Invasive Plant Management Section Research Program Newsletter

Division of Habitat and Species Conservation

AUGUST 17, 2009 VOLUME 1, NUMBER 2

Newsletter Index

FWC photo

The Impact:

After 4 biocontrol insect species were released by the USDA-ARS targeting Australian melaleuca trees in Florida, field evaluations and surveys indicate:

Melaleuca seed production reduced >90%

Stand densities reduced >70%

Sapling growth strongly curtailed

Regrowth from stumps reduced

Seedling survival reduced by >60%

Non-target effects - none

Source: Dr. Ted Center, USDA-ARS, Davie, FL

The Florida's Fish and Wildlife Conservation Commission's (FWC) Invasive Plant Management Section, along with the Division of Habitat and Species Conservation, hope that you will find this summer research program newsletter informative and useful. This research information, presented below, summarizes year end reports for studies that were funded for Florida's state government in fiscal year 2008-09 (July through June). Some of these studies are completed and are noted as such. Others are multi-year in nature and are continuing to receive FWC funding. Because these studies have not gone through the scientific peer-review process, and many are not complete, **please consider this information to be preliminary at best and do not cite.**

Please forward this research newsletter to ensure a wide distribution of this information. In addition, if there are avenues of research in the field of invasive plant management you believe are not being addressed, please email me your suggestions (my email address and contact information are at the end of this newsletter) – ***Don C. Schmitz, Editor***

Invasive Plant Research News

Dr. Ted Center, United States Dept. of Agriculture – Agricultural Research Service (USDA-ARS), was named **Florida Habitat Steward for 2009** by the Florida Wildlife Federation. Dr. Center's work at finding and releasing biological control insects targeting Australian melaleuca trees (*Melaleuca quinquenervia*) that infest much of South Florida including the Everglades wetland system has resulted in lowering the reproductive potential of this invasive tree and saved the State of Florida millions of dollars by reducing control costs. Dr. Center's research has been funded throughout the years by Florida's state government invasive plant management research program (now managed by FWC) along with the South Florida Water Management District and others. Because of his dedicated research efforts, Dr. Center was recognized at the Florida Wildlife Federation's 72nd Annual Conservation Awards Banquet held on June 20, 2009 in St. Augustine, Florida.

Dr. Ted Center, USDA-ARS, Davie, Florida
Florida Habitat Steward 2009

Aquatic Plant Research

Algae

Ultrasound control

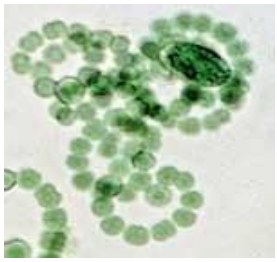
A study by the University of Florida (UF) showed **no significant impact of ultrasound treatment on the standing crop of *Lyngbya* mats** after nine weeks. Similarly, no significant effects of the treatment were observed in the microscopic and chlorophyll *a* content analyses. In addition, the consistent increases observed in estimated mat biomass over the study period, at both the control and test sites, indicate that the ultrasound treatment did not inhibit the growth of *Lyngbya.* Phlips, E.J. Control of *Lyngbya* Using Ultrasound, Department of Fisheries and Aquatic Sciences, University of Florida, 7922 N.W. 71st Street, Gainesville, FL. (COMPLETED)

Cyanobacteria and toxin transfer

Invasive aquatic plant species and a cyanobacterial epiphyte have been linked to the occurrence of an emerging wildlife disease, avian vacuolar myelinopathy (AVM), killing waterfowl and bald eagles in the southeastern United States. The cyanobacterium is a novel species within the order Stigonematales and grows densely on three plant species nonnative and invasive in North America; hydrilla (*Hydrilla verticillata)*, Brazilian elodea (*Egeria densa)*, and Eurasian watermilfoil (*Myriophyllum spicatum*). This field correlation has been validated with laboratory studies confirming the food chain transfer of the toxin from hydrilla with attached epiphytic Stigonematales species to mallard ducks. A research project at the University of Georgia expands this concept to additional cyanotoxins associated with hydrilla leaves and to important grazers of hydrilla - native and invasive apple snail (Pomacea). While the AVM suspect **Stigonematales species is not present or is very rare in Florida sites that have been screened, additional toxic species can be abundant on hydrilla** and other aquatic vegetation. The prevalence of hydrilla and native and invasive apple snails and dependence of snail kites on apple snails in Florida warrants additional screening to further investigate the potential for food chain linkages of epiphytic cyanotoxins. Initial results indicate that **consumption of hydrilla with toxigenic species was not directly lethal to the island apple snails, but the potential exists for the snails to concentrate and confer toxin to their predators, such as the endangered Everglades snail kites.** Wilde, S.B., and R.S. Haynie. Epiphytic cyanobacteria and toxin transfer from algae growing on invasive aquatic vegetation to threatened and endangered species, Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA.

Triggering cyanobacterial blooms

Scientists in St. Petersburg working for the Florida Fish and Wildlife Conservation Commission and the Florida Institute for Oceanography conducted a year‘s research on the interaction of physical, chemical, and biological factors that likely 'trigger' the occurrence and seasonal proliferation of toxic cyanobacterial blooms within Lake Eustis, Lake County, Florida. An assessment of over a decade of historical data (1996-2008, Florida Dept. of Environmental Protection data) for Lake Eustis denoted both inter- and intra-annual differences in water quality. Water quality in 1995 and 2008 exhibited the greatest distinction from other years, with differences arising initially due to greater turbidity and secondarily from deviations in phosphorus concentrations (and resulting N:P ratios) and water temperatures. A ‗seasonality‘ of water quality variables was apparent with warm, summer months (June through September) distinct from other months. Intra-annual variability of water quality appeared to mirror the system-level hydrologic regimes resulting from the ‗wet‘ and ‘dry‘ seasonality of tropical-temperate central Florida corresponding nutrient inputs (via storm-water inflows from watershed drainage). Cyanobacteria clearly dominated the lake‘s phytoplankton in all months, with (mean) monthly contributions of cyanobacteria to total chlorophyll concentrations typically approximating over 75%. *Planktolyngbya* sp. was dominant at one station



QI photo

Neurotoxic Algae

Research* indicates that some neurological disorders in humans may be linked to the ingestion of neurotoxic algae. Cyanobacteria nerve toxins have been found in the brain tissues of victims of amyotrophic lateral sclerosis-Parkinsonism-dementia complex in Guam.

(AVM related cyano-bacteria species Stigonematales sp. is also capable of producing this neurotoxin according to Dr. Susan Wilde, University of Georgia)

*Source: *Cox, P.A., Banack, S.A., and S. J. Murch. Biomagnification of Cyanobacterial Neurotoxins and Neurodegenerative Disease among the Chamorro People of Guam. Proceedings of the National Academy of Sciences of the United States of America, Vol. 100, (2003) No. 23, pp. 13380-13383*

with *Cylindrospermopsis raciborskii* comprising a significant component of the cyanobacteria assemblage. There appears to be a switch in the cyanobacterial assemblages during the dry and wet seasons (November thru April and May thru October) respectively, but further study of possible temporal relationships with precipitation is required. Scientists also conducted preliminary quarterly surveys on representative fish species in the lake's food web to determine if presumptive *Cylindrospermopsis* toxins (e.g. cylindrospermopsin, saxitoxin) or *Microcystis* toxins (microcystins) were present in their tissues. While cylindro-spermopsin and saxitoxin were not confirmed, low concentrations of microcystin-LR were found in the livers of gizzard shad, *Dorosoma cepedianum*, and bluegill, *Lepomis macrochirus*. Preliminary **results indicate that cyanotoxins in Lake Eustis are present in biota, but the extent to which they could cause chronic animal health problems or contribute to extensive acute animal mortalities during high bloom periods remains unknown**. However, unless strict management of anthropogenically-derived nutrient inputs is implemented throughout Florida, the threat of ever-increasing occurrence of cyanobacteria blooms, with potential cyanotoxin contamination of potable water supplies, remains. Landsberg[1], J., Millie[2], D., Pigg[1], R., Flewelling[1], L. and T. Lange[1]. An assessment of *Cylindrospermopsis raciborskii* within Lake Eustis: can selective nutrient influences be used to formulate strategies for controlling blooms and toxicity? [1] Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, 100 Eighth Ave. SE, St. Petersburg, FL and [2]Florida Institute of Oceanography, 830 First Street South, St. Petersburg, FL. (COMPLETED)

The Herbicide World Versus Hydrilla:

Herbicides applied to control submersed plants will be impacted by two key factors:

1. The concentration of the herbicide in water that surrounds the target plant.

2. The length of time a target plant is exposed to dissipating concentrations of herbicides in the water that surrounds the target plant.

Source: Dr. Mike Netherland, U.S. Army Corps of Engineer Research and Development Center, Gainesville, Florida

Hydrilla chemical control

Hydrilla and ALS herbicides

A series of laboratory and mesocosm studies have been conducted at the University of Florida to determine the baseline susceptibility of different hydrilla populations in Florida to several acetolactate synthase (ALS) inhibitors that are being evaluated for use in aquatics - bispyribac, imazamox, penoxsulam, and bensulfuron. Past studies indicate that different populations of hydrilla, including fluridone-resistant strains, show similar baseline susceptibility when an individual ALS inhibitor is evaluated. In contrast, this study noted significant differences in the threshold response of hydrilla to the different ALS herbicides. **Despite targeting the same enzyme, there are differences in the concentrations of the four ALS herbicides required to elicit a threshold or phytotoxic response by hydrilla.** In whole-plant assays, penoxsulam and bensulfuron have been the most active of the ALS inhibitors with complete growth inhibition noted when concentrations are sustained above 10 µg/l. Bispyribac treated hydrilla shows symptoms very similar to penoxsulam and bensulfuron at concentrations above 15 µg/l. Initial enzyme activity screening for the ALS inhibitor trifloxysulfuron-methyl and laboratory data suggest a high level of sensitivity of hydrilla to this herbicide.

There are observed differences in the response of hydrilla to imazamox when compared to the other ALS inhibitors. Results from both the lab and field trials provided evidence that following fairly short exposure scenarios to imazamox, hydrilla growth rates continued to be inhibited for an extended period of time. **The implications of this work are still under discussion, but the extended growth inhibition following short term exposures challenges the convention that all ALS herbicides would require an extended exposure to residual concentrations of the herbicides.**

As a result of this study, U.S. Army Corps of Engineers (USACE) and UF scientists are evaluating other ALS herbicides under a broader range of concentration and exposure scenarios. Whole-plant and laboratory assays are also starting to focus on combinations of the ALS herbicides with the contact herbicide endothall. While there are several field trials out using various combinations, the basic ratios of ALS herbicides to endothall and the required concentrations and exposure periods still remain unclear. The **current research in this area will help to establish a use**

FWC photo

Herbicide Evaluations at UF Continue

Dr. William Haller, University of Florida, began screening potential herbicides for use against hydrilla in 2004.

When asked and confronted about the economic and environmental harm the introduction of hydrilla caused in Florida, the man who first introduced this non-native submersed plant species into the state in the early 1950s via the Cypress Street canal in Tampa responded:

"Whoops."

Source: Don C. Schmitz, lunchtime interview with "anonymous," August, 1988, Tampa, FL

pattern for endothall and the different ALS inhibitors. This work is also being extended to evaluations on non-target submersed plants as well as emergent plants that have proven to be sensitive to the individual ALS herbicides. Netherland[1], M.D., A. Puri[2], and W.T. Haller[2]. Development of Baseline Susceptibility Data for Hydrilla to Three ALS Inhibiting Herbicides. [1]USAERDC, 7922 NW 71st Street, Gainesville, FL and [2]UF/IFAS Center for Aquatic and Invasive Plants, 7922 NW 71st Street, Gainesville, FL.

New herbicides

The development of hydrilla populations with resistance to fluridone was not expected of this vegetatively propagated clonal weed. Because of this resistance, UF scientists, with the cooperation of agrichemical companies, **began screening herbicides for activity against hydrilla**. As a result of this work, there are now two new fully labeled aquatic herbicides (penoxsulam and imazamox) and **5 more are undergoing expanded testing under Experimental Use Permits (EUP's)**, see table below. Haller, W.T., and A. Puri. Evaluation of New Herbicides for Hydrilla Control (Identifying new herbicides for possible use for control of Fluridone resistant hydrilla) UF/IFAS Center for Aquatic and Invasive Plants, 7922 NW 71st Street, Gainesville, FL.

Name (EUP-Date)	Company	Mode of Action	Status
Flumioxazin (2006)	Valent	PPO-Contact	EUP submitted for full EPA label
Bispyribac (2006)	Valent	ALS-Systemic	EUP submitted for full EPA label
Quinclorac (2007)	BASF	Auxin-Systemic	EUP - Current
Topramezone (2008)	BASF	Bleacher	EUP - Current
Trifloxysulfuron (2009)	Syngenta	ALS	EUP - Current

Flumioxazin and bispyribac-sodium testing

Flumioxazin and bispyribac-sodium, two new herbicides undergoing testing under EUPs, have demonstrated efficacy on the submersed weed *hydrilla verticillata*. Flumioxazin is a fast acting protoporphyrinogen oxidase (PPO) inhibiting herbicide that has reduced hydrilla biomass by 50% at concentrations as low as 56 $\mu g\ L^{-1}$; however, flumioxazin applied at this rate in field trials resulted in hydrilla re-growth in less than 2 months after treatment when water pH was > 8.0. Bispyribac-sodium is a slow-acting systemic herbicide that provides much longer control than contact herbicides such as flumioxazin. Unfortunately, hydrilla requires an extended exposure to bispyribac-sodium (>6 weeks). Terrestrial plants have developed resistance to herbicides in the acetolactate synthase (ALS) family. Combinations of other aquatic herbicides have been used successfully for hydrilla management to enhance efficacy or mitigate herbicide resistance. Therefore, this study by the United States Army Corps of Engineers (USACE) was conducted to determine whether additions of bispyribac-sodium (5, 10, and 20 $\mu g\ L^{-1}$) enhances flumioxazin (50 and 100 $\mu g\ L^{-1}$) activity and improve control of hydrilla. All flumioxazin and flumioxazin plus bispyribac-sodium combination treatments reduced hydrilla shoot dry weight 85 to 97% compared to the untreated controls. In contrast, the bispyribac-sodium alone treatments failed to reduce shoot biomass >70%. Shoot dry weight was reduced 94 to 98% in the second experiment by all flumioxazin alone or combination treatments except F50 + B20 (12 WAT). These **results indicate low concentrations of flumioxazin and bispyribac-sodium combinations may be effective in controlling hydrilla** and warrant further evaluation in mesocosm and field settings. Mudge, C.R., and L. S. Nelson. Flumioxazin and Bispyribac-sodium Combinations for Controlling Hydrilla, U.S. Army Engineer Research and Development Center, Environmental Laboratory, 3909 Halls Ferry Road, Vicksburg, MS.

Non-target impacts

Selectivity of an herbicide used for hydrilla control can be impacted by many factors including: 1) the plant species present; 2) the scale of the application; 3) the use rate

Highlighted Plant Species:

UF photo

West Indian Marsh Grass

Scientific name: *Hymenachne amplexicaulis*

Origin: Central, South America

Introduction: 1970s

Aquatic community: Emergent

Habitat: Wet soils to shallow water

Distribution: South & Southwest Florida,

FWC Management effort: Maintenance control

2007 public waters / plant acres: 22 / 751

selected; 4) the exposure period (often based on sequential treatments); 5) treatment timing; 6) stage of plant growth; 7) pre and post-treatment water quality and clarity; and 8) sediment type. Initial mesocosm trials with the ALS products that had received EUPs included penoxsulam, imazamox, and bispyribac-sodium and suggested that native grass species such as maidencane (*Panicum hemitomon*) and knotgrass (*Paspalidium geminatum*) are likely tolerant for most use rates and use patterns when used for control of hydrilla. Unfortunately, the lack of activity on native grasses also extends to the widely distributed invasive torpedograss (*Panicum repens*). **The early mesocosm trials also suggested that common emergent species such as pickerel-weed (*Pontedaria cordata*), sagittaria (*Sagittaria lancifolia*), and soft-stem bulrush (*Scirpus validus*) were generally sensitive to the ALS herbicides. Much of the initial concern regarding selectivity of the ALS herbicides was based on the assumption that long-term hydrilla control would occur following various ALS applications. This has not been the result following numerous experimental and operational hydrilla treatments with the ALS herbicides, and it does tend to confound our ability to assess selectivity.** For example, imazamox has performed more as a growth regulator of hydrilla and the rapid degradation of residues due to photolysis would suggest that long-term exposure patterns to this herbicide are very unlikely. Moreover, several operational penoxsulam treatments have resulted in reduction of hydrilla biomass and growth while active penoxsulam concentrations were being sustained (e.g. through bump applications), but fairly rapid hydrilla recovery followed once the residues were allowed to degrade. While the sensitive native plants generally recovered from these exposures, so did the target plant. Given the wide range of results noted in terms of hydrilla control and longevity of control, putting selectivity in a proper context has proven to be a significant challenge. Netherland[1], M.D., and W.T. Haller[2]. Evaluation of Non-target Impacts of New Aquatic Herbicides. [1]USAERDC, 7922 NW 71st Street, Gainesville, FL and [2]UF/IFAS Center for Aquatic and Invasive Plants, 7922 NW 71st Street, Gainesville, FL.

Large-scale experimental hydrilla management projects

In 2008 and 2009 treatments of endothall/penoxsulam, endothall/imazamox, and penoxsulam, imazamox, or endothall alone were conducted on several lakes across Florida. Results to date suggest that imazamox alone acts more as a growth inhibitor of hydrilla. This potential use pattern is still being discussed and imazamox may be a tool that can be integrated with other technologies. **Penoxsulam treatments have provided varying levels of hydrilla control and longevity, and the treatment results have generally been linked to the ability to maintain residues for an extended period of time in the target area.** Combination treatments of endothall with penoxsulam have resulted in initial reduction of hydrilla biomass and slow recovery. Nonetheless, the long-term efficacy and cost-effectiveness of this approach is still being evaluated. Large-block, cool-water herbicide applications of endothall have resulted in better hydrilla control than smaller block treatments in warmer water. Residue data suggests this is the result of decreased herbicide degradation rates and dilution following treatment. Monitoring and evaluations on Lake Toho have been particularly intense due to the presence of the endangered Everglades snail kite and potential concerns regarding large-scale plant management. Continued monitoring of this treatment and many others across the state remain ongoing so additional information should aid in developing more adaptive management plans. Slade[1], J.G., and M.D. Netherland[2]. Technical Support for Evaluating Large-scale and Experimental Hydrilla Management Projects, [1]UF/IFAS Center for Aquatic and Invasive Plants, 7922 NW 71st Street, Gainesville, FL and [2]USAERDC, 7922 NW 71st Street, Gainesville, FL.

Plant growth regulator's effects on hydrilla

Plant growth regulators (PGRs), such as flurprimidol, and herbicides with growth regulating properties, such as imazamox and bensulfuron methyl, have been reported to control/suppress hydrilla growth while maintaining vegetative structure important for fish and invertebrates. This change in vegetative structure created by the use of PGRs and herbicides with growth regulating properties have not been compared or quantified in terms of habitat complexity. USACE scientists investigated the effects of a static

exposure of flurprimidol (150 and 300 µg active ingredient (ai)/L) and bensulfuron methyl (5 µg ai/L), as well as a 14-day exposure of imazamox (50 and 100 µg ai/L) on hydrilla growth and aquatic habitat complexity (I_{hv}). Results at 12 weeks post-treatment indicate that all tested rates of flurprimidol, imazamox, and bensulfuron methyl significantly reduced hydrilla shoot length 46 to 69% compared to the untreated control. In addition, only imazamox (50 and 100 µg ai/L) and bensulfuron methyl (5 µg ai/L) significantly reduced hydrilla shoot biomass by an average of 68%. Habitat complexity was significantly reduced by all treatments compared to the control. **These results indicate that plant growth regulation may be a viable tool to decrease hydrilla's "weediness," while maintaining habitat complexity beneficial for fish and other aquatic fauna.** Theel, H.J., and L. S. Nelson. Effects of a plant growth regulator on hydrilla efficacy and aquatic habitat complexity, US Army Engineer Research and Development Center, Environmental Laboratory, Vicksburg, MS.

FWC photo

FWC's Present Research Effort:

FWC has funded 4 universities and 2 government agencies for 28 research projects in FY 09-10 to find more cost-effective means of controlling invasive plant species in Florida.

Hydrilla biological control

The search for biocontrol agents in China

In 2008, scientists in China focused on the Bagous weevil (*Bagous chinensis*) that was found on hydrilla in 2007. Host range testing showed that the weevil developed on four species, *Elodea nattallii, Egeria densa, Vallisneria natans*, and *Hydrocharis morsusranae,* indicating these species are in the physiological host range of the weevil. Intensive surveys searching for potential biological control agents of hydrilla in southwestern China at as many as 28 sites in 6 provinces included Sichuan, Chongqing, Hunan, Yunnan, Guizhou and Guangxi Provinces. These surveys identified a second Bagous weevil (*Bagous* sp.2) discovered in the Li River in Guangxi Province. A colony of this weevil (>40 adults) was established for further tests. Several moths in the genus *Parapoynx* were also found in Hunan and Sichuan Provinces –Ding[1], J., Zhang[1], J., Wheeler, G[2], and M. Purcell[3]. Exploration of natural enemies in southern China for biological control of Hydrilla in Florida. [1]Invasion Biology and Biocontrol Lab Wuhan Botanical Institute, Chinese Academy of Sciences, Wuhan, Hubei Province, China, [2]USDA—ARS, Invasive Plant Research Laboratory, 3205 College Ave., Ft. Lauderdale, FL, and USDA-ARS, [3]Office of International Research Programs, Australian Biological Control Laboratory (ABCL), 120 Meiers Rd., Indooroopilly, Queensland, Australia.

MT production

The USDA-ARS is working to develop an economic approach to the use of *Mycoleptodiscus terrestris* (MT) as a control tool for hydrilla. Studies focused on the scale-up of liquid culture production processes for microsclerotia of MT using deep-tank fermentation and on evaluating protocols for effective use of MT. Field trials in 2008 demonstrated enhanced hydrilla biomass reduction 3 months post application when MT and was used with an endothall/penoxsulam combination or with penoxsulam only. These studies have demonstrated that MT has potential to control hydrilla biomass accumulation when applied at water temperatures conducive to MT survival and growth with enhanced activity when applied with chemical herbicides. **Cost-effective approaches to MT production and application continue to be an impediment to the commercialization of MT**. Mark A. Jackson, M.[1] and Heilman[2] Laboratory and Field Demonstration of weed control properties of dry formulations of *Mycoleptodiscus terrestris*, a potential fungal bioherbicide for control of *Hydrilla verticillata*, [1] USDA, Agriculture research Service, National Center for Agricultural Utilization Research, Crop Bioprotection Research Unit, Peoria, IL, [2] SePRO Corporation, SePRO Research and Technology Campus, 16013 Watson Seed Farm Rd., Whitakers, NC. (COMPLETED)

The search for biocontrol agents in Africa

Surveys were conducted at 144 locations in ten lakes in Kenya, Uganda and Burundi to locate populations of *Hydrilla verticillata*. At all locations sampled, hydrilla plants typically had 30-80% of their growing tips missing, and leaves were often ragged or missing. Since no insects were found associated with these damage symptoms, the hypothesis that fish were feeding on hydrilla was tested. Gills nets were used in Lake Bisina, Uganda to trap 36 species of fish. Dissection and inspection of gut contents revealed the presence of hydrilla leaves in 4 species of cichlids. Analysis of chloroplast DNA revealed that Florida hydrilla was the same haplotype as plants from India and Africa, suggesting that African populations are the result of an introduction from India.

The Economy Impacts FWC Invasive Plant Management Research Funding Levels:

FY 03-04 - $613, 559

FY 04-05 - **$724,225**

FY 05-06 – **$1,368,657**

FY 06-07 – **$1,875,707**

FY 07-08 - **$2,367,941**

FY 08-09 – **$2,049,733**

FY 09-10 - **$1,485,060**

China had very high genetic diversity, supporting an hypothesized Asian center of origin for hydrilla. Examination of 8 nuclear microsatellites revealed a surprising amount of genetic diversity in Florida, which is believed to be the result of somatic mutations. Data suggests that a single clone of hydrilla was introduced into Florida ~50 years ago and has spread throughout Florida and the southern United States. Based on the **genetic studies, which strongly suggest that hydrilla is not native to Africa**, and the lack of insect herbivory, the prospects of finding host specific natural enemies of hydrilla in eastern/central Africa are low. The reasons that hydrilla does not reach high densities in east/central African lakes are unknown, but may be associated with fish herbivory, or to abiotic factors - Overholt[1] W.A., Copeland[2] R., Williams[3] D., Cuda[4] J., Nzigidahera[5] B., Nkubaye[5] E., Wanda[6] F., and B. Gidudu[6]. Exploration for natural enemies of *Hydrilla verticillata* in East/Central Africa and genetic characterization of worldwide populations. [1] Biological Control Research and Containment Laboratory, University of Florida, IFAS, 2199 South Rock Road, Fort Pierce, FL, [2] International Center of Insect Physiology and Ecology, Jinja, Uganda, [3] Department of Biology, Texas Christian University, [4] Department of Entomology and Nematology, Fort Worth, TX, University of Florida, Gainesville, FL, [5] Institute National pour l'Environnement et la Conservation de la Nature, Bujumbura, Burundi, [6] National Fisheries Resources Research Institute, Jinja, Uganda. (COMPLETED)

Hygrophila biological control

The search for biocontrol agents in India

Surveys for natural enemies of hygrophila were conducted in the states of Assam and West Bengal in the northeastern part of India. Several promising insects were collected during these surveys. One of these insects was a defoliating caterpillar of the butterfly *Precis alamana* L. (=*Junonia alamana*) (Lepidoptera: Nymphalidae). The common name of this butterfly is "Peacock Pansy" and it is widely distributed throughout India. It is usually observed in association with freshwater marshes and is known to feed on *Hygrophila auriculata*, a congener of *H. polysperma.* The native *Berleria cristata*, a critical test plant, was found to be an unsuitable host of this insect. An as yet unidentified caterpillar (Lepidoptera: Noctuidae) also was observed completely defoliating emerged hygrophila plants. This insect was collected at most sites in Assam and West Bengal. Preliminary host range studies revealed that this larva also feeds on *Hygrophila auriculata.* An aquatic leaf cutting moth (Lepidoptera: Crambidae) similar to our native *Synclita obliteralis* was found feeding on submerged leaves of hygrophila plants and a leaf mining beetle, *Trachys* sp. (Coleoptera: Buprestidae) was collected on hygrophila in both Assam and West Bengal. The beetle larva is a leaf miner and completes its life cycle within a single hygrophila leaf. The nematode fauna associated with hygrophila roots also was surveyed. Extraction and identification of plant parasitic nematodes was performed at Bidhan Chandra Krishi Viswavidyalaya University, West Bengal, India. Several common plant parasitic nematodes that are important crop pests were found to be associated with hygrophila roots, and have no biological control potential. These findings constitute the first record of nematodes associated with this aquatic invasive weed. – Cuda[1], J.P. and W. Overholt[2]. [1]Foreign Exploration for Natural Enemies of Hygrophila polysperma in India, University of Florida, Entomology and Nemotology Department, Gainesville FL and [2]Biological Control Research & Containment Laboratory, University of Florida, IFAS, 2199 South Rock Road, Fort Pierce, FL.

Nymphoides and Rotala chemical control

Best Management practices

During the past decade, non-native species of *Nymphoides* and *Rotala* have escaped the ornamental plant trade and become established in South Florida canals. Studies have been conducted by scientists at UF in mesocosms as well as in South Florida canals to develop best herbicidal management programs for these invasive plant species. Herbicide treatments included imazamox (50, 100, 200, 400 µg/l), endothall (0.25, 0.5, 1.5 and 2.5 mg/l), triclopyr (0.5, 1, 2, 2.5 mg/l), flumioxazin (50, 100, 200, 400 µg/l) and UF-20 (25, 50, 100, 200 µg/l). For *N. cristata*, endothall was the most effective herbicide and gave 98-100% control at 1.5 and 2.5 µg/l. UF-20 at 100 and 200 ppb gave 82 and 93% control, respectively. Flumioxazin was also effective at higher doses

It was reported in a 1944 journal article in the "Louisiana Conservationist" that waterhyacinth was imported from the Orinoco River in Venezuela by members of the Japanese Exhibit at the 1884-85 World's Industrial and Cotton Centennial Exposition held in New Orleans, La., and was given away to visitors as souvenirs. Curiously, there is no mention of waterhyacinth in the informational brochures from the exposition's Japanese and Venezuelan Exhibits that contain extensive information regarding imported horticultural species. This paper, published in the Louisiana Conservationist in 1944, is the first and earliest reference holding the Japanese as accountable for waterhyacinth introduction into the U.S. Coincidentally, anti-Japanese public sentiment was high when this paper was published in 1944.

Source: Schmitz, D.C., Nelson, B.V., Nall, L.E., and J. D. Schardt. Exotic Aquatic Plants in Florida: A Historical Perspective and Review of the Present Aquatic Plant Regulation Program. Proceedings of the Symposium on Exotic Pest Plants, November 2-4, 1988, University of Miami, Miami, Florida Technical Report NPS/NREVER/NRTR - 91/06 p 303-326

of 200 and 400 µg/l and gave 82 and 87% control of *Nymphoides*, respectively. Tryclopyr and imazamox were the least effective treatments and even the maximum labeled dose of tryclopyr (2.5 µg/l) gave only 55% control. Results of a field trial produced similar results. Submersed treatments of endothall at 2-3 µg/l gave about 80-90% control 8 weeks after treatment. For *R. rotundifolia*, triclopyr was the most effective herbicide in the mesocosm experiments. Further studies are being conducted in mesocosms and in the field to develop management programs for these weed species – Haller, W.T., and A. Puri. Best Management Practices (BMP's) for *Rotala* and *Nymphoides* Control. University of Florida Center for Aquatic and Invasive Plants and USAERDC, 7922 NW 71st Street, Gainesville, FL

Waterhyacinth biological control

Biocontrol evaluations in U.S. quarantine

The impact of *Megamelus scutellaris* on waterhyacinth was assessed in quarantine through a series of tests. Individual waterhyacinth plants exposed to two generations of feeding by *M. scutellaris* experienced a 66.9% suppression of biomass when compared to the controls. The mean number of leaves per plant increased 174.2% in the controls and declined 45.0% on plants exposed to *M. scutellaris*. A second year of pre-release field ecology studies was conducted at three sites in South and Central Florida. The existing biological control agents significantly reduced waterhyacinth biomass in the field, in amounts ranging from 50-68%. In addition, more than 98% of seeds were eliminated by their feeding. However, coverage was still high in many areas except for those with lower nutritional levels. ***Megamelus scutellaris* was approved for release by the Technical Advisory Group for the Biological Control of Weeds** - Tipping, P.W. Evaluating New Waterhyacinth Biological Control Agents in Quarantine, USDA-ARS Invasive Plant Research Laboratory, 3225 College Ave., Ft. Lauderdale, FL.

The search for biocontrol agents in South America

Another candidate for the biological control of waterhyacinth is *Thrypticus truncatus* Bickel & Hernández (Diptera, Dolichopodidae). Laboratory studies were conducted to estimate the impact of *T. truncatus* on various waterhyacinth variables. Results indicated that this species exerted minimal to no suppression of the plant. Ten sites in northern Argentina have been surveyed for waterlettuce herbivores. Various extraction methods have been tested and individual insect colonies have been created. Two weevil species in particular are being sought: *Argentinorhynchus breyeri* and *Onychylis cretatus* – Tipping, P.W. Foreign Surveys and Development of Biological Control Agents for Waterhyacinth and Waterlettuce, USDA-ARS Invasive Plant Research Laboratory, 3225 College Ave., Ft. Lauderdale, FL.

Wetland nightshade biological control

The host specificity tests

Multiple-choice & no-choice feeding-oviposition tests were conducted at the Florida Department of Agriculture and Consumer Services-Division of Plant industry Quarantine facility in Gainesville to determine the specificity of the Mexican/Central-American flower-bud weevil *Anthonomus elutus* Clark intended for biological control of *Solanum tampicense* Dunal (wetland-nightshade) in Florida. Eighty-seven plant species in 17 families were included in the feeding-oviposition multiple-choice tests including the target weed and the six major cultivated Solanaceae (*Capsicum annuum* L. *Capsicum frutescens* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., *Solanum melongena* L., and *Solanum tuberosum* L.). Results indicated that *A. elutus* fed and laid eggs only on the target weed. No eggs were deposited on any of the other 86 plant species tested. The host-specificity tests indicated that a host range expansion of *A.elutus* to include any of the major cultivated Solanaceae species is highly unlikely. **A petition for field release in Florida was submitted to the Technical Advisory Group for Biological Control Agents of Weeds (TAG) in December 2008.** Medal, J. Host specificity of *Anthonomus elutus* (Coleoptera: Curculionidae), a potential biological control agent of wetland nightshade (Solanaceae) in Florida. University of Florida, Department of Entomology & Nematology, Gainesville, FL.

Upland Plant Research

Air potato biological control

FWC photo

Some Air Potato Facts:
Dioscorea bulbifera

Has a growth rate of up to 8 inches/day:

- Although considered a species of yam, air potato is very toxic.
- Asia is the likely origin of this species.
- This species represents a significant threat to South Florida's endangered hardwood hammocks after a hurricane has passed because it can rapidly form dense canopies preventing the recovery of the hammocks.
- Because of its twining growth form, it increases the probability that the supporting plants will eventually collapse, resulting in a significant change in the vertical structure of the community.

Source: Air Potato Management Plan (2008) - FEPPC

Let's find a species name!

Following the discovery of the leaf beetle *Lilioceris* near *impressa* in Nepal, by a USDA-ARS scientist, a project was begun in 2005 to study its biology and host specificity. Beetles were obtained yearly from Nepalese cooperators at the Division of Entomology, Nepalese Agricultural Research Council in Katmandu. The biology of the beetle was studied primarily in Nepal and the host specificity in the Fort Lauderdale quarantine. Attempts to identify the beetle more precisely were made by USDA-ARS colleague Dr. Alex Kostantinov comparing the beetle with types from museums in London and Copenhagen. **The beetle is a voracious feeder of air potato leaves as both larvae and adults, consuming almost three square meters of leaf**. Both stages also eat the aerial bulbils**,** which are the only means of reproduction that the weed has in Florida. Host specificity testing determined that the beetle is an extremely narrow specialists able to develop only on the target weed. During the last year, a release petition was written and submitted in January 2009. However, the taxonomy of this beetle needs work**.** The comparison of the types of *Lilioceris impressa* from Copenhagen and London with the air potato feeding beetle from Nepal were inconclusive. The reproductive organs which are important for taxonomic determination of beetles also did not precisely match. Research is needed to compare Asian *Lilioceris* species to understand the variation observed. This is expected to result in either the identification of the Nepalese air potato beetle or a new description being written for the insect. (Note - the TAG reviewing the release petition may not approve the petition without a name and APHIS could also be reluctant to permit the insect for release without a more specific name). Pemberton, R.W. Host Specificity testing on *Lilioceris* sp.a Nepalese leaf beetle attacking air potato (*Dioscorea bulbifera*). Invasive Plant Research Laboratory, USDA-ARS, 3225 College Ave.,Ft. Lauderdale, FL.

Brazilian pepper biological control

The quarantine biocontrol labwork

The Brazilian pepper thrips, now correctly identified with both genetic and morphological methods, is undergoing testing for suitability in quarantine at the USDA-ARS Invasive Plant Research Laboratory (IPRL). Problems with rearing and testing protocols are being overcome with modifications of previous methods. **Most troubling is the finding of adult survival after 14 days when fed *Schinus molle*, *Metopium toxiferum* and *Pisticia vera* whereas the adults lived at least 30 days on their host *S. terebinthifolius*.** Additionally, complete development occurred on *Schinus molle*, and eggs (though few) were found on *S. molle*, *M. toxiferum* and *P. vera*. USDA-ARS scientists are determining the suitability of these and other species for thrips larval development. Within this single thrips species, enormous diversity is being found indicating the existence of 21 different genetic variants.

A leaf blotcher, *Leurocephala schinusae*, is being described as a new species and testing continues in quarantine at the USDA-ARS IPRL. Preliminary results in quarantine suggest this species may not be sufficiently host specific and field collections from Argentina and Brazil support this broad specificity within the family Anacardiaceae. Study of the leaf feeding caterpillar *Tecmessa elegans* was initiated to test suitability of North American Anacardiaceae species. These tests are still underway and indicate larvae will feed and complete development on many North American species including *Rhus copallinum* and *Cotinus obovatus*. Surveys for Brazilian pepper herbivores were conducted in South Amercia and results include the

discovery of several new species, most notably a new stem galling wasp, *Allorhogas* n.sp., (Hymenoptera: Braconidae) and the fruit-feeding *Lithraeus mutatus* (Coleoptera: Bruchidae). The identity of both is still under study. Wheeler[1], G., and D. Williams[2]. Quarantine risk assessment studies for classical biological control of Brazilian pepper. [1]USDA/ARS Invasive Plant Res. Lab, 3225 College Ave., Ft Lauderdale, FL and [2]Dept of Biology, Texas Christian University, 2300 S. University Dr., Ft Worth, TX.

At the University of Florida, **a petition to release the moth *Episimus unguiculus* (Lepidoptera: Tortricidae) is in preparation and should be submitted by early September**. The larvae of *E. unguiculus* are leaflet rollers and are capable of causing extensive defoliation of Brazilian peppertree. Host range testing of the weevil *Apocnemidophorus pipitzi* (Coleoptera: Curculionidae) is nearing completion. Adults resemble bird droppings and feed mainly on the upper surface of subterminal leaflets, where they produce a characteristic notching pattern. Females deposit eggs in the stems and larvae feed under the bark where they damage the vascular cambium. Cuda[1], J.P., J.C. Medal, J.L. Gillmore and W. Overholt[2]. Classical Biological Control of Brazilian Peppertree in Florida. [1]Biological Weed Control, UF/IFAS Entomology & Nematology Dept., Bldg. 970, Natural Area Drive, PO Box 110620 Gainesville, FL and [2]Biological Control Research & Containment Laboratory, University of Florida, IFAS, 2199 South Rock Road, Fort Pierce, FL.

"Federal and nonfederal officials identified funding, cooperation, and public education as key to effective weed management."

Source: Invasive Species – Cooperation and Coordination are Important for Effective Management of Invasive Weeds, U.S. GAO Report, 2005

Chinese tallow biological control

The search for biocontrol agents in China

In 2008, Wuhan Botanical Institute scientists conducted a complementary host range test for the leaf-rolling weevil, *Heterapoderopsis* (=*Apoderus*) *bicallosicollis* by including two congeners of Chinese tallow, *Triadica rotundifolia* and *T. cochinchinensis.* **Results indicated that adults could feed on the two plants in no-choice tests but preferred *T. sebifera* over either of other two species in pair-choice tests, indicating *H. bicallosocollis* is a specialist with a narrow host range in the genus *Triadica* but prefers Chinese Tallow**. Host range testing of the noctuid moth, *Gadirtha inexacta* using 51 plant species showed that only *T. sebifera*, *T. rotundifolia* and *Sapium chihsinianum* could support larval development while larvae did not feed or develop on the other species. Chinese scientists also conducted host specificity studies on the flea beetle, *Bikasha collaris.* The results indicate that in adult feeding and survival tests, *B. collaris* only fed on *T. sebifera, T. cochinchinensis* and *T. rotundifolia*. Adults were unable to survive more than a week on other test plants and larvae could not complete development on any of the test plants. In choice tests, the number of adult feeding holes on *T. sebifera* was significantly greater than on the *T. cochinchinensis* and *T. rotundifolia*. Field surveys on seed feeders suggest that some seed-feeders have potential for use in biological control of tallow in the United States. Tallow resistance and tolerance to herbivory was also evaluated and results indicate decreased resistance but an increased tolerance on invasive tallow populations, which may affect the efficacy of biocontrol agents. Ding[1], J., Wang[1], Y., Huang[1], W., Zhang[1], J., Wheeler[2], G., and M. Purcell[3]. Exploration of natural enemies in China for biological control of Chinese Tallow *Triadica sebifera (Sapium sebiferum)* in Florida. [1]Invasion Biology and Biocontrol Lab, Wuhan Botanical Institute, Chinese Academy of Sciences, Wuhan, Hubei Province, 430074 China, [2]USDA—ARS, Invasive Plant Research Laboratory, 3205 College Ave., Ft. Lauderdale, FL, and USDA-ARS, [3]Office of International Research Programs, Australian Biological Control Laboratory (ABCL), 120 Meiers Rd., Indooroopilly, Queensland, Australia

Coral ardisia chemical control

Herbicide screenings

Herbicides containing the active ingredient imazapic and triclopyr used alone or in combination were most effective in controlling coral ardisia (*Ardisia crenata*) at 12 months post-treatment. A tank mix of triclopyr + fluroxypyr also provided effective results in controlling coral ardisia, but it is unclear if fluroxypyr provided any additional control since this herbicide was not tested alone. **A single application of imazapic + glyphosate resulted in excellent control after 12 months and warrants additional screening.** Control of seedlings was similar to control of mature plants with products containing imazapic and triclopyr resulting in significantly higher control

FWC photo

Climbing Fern in South Florida is Becoming the Worst

Photo of Old World Climbing fern (*Lygodium microphyllum*) canopy (bright green) in the Loxhatchee National Wildlife Refuge. This species has emerged as one of the worst invasive plants in South Florida. Left unchecked, scientists have estimated that more than two million acres will be infested by 2014.

Source: Volin, J.C., Lott, M.S., Muss, J.D., and D. Owen. Predicting rapid invasion of the Florida Everglades by Old World Climbing Fern (Lygodium microphyllum. Diversity Distri. (2004) 10:439–446.

than products containing glyphosate. There was no significant difference between the control of seedlings using a tank mix of triclopyr + fluroxypyr compared to triclopyr use alone. Additional screenings should include fluroxypyr use alone. In another experiment, at three months post treatment in which products containing the active ingredient imazapic and triclopyr used alone or in combination were most effective in controlling coral ardisia. Imazapic used alone took longer to effectively control coral ardisia exhibiting only 59% control at three months. A basal bark application of 18% triclopyr diluted with Impel, which is commonly used for control of coral ardisia, resulted in 99% control three months post-treatment. At three months post-treatment, control of seedlings was similar to control of mature plants with products that contain imazapic and triclopyr resulting in significantly higher control than products containing glyphosate. Meisenburg, M., and K. Langeland. Evaluations of herbicides for controlling coral ardisia (*Ardisia crenata*). University of Florida, Agronomy Department, UF.IFAS Center for Aquatic and Invasive Plants, 7922 NW 71st Street; Gainesville, FL. (COMPLETED)

Lygodium biological control

Host specificity tests

Host range testing of the Thai sawfly *Neostromboceros albicomus* was completed. These tests included two developmental studies and three types of feeding studies. In the first two years, two types of larval transferral experiments were conducted, one using newly emerged larvae in a choice test and another test using larger larvae in a no choice test. During the past year, USDA-ARS scientists have completed the adult oviposition and subsequent larval development tests. This research determined that this sawfly is a narrow specialist. It is only able to develop on *Lygodium* species and has host range below the genus *Lygodium* level. It was able to develop fully on the 4 of 7 tested *Lygodium* species, including the two invasive (*L. japonicum* and *L. microphyllum*) and 2 of 5 tested *Lygodium* species native to the Americas (fully on *L. volubile* and marginally on *L. oligostachyum*). The North American *L. palmatum* was not a developmental host and it occurs in the cold temperate zone where the tropical sawfly can't live. The Cuban endemic climbing fern, *L. cubense*, did not support development. The oceanic barrier between Florida, the intended area of the sawfly's use, and Cuba and Hispanola will offer protection to *L. volubile* and *L. oligostachyum*. **Based on these research results and considerations, a petition requesting the release of this Thai sawfly, *Neostromboceros albicomus,* was written and submitted to the Technical Advisory Group for Biological Control of Weeds on July 28, 2009.** Pemberton, R.W. Biological Control of *Lygodium microphyllum*-Quarantine testing . USDA ARS Invasive Plant Research Laboratory, 3225 College Ave. Ft. Lauderdale, FL.

Lygodium biocontrol research in Australia included host-specificity testing of two *Callopistria* spp., comparative testing of north Queensland biotypes of the Lygodium mite *Floracarus perrepae*, and trips to Singapore, China, Hong Kong, Thailand, northern Australia and southeast Queensland for field-surveys and collections. Field surveys and quarantine testing of the Hong Kong stem-borer continued through this period and a new population of stem-borers was discovered in southern China. Advances were made in attempts to rear an adult of the weevil stem-borer in northern Australia and observational surveys of the seasonal fluctuations of the Singapore stem-borer, *Lygomusotima* sp. continued. Makinson[1], J., and R.W. Pemberton[2]. Lygodium Research in Australia. [1]Commonwealth Scientific and Industrial Research Organization and USDA-ARS Australian Biological Control Laboratory, Brisbane, Australia Invasive Plant Research Laboratory and [2]USDA-ARS, 3225 College Ave., Fort Lauderdale, FL.

Lygodium (*Lygodium microphyllum)* chemical control

Herbicides and prescribed fire

Following aerial and ground treatments with metsulfuron methyl to control *Lygodium microphyllum* on Everglades tree islands in 2006-2007, prescribed fire was applied to eight islands in August 2008. At six months post-fire, following two herbicide

applications, *L. microphyllum* was still common on all tree islands, accounting for 4.7% cover. UF scientists observed a loss of canopy cover from 45% pre-treatment (2005) to < 9% six months post-fire (2009). This was followed by a loss of dominant ground cover of native ferns to ground cover dominated by early successional and generalist plants, not typical of untreated tree islands. It is unclear how prescribed fire effects tree island ecology over time, but combining herbicide treatment and fire did not eliminate *L. microphyllum* while concomitantly changing the structure and composition of the tree islands. **UF scientists do not recommend prescribed fire following herbicide application**, unless the tree islands have no tree canopy and near complete cover of *L. microphyllum*. Hutchinson, J.T., and K. Langeland. Effects of Herbicide and Prescribed Fire on *Lygodium microphyllum* at A.R.M. Loxahatchee N.W.R. Agronomy Department, UF/IFAS Center for Aquatic and Invasive Plants, 7922 NW 71st Street, Gainesville, FL. (COMPLETED)

FWC photo

Biocontrol Insect Established that targets Old World Climbing Fern

Dr. Bob Pemberton, USDA-ARS, (left) and Mr. LeRoy Rodgers, South Florida Water Management District (SFWMD), celebrating the successful establishment of a biocontrol insect targeting Old World Climbing fern at a test release site in South Florida. FWC, along with the SFWMD, have funded this long-term research effort.

Cut and spray versus band spraying

UF research found no significant difference (P = 0.46) between cut and spray herbicide treatment compared to band spray (poodle-cut) herbicide treatments using glyphosate (2% product), metsulfuron methyl (2 oz./100 gal), asulam (2% product), and glyphosate (1% product) + metsulfuron methyl (1 oz./100 gal) for percent reduction of Old World climbing fern (*Lygodium microphyllum*) at six months post-treatment. There was no correlation (R = -0.32) between initial d.b.h. (diameter breast height) of Old World climbing fern and percent reduction of Old World climbing fern at six months post-treatment. Results indicated a higher number of Old World climbing fern fertile leaflets in un-cut plots compared to cut plots and that re-treatment was more effective on live fronds during re-treatment in the cut plots. Hutchinson, J.T., and K. Langeland. Comparison of cut and spray versus band spraying for ground treatments of *Lygodium microphyllum.* Agronomy Department, UF/IFAS Center for Aquatic and Invasive Plants, 7922 NW 71st Street, Gainesville, FL. (COMPLETED)

Herbicides and spore germination

The objectives of this UF research were to evaluate multiple herbicides with different modes of action to determine if any of these herbicides decrease or prevent Old World climbing fern spore germination. The herbicides used were: glyphosate, metsulfuron methyl, imazapyr, triclopyr, fluroxypyr, and asulam. Spore germination for all herbicide rates and exposure times was observed except for metsulfuron methyl at rates ≥ 1% in which spore germination rates were zero. Spore germination rates with metsulfuron methyl at 0.5% product were ≤ 0.6% for all exposure times. Germination rates of spores exposed to imazapyr were significantly different from controls at all rates and exposure. Spore germination with glyphosate only differed from control at the higher rates of 4% and 8 % product. In comparison of agar nutrient media plates mixed with metsulfuron methyl and controls there were significant differences (P ≤ 0.0006) in Old World climbing fern spore germination rates compared to the controls. These results provide some evidence that metsulfuron methyl residual activity impacts gametophyte development. Analysis of Old World climbing fern spore germination rates from 20 populations ranging in age from 5 to 432 weeks exposed on agar nutrient media plates mixed with metsulfuron methyl revealed a significant difference (P = 0.018) in gametophyte survival rates at 15 and 45 days (43% reduction). This experiment was only conducted once and further research needs to be conducted, but it provides some evidence on the residual activity of metsulfuron methyl. These **results indicate that of the metsulfuron methyl impacted spore germination of Old World climbing fern at a greater rate relative to the other herbicides tested**. In a comparison of Old World climbing fern spores from untreated and metsulfuron methyl treated Everglade tree islands, we observed deformities in some treated spores that were not observed in untreated spores. About 30% of the treated spores appeared to be irregularly shape with some damage to their cell wall, and contained dark colored bodies. These results may provide insight to why metsulfuron methyl has provided better results in some field herbicide trials to control Old World climbing fern than glyphosate or other herbicides. Hutchinson, J.T, and K. Langeland. Effects of Selected Herbicides on *Lygodium microphyllum* Spore Germination and Survival. Agronomy Department, UF/IFAS Center for Aquatic and Invasive Plants, 7922 NW 71st Street, Gainesville, FL. (COMPLETED)

The New Taxonomy

"With development of powerful genetic techniques, many old botanical classifications are being reevaluated. Where morphological and floral features once dominated, it is now chloroplastic DNA and other genetic markers that rule the day in classifying plant relationships. Morphological features are still being used for plant identification, but many species are organized into groups that are cryptic at best when just based on morphological features."

Source: Aquatic Plant Management Society 2009 Annual Meeting, presentation by Mr. Brett W. Bultemeier, University of Florida Graduate Student

Lygodium (*Lygodium japonicum)* chemical control

One year response to directed sprays

Six replicated studies examined the use of herbicides for efficacy in controlling Japanese climbing fern (*Lygodium japonicum*) and impacts to associated vegetation on conserved forest land in Florida. Three herbicides: glyphosate, imazapyr and metsulfuron, were applied over a wide range of rates alone and in two- and three-way combinations and compared to an untreated control. Herbicide treatments tested gave a high degree of control, but not complete control, suggesting the need for follow-up treatment. Percent cover at one year and percent control (percentage change in cover from initial values) were used to quantify efficacy at the 4 locations having matted fern. At the two locations using individual rootstocks the number of rachises and rachis length at one year, as well as percent control (percentage change from initial values) were used as dependent variables. At one year after treatment, the percent *L. japonicum* cover ranged from 0-13 for the various herbicide treatments and between 11 and 50 percent for the untreated control across the 4 study locations testing efficacy on matted fern areas. When averaged across the three herbicide rates, comparisons of the herbicides tested were not significant at two locations; whereas, at one location glyphosate alone or imazapyr alone resulted in significantly less *L. japonicum* cover than metsulfuron and at one location glyphosate alone resulted in significantly less cover than both metsulfuron and imazapyr. Initial **results at 60 days after treatment showed a significant herbicide rate response in *L. japonicum* control for glyphosate and imazapyr but not metsulfuron**; however, at one year a rate response was significant only for metsulfuron (at two locations). There was no significant improvement in *L. japonicum* control due to herbicide combinations and no significant imazapyr or metsulfuron rate effects when these were mixed with glyphosate at any location. Percent cover of non-target vegetation and species richness remained consistent or increased at all sites one year after herbicide treatment. Non-target vegetation responses were not significantly different between treatments, except at one site where percent cover of grasses increased significantly on plots treated with metsulfuron only. Increases in cover and diversity of non-target species was likely a response to the available growing space and resources following reduction of Japanese climbing fern. Phytotoxicity to woody stems was variable within and between treatments, ranging from no phytotoxicity to unusual growth and leaf necrosis. Phytotoxicity appeared related to herbicide selectivity (e.g. pines were more tolerant to imazapyr), and variability was likely due to level of exposure during the direct spray application. Minogue, P.J., Bohn, K.K., McKeithen, J., Osiecka, A., and D. K. Lauer. One year response to directed sprays of glyphosate, metsulfuron, and imazapyr herbicides for selective control of Japanese climbing fern (Lygodium japonicum) in Florida's natural areas. University of Florida, North Florida Research and Education Center, 155 Research Road, Quincy, FL. (COMPLETED)

Skunkvine biological control

The search for biocontrol agents in Asia

Field surveys resulted in the discovery of many insects including an unknown sawfly and a known polyphagous chrysomelid gall former in Thailand; and a fruit gall midge and stem-boring Lepidoptera in Hong Kong. Field investigations led to the discovery of the larvae of a regularly seen leaf-feeding staphylid beetle. Previously the larva was unknown and if it was predaceous or herbivorous. Learning that it is plant feeding creates much more interest in it. Small amounts of adult feeding cause considerable microbial decay of the leaf. The newly discovered stem-boring moth in Hong Kong kills the stem distal to the area of boring. The moth may have considerable potential as a biological control agent of skunk vine. Pemberton, R.W. Biological control of skunk vine, *Paederia foetida.* USDA ARS Invasive Plant Research Laboratory, 3225 College Ave. Ft. Lauderdale, FL. (COMPLETED)

General Research

Barcoding the invasive plants of Florida

Scientists at UF are DNA **barcoding invasive plant species** in Florida. The central premise of DNA barcoding is that each plant species has a distinctive set of DNA, of which a carefully chosen subset (a —DNA barcode"), can serve as a unique baseline reference for identification. Bar-coding has potential use as a tool for a wide variety of research issues such as developing a baseline of genetic barcodes for 136 of the Florida Exotic Pest Council's (FLEPPC) most invasive plant species. **This barcoding can provide a database** for comparative identification, with potential research and management implications, the strongest of which is being able to positively identify even tiny sterile fragments in the future. Traditionally, plant identification is based on morphology, with the limitation that many species must be collected at a very specific time of the year so that reproductive features can be used for accurate identification. With DNA data, once a reference library is built, barcode identification of plants is theoretically possible at all life stages, from seed to mature plant. Even a fragment of a leaf might be used to identify a species. DNA barcoding has practical utility for all research, wildlife and land management, and conservation efforts that rely on identifying plant species. It also has applications in forensics, biosecurity, trade in controlled species, foodstuffs, and herbal medicines, and in scientific questions involving evolution, biogeography, and population structure. DNA barcoding is not meant to replace detailed population genetic studies, but it will provide a baseline of genetic data for comparative analysis, and, with sampling of multiple populations, barcoding studies can provide a first glimpse at genetic differences that could reflect underlying patterns of cryptic or incipient species differences. DNA barcoding will enhance our understanding of the Florida flora and should become a useful tool for research and wildlife management. As a prelude of a larger —Barcoding the Flora of Florida" project, University of Florida scientists collected and sequenced *matK*, *rbcL*, and *trnH-psbA* spacer for all 136 species on the Florida Exotic Pest Plant Council list of invasive species. With 100% success at generating DNA barcode data for all FLEPPC species and a total DNA sequencing success rate of about 99% (on an individual gene region basis), this study provides compelling evidence that barcoding is logistically feasible. Further-more, this data generally shows excellent differentiation among the invasive species of Florida. Williams, N.H. Barcoding the flora of Florida: DNA identification of the invasive plants of Florida. Florida Museum of Natural History, University of Florida, Gainesville, FL. (COMPLETED)

"The outcome of any serious research can only be to make two questions grow where only one grew before."

Source: Quote from Thorstein Bunde Veblen, Norwegian-American Economist (1857-1929)

Parasites of native and non-native apple snails in Florida

At Florida International University (FIU), research is looking at the parasites infecting the non-native **island apple snail *Pomacea insularum*** in Florida and whether if any of these **parasites might negatively affect native species or human health**. Apple snails are the intermediate hosts for many parasites, and these parasites may have effects on apple snail populations themselves, on the animals that prey on apple snails, and on humans. FIU scientists examined populations of the native apple snail *Pomacea paludosa* and the introduced South American apple snail *Pomacea insularum* at 6 locations in Florida. Native apple snails were found at two of these sites, non-natives at three of these sites, and both species at one site. Densities were estimated of both native and non-native apple snails. At these sites, native apple snail densities ranged from 0.46 to around 1 snail per square meter. *Pomacea insularum* densities ranged from 0.18 to 0.62 per square meter. **There were no parasites in the *Pomacea insularum* sampled, although our sampling was somewhat limited.** Two parasites were found in *Pomacea paludosa* sampled, a digenetic trematode, and a parasitic mite. Light microscopy, scanning electron microscopy and DNA sequence analysis was used to identify these parasites. The digenetic trematode parasite is a new species in the genus *Echinostoma*. Echinostomatid parasites are cosmopolitan and found in the intestines of vertebrates, including humans. The mite parasite is also an undescribed species *Unionicola (Polyatax) n. sp.*, and seems to be correlated with increased

mortality in laboratory kept snails. Collins[1], T. and P. Darby[2]. Parasites of native and non-native apple snails in Florida. [1]Florida International University, Dept. of Biological Sciences, HLS 419D, University Park, Miami, FL and [2]University of West Florida, Dept. of Biology, 11000 University Parkway, Pensacola, FL. (COMPLETED)

A proactive approach to invasive plant management of landscape ornamentals

The majority of plants considered invasive today were originally introduced as ornamentals and there is often a lag time between when a species is determined to be invasive and when it is no longer produced commercially. Research is critical to provide scientific evidence of the potential negative impact invasives may have with regards to crossing with related native species, seedling trueness to type, invasive potential of cultivars, and geographic specificity. Five highly ornamental, FLEPPC Category I or II species (*Nandina domestica*, *Stachytarpheta* spp., *Ligustrum* spp., *Ruellia tweediana*, and *Lantana camara*) were selected for studies conducted at Fort Pierce, Quincy, Wimauma, and Gainesville. Nine selections of nandina, 8 selections of Stachytarpheta, and 12 selections of Ligustrum were obtained from various nurseries and planted in prepared beds located in north and south FL. They have been evaluated monthly for flowering and fruiting and visual quality. Upon maturity (year 2009/2010), seeds were collected, cleaned and subjected to viability and germination tests. In addition, seeds were subjected to four temperature regimes (20/10, 25/15, 30/20, 35/25 °C) in light and darkness. Germination varied widely among sites, temperatures, cultivars, and light provision. A completely sterile porterweed selection (*Stachytarpheta mutabilis* ‗Violacea') was identified. Tissue from 8 ruellia, 15 lantana, 8 porterweed, and 49 nandina accessions were collected and subjected to ploidy analysis. Two of the ruellia (‗Purple Showers' and ‗Snow White') were tetraploids; 5 of the lantana ('Confetti', 'Dallas Red', 'Gold', 'Pink Caprice', and 'Radiation') were tetraploids, all of the nandina were diploids; and two and five of the porterweed accessions were diploids and polyploids, respectively. More than three dozen triploid lantana lines have been produced and several highly sterile lantana triploids have been identified. Further tests are being done to evaluate their growth habits and flower colors. Nandina was treated with two chemicals (colchicine and oryzalin), two concentrations, and three treatment durations. Oryzalin appeared to be ineffective in inducing tetraploids in nandina. Colchicine promoted shoots to sprout, but inhibited shoot growth. Fifty-two out of 136 'Harbour Dwarf' plants survived colchicine treatments. Between 17.9% and 75.0% of the newly emerged leaves from treated 'Harbour Dwarf' contain tetraploid cells among diploid cells. Efforts are being made to promote tetraploid cells to develop into new shoots and minimize the elimination effects of the diplontic selection phenomenon. Hybridization potential between *R. caroliniensis* (native) and *R. tweediana* (invasive) was confirmed. Sterilization of *R. tweediana* cultivars and *R. coerulea* accessions was attempted by use of irradiation. Irradiation treatments performed in 2007 on rooted cuttings resulted in the selection of two plants that did not produce fruits from selfing: one pink-flowered *R. brittoniana* and one purple-flowered *R. coerulea*. Plants were propagated vegetatively and are currently being evaluated monthly at 4 different locations (Fort Pierce, Quincy, Balm and Citra). Wilson[1], S.B., Deng[2], Z, Knox[3], G., and R. Freyre[4]. A Proactive Approach to Invasive Plant Management of FLEPPC Category I and II Ornamentals. [1]Department of Environmental Horticulture, Indian River Research and Education Center, University of Florida-IFAS; 2199 South Rock Road; Fort Pierce, FL , [2]Department of Environmental Horticulture, Gulf Coast REC 14625 C.R. 672; Wimauma, FL, [3]Department of Environmental Horticulture, North Florida REC, 155 Research Road, Quincy, FL, and [4]Department of Environmental Horticulture, PO Box 110670, Gainesville, FL. (COMPLETED)

Testing the New Zealand Aquatic Weed Risk Assessment in Florida

Predictive tools for preventing introduction of new species with high probability of becoming invasive in the U.S. must effectively distinguish non-invasive species. The Australian Weed Risk Assessment system (AWRA), already demonstrated to meet this requirement for terrestrial vascular plants, weights aquatic plants heavily toward the conclusion of invasiveness. A second system, modified from a system developed in New Zealand for aquatic species (AqWRA), was tested at the state (Florida) and national (U.S.) scale. Fifty invasive and fifty non-invasive aquatic plants non-native to

FWC photo

Australian Pines: Concerning the Confirmed Hybridization between Casuarina glauca and C. equisetifolia

"The presence of novel hybrids in the Florida invasion may enhance evolution of invasive traits in these species. Novel *Casuarina* hybrids in Florida have no coevolutionary history with any insects or diseases, which may be problematic for (future) biological control efforts."

Source: Gaskin, J.F., Wheeler, G.S., Purcell, M.F., and G.S. Taylor. , Molecular evidence of hybridization in Florida's sheoak (Casuarina spp.) invasion. Molecular Ecology (2009) 18: 3216-3226.

"Invasive species that threaten agricultural crops or livestock are far more likely to elicit a rapid response than those affecting mainly natural areas."

Source: Invasive Species: Obstacles hinder federal rapid response to growing threat, U.S. GAO Report, 2001

the U.S. were assessed using both tools to evaluate the need for a specific aquatic plant system. The AWRA predicted that over 95% of the invaders would be invasive, with none predicted to be non-invasive and 4% requiring further evaluation. However, it predicted that only 2% of the non-invaders would not become invasive, with 60% predicted to be invasive and 38% needing further evaluation (Florida and U.S. results consistent). The resulting overall accuracy was 62%, dominated by scores for invaders. The AqWRA implemented for Florida with a threshold total score of 20 correctly predicted that 78% of the invaders would be invasive, 4% required further evaluation, and 18% were incorrectly predicted not to naturalize. For the non-invaders, 52% were accurately predicted not to become invasive, 26% required further evaluation, and 22% were incorrectly predicted to become invasive. Accurate assessments were made for 76% of the species. While the exact scores varied between the Florida and U.S. tests, the outcomes were the same so these percentages hold for the U.S. as well. Overall, **separate screening systems for terrestrial and aquatic species appear necessary**. With further refinement of the tool, scoring thresholds and data requirements, the AqWRA system may be promising for regional and national use.
Gordon, D.R. Testing the New Zealand Aquatic Weed Risk Assessment in Florida. University of Florida, Department of Biology, Gainesville, FL. (COMPLETED)

Don C. Schmitz, Editor
FWC Invasive Plant Management Research Program Newsletter
Research Program Manager
Invasive Plant Management Section
Florida Fish and Wildlife Conservation Commission
3900 Commonwealth Blvd. MS705
Tallahassee, Fl 32399
Tel. 850-245-2818
Don.schmitz@myfwc.com