern Phoebes have an abundance of natural nest-sites such as rock outcrops and cavities within cliffs and banks, as well as man-made nest-sites (i.e., bridges and culverts). Pairs nesting in natural areas did not differ significantly from those nesting in man-made structures in terms of aforementioned reproductive parameters, except for wooden bridges which showed significantly lower reproductive success. Since Eastern Phoebes reuse old nests (especially for second clutches) as well as build new ones (Klaas 1970; Weeks 1978), we examined the importance of old nests remaining from previous seasons and nest placement (vertical or horizontal) on reproductive success. After the 1990 nesting season, we removed old nests to assess the effect of a lack of existing nests on productivity and nest placement parameters in 1991. Significantly more attempts per nest were made in 1991 than in 1990, both in man-made and natural nest-sites. Success parameters were all significantly higher in 1990 than 1991. Vertical nests were more common than horizontal nests under bridges and culverts. Horizontal nests had a significantly higher clutch size than vertical nests. For 1990 and 1991 years combined, reproductive success appeared higher in horizontal than vertical nests though the difference was not significant. For all successful nests, however, fledging success was highest in vertical nests. There was no significant difference in fledging success between early and last nesting attempts; although, fledging success tended to be higher in early and peak season nests.

NICOLE L. TURRILL, DAN K. EVANS, AND FRANK S. GILLIAM, Dept. of Biol. Sci., Marshall Univ., Huntington, WV 25755. Identification of West Virginia members of the *Dentaria* complex [*D. diphylla* Michx., *D. heterophylla* Nutt., and *D. laciniata* Muhl. ex. Willd. (Brassicaceae)] using above-ground vegetative characters.

Long-term herb layer studies, especially those involving rare plants, require nondestructive sampling with species identified by above-ground characters. Spring herb communities of eastern deciduous forests, however, often contain members of the eastern North American *Dentaria* complex which show great intergradation of vegetative characters. The purpose of this study was to determine the above-ground vegetative characters that best differentiate West Virginia members of the complex: *D. diphylla*, *D. heterophylla*, and *D. laciniata*. Eleven morphological characters were

measured on West Virginia herbaria specimen and data were subjected to canonical discriminant analysis (CDA) and analysis of variance. CDA showed that the species fell into three distinct groups with some overlap in the ranges of *D. diphylla* and *D. heterophylla*. Total-sample correlation between character measurements and the first two canonical axes revealed that length and width of basal (BL and BW) and cauline (LL and LW) leaflets were the most important characters for identification. *D. laciniata* was easily identified by its lack of basal leaves and its long, narrow cauline leaflets (LL:LW = 4:1). The basal leaflets of *D. diphylla* were similar to but larger than its cauline leaflets (BW:LW = 2:1) where the basal leaflets of *D. heterophylla* were much wider than its cauline leaflets (BW:LW = 4:1). Further studies should apply these characters to simplify identification throughout the range of the eastern North American *Dentaria* complex.

EDWARD L. MOORE, ERICH B. EMERY, and DONALD C. TARTER, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755--Low pH tolerance under laboratory conditions of a larval population of the alderfly *Sialis hasta* Ross (Megaloptera: Sialidae).

A larval population of the alderfly *Sialis hasta* was experimentally tested under static bioassay conditions to determine its tolerance to low pH. Larval alderflies were exposed to four pH values (1.5, 3.0, 4.5, 7.0) in the laboratory investigation. The straight-line graphical interpolation method along with a linear regression analysis were used to determine the pH value at which 50 percent of the larvae survived after 96 hours (median tolerance limit). Additionally, the survival rate was monitored after 24, 48, and 72 hours. Larval alderflies of the genus *Sialis* have a wide tolerance range for pH (2.3-8.3). Results from this investigation will be compared to other low pH tolerance studies of larval populations of *S. itasca* Ross and *S. joppa* Ross from good water quality streams and *S. aequalis* Banks from Camp Creek, an acid mine stream, and Flatfoot Creek, a good water quality stream.

TIM P. HAYES and DONALD C. TARTER, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Food habits of a disjunct population of the central mudminnow, *Umbra limi* (Kirtland), in relation to seasonal changes and age groups, in the Green Bottom Wildlife Management Area, Cabell County, West Virginia.

The central mudminnow, *Umbra limi*, is mentioned on the Vertebrate Species of Concern list in West Virginia. The only population of

the central mudminnow in West Virginia is found in the 57 hectare wetland habitat of the Green Bottom Wildlife Management Area (ca. 378 ha) in Cabell County, West Virginia. This disjunct population is located 110 km from the nearest population in Hocking County, Ohio, and provides a unique opportunity for a seasonal food habit study outside its normal range. This wetland area is also currently under expansion by the U. S. Army Corps of Engineers in a mitigation project. Data collected for this study will provide baseline information for seasonal food habits prior to habitat expansion. Mudminnows (\bar{X} = 60/season) were collected monthly from October 1989 to December 1992 by seining the vegetated, littoral zones of the marshland. In the laboratory, quality and quantity of the stomach contents were determined using the following calculations: 1) percent frequency of occurrence, 2) average of volume percentages, and 3) percent of total volume. These calculations were accomplished using the point system of Hynes. Comparison of types of food found (e.g. copepods, ostracods, odonates) as well as seasonal changes will be made with other food habit studies in the literature.

DONALD C. TARTER, Dept. of Biological Sciences
Marshall University, Huntington, WV 25755 and DIANE
NELSON, Dept. of Biology, East Tennessee State Uni-
versity, Johnson City, TN 37601. First records of
tardigrades (Phylum: Tardigrada) from mosses in the
Cranberry Glades Botanical Area in the Monongahela
National Forest, Pocahontas County, West Virginia.

Nine species of tardigrades, including two state records and one new species, were collected from mosses in the Cranberry Glades Botanical Area located in the Monongahela National Forest in West Virginia. The Cranberry Glades area, located at an elevation of 3400 feet, comprises about 600 acres of land covered with bog forest, shrubs, and areas of sedges, mosses, and lichens. Most of the area is underlain with peat varying in thickness from a few inches to 11 feet. Soil in the area is acid (pH, 3.8-6.2). This bog, known as a "muskeg" in the northern sections of our continent, is a refugium for plants and animals whose ancestors were forced into the Southern Appalachians by glaciers during the Pleistocene period. The following species of tardigrades, including the state records, Macrobiotus pseudofurcatus Pilato and M. spectabilis Thulin, were identified from mosses: Diphascon n. sp., Hypsibius convergens (Urbanowicz), Isohypsibius sattleri (Richters), Macrobiotus harmsworthi Murray, Milnesium tardigradum Doyere, Minibiotus intermedius (Plate), and Ramazzottius oberhaeuseri (Doyere). The four year survey involving the distribution of tardigrades in West Virginia will be reviewed.

NANCY POWERS and DONALD TARTER, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Ecological observations of a disjunct population of the burrowing crayfish *Fallicambarus fodiens* (Cottle) in the Green Bottom Wildlife Management Area in southwestern West Virginia.

Fallicambarus fodiens is known only for two sites in West Virginia. Both localities on the Ohio River floodplain are in the valley of the preglacial Marietta River which was a tributary of the Teays River. The nearest population to the West Virginia sites is Jackson County, Ohio approximately 60 km northwest of Green Bottom Wildlife Management Area (GBWMA). Ecological observations were made on disjunct population from the GBWMA located 26 km northeast of Huntington, West Virginia. The area (ca. 338 ha) contains a valuable wetland habitat (ca. 57 ha). A proposed habitat alteration to add marshland by building dykes has prompted this study. The ecological information will establish a baseline prior to habitat perturbation. Burrow diameters were used to determine the age classes in the population. Stomach content analysis (25/season), including a grid method to estimate volume of detritus, was used to determine the seasonal food habits. The breeding cycle was documented for the population including attempted observations of amplexus, first form males, ovigerous females, hatching time, fecundity, and sex ratios. Meristic and morphometric data were compiled for males and females and correlated statistically by linear regression analysis using carapace and chela lengths as regressors. Information from this study of a disjunct population will be compared to the species within the normal range.

KATIE L. DANIELS and DONALD C. TARTER, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Reproductive biology of the bowfin, *Amia calva* Linnaeus, in the Green Bottom Wildlife Management Area, Cabell County, West Virginia.

The bowfin, *Amia calva*, is the only extant member of the family Amiidae. The Green Bottom Wildlife Management Area (GBWMA) is the location of the only reproducing bowfin population in West Virginia. The GBWMA (38° 35', 35"N, 82° 14' 55"W) is located along the Ohio River 26 km northeast of Huntington, West Virginia. The area (ca. 338 ha) contains a valuable wetland habitat (ca. 57 ha) in the southwestern portion of the State. A study of the reproductive biology of the bowfin became important when the U. S. Army Corps of Engineers proposed a habitat modification to add marshland by building dykes. Being the only reproducing population in the state also makes this population unique and in need of observation. The information from this study will establish a baseline for reproductive

activities of the bowfin prior to habitat perturbation so that their adaptation to the new environment can be accurately determined. The bowfin (39) were collected seasonally (spring, summer, fall) by hoop nets, pillow traps, and seines. Attempts were made to determine the duration of the reproductive season by: (1) calculating the seasonal gonosomatic index (GSI), (2) observing spawning in field and laboratory, and (3) noting the appearance of newly hatched individuals. Observations were also made on spawning colors, fecundity, egg diameters, sex ratios, and sexual dimorphism (meristics and morphometrics).

ERICH EMERY, DAN CHAFFIN, MARCIA HARRISON, DAN EVANS, and DONALD TARTER, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Acute and chronic toxicity of ichthyothereol, an ichthyotoxin from *Clibadium asperum* (Aubul.) DC of (Asteraceae) of Amazonia, to the fathead minnow, *Pimephales promelas* (Rafinesque).

Clibadium asperum, known locally as Masu, is used extensively by indigenous people of southeastern Ecuador to kill or stun fish. Upper leaves, flowers, and fruits from the low shrub are collected, crushed until moist and introduced into the stream or pool with the aid of a fiber basket passed back and forth in the water. Fish become disoriented in a few minutes and are easily caught and eaten without ill effects. The cyclic polyacetylene ichthyothereol is the active component in *C. asperum*. During this experiment, a crude extract was made from leaves and stems (fresh and dry). Both acute (96 hrs) and chronic (7 days) static bioassays were set up in the laboratory and tests were conducted according to EPA protocols. Fry of the fathead minnow which were less than 14 days old were held at varying concentrations to determine the lethal dose for 50 percent of the test population.

DON GASPER, West Virginia Department of Natural Resources, Elkins, West Virginia. Acid Rain in West Virginia.

West Virginia gets more acid rain than any other state (We share this with West Maryland and West Pennsylvania). It is 50 lbs/ac/yr as H_2SO_4 , or 1 lb/ac/yr as H^+ - the lightest thing in the universe. This dissolves out nutrients, impoverishing the soil, and the remaining acidity that is not thus neutralized by the soil, enters streams acidifying them. Nearly half of West Virginia trout streams

are in our high-elevation sandstone mountains. Streams draining sandstone watersheds were never rich. They have few nutrients and alkalinities to neutralize acid rain. They are easily acidified. Fish loss is occurring. This is biological evidence of stream and watershed acidification. Such loss may be "permanent" as these watersheds have been impoverished.

JASON P. RILEY, Moorefield High School, Moorefield, WV 26836, **AGGIE VANDERPOOL**, WVU Extension Service, P.O. Box 130, Elkins, WV 26241, and **SHARON ERICKSON HARMAN**, Moorefield HS and Shepherd College South Branch, Petersburg, WV 26847.

Effects of Density and Feeding on Growth and Behavior of Stream-Cultured Rainbow Trout.

Most aquaculture research has been conducted using tanks with carefully controlled flow rates, dissolved O_2 , and temperature. Our study examines the growth and behavior of cage-cultured domestic rainbow trout (Oncorhynchus mykiss) reared in a natural spring-fed stream in Hardy County, WV. Six-month-old trout were reared for 8 weeks in 1 m X 2 m mesh cages secured in the stream with an average water depth of 0.3 m. Fish were kept at densities of 25 and 50 g fish/L and fed a standard regime (2% of average body weight per day). No significant differences were found between the two density groups in fish length at 2, 4, or 6 weeks. At 8 weeks, fish at the lower density were slightly longer than the higher-density fish (18.3 ± 0.8 cm and 16.6 ± 1.9 cm, respectively). Mean weight of the lower-density fish increased 100% (40 g to 80 g) while the weight of the higher-density fish did not increase significantly. The fish reared at higher density had greater overall variance in length and weight. The higher-density fish were much more aggressive and competitive for food.

In a separate study, the importance of feeding stream-cultured trout was tested. Two groups of 30 caged trout were fed by the standard regime while two other groups were not supplementally fed. Fish that were fed gained 80-100% of their beginning body weight over an 8-week period while the fish feeding on their own actually lost weight. Our results indicate that cage culture of rainbow trout in natural streams is feasible only when the fish have adequate supplementary food.

General

HERBERT H. SNYDER, Department of Mathematics,
West Virginia Institute of Technology, Mont-
gomery, WV 25136. The calculus reform
movement: does the emperor have any clothes on?

It seems proper to speak of a movement associated with (so-called) reforms purporting to improve (= "modernize", "upgrade", and the like) the teaching of calculus, in view of the fact that NSF is spending large sums on it (with the blessing of National Academy of Sciences on its beginnings). The author develops the historical background of the reasons for the growth of dissatisfaction with the traditional calculus course (something the reformers never do), and concludes that students' difficulties with calculus arise from being caught in the scissors of (a) progressively greater ill-preparedness in prerequisite material, and (b) facing a calculus course that is much more demanding than it was before 1960.

HERBERT H. SNYDER, Department of Mathematics,
West Virginia Institute of Technology, Mont-
gomery, WV 25136. Further properties of
solutions of two nonlinear differential equations
arising in electron beam dynamics.

The author continues his studies of the nonlinear ordinary differential equation $y'' + \alpha(1 + \cos 2x)y - 2\alpha Ky^{-3} + \beta y^{-1}$ ($\alpha, \beta, K = \text{const.} \in [0, 1]$; vid. abs. in WV Acad. Proc. 63:30 and 64:41) as well as an associated equation,

$y'' + \alpha(1 + \cos 2x)y - 2\alpha Ky^{-3} + 2\beta y(1 + y^2)^{-1}$. It is shown that (a) no regular solution of the second equation can vanish unless $K = 0$; (b) all regular solutions of both equations are bounded. The latter result settles a hitherto open question.

HERBERT H. SNYDER, Department of Mathematics, West Virginia Institute of Technology, Montgomery, WV 25136. Computer algebra and Lambert's method applied to a nonlinear diffusion equation.

The partial differential equation in question is the Burgers' equation (B), $u_t + \beta u u_x = K^2 u_{xx}$ ($\beta, K = \text{const.} > 0$; J.M. Burgers, Proc. Royal Neth. Acad. Sci. Amsterdam B57:45-72), for which the author considers the initial- and boundary-value problem, find a solution $u(x,t)$ of (B) satisfying $u(x,0) = A \sin x$ ($A = \text{const.} > 0$), $u(0,t) = 0 = u(\pi,t)$. The author applies to the problem P. A. Lambert's method of successive analytic approximations. The method is made feasible by the availability of computer algebra, i.e., symbolic programming methods of algebra and analysis for handling expressions of manually prohibitive complexity.

ALPHA E. WILSON, Dept. of Physics, West Virginia Institute of Technology, Montgomery, WV 25136. Deriving the Kinematic Essence of Conservation of Angular Momentum.

The usual way of explaining the speeding-up and slowing-down of an object in orbital motion under a central force is conservation of angular momentum. Very quickly one sees that the mass of the object is superfluous and that the critical relationship is that the product of the radius and the velocity normal to it is a constant. This relationship was first derived by Kepler in his New Astronomy published in 1609 and has come down to us as Kepler's 2nd law of planetary motion or the law of equal areas. Newton gave a more rigorous proof of the law of equal areas in the Principia published in 1687. His proof generalized and validated Kepler's original discovery. This paper will review Kepler's derivation and then show how to obtain $rv = \text{constant}$ at the introductory physics level using simple vector addition.

ALAN D. SMITH, Dept. of Quantitative & Natural Sciences, Robert Morris College, 600 Fifth Ave., Pittsburgh, PA 15219. Spatial and mathematical modeling of geotechnical applications.

Three-dimensional computer modeling and statistical significance testing, through the use of hypothesis testing and model comparisons techniques were performed for selected borehole information of geotechnical interest derived from engineering reports and loggings from construction sites. The major objective was to determine through visual and statistical models a description of the bore-

hole variables of surface elevations of boreholes, elevation of and depth to bedrock, presence of water level after completion of borehole and 24 hours later, and selected similar variables for a particular building site within the study area. The results of the significance testing and model comparisons of trend surface equations suggest that: the elevation of bedrock surface was best described by the third-order trend surface; bedrock depth was best described by the second-order trend surface; elevation of water level after completion, by third-order trend surface. The variable borehole depth to water level after 24 hours and depth to water level after completion at selected construction sites could not be significantly described by a trend-surface equation, and bedrock depth at the same sites were significantly predicted by the second-degree trend equation. Although the techniques and models vary in their effectiveness and amount of variance accounted for in the criteria, they should provide the construction planner and geotechnical engineer valuable insights in predicting and eventually extending placement of exploratory boreholes in the future.

Illustrated below is an example of the types of statistical model comparisons utilizing the full and restricted models concept of multiple linear regression. Notice in this example, the third-degree polynomial trend surface accounted for a significant amount of explained variance over the second-degree response surface. Graphical representations of each response surface and their residual or error surface will be presented.

Model Comparison of Third Degree Polynomial Trend Surface with Second Degree Surface for Elevation of Bedrock.

Explanation of Models	Models	df	R ²	Alpha	F	P	Sign.
<p><u>Model 3:</u></p> $Z = a_0U + a_1X + a_2Y + a_3X^2 + a_4XY + a_5Y^2 + a_6X^3 + a_7X^2Y + a_8XY^2 + a_9Y^3 + E$	Full	4/129	.2773	0.05	6.4249	.0001	S
<p><u>Model 2:</u></p> $Z = a_0U + a_1X + a_2Y + a_3X^2 + a_4XY + a_5Y^2 + E$	Restricted		.1333				
<p>Where; Z = Elevation of Bedrock (Feet) U = Unit Vector a₀ - a₉ = Coefficients XY = Geographical Coordinates E = Error Vector (Residuals)</p>							
<p><u>Hypothesis:</u> Does the third degree trend surface account for a significant amount of variance in predicting bedrock elevation over and above what can be accounted for by the second degree trend surface?</p>							

ROBERT FISCHER, Marshall University
Community and Technical College
Huntington, WV 25755 and LEE OLSON,
Division of Human Development and Allied
Technology, Marshall University,
Huntington, WV 25755. "Tech-Prep"
Curriculum at Huntington East High School
Developed Winners in 1965.

Twenty-five years before "tech-prep" was a buzz word in educational circles, the concept was applied in Huntington, West Virginia.

Huntington East High School graduated it's first class with "Electronics Technology" certificates in 1965. A 25-year follow-up study documents phenomenal success of the class in both academic and business pursuits.

Eighty percent of the class members have earned college degrees, over half of them have electrical engineering credentials, 20% have advanced degrees and 26% of the group hold professional titles. Most of the graduates are employed by high-technology, industry-leading companies. Several operate their own businesses.

High entry standards, a rigorous curriculum with strong math and science co-requisites, counseling less interested and low performers out of the program, a state-of-the-art lab and a competent instructor are among the factors identified as responsible for making this an exemplary program.

W. HUNTER LESSER, Monongahela National Forest,
Elkins, West Virginia 26241. A Predictive Model
for Cultural Resource Site Location on the
Monongahela National Forest

A model for predicting prehistoric and historic site locations on the Monongahela National Forest was developed and implemented in 1992. The model is based on preliminary correlations first proposed in the Forest Cultural Resource Overview in 1978 and refined by field inventory data over the past decade. The predictive model may be used as a planning tool to be quantified, tested and refined to enable land resource managers to focus cultural resource inventory efforts in areas most likely to contain significant sites.

EBERHARD WERNER, GeoAnalysis, P. O. Box 795,
Morgantown, WV 26507. **Water quality in an area
mined for Waynesburg coal in southwest Pennsylvania.**

Routine water monitoring in connection with surface coal mines in the Waynesburg coal has provided considerable data on water quality for portions of Greene and Fayette counties, for periods of up to ten years at some sites. All samples were analyzed for the parameters mandated by the state's mine regulations: pH, specific conductance, and temperature in the field, and alkalinity, acidity, sulfate, total iron, total manganese, and suspended solids in the laboratory. Other analyses were also performed on a small number of samples. In general, although some water flowing from the mine sites was high in acid mine drainage components, these components produced relatively little change in the receiving streams, unlike the situation reported for other coals or for other areas. Except for sulfate, most of the contaminants usually reacted rapidly with water and sediment of the receiving stream and were neutralized or otherwise removed. Because most of the mine sites are set topographically high, there was relatively little drainage from the sites, resulting in relatively low flux rates for acid-mine-drainage-contaminant components. Generally, impact from other activities in the drainage basin, especially the impact of drainage from deep mining of the Pittsburgh coal was much greater and tended to mask any contribution from the Waynesburg sites. At some sites, sampling was begun before mining and terminated after reclamation. There was little change apparent in the permanent streams draining these sites or in wells or springs in the vicinity over that period. The greatest variations observed in the water quality of the larger receiving streams are attributable to changes in the discharge caused by climatic cycling. While discharge varied by as much as three orders of magnitude, mine-drainage-related components varied by one order of magnitude or less, which was still much higher than the change between before mining and after mining.

GREGORY M. DAMICO and JOHN H. HULL, Dept. of
Psychology, Bethany College, Bethany, West
Virginia 26032. Winning Isn't Everything, If
You're 10.

Female and male youth soccer players (aged 6 to 16) and female and male college varsity soccer players (aged 18 to 24) answered questionnaires designed to measure on Likert-type scales their attitudes on issues related to playing soccer. Subsequent 2 (subject gender) by 2 (age category) analyses of variance run on the questionnaire items showed that youth soccer players agreed significantly more strongly with these statements: Soccer is fun even when my team loses; my coach tells me when I do a good job in a game; my soccer skills have improved this past season; I want to play soccer again next season. College soccer players - and male respondents, overall - agreed significantly more strongly with these statements: Winning games is very important to me; when my team loses a game, I am disappointed. Results are discussed as they relate to child development and participation in competitive sports.

Zoology

CAROL POLLIO, West Virginia Graduate College, Institute, WV and National Park Service, Glen Jean, WV and THOMAS K. PAULEY, Department of Biological Sciences, Marshall University, Huntington, WV. Interactions of Adult and Larval Salamanders, *Desmognathus quadramaculatus* and *Eurycea cirrigera* in Keeney Creek, Fayette County, WV.

Studies were conducted on Keeney Creek in Fayette County, West Virginia during the summer and fall of 1992 to determine the status of adults and larvae of the two dominant species, *Desmognathus quadramaculatus* and *Eurycea cirrigera*. All specimens were captured in funnel traps (10 cm x 30 cm) constructed of aluminum mesh. The study site was divided in two sections, an upper area that consisted of approximately 22.5 meters and a lower area of 33 meters. Fifteen traps were set in each area and monitored weekly from July through October. Parameters considered included interspecific and intraspecific interactions, seasonal patterns, and influence of water temperature and pH. *Desmognathus quadramaculatus* larvae were captured until mid-August at which time they apparently either took refuge in the substratum or moved downstream. Adult *D. quadramaculatus* decreased in surface density in late August as water temperature decreased. Surface density of *Eurycea cirrigera* larvae showed a positive correlation with water temperature, i.e., larvae decreased as water temperature increased and, conversely, increased as water temperature decreased. Adult *E. cirrigera* did not appear in the study site until late October. There was no significant difference in the occurrence of either species relative to water pH.

THEODORE M. VITELLI and JOHN T. BURNS, Dept. of Biology, Bethany College, Bethany, West Virginia 26032. Temporal synergism of corticosterone and prolactin in regulation of body weight and testis weight in *Necturus maculosus*.

Temporal synergisms of circadian rhythms of hormones may regulate the seasonal physiology of vertebrates (Meier, A. H., Chronoendocrinology of Vertebrates. In *Hormonal Correlates of Behavior*. Edited by B. E. Eleftherion and R. L. Sprott. Plenum Press, N.Y. 469-549, 1975). Forty-seven adult salamanders were received December 2, 1992 from the supplier and maintained in plastic tanks on continuous light. There were four experimental groups of 8 salamanders each to receive hormones and four control groups of 4

salamanders each (except for one group of 3) to receive saline injections. Beginning on December 9, the experimentals received daily injections of 0.1 mg of corticosterone in 0.05 ml of 0.65% saline at 0600 and daily ovine prolactin injections of 0.075 mg of prolactin (31 I.U./mg) in 0.05 of 0.65% saline at either 0600, 1200, 1800, or 2400 for 10 days. Similarly timed injections of saline were made in the controls. Salamanders receiving hormone injections had a loss ($0.0005 < P \leq 0.005$) in body weight, as did the controls ($0.025 < P \leq 0.05$). The 12-hour relation of hormones was associated with the greatest loss in body weight ($0.025 < P \leq 0.05$). The testis weight for the combined experimentals was greater ($0.025 < P \leq 0.05$) than the testis weight for the combined control groups. The 12-hour relation of hormones was associated with the greatest increase ($0.025 < P \leq 0.05$) in testis weight as compared to the 12-hour controls. This is evidence that temporal synergisms of hormones may regulate the physiology of *Necturus maculosus*.

THOMAS K. PAULEY and KAREN MCCLURE, Department of Biological Sciences, Marshall University, Huntington, WV and CAROL POLLIO, National Park Service, Glen Jean, WV. Toads and frogs of the New River Gorge National River.

Herpetological surveys conducted in the New River Gorge National River from 1989 through 1992 resulted in the observation of 2 species of bufonids, 3 species of hylids, and 4 species of ranids. Bufonids included the eastern American toad (*Bufo a. americanus*) and Fowler's toad (*Bufo woodhousii fowleri*); hylids included the northern spring peeper (*Pseudacris c. crucifer*), mountain chorus frog (*Pseudacris brachyphona*), and gray treefrogs (*Hyla chrysoscelis/versicolor*); and ranids included the bullfrog (*Rana catesbeiana*), green frog (*Rana clamitans melanota*), wood frog (*Rana sylvatica*), and the pickerel frog (*Rana palustris*). *Bufo w. fowleri* was generally confined to the floodplains while *B. a. americanus* inhabited the higher elevations. *Pseudacris c. crucifer* and *Hyla chrysoscelis/versicolor* were found in road puddles and roadside ditches throughout the Gorge, while *Pseudacris brachyphona* was more common in road puddles and roadside ditches in higher elevations. *Rana catesbeiana* was confined to the New River and *R. c. melanota*, *R. sylvatica*, and *R. palustris* were found in vernal and permanent pools and road puddles throughout the Gorge.

THOMAS K. PAULEY and JEFFREY E. BAILEY,
Department of Biological Sciences, Marshall
University, Huntington, WV and Carol
Pollio, National Park Service, Glen Jean,
WV. Occurrence of the cave salamander,
Eurycea lucifuga, in abandoned coal mines
in West Virginia.

The total range of the cave salamander, Eurycea lucifuga, extends from western Virginia and eastern West Virginia south to northern Georgia and west to Missouri, Kansas, and Oklahoma. In West Virginia, it was thought to be restricted to natural, limestone caves in Pocahontas, Greenbrier, Summers, Monroe, and Mercer counties. Surveys in the New River Gorge National River in 1991 and 1992 revealed that E. lucifuga occupies abandoned coal mines in sandstone formations. Forty nine portals were examined and salamanders were located in 9 (22.5%). The occurrence of E. lucifuga in the New River Gorge extends its range in West Virginia west to near Ames in Fayette County. The occurrence of E. lucifuga in Fayette County establishes a new county record.

STEPHANIE BURKE, ROBERT DEAL and DAVID TODT, Dept.
of Natural Sciences, Shawnee State University,
Portsmouth, OH 45662. Flight Activity of Moths
Correlated with Dates, Times and Environmental
Conditions during 1991 and 1992 in a Hollow in
Scioto County, Ohio.

From April through October, 1991 and 1992, moths were collected at least one night each week for one-half hour at two hour intervals from 8:00 pm through 6:00 am over a one and one-third acre of yard, flower beds and house porch illuminated by a single 75 watt mercury vapor pole light. The area is located in a narrow hollow surrounded by steep, wooded hills. During each collecting period temperature, atmospheric, wind and sky conditions were recorded.

Five hundred fifteen specimens were collected from flight or perch and identified to Family, all except 42 badly worn or damaged individuals were identified to species. The moths were distributed among twelve Families: Apatelodidae (1), Arctiidae (44), Geometridae (163), Lasiocampidae (5), Megalopygidae (2), Noctuidae (154), Notodontidae (2), and Yponomeutidae (7). Information on the most frequently collected species was analyzed to determine first flight (capture), peak flight as determined by number of captures and last flight (capture) as each of these events was correlated with date, time of night and environmental conditions. In general, for most species peak flight times were 8:00 pm and 6:00 am with clear skies, no/low wind and warm temperature.

THOMAS K. PAULEY and GLENN MILLS, Department of Biological Sciences, Marshall University, Huntington, WV and CAROL POLLIO, National Park Service, Glen Jean, WV. Status of the blackbelly salamander (*Desmognathus quadramaculatus*) in the New River Gorge National River.

Surveys in the New River Gorge National River from 1989 through 1992 in 103 streams revealed that the blackbelly salamander (*Desmognathus quadramaculatus*) occurred in 59 (57%) streams. Streams varied from intermittent to third order creeks. *Desmognathus quadramaculatus* is a southern species with the most northern part of its known range extending to Fayette County, West Virginia. The total range in New River Gorge includes the counties of Fayette, Raleigh, and Summers and extends from Marr Branch in the north to an intermittent creek north of Brooklin in the south. Prior to this survey, the status of *Desmognathus quadramaculatus* in West Virginia was unknown. This study showed that while the salamander has a very limited distribution in the state, it is more abundant than previously known.

THOMAS K. PAULEY and GINGER KEES, Department of Biological Sciences, Marshall University, Huntington, WV and CAROL POLLIO, National Park Service, Glen Jean, WV. Status of the green salamander (*Aneides aeneus*) in the New River Gorge National River.

Aneides aeneus (green salamander) is listed as a species of concern by the West Virginia Division of Natural Resources. Surveys in the New River Gorge National River from 1989 through 1992, resulted in 28 previously unknown populations. Habitats that supported *A. aeneus* within the Gorge included emergent rocks and high, rock outcrops on ridges. In West Virginia, *A. aeneus* occurs in the Allegheny Plateau from Monongalia and Preston counties to the southwestern border. This study showed the range of *A. aeneus* in the New River Gorge National River to include the area south of Ames near the northern park boundary south to Fall Branch near Sandstone Falls.

THOMAS K. PAULEY and CONLEY MARCUM, Department of Biological Sciences, Marshall University, Huntington, WV 25755. Salamanders of the West Virginia University Forest.

Herpetological surveys were conducted in the West Virginia University Forest located in Monongalia County from June through September 1990. Habitat surveys included mixed deciduous forest, coniferous forest, emergent rocks, seeps, vernal and permanent pools, and creeks. Dominant species in forest habitats included the redback salamander (Plethodon cinereus) (both redback and leadback phases), slimy salamander (P. glutinosus), mountain dusky salamander (Desmognathus ochrophaeus), and the red eft (Notophthalmus v. viridescens). Species observed on/in emergent rocks included the green salamander (Aneides aeneus) and the slimy salamander (P. glutinosus). Vernal and permanent pools were habitats for the red-spotted newt (Notophthalmus v. viridescens) and spotted salamander (Ambystoma maculatum). Salamanders in seeps and creeks included the northern two-lined salamander (Eurycea bislineata), northern dusky salamander (Desmognathus f. fuscus), mountain dusky salamander (D. ochrophaeus), and the seal salamander (Desmognathus monticola).

THOMAS K. PAULEY and LINDA ORDWAY, Department of Biological Sciences, Marshall University, Huntington, WV and JAMES KOCKENDERFER, Fernow Experimental Forest, Parsons, WV. Terrestrial and Stream Salamanders of the Fernow Experimental Forest, Parsons, WV.

Studies in 5 watersheds from 1986 through 1992 have disclosed that 4 terrestrial species and 4 aquatic species make up the salamander fauna in the Fernow Experimental Forest located in Parsons, West Virginia. Terrestrial species included the redback salamander (Plethodon cinereus), mountain dusky salamander (Desmognathus ochrophaeus), slimy salamander, (Plethodon glutinosus), Wehrle's salamander (Plethodon wehrlei), and the red eft (Notophthalmus v. viridescens). Stream salamanders included the seal salamander (Desmognathus monticola), northern two-lined salamander (Eurycea bislineata), northern spring salamander (Gyrinophilus p. porphyriticus), and northern red salamander (Pseudotriton r. ruber).

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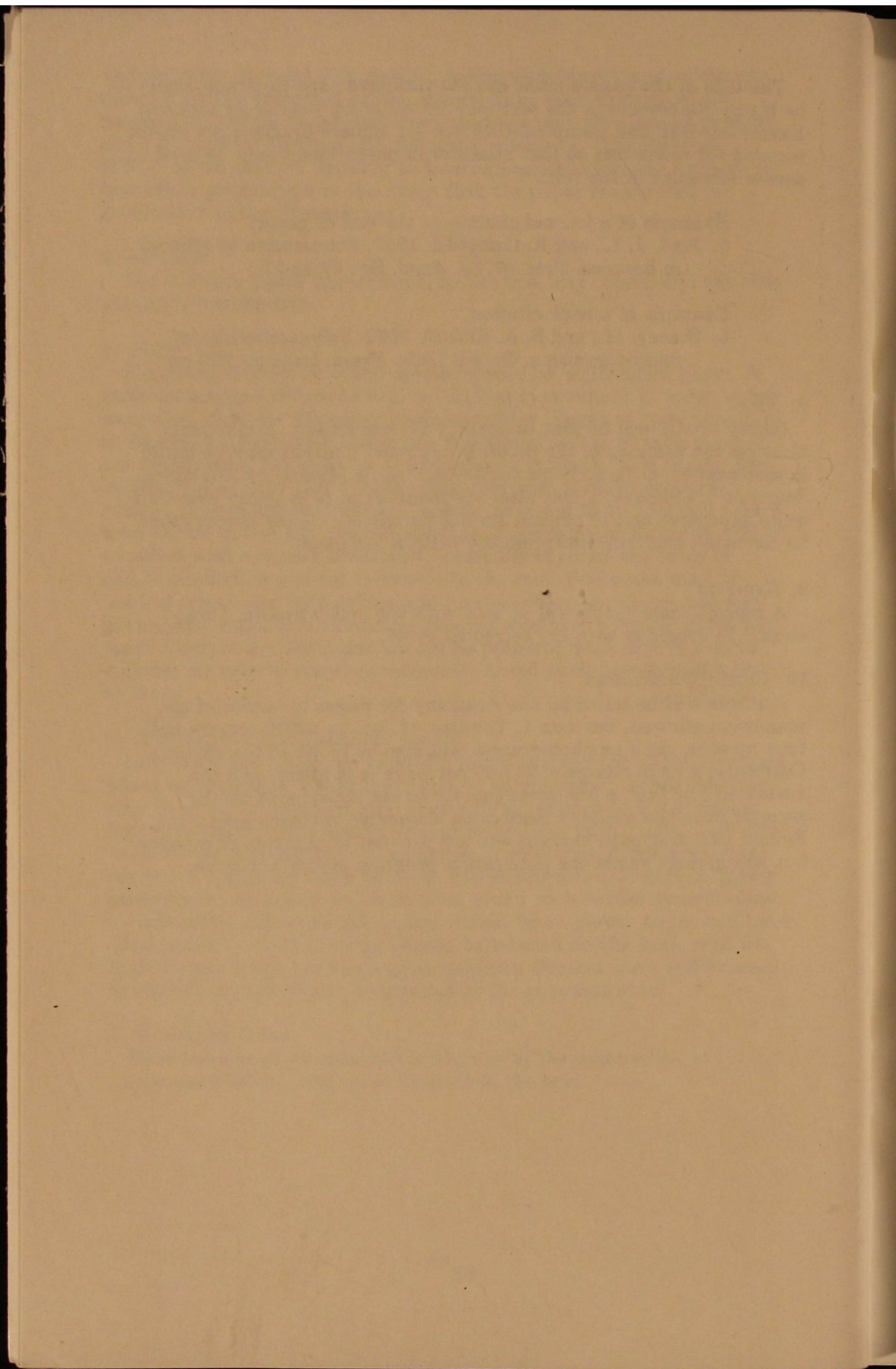
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