Engineering, Structures and Innovations

Gary Bachman
Section Editor and Moderator

Biomass Productivity Potential by Selected Cellulosic Herbaceous Perennials in Acid Impacted Soil

E. Kudjo Dzantor[1], Vallaban Murugesan[2], Roger Painter[2] and Dafeng Hui[3]

[1]Department of Agricultural Science, [2]Department of Environmental Engineering and
[3]Department of Biological Sciences, Tennessee State University,
3500 John A. Merritt Blvd; Nashville TN 37209

edzantor@tnstate.edu

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Significance to Industry Global climate change, energy supply and security, concerns over land, air and water degradation have converged to intensify debate on *renewable energy*. Central to this debate is *bioenergy*, renewable energy derived from biological sources, including biomass from plant material, which can be converted to heat, electricity, or vehicle fuel. The focus and national support for the development of bioenergy alternatives to fossil fuels offer the agricultural sector of the nation a crucial role in the establishment and growth of the bioenergy and biobased product industries. The nursery industry has long addressed issues of energy and environmental sustainability, including reduced consumption of non-renewable energy resources, and conversion of biomass wastes to materials for the production of floral and nursery crops (http://aggiehorticulture.tamu.edu/GREENHOUSE/nursery/environ/2000.html). More recently, undoubtedly due in part to the enthusiasm surrounding bioenergy, UDSA investigators have reported that switchgrass, the model bioenergy crop, may be a viable nursery container substrate (1). This finding offers the nursery industry a welcome solution for a looming shortage of pine-bark based container media in certain parts of the country. Additionally, the industry has began direct involvement in issues pertaining to dedicated biomass crop production, including assessment of agronomic suitability of alternative feedstocks (2), their breeding, propagation and evaluation (3) as well as forecasting the effects of insects and other pests on perennial biomass crops (4).

We recently began explorations issues of large-scale, sustainable production of bioenergy feedstock. This report describes the initial stages of these explorations that started with determining boundary productivity potentials of selected native grasses in acid impacted soil.

Nature of Work *Background.* Bioenergy is one solution to the global problem of energy security and environmental degradation. However, it can be limited by availability of suitable land that does not compete with growing food, feed and fiber. Currently, grain-based crops, notably corn, account for over 90% of ethanol production in the U.S. However, high input requirements of corn production on the one hand, and on the other, the requirement of production on prime land has caused attention to be focused on cellulosic materials such as herbaceous perennials as well woody species for bioenergy production. The most well-known cellulosic herbaceous perennial (CHP) is switchgrass, which was selected by the US Department of Energy (DOE) as the model bioenergy crop because of desirable qualities including potential high biomass productivity, nutrient use efficiency, environmental enhancement, wide geographic distribution and ability grow well on marginal lands (5) . It is the goal of DOE to replace 30% current US petroleum consumption with bioethanol by 2030 using CHPs. Achieving this goal will require production of billions of tons of biomass annually (6). Not only will this necessitate enhancement of productivity of switchgrass, but also it will be necessary expand the collection of potential feedstocks as well as their cultivation on marginal or less than prime lands.

One limitation to crop productivity worldwide is soil acidity. It is estimated that up to 30-40% of the world's arable land has been rendered unproductive by soil acidification and this trend increasing (7). Soil acidification generally results from a sequence of events some of which can be naturally occurring but can be exacerbated by human activities including agriculture. Acid soils generally result from parent materials that are naturally low in base forming cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) to begin with, or because these elements have been leached from soil, replaced (exchanged) by Al^{3+} and H^+. When pH is low enough, dissolution of Al- and then Fe-containing minerals occurs, releasing toxic metals. A major appeal of CHPs as bioenergy feedstock stems from the fact that they can be produced on marginal land, thereby saving prime cropland food and feed.
In this study, we report pot experiments that were conducted in the summer 2010 to determine boundary productivity measures of biomass yield and photosynthesis rate of selected CHPs growing on acid impacted soil.

Approaches. The soil investigated was Amour silt loam collected from the Tennessee State University Agricultural Research Farm in Nashville, TN. The cellulosic herbaceous perennials evaluated were switchgrass (*Panicm virgatum*), eastern gamagrass (*Tripsacum dactyloides*) and big bluestem (*Andropogon gerardii*).

Biomass productivity of the grasses was assessed at three soil pH levels, namely 6.6, 5.0 and 4.5. The soil pHs were adjusted to the desired levels following modifications of protocols described by Islam et al., 2004. Briefly, increments of $AlK_2(SO_4)_3.18H_2O$ or $CaCO_3$ were added to soil and dose-response curves were generated that enabled estimation of amounts of the chemicals required to bring a known amount of soil to pH_{Ca} levels evaluated (1:5, w:v, soil:10mM $CaCl_2$). For acidification, 0, 15, 30, 60, 120mg [$AlK_2(SO_4)_3.18H_2O$] were weighed into 125 ml plastic bottles, each containing 10g of air dried soil. Fifty milliliters (50) of 10 mM $CaCl_2$ were added and bottles were agitated on a rotary shaker at 200 rpm for 24 h. For raising pH, similar increments of $CaCO_3$ were added to 10g air dry soil in each bottle and shaken on rotary shake for 24h Plots of pH and $AlK_2(SO_4)_3.18H_2O$ or $CaCO_3$ that were used to determine exact amounts of chemicals used to bring soil pH levels to desired levels (Figure 1a, 1b).

Seeds of each plant were initially sown in germination trays filled with potting mix (Fafard® #2 mix). Seedlings of the grasses were transplanted into 5 inch pots containing test soil to give four plants per 5 pot (because of low germination efficiencies of seedlings of eastern gamagrass (GG) were transplanted three days later than those of switchgrass (SG) and big bluestem (BB). Each treatment was replicated four times. The plants were watered as needed and the pot locations were randomly rotated at least every 2-3 days to minimize random error. After two months in pots, the grasses were harvested by cutting their tops to heights of 15 cm. Leaves from each treatment were bagged separately and dried at 70^oC over five days and weighed. Biomass data were analyzed by ANOVA.

Light-saturated photosynthetic rates (A_{max}) of leaves of the grasses under three pH treatments were measured on September 16, 2010 and September 30, 2010. The measurements were made using a Li-6400 Portable Photosynthesis System (Li-Cor, Lincoln, NB) with a 2 x 3-cm cuvette with a LED light source (Li-6400 02B). A single leaf on each of the two plants in the pot was measured. The leaf was marked and the area was measured for each leaf and used for leaf photosynthesis correction. Full spread young leaves were selected. The measurements were made with CO_2 in the reference chamber set to 390 ppm and the light was set to 1500 µmol m-2s-1. Temperature in the greenhouse was controlled using air conditioners and we set the leaf temperature at the ambient air temperature (32^oC). Leaf photosynthesis data were analyzed using a two-factor random design ANOVA considering both grass species and pH treatment with 4 replications.

Results and Discussion Results showed that SG produced significantly higher amounts of biomass than GG or BB. Furthermore, soil acidity had no apparent on biomass productivity between pH 6.5 to 4.5. Average biomass production was 3.9, 4.2 and 3.8 grams per pot for plants cut to 15cm height. Biomass productivity by GG at pH 6.5 and 5.0 did not differ significantly from the productivity by BB at the corresponding levels of pH (Table 1). In contrast to what was observed with SG, soil acidity significantly caused decreases in biomass productivity of GG and BB. Average biomass production of GG was 2.5 and 2.8 grams per pot at pH 6.5 and 5.0 respectively this decreased to 0.7 grams at pH 4.5. Similarly, average biomass production of BB was 2.0 and 2.1 at pH 6.5 and 5.0 respectively to 1.0 gram per pot (Table 1).

Cellulosic crops are expected to become more important than corn for bioethanol production with progressive advances in conversion technologies. Although the DOE selected SG as the model herbaceous perennial for production of bioenergy other crops are needed to add to the collection of plants that can be used for bioenergy production. The appeal of biomass crops is their environmental protection and enhancement qualities. We previously investigated the same native CHPs for abilities to remediate environmental contamination, through phytoremediation (9, 10). Accordingly, our current focus represents efforts at coupling environmental remediation to biomass (and bioenergy) production with emphasis on enhancing biomass production on degraded lands.

Modern crop technologies are increasingly providing insights about how crops may be endowed with enhanced traits including biomass productivity and tolerance to stresses that limit the attribute thereof. While these technologies are still in their infancy, we have begun to explore feasibility enhancing biomass productivity the crops tested using traditional approaches. One such approach capitalizes on the symbioses between grasses and mycorrhizae. Arbuscular mycorrhizal fungi are ubiquitous soil inhabitants that form close associations plant roots where they confer on the plant numerous benefits including improved tolerance to soil acid and heavy metals toxicity (11) Experiments are underway evaluate strains of mycorrhizae to be used to counteract. The boundary biomass productivity measures from this study provide us a starting point.

Results of photosynthesis measurements between September 16 and September 30 are shown in Table 2. We found significant differences among the grass species, but no differences were found among the pH treatment and the species and pH interactions. Bluestem and gamagrass had higher photosynthetic rates than switchgrass (Table 2). During the period, photosynthetic rate of switchgrass increased while these of bluestem and gamagrass decreased. The short duration of our measurements do not allow us to draw definitive conclusions about the relative efficiencies of the grasses to capture and convert light energy. Experiments are ongoing to monitor photosynthesis for an extended duration and develop photosynthetic responses to temperatures and CO_2 concentrations. One consequence of the global climate change is potentials of elevated atmospheric CO_2 and consequently impacts on biomass productivity. It is important to understand how this CHPs will respond to elevated atmospheric CO_2.

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Table 1a. Biomass yield and plant of switchgrass eastern gamagrass and big bluestem

Cellulosic Herbaceous Perennial	Biomass Yield (g) at Indicated pH		
	6.5	5.0	4.5
Switchgrass	3.9a	4.2a	3.8a
Eastern Gamagrass	2.5b	2.8b	0.7b
Big Bluestem	2.0b	2.1b	1.0b

Mean values

Table 1b. Light-saturated photosynthetic rate of leaves of three grass species.

Species	9/16/2010	9/30/2010
Switchgrass	9.97b	16.02b
Eastern gamagrass	24.47a	19.71a
Big bluestem	25.29a	20.73a

Mean values with same letters are not significantly different.

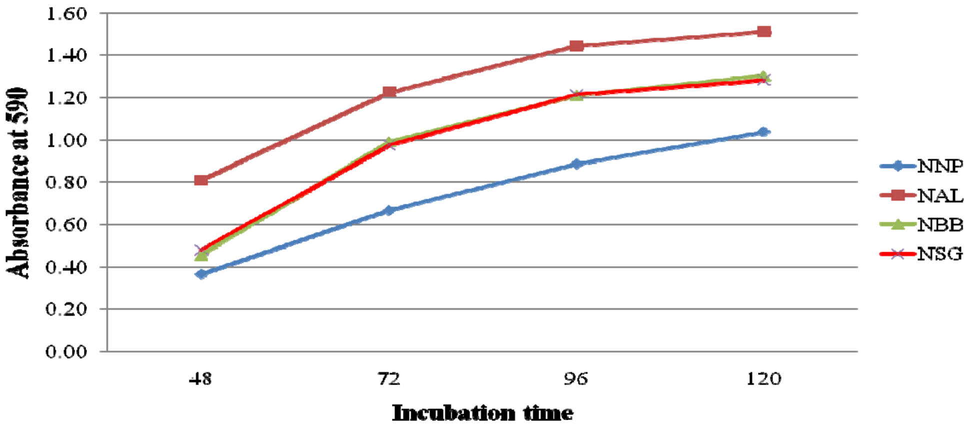

Figure 1. Mean carbohydrate utilization pattern of microbial populations in Armour rhizosphere soil after 10 weeks of planting.

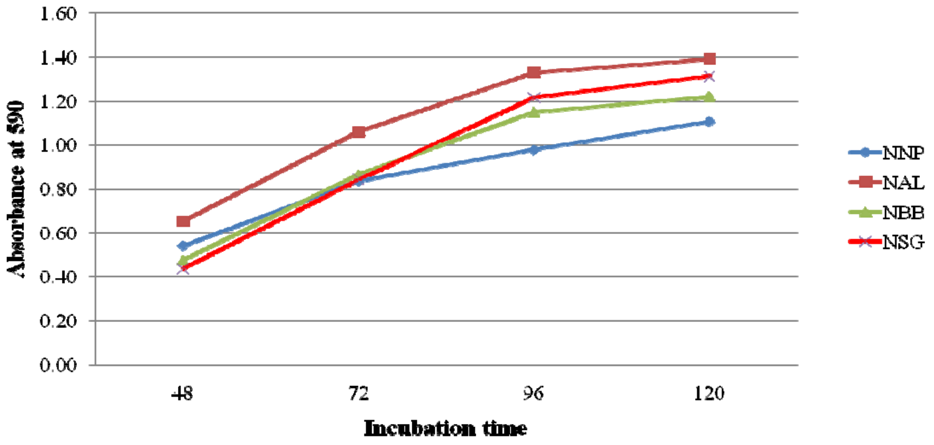

Figure 2. Mean carboxylic acid utilization pattern of microbial populations in Amour rhizosphere soil after 10 weeks of planting.

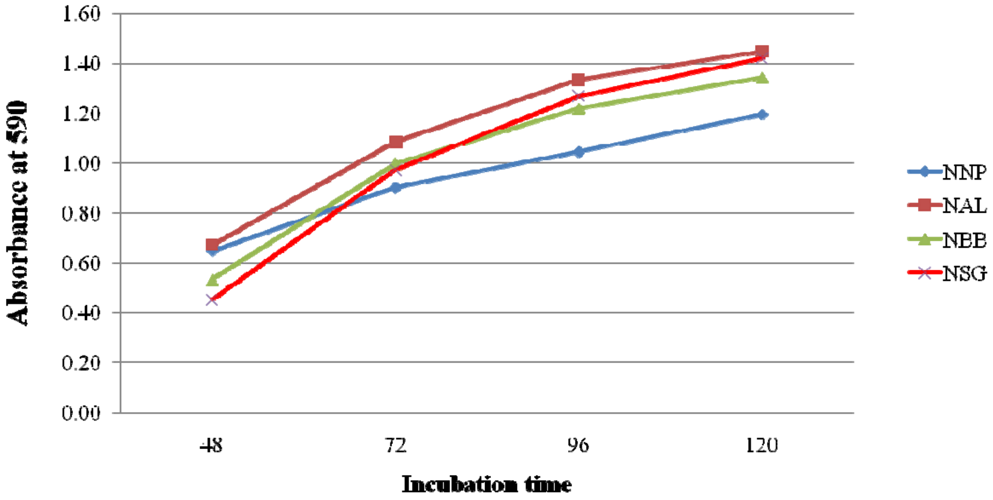

Figure 3. Mean amino acid utilization pattern of microbial populations in Amour rhizosphere soil after 10 weeks of planting.

Design and Construction of a Machine for Grafting Prickly-pear Cactus (*Opuntia* spp., Cactaceae) Cladodes

Andrés Adolfo Estrada-Luna[1]; Francisco Javier Martínez Serrano[2]; Sergio Ortiz Mendoza[2]; Manuel Domingo Vargas Aguayo[2], Carlos Patricio Achurra Sánchez[2]

[1] Escuela de Agronomía, [2] Escuela de Ingenierías. Universidad De La Salle Bajío. Av. Universidad 602, Col. Lomas del Campestre. León, Gto., México. C.P. 37150.

aestradaluna@yahoo.com

Index Words: cacti, seed germination, prototype, asexual propagation, Cactaceae, graft, grafting machine.

Significance to Industry: Cactus pear or Prickly pear cactus (*Opuntia* spp.) is the most important species in the Cactaceae. It is a multi-purpose plant widely disseminated in America and many countries through the world (1), which is primarily cultivated for its fruit production; however, young cladodes are also used as vegetables or consumed as relish or for animal feed (2,3). Other valuable byproducts obtained include candies, wine, and the red dye acetocarmine obtained by the cultivation of dacti (*Dactilopius coccus* Costa) (4). Traditional propagation systems of this plant rely on several asexual techniques including rooting of single or multiple cladodes (5), rooting small portions of mature cladodes derived from the dissection of tissues comprising two or more areoles, or by using fruits as propagules (6). Other available asexual methods include apomixis, micrografting, and tissue culture (7,8,9). Commercial propagation of prickly-pear cactus through grafting has not been implemented. This method of propagation may be used to rejuvenate old plantations, produce high quality plants, to establish ornamental combinations with increased commercial value or to enhance productivity, extent the ecological limits of selected genotypes or to program fruit harvest. Because of this, our objectives were to design and build a machine capable of making accurate tissue cuts to establish inverted wedge grafts. Through the mechanization of this process the mexican growers, propagators, and the whole nursery industry will benefit, since selected genotypes might be conserved and more uniform and healthy material would be propagated for commercial exploitations.

Nature of Work: The project was divided in two stages: 1) designing and construction, and 2) evaluation and modification of the machine. For the designing and construction stage, we establish several important considerations regarding the functions of the machine including the type of cut to be performed (inverted wedge), the construction materials to obtain a strong but light machine, and the force needed to operate it. Because of this, we initially obtained data to determine the force needed to cut the prickly pear cladodes and to determine whether the machine could be manually operated. A texture analyzer (TAXTT2i, brand Stable Microsystems) was used to get the information. At the same time, we characterize the morphology of mature cladodes (one-year old) to be used in the grafts (stocks and scions) from different cultivars and species in order to establish the dimensions and some others parameters to build the prototype. To sketch the prototype, our research team discussed all details before making the layouts and manufacturing the pieces to build the prototype. To evaluate the assembled machine, we confirmed the functioning of each part and elements through computer simulation or performing real cuts. Then, homo, auto, and heterografts have been performed to establish particular details regarding the wounding process and the cladode handling to optimize the process.

Results and Discussion: Data obtained from the morphological characterization showed that cladodes dimensions largely vary and range from 4 to 35 cm in diameter and from 5 to 42 cm for the different *Opuntia spp.* evaluated. The cladode thickness, which is an important trait to be considered to establish accurate and intimate union areas, also varied from 0.7 to 3.42 cm and from 0.5 to 2.89 cm at the central and edge areas, respectively (Table 1). Data obtained from the texture analyzer help us to design the knives for dissecting the cladodes and to determine that the cuts could be manually performed (Table 2). We end with an assembled prototype that includes a structure to support the whole device and three mechanisms for cutting (knives) and transmit movement to knives, immobilize the cladodes and to raise and control the position (angle) of the cladode to be dissected. (Figure 1: a, b). We can conclude that the machine built is a light and easy to handle tool, which meet all the requirements established to perform accurate and fine cuts that facilitate an intimate contact between stock and scion tissues.

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Table 1. Data of the morphological characteristics obtained from one-year old cladodes of prickly-pear cactus (*Opuntia* spp.).

Scientific name (Cultivar name)	Central zone thickness (cm)	Edge zone thickness (cm)	Cladode diameter (cm)	Cladode lenght (cm)
O. ficus-indica (pelón forrajero)	2.46	1.84	40	48
O. ficus-indica (pelón tunero)	3.49	2.78	22	34
O. spp (espinoso)	2.15	1.70	17	28
O. mycrodasis (cegador)	0.70	0.50	4	5
O. robusta (tapón)	3.42	2.89	32	39
O. streptacantha (cardón)	2.88	2.54	28	35
O. amyclaea (fafayuco)	3.20	2.80	34	43

Table 2. Data of the force required to cut one-year old prickly-pear (*Opuntia* spp.) cladodes.

Scientific name (Cultivar name)	Force at central area (Newtons)	Force at edge area (Newtons)
O. ficus-indica (nopal pelón forrajero)	756.76	782.23
O. ficus-indica (nopal pelón tunero)	1,245.12	720.75
O. spp (nopal espinoso)	1,213.35	932.02

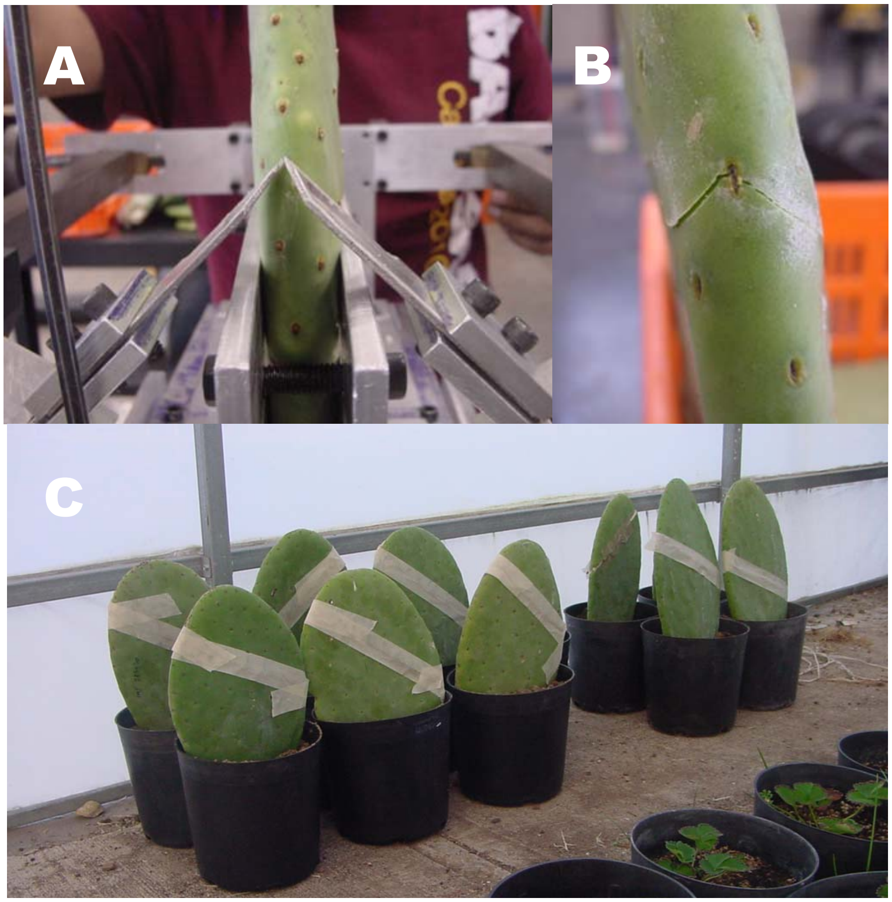

Figure 1. Details of the pricly-pear cactus grafting machine. A. Knives performing cuts to produce inverted wedge grafts, B. Overview of cuts produced to cladodes. C. Homografts cultivated on glasshouse.

Elucidating Rhizodegradation for Use in Phytoremediation of Synthetic Pyrethroids

Le, X., D[1]. Hui[2], and E.K. Dzantor[1]
Department of Agricultural Science[1] and Department of Biological Sciences[2]

Tennessee State University, 3500 John A. Merritt Blvd; Nashville TN 37209

edzantor@tnstate.edu

Index Words: Pyrethroids, bifenthrin, phytoremediation, rhizodegradation, microbial communities, carbon utilization profiles.

Significance to Industry: Two important insect pests of the nursery industry in Tennessee and other parts of the nation are the Japanese beetle and imported fire ants. Both the US Domestic Japanese Beetle Harmonization Plan and the Federal Imported Fire Ant Quarantine Program require quarantine treatment of nursery stock from areas infested by these two pests (1, 2). One insecticide that is recommended for quarantine treatment for Japanese beetles and imported fire ants is *bifenthrin.* Bifenthrin belongs in the family of synthetic pyrethroids, which are noted for their efficacy at low concentrations. The family also contains such members as cyfluthrin, cyhalothrin and cypermethrin that are under investigation for nursery production. These insecticides are becoming increasingly important in the industry as the older, well-known organophosphate insecticides such as chlorpyrifos (dursban) have come under increasing scrutiny because their environmental pervasiveness. The environmental behaviors of the new generation pyrethroids have not been have thoroughly evaluated; our study provides information that contributes to our understanding of behavior of bifenthrin and possibly related synthetic pyrethroids in nursery production. This information may be used for developing plant-mediated strategies for mitigating unwanted impacts of insecticides in the environment.

Nature of Work: *Background:* The nursery industry relies quite heavily on pesticides, nutrients and water to ensure quality production. The combination of frequent applications of water, nutrients and pesticides can generate discharges that threaten human and ecosystem health. Japanese beetles and imported fire ants are two important insect pests of the nursery industry in Tennessee and other parts of the nation. Traditionally, the industry relied on organophosphate insecticides, like chlorpyrifos for quarantine treatments for these pests. However, the over-reliance on the insecticide has become a major environmental concern because of its pervasiveness. A survey by the U.S. Geological Survey a little over 10 years ago cited chlorpyrifos as the third most frequently detected insecticide in urban streams and it was one of the top 15 pesticides routinely detected in surface water surveys (3). As chlorpyrifos has come under increasing scrutiny, alternatives continue to be vigorously sought. One alternative is *pyrethroids*, a family of synthetic chemicals that are manufactured based on the natural pesticide pyrethrum, which is produced by chrysanthemum flowers. The first pyrethroid, *alletrin* was produced in 1965. Since then, scientists have progressively enhanced the pesticidal activities of pyrethroids to what is referred to as the fourth and current generation of 17 chemistries that include *bifenthrin* and *cyfluthrin* (http://ipmworld.umn.edu./chapters/ware.htm)

Bifenthrin and related pyrethroids are characterized by their effectiveness in the 0.01-0.05 lb acre active ingredient ai/Ac range. They are relatively insoluble in water and they partition preferentially partition into lipophilic phases so concerns about their leaching into groundwater are minimal. These characteristics make bifenthrin a valuable insecticide in nursery production; however, they can make the insecticide persistent in contaminated matrices. *Phytoremediation* is the use of plant systems for cleaning up unwanted concentrations of pesticides and other contaminants. The mechanisms that facilitate use of phytoremediation of organic compound are phytodegradation (4, 5), phytovolatilization (6), plant assisted bioremediation, also known as *rhizodegradation* (7). Information available in the literature indicates that bifenthrin is not translocated in plants (8, 9). This suggests that application of plant-mediated strategies for removal of unwanted concentrations of the insecticide must proceed via rhizodegradation.

Rhizodegradation is a process in which substrates supplied by plants stimulate soil microbial populations in plant root zones (rhizospheres) to cause removal (biodegradation) of undesirable levels of contaminants. In order to take advantage of phytoremediation, it is important to understand the components (microbes, root exudates) and root processes (biodegradation) that facilitate rhizodegradation. Here, we describe experiments aimed at establishing correlation between microbial communities and dissipation of bifenthrin to allow us to enhance processes that mitigate unwanted impacts of insecticide in soil and water.

Approaches: The formulation of bifenthrin used was Talstar® P, 7.9%, EC distributed by FMC Corporation. Plants selected for these investigations were switchgrass (*Panicum virgatum* L) big bluestem (*Andropogon gerardii* Vitman) and alfalfa (*Medicago sativa* L). They were selected from previous experiments that screened abilities of different plant rhizospheres to enhance dissipation of different pesticides (10). The plants were grown in two soil types: Armour silt loam collected TSU agricultural experimental station in Nashville TN, and Sullivan sandy loam collected from Tennessee Technological experimental station in Cookeville TN. Soil preparation and incubation were similar to those described previously for other soil contaminants (11). Briefly, Talstar® was added to soil to provide a nominal concentration of ~ 10ppm of bifenthrin in soil. Approximately 50g aliquots of contaminated soil were incubated in the greenhouse in soil microcosms for 10 weeks.

We used the Biolog carbon utilization method for characterizing microbial communities in our treatments. The carbon utilization profile approach (CUP) involved incubation of a soil suspension in 96 well plates and assessing the abilities and extent of utilization of selected substrates by particular communities, as measured by the absorbance (A_{590}) of respiratory dye at 590nm during incubation. The Biolog method was originally developed for classifying bacteria based on relative abilities to metabolize 95 substrates on a 96-well plate (one control). It was since been adapted to provide valuable information on physiological profiles of microbial communities in a given sample (12). In its modified version, 31 of the original 95 substrates are replicated three times on a plate (EcoLog) to allow quantitative analysis of microbial community profiles (13) In selecting the 31 substrates on the EcoLog an attempt was made to chose those represented in environmental matrices.

To assess dissipation of bifenthrin, parent residues were extracted from soil with ethyl acetate using a rotary shaker and the extracts were analyzed by electron capture gas chromatography using an Agilent 6890 system with a JW Scientific DB 608 capillary column (30m x 0.25mm x 0.25µm). Oven temperature was programmed from an initial 80^oC rising at a rate of 15^oC to 320^oC. Injector and detector temperatures were 250^oC and 300^oC respectively. Carrier gas was He at a constant flow rate of 2 ml/min and make-up gas was $Ar\text{-}CH_4$ at 58 ml/min. Dissipation of bifenthrin was analyzed SAS.

Results and Discussion Plots of rhizosphere substrate utilization profiles (measured as absorbance in EcoPlate at 590nm) are shown for carbohydrates, carboxylic acids and amino acids (Figures 1-6). These representations provide a qualitative measure of the abilities of different rhizosphere communities to metabolize 31 substrates in a Biolog plate. In general, utilization of each substrate category by rhizosphere microbial communities was greater in planted soils than in corresponding unplanted soil types. In both soil types, utilization of carbohydrates provided the greatest separation between microbial community profiles in plated and unplanted soil.

The carbon utilization profiles assays are a useful approach for differentiating between microbial communities in different ecosystems. The approach has found applications from a wide range of habitats; its value lies in its ability to establish a link between microbial communities and some function in that community (functional measure). In our case, this link is between substrate utilization profiles to the communities' ability to cause dissipation of unwanted levels of bifenthrin. Such a link is necessary to identify individuals or consortia of microorganisms that are most responsible for dissipation of bifenthrin.

Dissipation of bifenthrin in Armour and Sullivan soils after 10 weeks of incubation in the greenhouse is shown in Table 1. The results show that significantly more bifenthrin was recovered from both unplanted soil types than recoveries in planted soils. Different recoveries of bifenthrin were observed in planted Armour soil but the levels were not significantly different. Recoveries of bifenthrin in planted Sullivan soil were different but in contrast to observation in Armour soil differences in bifenthrin recoveries from planted Sullivan soil were statistically significant.

We used the multivariate canonical discrimnant analysis procedure to attempt to link bifenthrin dissipation and microbial community profiles. Preliminary results shows that substrate utilization were sufficiently separated into three distinct groups: 1) all unplanted soils, 2) Sullivan soils planted with AL and 3) Amour soils planted with SG (Figure 2). The canonical discriminant analyses are ongoing to resolve further other groupings and to allow us to establish relationships between dissipation microbial community profiles bifenthrin. This information will be used together with data on microbial community structure to allow rhizospheres to be modified to favor removal of unwanted chemicals.

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Table 1 Dissipation of bifenthrin in rhizospheres of two soils

	% of initially added bifenthrin in two rhizosphere soils of three crops after 10 weeks	
	Armor Soil	Sullivan Soil
No plant	11.07 (2.7) a	83.49 (3.7) a
Alfalfa	70.30 (8.9) b	59.93 (10.5) abc
Big bluestem	6.7.09 (7.2) b	41.15 (4.7) b
Switchgrass	83.49 (12.1) b	62.31 (3.5) c

Means of four replicates; means in within a column followed by different letter are significantly different ($p<0.05$)

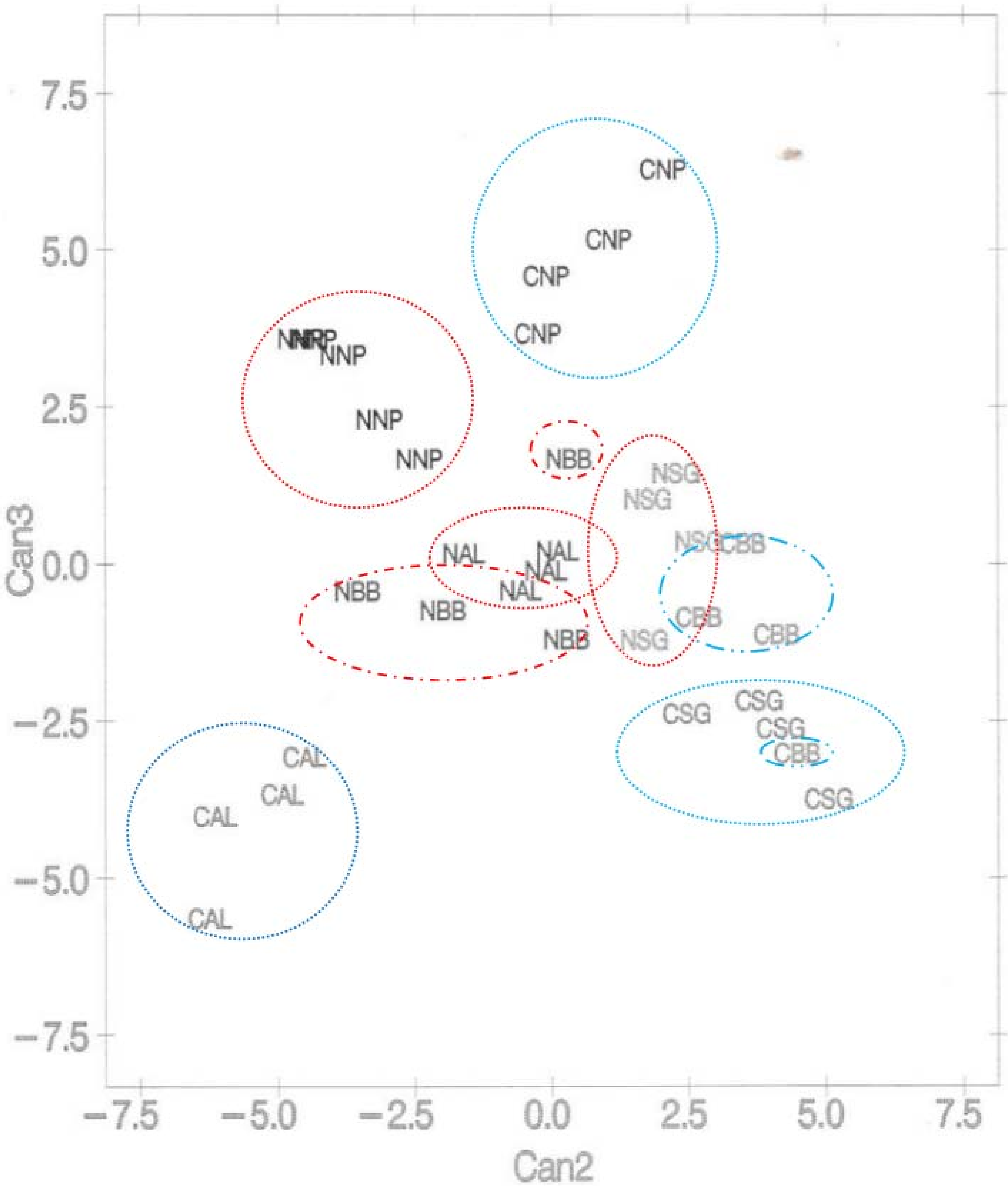

Figure 2. Separation of carbon substrate utilization profiles by microbial communities in Armour and Sullivan soils using canonical discrininant analysis. NNP- Nashville (Armour) soil/ No plant; NAL-Nashville/alfalfa; NSG-Nashville/switchgrass; NBB-Nashville soil/big bluestem. CNP-Cookevill (Sullivan) soil/no plant; CAL-Cookeville/alfalfa; CSG-Cookeville/switchgrass, CBB-Cookeville/big bluestem

Demonstration Results From Greenhouse Heating with Liquified Wood

Philip Steele, Don Parish and Jerome Cooper

Department of Forest Products, Mississippi State University
Mississippi State, MS 39762 USA
psteele@cfr.msstate.edu

Index Words: Greenhouse, heating, fuel, bio-oil, pyrolysis, wood

Significance to Industry A boiler fuel known as Lignocellulosic Boiler Fuel (LBF) was developed at the Department of Forest Products, Mississippi State University for potential application for heating agricultural buildings. LBF was field tested to heat green houses in cooperation with Natchez Trace Greenhouses (NTG) located in Kosciusko, Mississippi. MSU modified an idled natural gas boiler located at NTG to combust the LBF. Thirty gallons of bio-oil were produced at the MSU Bio-oil Research Laboratory. The bio-oil was produced from the fast-pyrolysis of southern pine (15 gal) and white oak (15 gal) feedstocks and subsequently upgraded by a proprietary process.

Preliminary field testing was conducted at (NTG). The LBF was produced from each wood species was tested separately and co-fed with diesel fuel to yield three fuel formulations: (1) 100% diesel; (2) 87.5% LBF from southern pine bio-oil co-fed with 12.5% diesel and (3) 87.5% LBF from white oak co-fed with 12.5% diesel fuel formulations. Each fuel formulation was combusted in a retrofit NTG boiler. Fuel consumption and water temperature were measured periodically. Flue gas from the boiler was analyzed by gas chromatograph.

The 100% diesel fuel increased water temperature at a rate of 4 ^{o}F per min. for 35 min. to achieve the target 140 ^{o}F water temperature increase. The 87.5% pine LBF fuel co-fed with 12.5% diesel attained the 140 ^{o}F water temperature increase in 62 min. at a rate of 2.3 ^{o}F per min. The 87.5% white oak LBF fuel co-fed with 12.5% diesel reached the 140 ^{o}F water temperature increase in 85 min. at a rate of 1.6 ^{o}F per min.

Fuel that contained 87.5% pine LBF co-fed with 12.5% diesel yielded nitrogen and oxygen at a ratio of 5.3 and carbon dioxide and carbon monoxide at a ratio of 22.2. Fuel formulations that contained 87.5% white oak LBF co-fed with 12.5% diesel yielded nitrogen and oxygen at a ratio of 4.9 and carbon dioxide and carbon monoxide at a ratio of 16.4. Neither the pine LBF nor the white oak LBF fuel showed any measureable methane emissions from the NTG boiler flue gas. These results indicate a viable potential for mildly upgraded bio-oil to become an alternative fuel source for greenhouse operations.

Nature of Work Natural gas ranks second in U.S. energy consumption after petroleum, providing 38% of total energy demand (4). Natural gas price increases accelerated over the decade through July of 2008 with the peak price reaching a level above $14 per MM Btu. Following this peak price, technological advances have increased supply and economic recession has reduced international demand for natural gas; price has, therefore, declined to an approximate current mean price of just above $5.00. However, long-term expectations are that price increases will resume in following decades as the fields in which new production technology can be applied decline in number and international economic activity resumes to pre-recession levels (4).

Historically, the greenhouse industry has depended on natural gas as a convenient and low-cost energy resource for greenhouse heating. Energy cost ranks second, behind labor, as the largest cost for the greenhouse industry, comprising 60% of total production costs. Increased costs through 2008 had forced a contraction in greenhouse businesses nationwide as some entities could not compete effectively because of the high level of energy costs they were required to absorb. Some greenhouse operations have converted to heating with biomass but this source of energy is as convenient to use as a liquid fuel.

Development of lower-cost fuels as a future supplement or replacement for natural gas is important for preserving the economic viability of the greenhouse industry. A potential alternative fuel for greenhouse heating is the use of bio-oil produced from the fast pyrolysis of biomass. Fast pyrolysis processes thermally convert biomass materials to produce a liquid fuel, generally referred to as bio-oil. In a typical pyrolysis process biomass particles are heated to between 400 and 550°C very rapidly in the absence of oxygen followed by cooling to condense the pyrolysis vapors to a liquid. This treatment fractures plant cellular material molecular bonds converting the biomass to the final bio-oil. The yield of bio-oil can be relatively high at about 65% dry weight basis or higher depending on the production process.

Bio-oil chemical properties vary with the feedstock pyrolyzed but woody biomass typically produces a mixture of 30% water, 30% phenolics, 15% aldehydes and ketones, 15% alcohols, and 10% miscellaneous compounds. As a fuel, bio-oil has environmental advantages when compared to fossil fuels producing no SOx and half the NOx; because bio-oil is derived from biomass it is CO_2 neutral (3). In general, liquid fuels are more convenient to transport, store, and combust than gas or solid fuels. Thus, a primary benefit offered by fast pyrolysis is the production of a more convenient and more readily marketable liquid fuel. One drawback to bio-oil is that the chemical mix is water soluble and is, therefore, immiscible in petroleum products. This precludes an easy route to utilization of bio-oils through mixing with diesel or gasoline to extend petroleum product supply. A method of producing a fuel from bio-oil without mixing with petroleum fuels is required.

Cernik and Bridgwater (1) have published the most recent description of utilization of bio-oil for heating fuels. They point out that limitations to utilization of raw bio-oils include poor volatility and corrosiveness. These limitations are produced by the bio-oil acidity, relatively high water content and the oxygenated nature of the bio-oil chemical compounds. Nearly all of these limitations can be eliminated if bio-oil is catalytically hydrodeoxygenated (HDO). However, catalytic HDO has proven very difficult to apply to bio-oil, mainly due to rapid catalyst coking (1). No reports of successful commercial application of HDO to produce an upgraded bio-oil have been reported.

A U.S. producer of liquid smoke from pyrolysis of wood reports utilization of the non-commercial bio-oil fraction as a fuel for their 5 MWth swirl burner citation. Space heating is provided by a heat-exchanger system incorporated into the burner. Finnish researchers have burned bio-oil in a dual-fuel burner that required only minor modifications to account for lower combustibility of the mix. A second Finnish research project tested an additional furnace configuration. Main findings were that some modifications were required to improve combustion, a secondary support fuel was required for startup, and emissions were within the environmentally acceptable range (1).

Researchers at MSU have developed a bio-oil upgrading process that increases bio-oil energy content, reduces acidity and stabilizes the fuel over time. The yield of this product is 100% because the reaction is produced by addition of chemicals and the reaction produces no production of gas as does the HDO process. This upgraded product is known as Lignocellulosic Boiler Fuel (LBF). Table 1 gives the properties of LBF compared to raw bio-oil prior to upgrading. Water content is increased by 1.2 percent from 24.2 to 24.5; acid value decreased by 44.4% from 99.3 to 55.2; higher heating value (HHV) increased by 36% from 17.5 MJ/kg to 23.8 MJ/kg; viscosity was reduced by 56.6% from 29.7 cSt to 12.9 cSt. A decrease in acid value will eliminate the corrosion issue involved with using bio-oil as a liquid fuel. While the water content increased slightly based on a chemical reaction the total energy value of the upgraded bio-oil was significantly higher. The increase in HHV moves bio-oil to an energy level of just over half that of diesel fuel making LBF much more competitive as a liquid fuel than is raw bio-oil. The ash content of raw bio-oil is a very low 0.04% and LBF maintains this low level. Low ash content will eliminate a source of coking on boiler parts.

The exact upgrading process applied in the MSU bio-oil upgrading process is currently proprietary but is somewhat similar to the process applied to produce bio-diesel. The process is relatively simple and much more cost effective than application of HDO with its process requirements for high pressure and heat combined with relatively expensive catalysts and hydrogen. MSU has developed both batch and continuous processes for upgrading raw bio-oil to LBF and a patent protecting the technology is pending.

The bio-oil that is upgraded to LBF is currently produced in the MSU auger pyrolysis reactor. Characteristics of the bio-oil produced by this auger reactor have been described in detail by Ingram et al. (2). The MSU bio-oil reactor typically pyrolyzes wood particles produced from hammer milling wood to particles of 1 to 3 mm diameter. These particles are very close in size to those produced in sawmilling.

The Department of Forest Products performed the boiler conversion and testing in cooperation with Natchez Trace Greenhouses (NTG), located in Kosciusko, Mississippi. NTG is a medium-sized wholesale producer of plant products with 20,000 square feet of plant material under glass.

NTG provided access to an idle natural gas hot water boiler which was converted by MSU researchers to burn bio-oil. An adjustable output waste oil burner was retrofit into the boiler. The new burner has the capability to combust fuel types ranging from No. 2 heating oil up to 90 wt. gear oil. The waste oil burner used compressed air to accomplish fuel atomization starting at 2 psi.

Thirty gallons of bio-oil were produced by the MSU auger pyrolysis reactor. Fifteen gallons of bio-oil were produced from the fast pyrolysis of southern pine and fifteen gallons from white oak feedstocks. Both fuels were subsequently upgraded to LBF. Initial burn tests showed that intermittent failure of the LBF flame resulted from increased water content and higher flash point temperature. To prevent intermittent flame failure, the three fuel-nozzle burner system incorporated into the output waste oil burner was modified to inject upgraded bio-oil through two injectors and diesel through the third injector. This resolved the flame failure problem and enhanced the combustion of the LBF with the result shown in Figure 1.

Preliminary field testing was conducted at NTG. LBF from southern pine and white oak biomass types were co-fed with diesel fuel in varying ratios to yield three different fuel formulations: (1) 100% diesel; (2) 87.5% LBF from southern pine co-fed with 12.5% diesel and (3) 87.5% LBF from white oak co-fed with 12.5% diesel. Each fuel formulation was combusted in the retrofit boiler at a rate of four gallons per hour. Fuel consumption and water temperature were measured periodically. The time needed to increase the boiler water temperature to 140^{o}F was measured. Flue gas from the boiler was analyzed by gas chromatograph (GC) to identify its chemical components for the fuel formulations containing 87.5% pine LBF with 12.5% diesel and 87.5% white oak LBF with 12.5% diesel. The flue gas was tested for oxygen, nitrogen, methane, carbon monoxide and carbon dioxide.

Results Each fuel tested was capable of producing enough heat to achieve a 140 ^{o}F water temperature increase. Bio-oil is a lower energy fuel, containing approximately 50 percent of the heat content of diesel fuel oil and therefore, provides about half the water heating capability. Thirty gallons of LBF were consumed during the boiler testing phase. Figure 2 shows the time required for a 140 ^{o}F water temperature increase by boiler fuel type. The 100% diesel fuel required 35 min. to achieve the 140 ^{o}F increase in water temperature. The 87.5% pine LBF and 87.5% white oak LBF each separately co-fed with 12.5% diesel required 62 min. and 85 min. to reach the 140 ^{o}F water temperature increase, respectively.

Figure 3 gives the rate of water temperature increase by fuel type. The 100% No. 2 diesel fuel yielded a water temperature increase of 4.0 ^{o}F per min. The 87.5% pine LBF and 87.5% white oak LBF each separately co-fed with 12.5% diesel resulted in respective water temperature increase rates of 2.3 ^{o}F/min. and 1.6 ^{o}F/min.

Table 2 shows flue gas comparison data from heating experiments with the retrofit NTG boiler. Fuels that contained 87.5% pine LBF co-fed with 12.5% diesel yielded nitrogen and oxygen at a ratio of 5.3 and carbon dioxide and carbon monoxide at a 22.2. Fuels that contained 87.5% white oak LBF co-fed with 12.5% diesel yielded nitrogen and oxygen at a ratio of 4.9 and carbon dioxide and carbon monoxide at a ratio of 16.4. Neither the pine LBF nor the white oak LBF fuel contained any measurable methane emissions from the NTG boiler flue gas.

It was determined from the Table 1 and 2 data that approximately 100% more bio-oil will be needed to provide the same energy as diesel fuel. While additional bio-oil will be required to equal the performance of diesel fuel it will also be true that the bio-oil fuel will be marketed at a price reflecting the reduced energy value. Therefore, the reduction in the amount of diesel fuel required when used in combination with LBF should be a viable environmentally friendly alternative future fuel source for greenhouse operations.

Table 1. Physical and chemical properties of raw bio-oil and bio-oil upgraded to Lignocellulosic Boiler Fuel.

	Raw bio-oil	Upgraded bio-oil
Water (%)	24.2	24.5
Acid value (% acetic acid)	99.3	55.2
Higher heating value (MJ/kg)	17.5	23.8
Viscosity at 40^oC (cSt)	29.7	12.9
Ash (wt %)	0.04	0.04
Specific gravity at 20^oC (g/ml)	1.25	1.12
Metals (ppm)	< 15	< 15

Table 2. Flue gas chemical compounds produced during combustion of LBF in the retrofit NTG boiler.

	Molar percent (%)	
Compound	87.5% pine LBF co-fed with 12.5% diesel	87.5% white oak LBF co-fed with 12.5% diesel
Oxygen	12.66	14.03
Nitrogen	67.41	68.62
Methane	0.00	0.00
Carbon Monoxide	0.30	0.35
Carbon Dioxide	6.65	5.73
Unknown	12.98	11.27
Total	100.00	100.00

Figure 1. Flame produced from combustion of LBF in the retrofit NTG boiler.

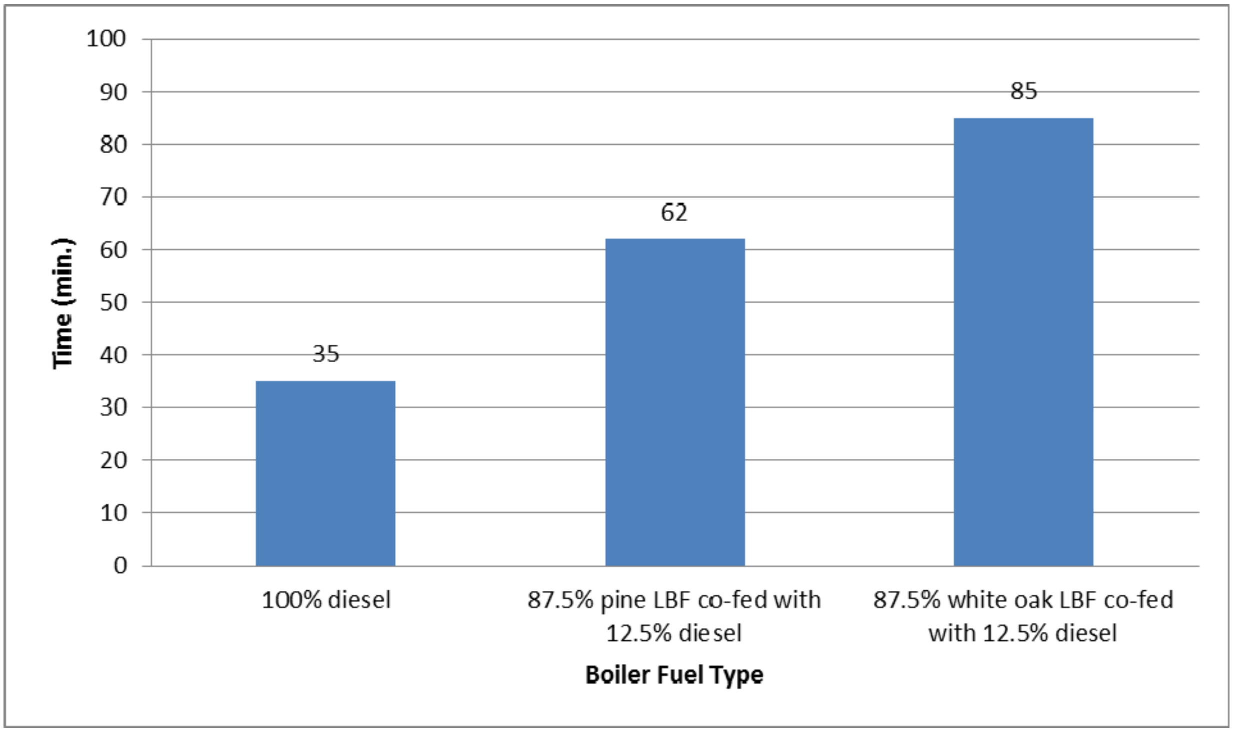

Figure 2. Heating time required to reach 140 ^{o}F water temperature by boiler fuel type.

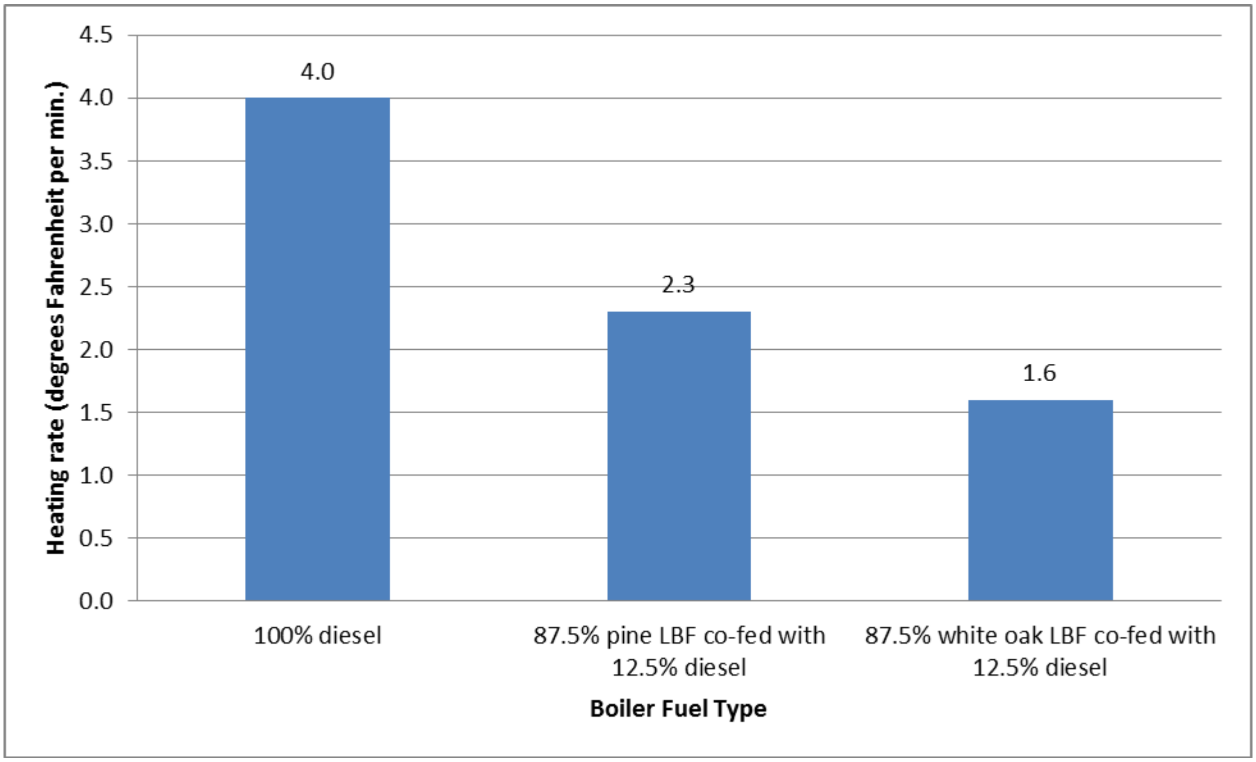

Figure 3. Mean time in degrees F per min. to bring boiler water temperature to 140 ^{o}F.

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Integration of Aquaculture Waste with Horticulture Crop Production

Jeremy M. Pickens[1], Jesse A. Chappell[2], Jeff L. Sibley[1], Adam M. Sleeper[1]
Wheeler G. Foshee[1] and Sami Abdul Rhaman[2]

[1]Department of Horticuture, [2]Department of Fisheries and Allied Aquacultures
Auburn University, Auburn AL 36849

Index of words: sustainability, water efficiency, nutrient recovery

Significance to Industry Agriculture is pressured to increase production while improving sustainability to meet the demands of natural resource conservation and issues feeding, clothing, and providing shelter for an ever increasing population. Of all agricultural industries, two are considered the most intensive, aquaculture and horticulture. Aquaculture is the fastest growing animal production industry worldwide (3). Horticulture involves many facets spanning thousands of crops, each crop requiring specific cultural needs. While aquaculture and horticulture can be very intensive they can also be highly adaptable. The flexibility and adaptability of horticulture and aquaculture crops allows for many areas of integration. Integration can provide opportunities for cost sharing and increased sustainability, while also diversifying products and markets. Large quantities of water used in both intensive aquaculture and the nursery and greenhouse industries provide many opportunities for integrating on several scales.

Intensive fish production usually involves concentrations significantly more concentrated (20,000-600,000 pounds per acre) than conventional pond culture (7,000 to 8,000 lbs per acre) (2, 5). Concentrations of fish at these levels require constant water quality monitoring and management due to the buildup of toxic nutrients to the cultured fish species. The primary nutrient toxicity of concern in regards to fish health is un-ionized ammonia and nitrite. Un-ionized ammonia NH_3 is the toxic form of what is collectively called total ammonia nitrogen (TAN) with its concentration heavily dependent upon the culture water pH. Nitrite (NO_2^-) is an intermediary form of nitrogen that exists in between ammonium (NH_4) and nitrate (NO_3) synthesis through the biological process of nitrification. Both NH_4 and NO_3 are relatively safe for fish within reasonable concentrations. There are many culture techniques that deal with these toxic nutrients but the least difficult is through water exchange. In most parts of the world were environmental restrictions are almost nonexistent, water exchange is the least difficult and in some cases the least expensive means of water quality treatment. In some culture systems such as trout culture, water is completely exchanged several times during the day in flow-through raceways.

A more sustainable approach is utilizing the integration of plant culture with fish culture. Integration of plant/fish production systems has been researched extensively in an effort to reduce the nutrient loads (nitrogen and phosphorus) of fish culture water and to conserve water in arid regions of the world. This research usually involves zero or minimum discharge systems in which the fish culture water is recirculated through hydroponic culture of vegetable crops, usually leafy greens. Although water is becoming scarcer, there are some parts of the world that still have an ample supply of irrigation water -- be it ground water, or collected water from precipitation. Areas with ample water supplies still face water concentration concerns due to industrial activities and population needs. The container nursery industry in the southeast U.S. is known to use ample amounts of water through overhead irrigation and water usage in this industry is likely to become more stringent in the near future.

Nature of Work: Several advantages exist in integrating fish production with container nursery production. The sustainable benefits are likely the greatest as water use efficiency increases per gallon as well as the reduction of nutrient loading into surrounding water sheds as plants have the ability to assimilate a large percentage of the nutrients contained in the fish culture water. Fish culture water in intensive systems can reach nitrate concentrations greater than 400 mg/l (4). Several small experiments have evaluated multiple plant species to determine the effects of fish effluent from an intensive system when used as a fertilizer source. Table 1 shows summaries of several experiments conducted at the Auburn University Integrated Aquaculture Research Facility at the E.W. Shell North Auburn Fisheries Station, Auburn, Alabama where fish effluent is directly compared to what was considered conventional fertilizer rates.

Discussion: These experiments demonstrate the potential use of fish culture effluent as a fertilizer source and adaptability over a wide array of crops. It has been observed through other experiments (data not shown) how stocking densities and feeding rates largely influence the concentrations of nutrients contained within the culture water. Water exchange also plays a significant role in the concentration of nutrients. The more frequent water is exchanged or the greater the percentage of exchange the less concentrated nutrients become. The rate of exchange will dictate its use and the scale of the integration. A system that utilizes lower exchange rates will be better able to utilize nutrient laden waters as a fertilizer source but will only be able to use it on a small area of plant production due to the lower volume of water available. Higher exchange rates in turn lend themselves to larger areas, but in this situation nutrient recapture is not of primary concern since nutrients are likely to be at minimal concentrations.

Container nurseries are reported to use as much as 13,577 gallons of water per acre daily (0.5 inch of water per acre) during peak production times (1). This is a considerable amount of water when considering the vast acreage in production today (6). Double cropping water, with fish culture as the first use and plant production as the second use increases the use efficiency of water on a per gallon basis. Most intensive fish production tanks are about 4 feet deep (equivalent to 30 gallons per ft^2). Using 30 gallons per ft^2 with a liberal 100% exchange daily, the water requirements for one acre of nursery production could require 450 ft^2 of fish production. Using these rough figures would present a ratio of a 100:1 nursery area: fish production area at a 100% exchange. In this case a 30 x 96 ft fish production greenhouse (2880 ft^2) could water 6.36 acres of nursery daily at a 100% exchange. Logistically, to water direct from a fish production tank using overhead irrigation would require two pumps, pumping equal volumes: one pumping water out of the tank and one pumping water back into the tank from a well or reservoir. In this case a cool water species such as trout could be grown. A less complicated approach is to pump water into a production tank and let the overflow be used to make up the water in the reservoir that is used for runoff and irrigation. This method could be used with a variety of fish species including tilapia and yellow perch. Using runoff from container nurseries in fish production would be limited due to aquatic species sensitivity to most pesticides. More work is needed to evaluate integration of intensive aquaculture with container nurseries in regards to water budgeting, effects of fish waste as a fertilizer source, and economic cost analysis. There is also potential to integrate other intensive animal production facilities that utilize lagoon technology such as swine, dairy and in some cases poultry.

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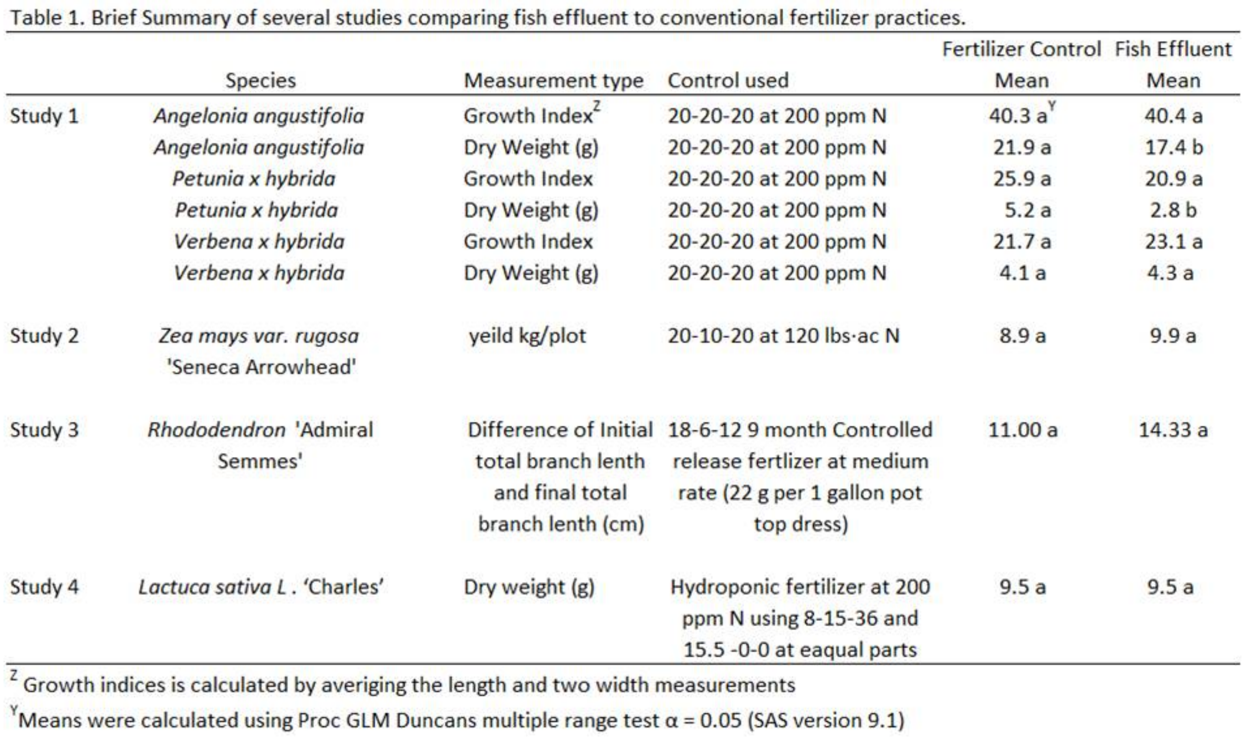

Table 1. Brief Summary of several studies comparing fish effluent to conventional fertilizer practices.

	Species	Measurement type	Control used	Fertilizer Control Mean	Fish Effluent Mean
Study 1	*Angelonia angustifolia*	Growth Index[Z]	20-20-20 at 200 ppm N	40.3 a[Y]	40.4 a
	Angelonia angustifolia	Dry Weight (g)	20-20-20 at 200 ppm N	21.9 a	17.4 b
	Petunia x hybrida	Growth Index	20-20-20 at 200 ppm N	25.9 a	20.9 a
	Petunia x hybrida	Dry Weight (g)	20-20-20 at 200 ppm N	5.2 a	2.8 b
	Verbena x hybrida	Growth Index	20-20-20 at 200 ppm N	21.7 a	23.1 a
	Verbena x hybrida	Dry Weight (g)	20-20-20 at 200 ppm N	4.1 a	4.3 a
Study 2	*Zea mays var. rugosa* 'Seneca Arrowhead'	yeild kg/plot	20-10-20 at 120 lbs·ac N	8.9 a	9.9 a
Study 3	*Rhododendron* 'Admiral Semmes'	Difference of Initial total branch lenth and final total branch lenth (cm)	18-6-12 9 month Controlled release fertlizer at medium rate (22 g per 1 gallon pot top dress)	11.00 a	14.33 a
Study 4	*Lactuca sativa L*. 'Charles'	Dry weight (g)	Hydroponic fertilizer at 200 ppm N using 8-15-36 and 15.5 -0-0 at eaqual parts	9.5 a	9.5 a

[Z] Growth indices is calculated by averiging the length and two width measurements

[Y] Means were calculated using Proc GLM Duncans multiple range test α = 0.05 (SAS version 9.1)