

## Second Annual Truffle Biotechnology Workshop

The 2<sup>nd</sup> annual truffle biotechnology workshop hosted by Duke University and North Carolina Agriculture and Technology (A&T) took place from June 3-29, 2008. This three-week course in molecular mycology provided hands-on mycological and molecular training for undergraduate and masters level students, through an NSF-sponsored\* collaboration between Duke and North Carolina Agricultural and Technical Universities.

During the first week the course presented students with an overview of current mycology of cultivated saprophytic and ectomycorrhizal fungi. Lectures demonstrated current and traditional methods, combined with field trips for collecting, identifying and culturing of fresh collections (mushrooms, rhizomorphs, soils, mycorrhiza). A variety of fungi were successfully cultured, including *Pleurotus ostreatus*, *Trametes* sp., bird's nest fungi, and numerous anamorphic fungi. Fresh truffles (*Tuber aestivum*, imported from Europe for the occasion) were also studied in detail

Over the following two weeks, students collected ectomycorrhizal roots from pine, oak, hazelnut and birch species to get a firsthand glimpse and comparison of these amazing symbioses using stereo- and compound microscopy. Some students also experienced other elements of nature for the first time (including yellow-jacket stings, poison ivy, and tick and chigger bites).

The highpoint of the course was a field trip to Chuckey, Tennessee where the class visited several truffle orchards which were planted by Dr. Tom Michaels. Tom's orchards are located on a mountainside that overlooks Davey Crockett's birthplace, with breathtaking views of several peaks in the southern Appalachian Mountains including Roane Mountain and Klingman's Dome (in the Great Smoky Mountains). After a brief overview of the biology of truffles and the methods used to inoculate and cultivate mycorrhizal fungi, the class collected soil samples and mycorrhizas from several productive and non-productive sites where Tom had successfully collected *Tuber melanosporum*. Later in the lab, students assessed the mycorrhizal status of Tom's trees and used molecular systematics to identify the fungal community associated with Michaels' truffiere. Ectomycorrhizae were morphotyped and assessed using PCR and sequence-based methods. Through this assessment, students became familiar with the principles of PCR, gel electrophoresis, systematics, and bioinformatics with hands-on experience gathering and interpreting gel and sequence data.



**Participants of the 2008 Truffle Biotechnology Workshop in the field after sampling mycorrhizae from hazelnut and oak orchards that have been inoculated with the black truffle species *Tuber melanosporum*. Top row (left to right): Sheriff Amusa, Morgan Kearse, Tom Michaels, Rytas Vilgalys, Maryna Didukh, Belgis E. Mansour, Omon Isikhuemhen. Bottom row (left to right): Mansuru Usif, Claudio M. Donoso, Greg Bonito and Richard Farris.**

Students successfully recovered sequences from the majority of roots which they collected. The majority of the class samples confirmed that *Tuber melanosporum* can establish and perenate in North American soils. In addition to *T. melanosporum*, students also detected several endemic mycorrhizal species (including other *Tuber* species). The fact that *Tuber melanosporum* mycorrhiza were found in both "productive" and "non-productive" sites nearby to each other suggests that ecological factors may be at play a role in fruiting.

This is the second year that we have run this workshop and it has been a big success. It has served both as a training ground in biotechnology for minority students and provides a service to agriculturists experimenting with alternative crops. It is exciting to explore these mycological frontiers with such a diverse group of people. We hope to continue such workshops in the future.

—Gregory Bonito  
—Rytas Vilgalys  
—Omonanghe Isikhuemhen

\*This workshop was funded by NSF award # 0641297 'REVSY: Phylogenetic and Revisionary Systematics of North American Truffles (*Tuber*, Ascomycota)' awarded to R. Vilgalys at Duke University.