

**Meeting Abstracts  
of Graduate Students in the  
Department of Environmental & Plant Biology  
Summer 2007**

**American Society of Plant Biologists**

**Developmental control of leaf ad-/abaxial polarity and leaflet initiation in *Eschscholzia californica*.**

Conny Bartholmes, Sabrina Hutter, Sebastian Pape, Stefan Gleissberg

Leaves grow as bifacial organs through the establishment of a primordial margin early in their development. In angiosperms, this primordial margin forms at the border between adaxial and abaxial domains, which are defined by gene expression patterns. In plants with dissected and compound leaves, these margins later on also play a role in segment formation. The genetic interactions involved in ad-/abaxial polarity are well characterized in *Arabidopsis thaliana*. However, this system does not lend itself to the study of leaf dissection, as genes typically involved in marginal segment formation are not expressed during serration formation in this species. Data from the dissected-leaved *Lycopersicon esculentum* indicate that some leaf polarity genes may also be involved in leaf dissection. This project aims to broaden the evolutionary understanding of angiosperm leaf polarity and how it relates to the regulation of leaf dissection. Our focus on the basal eudicot *Eschscholzia californica*, a bifacial and dissected-leaved taxon, allows us to take a comparative approach, taking into account data from both monocots and core eudicots. We are interested in (i) characterizing the establishment of leaf polarity in this species, (ii) determine which of the relevant genes play a role in leaf dissection. Here, we present preliminary data on *Eschscholzia* homologs of several leaf polarity genes and show that a YABBY-like gene exhibits prolonged expression during leaflet formation.

**Identification and characterization of a glucuronosyltransferase activity involved in glucurono(arabino)xylan biosynthesis in Wheat**

Mohor Chatterjee, Wei Zeng, and Ahmed Faik

Cell walls in cereals are classified as type II walls and predominantly have glucurono(arabino)xylan (GAX) as a major hemicellulose (~35%). GAX consists of a  $\beta$ -(1,4)-D-xylan backbone that is substituted mainly by  $\alpha$ -glucuronic acid residues on C-2 positions and by  $\alpha$ -arabinofuranose on C-3 positions. Cellulose microfibrils (~20%) in type II walls are cross-linked by  $\beta$ -(1,3)(1,4)-glucan (~30%). In contrast to many hemicelluloses for which the biosynthetic pathways have been elaborated and genes cloned, the biosynthesis of GAX have received relatively little attention. The xylan synthase (XylS) and glucuronosyltransferase (GlcAT) genes have not been identified yet.

The goal of this work is to understand the enzymology of GAX biosynthesis by focusing on the identification and characterization of the glucuronosyltransferase activity that is specific for GAX biosynthesis in wheat. Golgi-enriched microsomes from etiolated wheat seedlings incorporated [ $^{14}$ C]GlcA from UDP-[ $^{14}$ C]GlcA into ethanol-insoluble products in presence of UDP-Xyl. The products formed are degraded by an endoxylanase specific to wheat arabinoxylan but not by other hydrolases. The [ $^{14}$ C]-radiolabel released by total acid hydrolysis was solely associated with glucuronic acid as indicated by analysis on CarboPac PA10 columns using a Dionex system. The activity was partially solubilized with detergents but most of the activity remained tightly associated with the membranes. The activity transferred [ $^{14}$ C]GlcA specifically onto

xylans added exogenously suggesting that glucuronosyltransferase can act independently from XylS. Purification of this activity is underway.

### **Xyloglucan Biosynthesis in grasses: Bioinformatics, cloning and expression of putative xylosyltransferases from wheat and rice.**

Ramya Nadella and Ahmed Faik,

Xyloglucans are the major hemicellulosic polysaccharides in the primary cell walls of dicots and non-graminaceous monocots. Small amounts of xyloglucan are also seen in the cell walls of grasses. Xyloglucan cross links the cellulose microfibrils and maintains the structural integrity of the cell. Considerable amount of progress has been made in identifying the genes involved in the biosynthesis of xyloglucan in dicots. But the biosynthesis of xyloglucan in grasses is not well characterized in detail, which may be because of its presence in low amounts in grasses. Therefore, the current study aims to identify the genes involved in the xyloglucan biosynthesis in grasses, particularly wheat and rice. Two homologs of the xylosyltransferase gene, AtXT1 (*Arabidopsis thaliana* Xylosyltransferase1), were identified in wheat and rice. The putative rice and wheat xylosyltransferases show ~75% similarity to AtXT1 in the amino acid level. Bioinformatic analyses revealed that both the putative rice and wheat xylosyltransferases have a single transmembrane domain at the amino terminal region, which is a characteristic feature of the Golgi-localized glycosyltransferases. A DXD motif, which is conserved in all the members of the glycosyltransferase 34 family, is also present in the rice and wheat xylosyltransferase homologs. Thus, the bioinformatic analyses provided strong evidence for their identity as the putative xylosyltransferases. Therefore, the cDNA's encoding the two putative xylosyltransferases were cloned into *Pichia* and *Drosophila* expression vectors for the expression of proteins. The expressed proteins will be biochemically analyzed for the xylosyltransferase activity. Also the biochemical requirements necessary for higher activities of these enzymes will be investigated. The results of this study will certainly help in understanding the xyloglucan biosynthesis in grasses and fill the existing gaps in the xyloglucan biosynthesis.

### **gravity persistence signal 1 reveals two novel cytochrome P450s involved in plant gravitropism.** J.C. Withers and S.E. Wyatt.

Understanding gene expression that occurs during gravitropism is important for studying the processes that link the perception of gravity to the growth response. *Arabidopsis* plants with a mutation in the GRAVITY PERSISTENCE SIGNAL (GPS)1 locus show a "No Response" phenotype during gravistimulation experiments. GPS1 encodes CYP705A22 (A22), a cytochrome P450 protein (P450) of unknown function. Microarray data collected from *Arabidopsis* root tips indicated that CYP705A5 (A5) is increased following a gravity stimulus in roots. The GPS1 gene was transformed into the *gps1* mutant background, and subsequent gravity stimulation experiments showed that the transformation successfully rescued the mutant phenotype. An expression profile was generated for A5 using real-time quantitative PCR, and the data indicate that A5 is up-regulated nearly five fold within minutes of gravity stimulation. Reporter gene constructs that link the A5 gene to the green fluorescent protein (GFP) have shown that A5 is expressed in the root zones of elongation and maturation. Computer modeling of the catalytic domain and screening of potential substrates has generated a list of 130 compounds that may have the ability to bind to A22 and A5, and many of the compounds are phenylpropanoid derivatives. Protein expression constructs were created and expressed in cell culture to determine the specific biological substrate for each. (Supported by NASA: NAG2-1608 and NSF: 0618506 to SEW)

### **Identification of putative arabino(glucorono)xylan (AGX) biosynthetic genes in wheat**

Wei Zeng, Mohor Chatterjee and Ahmed Faik

Arabino(glucorono)xylan (AGX) is an important hemicellulose in type II cell wall. It consists  $\beta$ -(1,4)-D-xylan backbone substituted mainly with  $\alpha$ -Araf and  $\alpha$ -glucuronic acid residues. In cereal grains, AGX makes up to 70% of the endosperm cell wall. Up to date, nothing is known about of the AGX biosynthesis pathway and none of the biosynthetic genes, i.e. xylan synthase (XylS), arabinosyltransferase (AraT) and glucuronosyltransferase. (GlcAT) has been identified in plants.

We used two strategies to identify putative AGX biosynthetic genes in wheat. The first one is studying the gene expression profiling using developing wheat seeds. Microscopy section shows that endosperm develops in specific stages and total RNA were extracted from each developmental stages. Twenty four genes from glycosyltransferase (GT) subfamilies GT8, GT43, GT47 and CSL were identified via bioinformatics and their expression profiles were analyzed with RT-PCR. At least ten genes were highly expressed in specific stages, which indicated that they might be involved with AGX biosynthesis. Some of these full length genes have been cloned. The second strategy is enzymatic assay for AGX biosynthesis. Golgi-enriched microsomal particles were extracted from etiolated wheat seedlings. [ $^{14}$ C] labeled UDP-Xyl, UDP-Araf and UDP-GlcAc were used in enzymatic analysis. The [ $^{14}$ C] small oligosaccharide formed by microsomal membranes can be degraded by wheat AX-specific xylanase. Several detergents were used to solublize the enzymatic activities and the solublized protein were stained on SDS-PAGE gel. The proteins with the molecular weight between 40 and 120 kDa might be involved in AGX biosynthesis. More proteomics analysis and the expression of the candidate gene are being conducted to characterize the functions of the candidate genes.

## **Botanical Society of America**

### **Evolutionary mechanisms of pre-zygotic isolation in two replicate sublineages of Hawaiian Violet (*Viola*, *Violaceae*).**

Havran, J. Christopher, Ballard, H. E.

On the Hawaiian Islands, two sublineages of Violets, containing montane bog and swamp forest ecomorphs, are distributed on the islands of Kauai (*Viola wailenalenae* and *V. kauaensis*) and Molokai (*V. robusta* and *V. maviensis*). The two sublineages represent evolutionary replicates, separated by approximately 3 million years, and offer a unique opportunity to study the evolution of pre-zygotic isolation. During the summers of 2005 and 2006, the potential temporal, ethological and mechanical isolation mechanisms maintaining species integrity and limiting gene flow between intra-island taxa were investigated. On Kauai, observations of flowering phenology during July and August revealed that *V. kauaensis* produces cleistogamous flowers throughout most or all of this time period while *V. wailenalenae* produces only chasmogamous flowers. On Molokai, *V. robusta* and *V. maviensis* produce chasmogamous flowers concurrently. On both Kauai and Molokai, no Violet species were visited by insect pollinators. Pollinator exclusion experiments indicated that each species is capable of producing fruit in the absence of insect pollinators. Anatomical investigations of flowers that developed inside pollinator exclusion bags showed that the Hawaiian Violets are capable of producing fruit autogamously, perhaps by the delayed selfing mechanism characterized in eastern North American *V. pubescens*. On Molokai, the hybrid *V. x luciae* (*V. maviensis* x *V. robusta*) is confined to the bog-swamp forest ecotone. The hybrid has 37% pollen stainability, indicating that it has substantially depressed fertility, and produces malformed carpels and seeds (and is probably an  $F_1$ ). The presence of the hybrid, and lack of temporal isolation on Molokai, indicate that pre-

zygotic isolation on this island are not as fully developed as on Kauai. Because no differences were observed to confirm other pre-zygotic isolation mechanisms (ethological and mechanical), temporal isolation may represent the last step in the establishment of complete pre-zygotic isolation among co-occurring sister species of Hawaiian Violets.

**RACE TO THE TOP: AILANTHUS ALTISSIMA SAPLING GROWTH RATES OUTPACE NATIVE OHIO HARDWOODS.** S. Polgar, K. Brown.

*Ailanthus altissima* (Tree of Heaven, AIAL), an invasive tree distributed throughout most of the United States, threatens rare community types at the Edge of Appalachia Nature Conservancy preserve in Adams County, Ohio. Previous research indicated that seedling establishment and juvenile growth rates of AIAL, while substantial, were limited by light availability. However, comparisons of AIAL growth rates with those of native tree species are limited. Understanding interspecific competition in height growth between native and invasive species allows us to estimate the magnitude and rates of invasive dominance. Our main questions were: (1) Do height growth rates of AIAL vs. native saplings respond differently to increasing amounts of canopy openness? (2) How do soil properties and canopy structure influence AIAL sapling growth and biomass? In 2006, 20×50 m plots were placed in 12 AIAL populations and 12 randomly-selected control plots. Sapling density was measured in 3 subplots. In the AIAL plots, ten AIAL saplings were harvested to determine: annual growth rate of main stem, age, diameter, and dry biomass. Native sapling growth rates for 9 species were determined *in situ* for 5 saplings within each plot. AIAL sapling density was 583 stems ha<sup>-1</sup>. The mean sapling growth rate from 2002-2006 for AIAL saplings was 41.42 cm\*yr<sup>-1</sup>. The fastest-growing native hardwood species (*Liriodendron tulipifera* and *Sassafras albidum*) had much lower growth rates than AIAL (16.67 cm and 15.72 cm, respectively). Yearly means for slower-growing native hardwoods ranged between 6.27 and 13.08 cm of mean annual growth. These results will be evaluated with data on leaf area index, soil properties, and site history to assist the Nature Conservancy with their management efforts of AIAL, in order to understand what factors are contributing to the intensity of the AIAL invasion at the Edge of Appalachia.

**On vascular development in the leaves of *Isoetes*.**

Sanders, Heather, Karrfalt, Eric, Rothwell, Gar W..

Auxin flow lays down the pattern of vascular tissue differentiation. Serial sections of *Isoetes tuckermanii* show that tracheids differentiate acropetally into the leaf traces. This indicates that the microphyllous leaves of *Isoetes* act as a permanent sink to auxin. In angiosperms, auxin flows from the developing leaf primordium towards the stele, correspondingly, xylem differentiation occurs from the base of the leaf primordium, basipetally. The difference in physiology may reflect the different evolutionary origin of microphylls.

**Physiological mechanisms of leaf shape in *Ceratopteris*.**

Sanders, Heather

While our knowledge of the developmental basis of morphology in angiosperms is extensive, we are lacking comparable information on morphogenesis in other vascular plant groups. Comparative studies of organogenesis in ferns and seed plants will differentiate homology from parallel evolution of form. As such, studies of leaf genetics, development and physiology in ferns have the potential to identify the developmental regulatory pathways that are the same as, or different from seed plant leaves. The fern *Ceratopteris richardii* is used as a model organism for genetic studies and is ideal for examining leaf morphogenesis in ferns because it has a heteroblastic series of leaf

development. Leaves in *C. richardii* range from entire ovate leaves on sporelings, to highly lobed leaves produced on older rhizomes. This study examined the effect of auxin transport inhibitors on leaf morphogenesis in *C. richardii* to identify similarities and/or differences from effects on leaf morphology in seed plants. Auxin transport inhibitors, N-(1-Naphthyl) phthalamic acid (NPA) and 2-3-5-triodobenzoic acid (TIBA) were applied to sporophytes of *C. richardii*. The sporophytes showed radical alterations in leaf morphology. In the plants that were treated with auxin transport inhibitors, leaves that were normally ovate became lanceolate, sometimes precociously lobed. These leaves had 1-2 vascular strands, in contrast to reticulate venation of normal leaves. Spatial alterations in auxin accumulation were correlated with morphological alterations. The morphological changes observed in *C. richardii* leaves were different from morphological changes in seed plant leaves that result from reductions in auxin transport, suggesting parallel evolution of leaf morphology.

### **Upside down auxin suggests the evolutionary origin of isoetalean rhizomorphs.**

Sanders, Heather, Rothwell, Gar W., Wyatt, Sarah

Lycopsids, including quillworts and clubmosses, are the sister group of all other vascular plants. Paleozoic lycopsids included giant trees that consisted of an aerial and an underground shoot system (rhizomorph) that rooted the plant. In stems of vascular plants, and living lycopsids, the plant growth regulator auxin usually flows from the apex to the base of the shoot. Tracheary elements differentiate along a polar auxin transport (PAT) gradient. The direction of PAT is evidenced by the positions of swirled tracheary elements. When the flow of auxin through differentiating tracheary elements is disrupted by a branch or a node, the auxin pools and the tracheids differentiate in swirls. In stems of both seed plants and lycopsids, where PAT is basipetal, these circular tracheids occur immediately above branches or vascular traces (toward the shoot apex). The rhizomorph also produces swirls of tracheary elements that are a structural fingerprint for the direction of auxin transport. However, they occur away from the apex in the rhizomorph. The position of these swirled tracheids indicates that auxin is flowing backwards in this modified shoot system. Therefore, auxin flow is towards, not away from, the apex in the differentiating tracheary elements of the rhizomorph. This reversed directionality of PAT is correlated with the downward growth and the modification of the rhizomorph for rooting the plants.

### **Species Diversity of *Gomphandra* (Stemonuraceae) in the Philippines**

Melanie Schori

*Gomphandra* (Stemonuraceae [Laciniaceae s.l.]) was revised by Sleumer in 1969 prior to its inclusion in volume 7 of Flora Malesiana. At that time, he recognized eight species in the Philippines but wrote that "Specific distinction... is far from satisfactory." His revision was hampered by a lack of reproductively complete and mature herbarium specimens. Since 1969, approximately 60 collections of *Gomphandra* have been made in the Philippines. These specimens, combined with uncited older materials and recent field work, suggest that species diversity may be at least twice as great as previously believed.

Current comparisons to type images and emended descriptions indicate that many specimens cannot be assigned to previously described species, suggesting there may be eight undescribed and possibly endemic species of *Gomphandra* in the Philippines. Key distinguishing features include inflorescence structure, fruit shape, and pubescence distribution. However, a final determination of species identity and diversity must await detailed examinations of type specimens.

## **Identification and characterization of the floral genes in a cleistogamous species *Viola pubescens* (Violaceae)**

Yunjing Wang, Harvey E. Ballard, R. Ryan McNally and Sarah E. Wyatt

Many plants, including most species in the genus *Viola*, can produce both open (chasmogamous) and closed (cleistogamous) flowers. Chasmogamous and cleistogamous flowers produce seeds by outcrossing and selfing respectively, and the mixed breeding system is considered a successful reproductive strategy. But the underlying molecular basis of the floral dimorphism is not known. Gibberellic acid (GA) functions in triggering flowering and has been suggested to play some roles in the floral dimorphism. The *LEAFY* (*LFY*) gene of *Arabidopsis* and its orthologs in other plants are responsible for the initiation of floral meristems and the regulation of the downstream flower development. Afterward, the ABCE classes of floral organ identity genes collaborate to give rise to a flower. The GA20 and GA3 oxidases (two key enzymes in GA biosynthesis), *LFY* and most of the ABCE floral gene orthologs of a widespread North American violet, *V. pubescens*, were identified by polymerase chain reaction (PCR). Semi-quantitative reverse transcriptase-PCR indicated that all the genes were expressed in both types of flowers, but the expression levels showed significant differences. Both *VGA20* and *VGA3 oxidase* genes were expressed more in chasmogamous flowers than in cleistogamous flowers. At least four *VLFY* gene transcripts were detected, and different transcripts displayed different expression patterns between the two types of flowers. The A class genes were expressed equally in both types of flowers. Expression of B class genes was increased in chasmogamous flowers as compared to cleistogamous flowers, while the C class genes' expression was much more reduced in chasmogamous flowers than in cleistogamous flowers. Overall, the differential expression of the floral genes explained the morphological differences between the two flower types. Our study provided the first step to understanding the molecular control of the floral dimorphism. We are currently investigating the regulation of the floral genes of *Viola pubescens*.

## **Gordon Research Conference**

### **GPS2, a v-SNARE, involved in gravity response**

Betsy Justus, Darron Luesse, Jonathan McKenzie and Sarah Wyatt

Three *gravity persistent signal* (*gps*) mutants were identified using a cold gravistimulation technique to study the process of signal transduction. One of them, the 'wrong way' mutant, *gps2*, bends in the opposite direction when compared to the wild type, upon Gravity Persistent Signal (GPS) treatment. In *gps2*, statolith sedimentation occurs normally in the inflorescence stems after reorientation at 4°C. Pulse chase studies using <sup>3</sup>H-IAA indicated that auxin transport was not significantly different from that in the wild type. Auxin redistribution, as indicated by an auxin responsive promoter-GUS fusion, was found to be reversed which resulted in the wrong way phenotype in *gps2*. Thermal Asymmetric InterLaced (TAIL) PCR identified the gene that was mutated in *gps2* to be At5g11150. This gene encodes AtVamp713 which is a vesicle soluble N-ethylmaleimide-sensitive factor adaptor protein receptor (v-SNARE). Sub-cellular localization studies using a GFP fusion shows that the protein is associated with a cellular compartment in the cytoplasm. We hypothesize that GPS2 is involved in trafficking of auxin efflux facilitators like the PIN proteins, whose improper delivery could result in the aberrant redistribution of auxin after gravistimulation. Colocalization studies of fluorescently tagged GPS2 and PIN proteins (PIN1, PIN2 and PIN3) are being performed to test this hypothesis. Bimolecular Fluorescence Complementation (BMFC) is underway to identify the binding

partners of GPS2. *In planta* tracking of GPS2 would elucidate the significance of vesicle transport in gravity signal transduction.

### **Characterization of Cytochrome P450 proteins that Regulate Plant Gravitropism.**

J.C. Withers and S.E. Wyatt

An understanding of gene expression that occurs during the gravity response is of central importance to studying the cellular mechanisms linking the physical perception of gravity to the biochemical activities governing the growth response. Plants with a mutation in the GRAVITY PERSISTENCE SIGNAL (GPS)<sup>1</sup> locus of *Arabidopsis thaliana* show a "No Response" phenotype during cold gravistimulation experiments. Cloning of GPS1 revealed that it encodes CYP705A22 (A22), a cytochrome P450 protein (P450) of unknown function. Microarray data collected from *Arabidopsis* root tips indicated that the expression of a closely related family member, CYP705A5 (A5), is increased following a gravity stimulus.

An expression profile was generated for A5 using real-time quantitative PCR, and the data indicate that A5 is up-regulated nearly five fold within the first five minutes of gravity stimulation. Reporter gene constructs that link the A5 gene to the green fluorescent protein (GFP) have shown that A5 is expressed in the root zones of elongation and maturation. To further investigate the role of P450s in regulating gravitropism, plants containing a T-DNA insertion at the A5 locus were obtained and characterized with respect to their gravity response. Homozygous mutants showed an attenuated rate of curvature that was able to be rescued by the addition of dihydroquercetin, a flavonol known to be the product of a P450 hydroxylation event.

Computer modeling of the catalytic domain and screening of potential substrates has generated a list of 130 compounds that may have the ability to bind to A22 and A5, and nearly 50% of the compounds are derivatives of the phenylpropanoid biosynthetic pathway. Protein expression constructs were created using the cDNA sequence encoding each of the proteins and expressed in cell culture in order to isolate the proteins and determine the specific biological substrate for each.

## **North American Benthological Society**

### **Use of a Diatom Community Model as an Index for Assessing Stream Impairment in Southeastern Ohio**

Nathan J. Smucker, and Morgan L. Vis

Periphyton, water chemistry, and qualitative habitat data from 44 stream segments in the unglaciated Western Allegheny Plateau ecoregion of southeastern Ohio were collected to determine the interrelationship of physical, chemical and biological data for monitoring impairments. For comparison purposes, an epilithic riffle sample and a multi-habitat sample were collected. A diatom community model using genus level taxonomy was created from 10 reference sites distributed throughout the region. Reference sites had high quality habitat, minimal anthropogenic disturbance, and fulfilled OEPA water chemistry standards. Scores were derived from percent similarity to the model ranging from 0 - 100 with higher percent similarity indicating less impaired conditions. Several chemistry variables were correlated with index scores. Discriminant analysis was used for classifying minimal impairment, moderate impairment, and severe impairment represented by upper quartile, interquartile, and lower quartile scores, respectively. Riffle scores had 92% correct assignments, and multi-habitat scores had 85% correct assignments. The index will be tested on 66 sites sampled during summer 2006. Species models were also used for scoring sites in a similar manner, but require

more taxonomic expertise by users. Use of diatom community models shows great promise with ascertaining severity of stream impairment by various stressors.

### **The Spatial Heterogeneity of Periphyton in Eight Southeastern Ohio Streams: How Far Can One Sample Take You?**

Emily K. Hollingsworth and Morgan L. Vis.

Periphyton is commonly used as a biomonitoring tool for stream research. Often only one or few sites are sampled and assumed to be representative of an entire stream reach. Current literature focuses on periphyton heterogeneity at a smaller scale, on individual rocks within a riffle, and larger scales, within a watershed or ecoregion. For the purpose of this research, the understudied intermediate scales, within a single riffle and among riffles, were sampled. Periphyton was collected at four nested spatial scales: eight reaches (each nested within a different stream), ten riffles (nested within a reach), five samples (nested within a riffle), and ten rock scrapings (nested within a sample). The eight streams included three highly impacted, three moderately impacted, and two unimpacted by acid mine drainage. Scores from a diatom index of biotic integrity and species composition data was analyzed using UPGMA cluster analysis and DGA to determine heterogeneity of periphyton communities within and among riffles. As expected, these data show little variation within riffles and among riffles of highly impacted streams. The length of a stream represented by the biological monitoring of a single point and whether the represented length is dependent upon the health of the stream is discussed.

## **Phycological Society of America**

### **COMPARISON OF THREE ORGANELLE MARKERS FOR PHYLOGEOGRAPHIC INFERENCE IN *BATRACHOSPERMUM HELMINTHOSUM* (BATRACHOSPERMALES, RHODOPHYTA) FROM NORTH AMERICA**

House, Denise L., Sherwood, Alison R. & Vis, Morgan L.

Phylogeographic signal provided by the newly developed 23S plastid rRNA marker (UPA) and the cytochrome oxidase subunit 1 marker (COI) in the freshwater red alga *Batrachospermum helminthosum*, throughout its range in North America, was investigated for intraspecific variation. These markers were compared in individuals from a previous study using the cytochrome oxidase 2-3 spacer region (*cox2-3*), which has yielded the most useful data to date with thirteen haplotypes among geographic locations. Five haplotypes were resolved for the UPA, differing by only 1-2 base pairs (bp), and we conclude that this marker may be more appropriate for studying interspecific variation. In contrast, the COI gene revealed 14 haplotypes, differing from 1-46 base pairs or up to 6.9% sequence variation. The intraspecific variation of COI in this taxon is much greater than that reported thus far for marine red algae (generally <5 bp). The intraspecific variation within *B. helminthosum* is in accord with levels shown in *B. macrosporum* (48 bp within distant locations in Brazil). The COI gene is comparable to the *cox2-3* spacer for phylogeographic studies as the haplotype networks were similar and showed the same geographic patterns. To our knowledge, this is the first comparison of these 3 regions for phylogeographic research in the red algae.

## **Ecological Society of America**

**Movement corridors as avenues of invasion of a deciduous forest by a non-native annual grass, *Microstegium vimineum***

Nathaniel P. Miller and Glenn R. Matlack. Ohio University

Shade-tolerant invasive plant species are threatening deciduous forest communities of the eastern United States, but their method of invasion remains unclear. We test the idea that arrival of one such species, the non-native annual grass *Microstegium vimineum*, is facilitated by movement of humans or animals by direct observation of distributions along movement corridors over two years. Weak colonization of experimental plots shows that *Microstegium* is limited by seed availability, and to a lesser extent litter cover, in the absence of human or animal movement. Extension of populations along off-road-vehicle trails, small roads, and stream sides implies movement of seeds by vehicles, road graders, and water movement, with maximum rates approaching 80m per year. Saturation of isolated roadside sites demonstrates that within-site colonization is very rapid relative to colonization between sites. Little extension along footpaths and horse trails implies that pedestrian and horse movement are not important mechanisms of invasion. These results suggest that *Microstegium* invasions are spatially hierarchical, with different mechanisms at different scales, and that rapid within-site movement is caused by substrate-moving processes.

### **Transient land cover in small developing watersheds as disturbance of water quality parameter**

Peter E. Schweizer and Glenn R. Matlack. Ohio University

Land cover change from residential sprawl and urban expansion alter or disturb existing watershed dynamics. Transient land cover, such as forest clearings, ground preparation for construction of residential or commercial sites, or road expansions are reoccurring forms of disturbances in developing watersheds and change energy flow and ecosystem functions. We investigated the influence of transient land cover as a form of disturbance in watershed condition by measures of water quality, stream dynamics and stream fish assemblages. We utilized a geographic information system (GIS) and characterized the spatial distribution of land cover in 17 subwatersheds. Land cover type, their spatial extent and position, varied in influence on surface hydrology, water chemistry and stream geomorphology, and between low flow and pulse flow. Watersheds with forest as dominant land cover yielded lower concentrations for nitrate nitrogen, nitrite nitrogen and total phosphate and more stable streambed geomorphology compared to residential and urbanized catchments. Managed green areas and impervious surfaces exerted strongest influence on hydraulic pulse and water chemistry, with elevated total phosphate concentrations, by magnitudes greater suspended loads of settleable solids during pulse flow and electric conductivity during low flow conditions. Transient land cover differed in their influence on water quality based on spatial position within the catchment, water quality parameter measured, and flow condition. Their strongest effects on stream water parameter were found within 600 meter Euclidean distance upstream from sampling sites for turbidity measures during low flow, and within 90 meter Euclidean distance for electric conductivity and total phosphate concentrations during pulse flow. During low flow conditions transient land cover within a 90 meter wide riparian buffer for the entire stream network correlated strongest with stream condition. The importance of spatial position of transient land cover should be taken into consideration at initial stages of land use planning for developing watersheds.