Symbiosis Biofeedstock Conference: *Expanding Commercialization of Mutualistic Microbes to Increase Biofeedstock Production*

December 2013

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Executive Summary

Mutually beneficial interactions between plants and other organisms are important to plant health and productivity. Through numerous mechanisms, including—but not limited to—the production of defensive compounds and antioxidants, mutualistic microbes can increase plant performance and resilience to a variety of perturbations. One example is the commercial use of root microbes (mycorrhizae and rhizobia) to increase the plant host's ability to uptake nutrients in nutrient-poor soils or directly from the atmosphere. Because the microbial symbiont can also increase plant resilience to, or tolerance of, drought, salty soils, or soils low in nutrients, adding mutualistic microbes (native to and ubiquitous in plants) is a warranted strategy for bioenergy crop production in general, especially in response to climate change and the concomitant increase in weather variability. Microbial mutualists have the potential to increase land yield and reduce production risks. The goal of the Symbiosis Biofeedstock Conference (SBC) was to explore the state of science and commercial product development specific to applications in biofeedstock crop production.

Microbial mutualistic symbionts (mutualists) can increase plant (and other organismal) host productivity through a number of mechanisms, such as increasing herbivore resistance and stress tolerance (Clay 1988, Redman *et al.* 1999 and 2011, Harman *et al.* 2004, Franzluebbers & Hill 2005, Lorito *et al.* 2010, Hamilton and Bauerle 2012). Microbial symbionts can change plant physiological parameters, increasing drought tolerance and/or nutrient absorption (Rasmussen *et al.* 2009, as reviewed by Mei & Flinn 2010, White *et al.* 2012, see also Bücking *et al.* 2012). Some causal mechanisms for these phenomena have been described. Changes in plant gene expression due to mutualistic symbioses increase the plant's innate defense system in response to both abiotic and biotic challenges (Harman *et al.* 2004, Tanaka *et al.* 2006 and 2008, Zhang & Nan 2007, Brelles-Mariño & Ané 2008, Kevei *et al.* 2008, Venkateshwaran *et al.* 2008, Hamilton *et al.* 2012, Mastouri *et al.* 2010, Tian *et al.* 2010, Fellbaum *et al.* 2012). An increase in a symbiotum's ("symbiotum" refers to combined microbe-plant genotype and resulting phenotype, see Appendix A) induced resistance response to pests includes a broad array of plant taxa (monocots and dicots). This resistance is exhibited at all life stages, from seedling emergence to reproductive maturity (Schardl *et al.* 2004, as reviewed by Rodriguez & Redman 2008 and Mei & Flinn 2010). In addition, the fungal or bacterial partner of the symbiotum is capable of producing a suite of chemicals, resulting in reduced losses to pathogens and parasites (Bush *et al.* 1997). Changes in carbon to nitrogen and lignin to nitrogen ratios in the symbiotum compared to uncolonized counterparts have also been documented (Lyons *et al.* 1990, Omacini *et al.* 2004, Cameron *et al*. 2006, Wu *et al.* 2009, Danielsen *et al.* 2013). All of these phenotypic changes in response to mutualistic interactions have been utilized to improve food and horticultural crop production (Bouton *et al.* 1993 and 2002, Waller *et al.* 2005, Assuero *et al.* 2006, Govindarajan *et al.* 2006 and 2008, Weyens *et al.* 2009, Mei & Flinn 2010, Popay & Hume 2011, Ker *et al.* 2012, Kim *et al.* 2012, Mei *et al.* 2012, Duhamel & Vandenkoornhuyse 2013). Mutualist-plant interactions need to be considered in light of the complexity of interacting organisms living in environments typified by change. Microbial-plant interactions are inherently complex. Like all ecological phenomena, they are a result of, and responsive to, evolution and phenomena operating at multiple geographic scales. To ensure stable states for successful commercial application of these products, context-dependent phenomena must be considered.

The SBC was designed to explore commercial opportunities utilizing microbial-based products in bioenergy crop production. Conference goals included identifying research supporting this approach in agronomic crops, identifying limitations to current products and using this information to inform future commercial production, and identifying the issues specific to utilizing mutualists in bioenergy crop production. The latter point was included to determine if there is sufficient research to warrant field exploration of microbial mutualists in bioenergy crops, and how such research can be facilitated via agency and national laboratory involvement to aid the advancement of commercial products.

Acknowledgements

This report is the product of the Symbiosis Bioenergy Conference (SBC), hosted by the U.S. Department of Energy's Bioenergy Technologies Office (BETO), which took place at Cornell University on June 20–21, 2013. We thank all who participated in the conference. Please visit BETO's Past Meetings Web page at bioenergy.energy.gov/past_meetings.html for more information about the SBC. We thank Dr. Erin Searcy, Dr. Vanessa L. Bailey, Morgan Evans (BCS, Incorporated), and Morgan Smith (BCS, Incorporated) for providing significant editorial guidance. We thank reviewers Drs. Catherine Ronning, Alan Franzluebbers, and Tim Widmer for providing insightful comments that led to an improved report.

Introduction

The Importance of Biobased Fuels

Energy is integral to the U.S. economy. A diversified energy portfolio, including domestic energy sources generated from renewable energy technologies, is critical to reducing the nation's dependence on foreign energy sources, particularly oil. Renewable energy resources have benefits beyond energy security. Renewables lower greenhouse gas emissions (GHGe), generate jobs in America, and revive rural economies. Recognizing these benefits, the U.S. government is funding efforts to increase renewable energy use in America. The Bioenergy Technologies Office (BETO) is one of several development programs within the U.S. Department of Energy's Office of Energy Efficiency and Renewable Energy focused on renewable energy. BETO's mission is to "Develop and transform our renewable biomass resources into commercially viable, high-performance biofuels, bioproducts, and biopower through targeted research, development, demonstration, and deployment supported through public and private partnerships" (Bioenergy Technologies Office 2012).

U.S. Department of Energy Support of Biobased Fuels

To meet long-term bioenergy production goals, BETO has a diverse portfolio of applied technologies involving research, development, demonstration, and deployment efforts (see Figure 1). The first element in bioenergy production is the biomass feedstock: biorefineries require a sustainable, consistent supply of quality biomass.

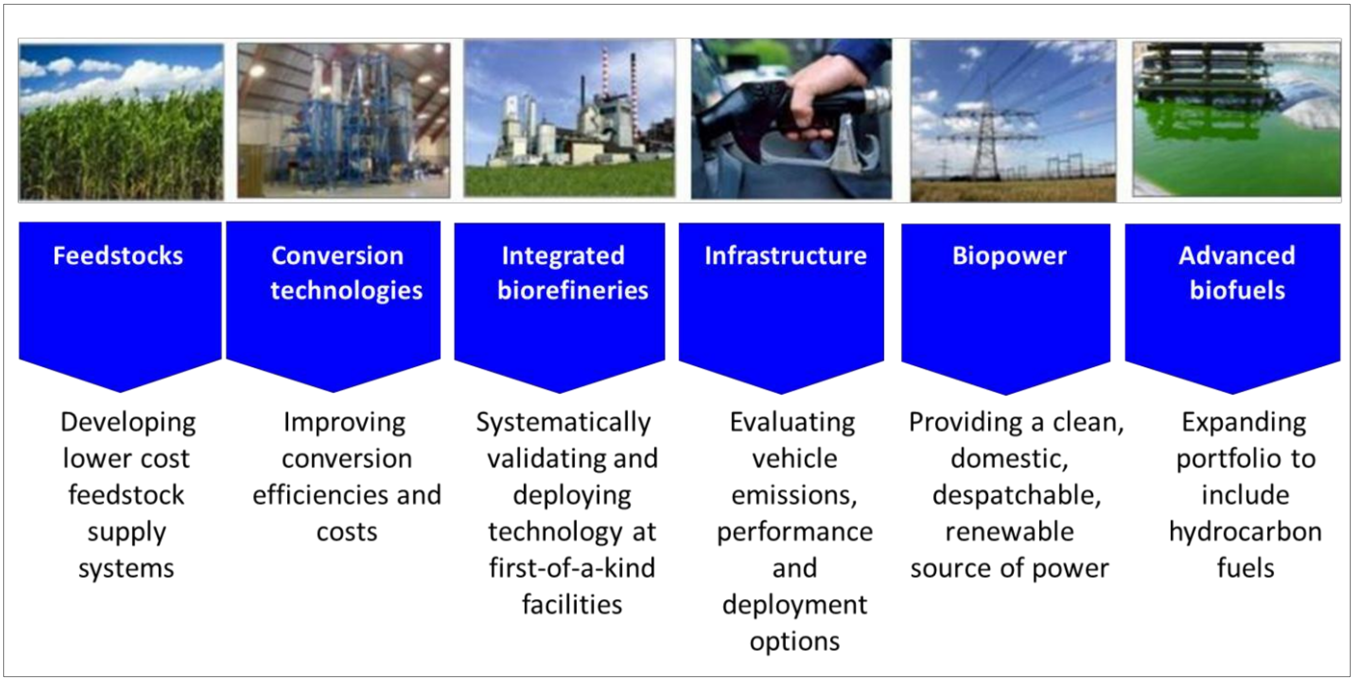

Figure 1. Bioenergy Technologies Office, bioenergy.energy.gov/biomass_feedstocks.html.

In this report, 'biofeedstock' is used as a collective term for field- or forest-grown biomass used in energy production (see Appendix A). Biofeedstocks include agricultural residues, woody biomass, and dedicated energy crops (i.e., crops grown specifically for energy production and not used as food or animal feed grain stocks). The bioenergy supply chain has many challenges, ranging from conversion efficiency to variability in biomass quality. Availability and accessibility are major issues in biofeedstock supply.

By supporting research in bioenergy, BETO can assist the public sector and industry by reducing the risk associated with commercializing new technologies based on biofeedstocks. This reduced risk can translate to the commercial market, providing positive impacts to biofuel commercial development and expansion of biofuel utilization through increased commercial investment and market realization.

Microbial Symbionts and Their Relation to Biobased Fuels

Symbiotic interactions are complex, reciprocal interactions between two or more organisms spanning a continuum of positive to negative interactions. Some popular examples of positive symbioses (mutualistic) include plants and their pollinators, clownfish and sea anemones, and oxpecker and zebra. The focus of the Symbiosis Bioenergy Conference (SBC) was on mutualistic, microbial symbionts (fungi and bacteria) of plants documented to repeatedly increase plant performance. These interactions result in phenotypic and genotypic changes to both micro- and macro-symbiont, referred to collectively as the symbiotum (Table 1).

All plants are colonized by a suite of microbes; this microbiome is composed of multiple bacterial and fungal strains known to enhance plant performance in general, and in response to stress (Clay 1993, Bush *et al.* 1997, Johnson *et al.* 1997, White & Torres 2010, Denison & Kiers 2011, Redman *et al.* 2011, Hamilton & Bauerle 2012). For several decades, endo- and ecto-symbionts have been researched and demonstrate a consistent ability to increase plant performance. Utilization of mutualistic microbes in agronomic settings is also several decades old. The questions are as follows:

1. Are microbial mutualisms underutilized in agronomic and, more specifically, biofeedstock production settings?
2. Can they address economic and sustainability concerns specific to biofeedstocks?
3. Are commercial products on the market ready for market expansion and inclusion in biofeedstock production?

The current biofuels industry predominately uses grain and stover as feedstock (Sedjo & Sohngen 2009, Wang *et al.* 2012). Other non-grain feedstocks, most notably wood, are used for bioenergy production; however, new energy crops are being developed and tested specifically for use in the bioenergy industry (Sedjo & Sohngen 2009). Very few producers are currently growing these biofeedstocks for a number of reasons, some of which can plausibly be addressed by incorporating mutualistic symbioses. Examples include (1) increasing producer profit margins relative to food crop profit margins, (2) successfully addressing public concerns about displacing land dedicated to food crops with biofeedstocks by increasing yields per acre, and (3) successfully addressing public concerns about environmental degradation by reducing resource and chemical use on biofeedstocks crops. How mutualistic symbionts can be incorporated to address items 1 through 3 will be addressed later in this report.

The information and conversations resulting from the SBC inform the topics of this report. This document provides a review of the state of basic research and the efficacy of commercializing mutualistic microbes to increase biofeedstock production. The conference provided an opportunity for diverse members of the research and commercial communities to review the basic scientific accomplishments, to-date, in microbial-plant mutualism. It also provided an opportunity to discuss the potential benefits of using native, mutualistic systems to address biofeedstock production via novel paradigms being developed and utilized in the food agronomy sectors (e.g., precision agriculture).

Report Goals

This report reflects the goals of the SBC within the context of BETO's mission and suggests avenues to realize demonstration and deployment of mutualists to increase biofeedstock production. Specifically, the report addresses why the utilization of mutualistic, symbiotic organisms in biofeedstock production is important to accomplish the following:

- Improve biofeedstocks economics by increasing and/or stabilizing biofeedstock yields and decreasing biofeedstocks' grower costs.
- Increase biofeedstock adoption by producers (farmers/growers).
- Decrease environmental impacts from biofuel production by
    - Reducing GHG emissions
    - Increasing positive environmental impacts of biofeedstock production by incorporating mutualistic, microbial symbionts (mutualists) with precision agronomy and breeding.
- Preemptively and proactively address impacts of climate change to biofeedstocks production by
    - Decreasing chemical requirements (e.g., pesticides, herbicides, and fertilizers)
    - Increasing plant resilience to climate variability
    - Increasing plant resistance to herbivores, parasites, and pathogens.
- Expand rural and national economic development by supporting the displacement of imported fuels with energy produced from biofeedstocks.

Roles for Mutualistic, Microbial Symbionts in Biofeedstock Production

The SBC and this report are specifically addressing how to effectively employ mutualists to accomplish the following:

1. Increase biofeedstocks yields
2. Increase producer profit
3. Decrease chemical additions
4. Decrease irrigation demands
5. Reduce the average amount of resources to produce biofeedstocks
6. Stabilize yields across years
7. Stabilize yields in response to extreme climate events
8. Increase sustainability of biofeedstock production (e.g., reduce chemical inputs and increase carbon dioxide sequestration).

Many of these items are interrelated. For example, by reducing resources used to grow biofeedstocks, the producer's profit margin is potentially increased and the market-driven competition between foods versus biofeedstock crops is reduced, while environmental sustainability is improved.

Mutualists could potentially alleviate a variety of environmental concerns, including public concerns about land-use change for biofeedstocks. The biofeedstocks considered are plants with a comparatively high tolerance for poor resources (e.g., nutrients and water). Because mutualists increase plant productivity in resource depauperate habitats (as reviewed in Mei *et al.* 2010, see also Rodriguez *et al.* 2009, Resat *et al.* 2012), the amount of land in production could be decreased while maintaining yields (Duhamel & Vandekoornhuyse 2013).

Mutualists can enhance plant growth on toxic soils where food crops cannot and will not be grown (Bücking 2001), potentially opening up novel land types for biofeedstock production. Other considerations include the impacts of increased root biomass and depth and associated effects produced by symbiota. Increased root biomass and depth leads to increases in carbon sequestration (in the soil), especially in crops with decadal harvest rotations, reduced tillage, and staggered plantings (to account for harvest rotations and plant growth rates). Reduced utilization of chemicals that are known to increase GHGe will increase the positive impacts of employing mutualists. This will also reduce the indirect negative impacts on primary consumers and additional trophic levels, as well as downstream phenomena related to fertilization of ground and surface waters.

The commercial readiness of mutualistic microbes is supported by patents, products, and research being conducted by the private sector. Companies such as Symbiogenics and Novozymes are creating microbial-based products for agricultural crops, potentially translatable to biofeedstocks. The BioAg division of Novozymes produces several microbial-based products, including Optimize® and a suite of microbial- based products, through its recent acquisition of TJ Technologies (tjtechnologiesinc.com). These microbial-based soil additions can potentially benefit a wide variety of plant taxa. The number of microbial strains patented by national and academic laboratories provides additional illustration of the commercial potential of microbial mutualists (see Appendix B).

Conference Discussion

Increasing Resistance to Disease and Herbivores

Pathogen- and herbivore-plant coevolutionary phenomena produce significant challenges to breeding regimes. Pathogenic fungi and herbivores have faster generation times and are not limited by breeder selection; instead, they are fostered by host cultivation. Meaning, selection continues to act on the pathogen or pest, but the host is removed from evolutionary selective forces via breeding/cultivation. This is explained best perhaps by evolutionary game theory (Weyl *et al.* 2010, Burdon & Thrall 2009, Doebeli & Knowlton 1998). In order for the two entities to enjoy fair play in game theory, they must be able to exert selection pressure reciprocally. In native habitats, plant populations and their antagonists (herbivores, pathogen) will have high levels of genetic variation relative to highly cultivated monocultures. If the plant’s defense system cannot respond to attack, then it is at a disadvantage. In a population of plants with high genetic diversity, some will succumb and some will not. Those that do not succumb go on to grow and reproduce. However, if the cultivated plant becomes a stable target (due to clonal propagation and reduced genetic variation) for the pathogen/herbivore, it will not succeed (Grisham & Pan 2004 and 2007). No longer is it a moving genetic target; the plant population now composed of highly similar (in terms of genotype) cultivars increases the probability of pests encountering an accessible host. A metaphor would be a burglar learning a single alarm system (or code) and then discovering an entire block or neighborhood is using the same alarm system. Alternatively, if the neighborhood contains a multitude of diverse alarm systems, thievery will be retarded. This is why it is important to retain as much genetic diversity as possible when breeding cultivars for selected traits (Pan *et al.* 2004).

Many mutualistic fungi provide host resistance across cultivars and even taxa (Schardl *et al.* 2007, Torres *et al.* 2012, Shoresh *et al.* 2010). Taking advantage of the natural variation in mutualist-plant interactions is a strategy that accounts for and utilizes both evolutionary and ecological processes (Ewald 1994). The symbiotum can respond to a novel pathogen/herbivore attack because it has not been removed from the selective forces of the pathogen via cultivation. Instead, the symbiotum has a unique genotype, as will its offspring. This creates a moving target that the pest's genetic information has to track in order to evade the symbiotum's defense systems.

Using Mutualism to Address Land-Use Change, Competition for Resources, and Resilience to Climate Variation

Another barrier to biofeedstock production identified from the SBC is the amount and type of land used to grow dedicated energy crops. Questions asked include: is it land once used for food or range cattle, and if so, will changing to biofeedstocks be feasible (economically) and environmentally sustainable? According to a recent U.S. Department of Agriculture assessment of land available for agricultural and nonagricultural uses, 37 million acres of potential cropland is idle; potential cropland includes land formerly used to grow crops, as well as rangelands used for ruminants. The projected average increase in food crop yields of 4% annually up to 2022 (Perlack & Stokes 2011) combined with the utilization of precision agriculture and intercropping with biofeedstocks support the argument that land-use change for biofeedstocks will be limited to nonexistent (Perlack & Stokes 2011).

In the recent past, there have been dissenting views regarding the productivity of various crops in the United States, which have led to questions about the assumption of a 4% average increase in crop production in the future (Perlack & Stokes 2011), especially since these predictions do not include climate change scenarios (Lobell *et al.* 2011). The presumption of unlimited yields for any crop are challenged by basic physiological limitations of various plant taxa, in addition to the complications of global climate change on crop productivity due to limited or unpredictable resource availability, increased pest and pathogen pressures, and increased competition for economic resources, to name a few (Food and Agriculture Organization of the United Nations 2012, Intergovernmental Panel on Climate Change 2013, Organisation for Economic Co-operation and Development 2012). What Lobell *et al.* (2011: Figure 2) indicate are significant reductions in major food crops worldwide utilizing data from multiple years. Long *et al.* (2006) reviewed the literature in which the meta-analysis concluded crop yields would likely increase in response to elevated carbon dioxide (CO_2). This is a bit like comparing apples with oranges. Increased CO_2 is not equivalent to increased climate change; climate change is a box within which numerous phenomena exist, including the amount of CO_2. This illustrates the need to consider the context and utilize multiple models when developing prediction to crop production in response to climate change.

There are clear benefits to the plants selected for biofeedstocks, namely low resource usage, fast growth rates, and high biomass production. To increase or maintain yields per acre, and thus increase crop adoption and environmental sustainability, it is important to find a means of increasing yields despite environmental perturbations.

One means of increasing plant resiliency and robustness is through maintenance of beneficial soil microbial communities. Conservation of mutualistic microbial communities by reducing pesticide and nutrient applications was explored during SBC discussions. Not unlike the recommendations for soil management recently reported by the Global Environmental Fund (Govers *et al.* 2013, see also Houghton *et al.* 1983, DeGraaff *et al.* 2006), SBC conference participants concluded that management to foster mutualistic soil communities would feed back to producer profits and positively impact the environment. Mutualistic interactions, including their degree of mutualism, are influenced by a variety of factors (Wardle *et al.* 2004, Verbruggen *et al.* 2010, Wallis *et al.* 2010, Bailey *et al.* 2011 and 2013), which can be negatively impacted through a variety of agricultural management techniques, resulting in changes to atmospheric concentrations of CO_2 and nitrogen oxide (Angers & Eriksen-Hamel 2007, Crow *et al.* 2009, Pan *et al.* 2009, Franzluebbers 2010, Ayuke *et al.* 2011, Bakarr 2012). Mutualists provide a means of addressing plant performance in response to pest pressures and, as such, can lead to increases in yield per acre. Increased yield per acre (reducing land-use change potential) is likely to facilitate adoption of these crops by producers, provided utilization and application of mutualistic microbes does not increase production costs. Increased yields per acre will also address potential issues of land used for biofeedstock production by reducing the amount of land needed and the amount of energetically expensive chemicals needed to thwart plant pests.

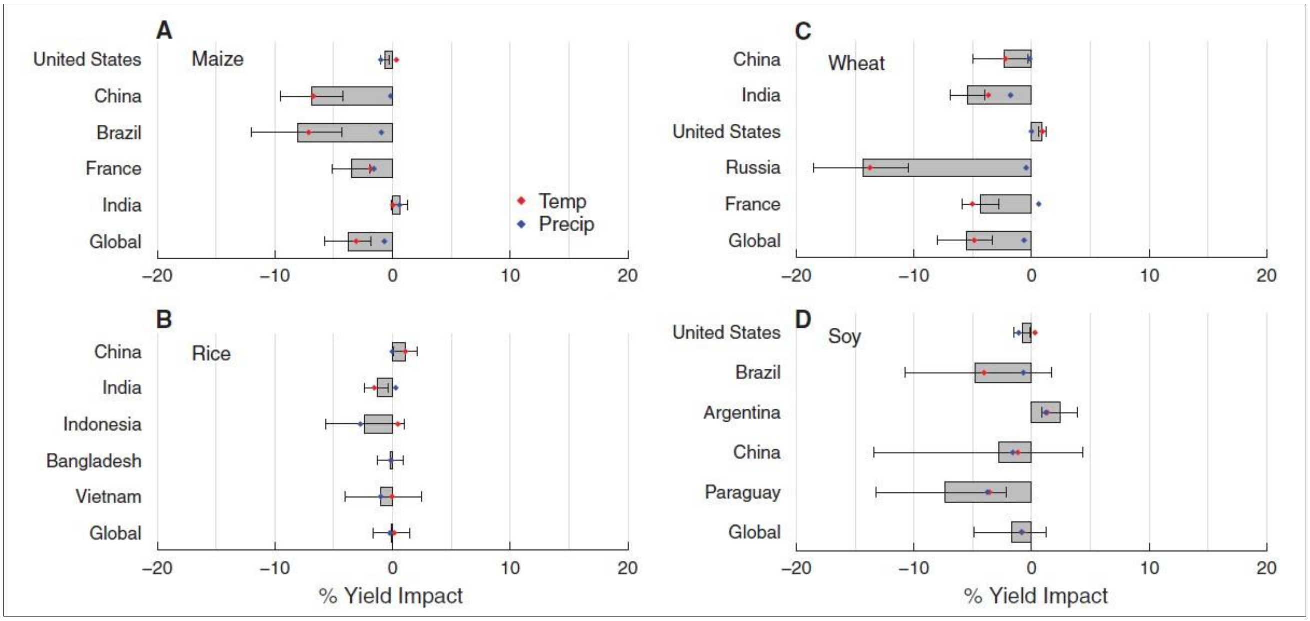

Figure 2. Estimated net impact of climate trends from 1980 through 2008 at national, regional scales. Gray bars show medians with error bars for 5% to 95% confidence intervals from bootstrap resampling, 500 replicates. Red and blue dots show median estimate of impact for predicted trends resulting from either temperature or precipitation. Source: Lobell *et al.* 2011.

Decreasing GHGe of Biofuel Production via Microbial Mutualists (absolute and relative to fossil fuels)

Reducing chemical inputs (fertilizers, pesticides, etc.) has a positive environmental impact by decreasing GHGe (Tilman *et al.* 2002, Wullshleger *et al.* 2010). Many models of GHGe from food crop production fail to include the sevenfold increase in nitrogen fertilizers and the three-and-a-half-fold increase in phosphorus use between 1960 and 1995, or contemporary increases and the resulting impacts to GHGe (Tilman *et al.* 2002). There are valid criticisms of models showing increases in GHGe from bioenergy relative to fossil fuel-based energy, including model exclusion of harvest rotation timing and other components of precision agriculture, or the lack of full life-cycle analysis comparisons with fossil fuels' energy consumption and resulting GHGe (Cherubini *et al.* 2009, Searchinger 2010, Loeffler & Anderson 2014). Additionally, corn or soybean crop production demands are not a good proxy for biofeedstocks, which are C4 annual grasses, perennial grasses, or short rotation woody crops that require significantly lower fertilizer, water, and total energy input (Hill *et al.* 2006). Also, perennial crops and woody crops retain root systems after harvest, and these root systems are carbon (C) sinks (Tuskan *et al.* 2001, Bücking *et al.* 2008, Jansson *et al.* 2010).

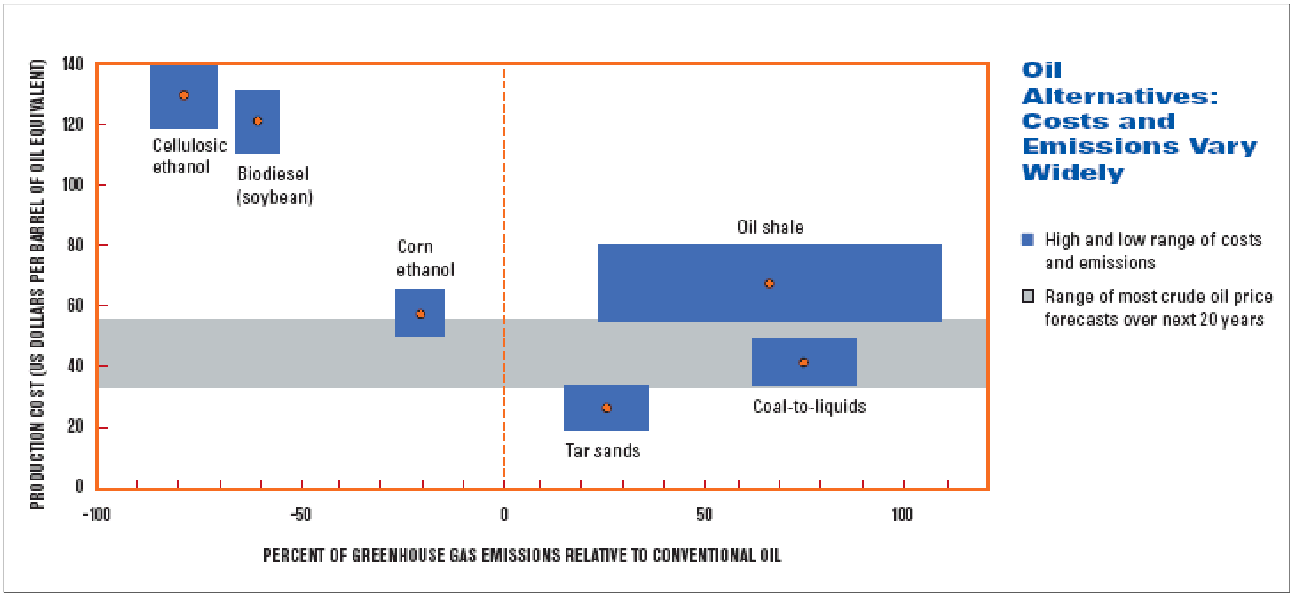

Figure 3. Original Source: Newell (2006) "What's the Big Deal about Oil" (copyright Resources for the Future).

Pertinent to this report is the role of mutualists to directly and indirectly reduce GHGe from biofeedstock production. To successfully decrease GHGe via biofeedstock production, strategies must result in the following:

1. Reduced chemical application (e.g., fertilization, fungicide, and other pesticide applications).
2. Increased biomass production (yield) on average per acre.
3. Overlapping and rotation harvests to maximize C sinks (sinks increase C sequestration in the soil) and minimize C source (sources release C from the soil to the atmosphere) phenomena.
4. Reduced tilling to optimize microclimate and soil microbial communities, as well as minimize C source phenomena.
5. Soil C sequestration via root retention and in aboveground plant tissues.
6. Soil C sequestration via physical and chemical protection of microbial community through precision agricultural practices (e.g., low/no-till, low/no-nutrient applications).

Mutualists can facilitate items 1, 2, and 5 in this list, and they require item 4 to ensure the presence of inocula in the soil and plant tissues.

Items 1 and 2—Reducing Chemical Applications via Microbial Symbiosis and Increasing Yields

Increased disease and herbivory in response to elevated CO_2 or increased immune expression in response to herbivory and disease, relative to ambient CO_2, has been documented (Lau & Tiffin 2009, Lake & Wade 2009, Newton *et al.* 2011, Altizer *et al.* 2013). Although, the response is dependent on plant life stage and feeding taxonomy of the herbivore (as reviewed by Bezemer & Jones 1998, Massad & Dyer 2010, Klaiber *et al.* 2013). Mutualistic endophytes can reduce herbivory, herbivore fitness, or the consequences (on host performance) of herbivory in response to CO_2 exposure (Newman *et al.* 2003, Hunt *et al.* 2005, Alberton *et al.* 2010, Resat *et al.* 2012). Reduced herbivory via production of defensive compounds by mutualistic endophytes is well documented (as reviewed in Clay & Schardl 2002, Compant *et al.* 2005). As previously reported, there are decades of literature supporting increased plant performance in response to microbial symbioses, resulting in reduced nutrient application and pesticide/herbivore applications (see also Shoresh & Harman 2008, Rodriguez *et al.* 2009, Torres & White 2010, White & Torres 2010, Mastouri *et al.* 2012). The point being—many mutualistic microbes tolerate or even require lower nutrient inputs to retain a mutualistic symbiosis, and this has the potential to significantly reduce fertilizer application on biofeedstock crops (Figure 4) with multiple beneficial, indirect environmental impacts.

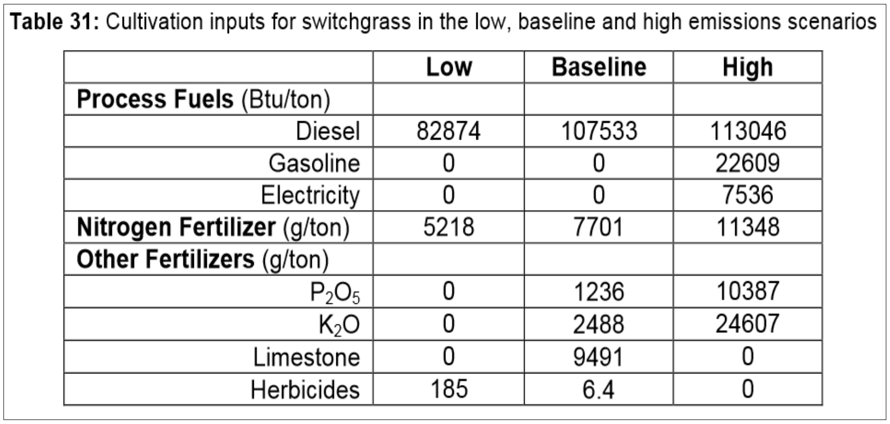

Table 31: Cultivation inputs for switchgrass in the low, baseline and high emissions scenarios

	Low	Baseline	High
Process Fuels (Btu/ton)			
Diesel	82874	107533	113046
Gasoline	0	0	22609
Electricity	0	0	7536
Nitrogen Fertilizer (g/ton)	5218	7701	11348
Other Fertilizers (g/ton)			
P_2O_5	0	1236	10387
K_2O	0	2488	24607
Limestone	0	9491	0
Herbicides	185	6.4	0

Figure 4. Lifecycle GHGe from alternative jet fuels. Source: Stratton *et al.* 2010.

Items 3 through 5—Incorporation of Mutualists with Precision Agronomy and Breeding

Precision agriculture is defined by a suite of best practices, including incorporation of crop management practices measuring inter- and intra-field variability in crops with consideration of both spatial and temporal variation. At the field scale, precision farming involves spatially explicit evaluation of soil and landscape conditions, including—and resulting from—soil characteristics, drainage patterns, land aspect, land-use history, and land tenure (Dale 2013). Based on this information, the plant genetic source, timing and degree of cultural operations, till or no-till, and the quantity and quality of chemical applications are determined (Kitchen *et al.* 2005, Cabot *et al.* 2006, McConnell & Burger 2011). The goal of precision agriculture is two-fold: increase environmental sustainability and positively impact producer economics (Dale *et al.* 2013).

Consistent in the microbial-plant symbioses literature, as well as during the SBC discussions, is the recognition that where the symbiosis falls upon the continuum of interactions—from antagonistic to mutualistic—is a context-dependent phenomenon. Context is defined as the microbial community present in and outside the plant, unique environmental differences the plant population and symbiotum experience (e.g., microclimate and localized soil variation). These phenomena add complexity and uncertainty to the symbiotic outcome in the field at large spatial scales and over multiple plant generations (Bever *et al.* 1997, Meijer & Leuchtmann 2001, Wullschleger *et al.* 2005, Hamilton *et al.* 2009, Furseth *et al.* 2011, Kiers *et al.* 2011, Furseth *et al.* 2012, Moebius-Clune *et al.* 2012, Verbruggen *et al.* 2012, Weston *et al.* 2012, Hart *et al.* in press). For example, Dr. Heike Bücking's laboratory has found mycorrhizal communities demonstrating increased biomass production regardless of mycorrhizal strain or nutrient application; although, symbiotum performance was complicated in response to nutrient treatments. Symbiotum produced decreased biomass in higher phosphorous (P) treatments relative to lower P additions (Verbruggen *et al.* 2012, Hart *et al.* in press). It is known that there are carbon costs to symbiosis—it's the plant's exchange currency with the microbial symbiont. In return, the plant obtains P and nitrogen (N). Bücking's work suggests that integrating mycorrhizae in breeding programs could lead to increased efficiencies and yields (Bücking & Sacher-Hill 2005) and greater control of the C costs incurred by the plant. This illustrates the need to consider previous agricultural practices to ensure mutualistic, microbial symbionts (soil or in planta) are successful. For example, if the symbionts being employed are fungal, then fungicides cannot be used. If the symbionts are retained in root tissues following aboveground harvest, then no- and low-till is warranted.

Utilization of engineering and breeding to create novel plant microbial associations exhibiting a mutualistic phenotype are also under exploration (Goodrich-Blair *et al.* 2010, Jayaraman *et al.* 2012, Delaux *et al.* in press). The challenge to this approach is largely regulatory and involves public perception of genetically modified crops. Consensus at the SBC included utilization of a combined approach, integrating breeding, engineering, and native microbial mutualists.

Preemptively and Proactively Addressing Negative Impacts of Climate Change to Biofeedstocks Production via Microbial Mutualists

Under current policies and energy sources, GHGe are projected to increase cumulative GHG concentrations by an additional 40 gigatons by 2030 (U.S. Energy Information Administration 2007). Recent reports show increases in average global temperatures by approximately 2°C to 3.5°C by midcentury or the end of the century (Figures 5a and 5b). Rosenzweig & Parry (1994) used three global climate models to explore warming of 1.5°C to 4.5°C, finding decreased grain yields of 10%–70%. Variation in crop reductions are a consequence of (1) which model is used; (2) regional variation; and (3) whether or not direct impacts of climate change on plant water use, plant physiology, and changes in pest pressures were included, to name a few. Though variable in predicted outcomes, consistent crop yield reductions are greater than or equal to 10% (Organisation of Economic Co-operation and Development 2012, Schlenker & Roberts 2009).

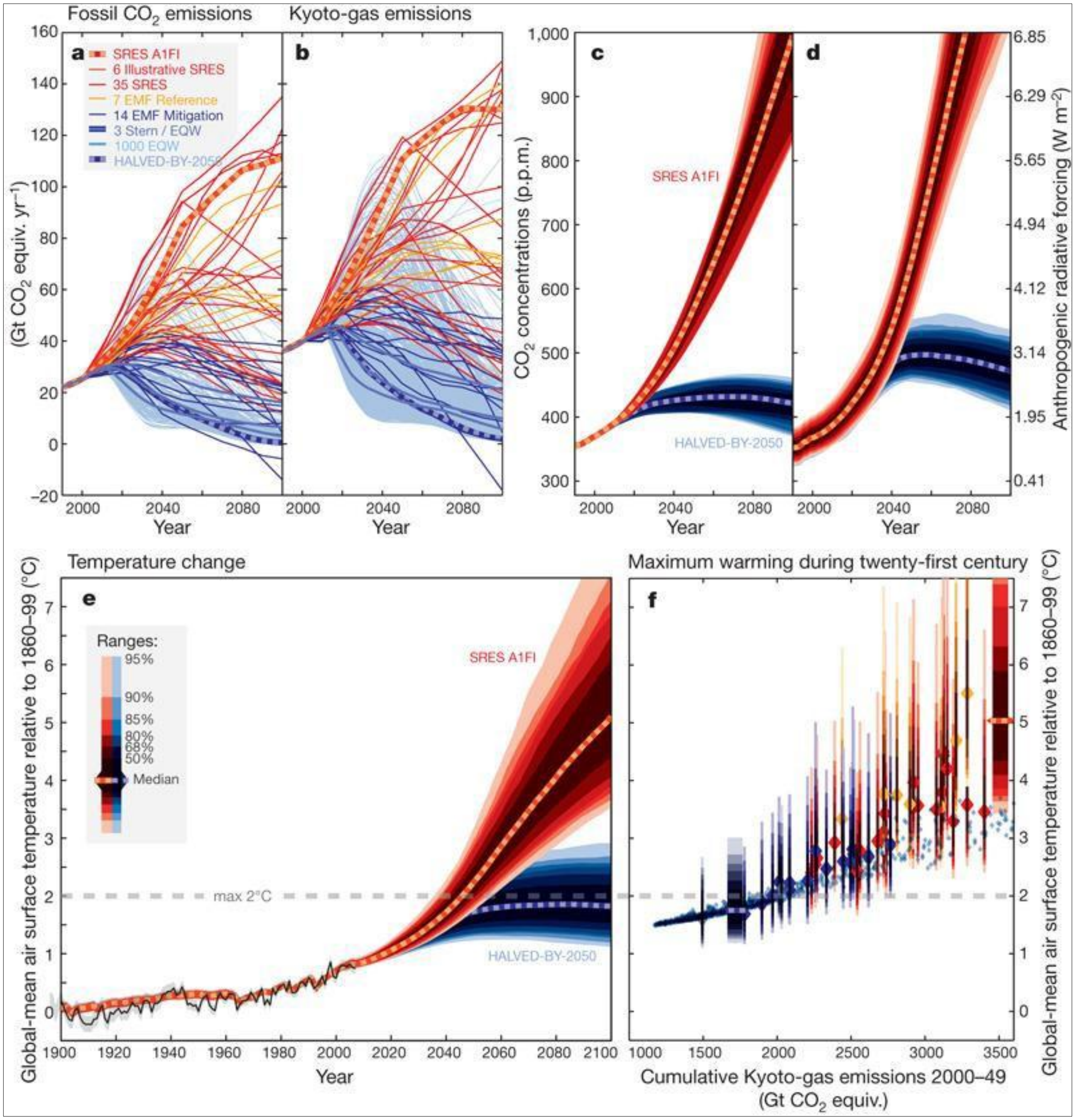

Figure 5a. GHGe targets for limiting global warming to 2°C. Source: Meinshausen *et al.* 2009.

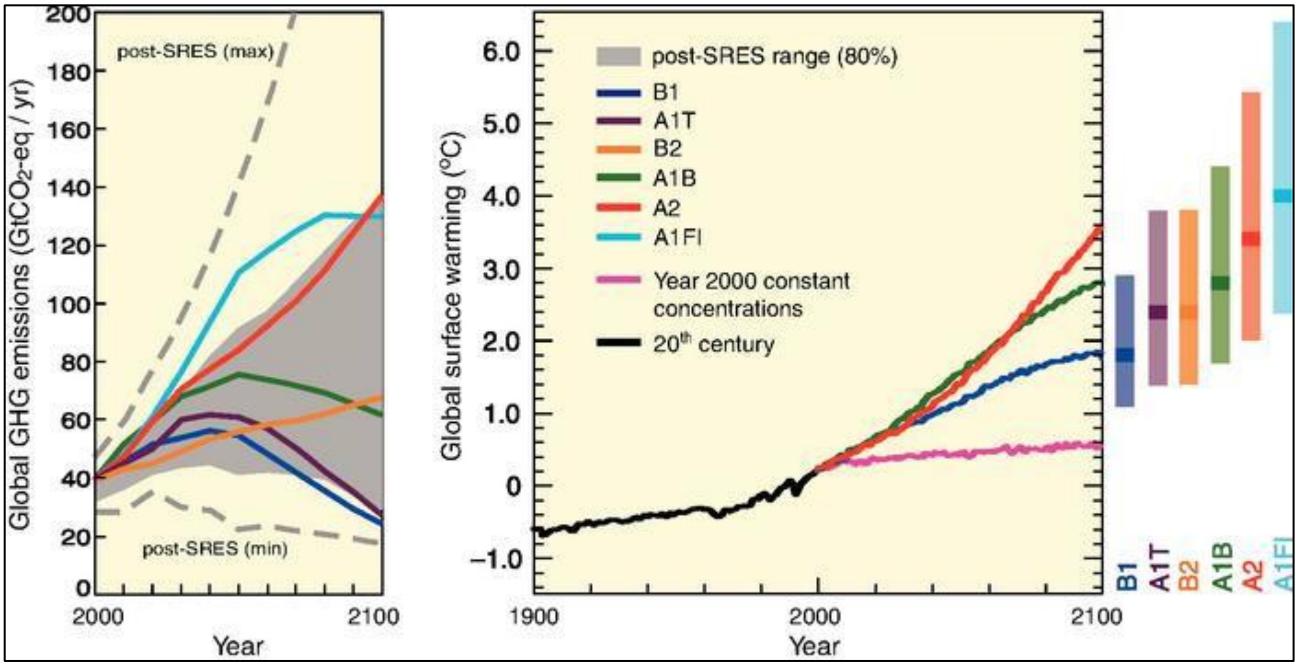

Figure 5b. Intergovernmental Panel on Climate Change estimates of global GHGe and increases in average global temperature through 2100.

Climate change is not a single parameter phenomenon. Along with increased GHGe comes increased average global temperatures, changes in precipitation patterns, various outbreaks of plant and animal disease, shifts in ecological regions, and variations in socio-economic conditions all occurring in conjunction with human population increases. All of these factors will lead to increased demands for food and other basic resources (e.g., energy, land area for food production and housing) (Duhamel & Vandenkoornhuyse 2013).

Climate change and concomitant environmental changes can significantly impact plant yields depending on when and what events occur in a plant's growth stage (emergence—seed set; Figure 2). For example, the impact of flooding or drought in the early spring when seeds are germinating or plants are emerging will reduce yields. Drought and increased herbivore numbers during summer months when plant biomass is peaking will also reduce yields. These complex factors are important to agriculture and necessary considerations when evaluating climate change impacts on future crop yields (Figure 6). This is not only because regional and intra-annual vagaries are likely to impact plant production, but also because they will make breeding programs more difficult (Wheeler *et al.* 2000, Sinclair *et al.* 2004). Breeders will have to incorporate extreme events, such as flooding, higher summer night-time temperatures, increased frequency of critical temperatures (Gourdji *et al.* 2013), unpredictable and recurrent droughts, increased exposure to soil pathogens, and more. Current breeding regimes assume stable environments with relatively high soil quality (Duhamel & Vandenkoornhuyse 2013). Plant engineering poses challenges as well; creating a cassette of genes capable of producing a suite of phenotypes adaptive to numerous, distinct pressures (Fedoroff *et al.* 2010). However, inserting a gene or suite of genes capable of increasing adaptation to variability—i.e., increasing phenotypic plasticity—will be challenging (Hoffman & Sgrò 2011). This strategy requires a robust understanding of the mechanisms foundational to phenotypic plasticity, as well as the metabolic components possibly shared by multiple defense responses (e.g., drought and pathogen resistance). This is where microbial mutualists can provide a solution—be it a bridge (short-term) or a long-term strategy incorporating novel technologies and classical breeding.

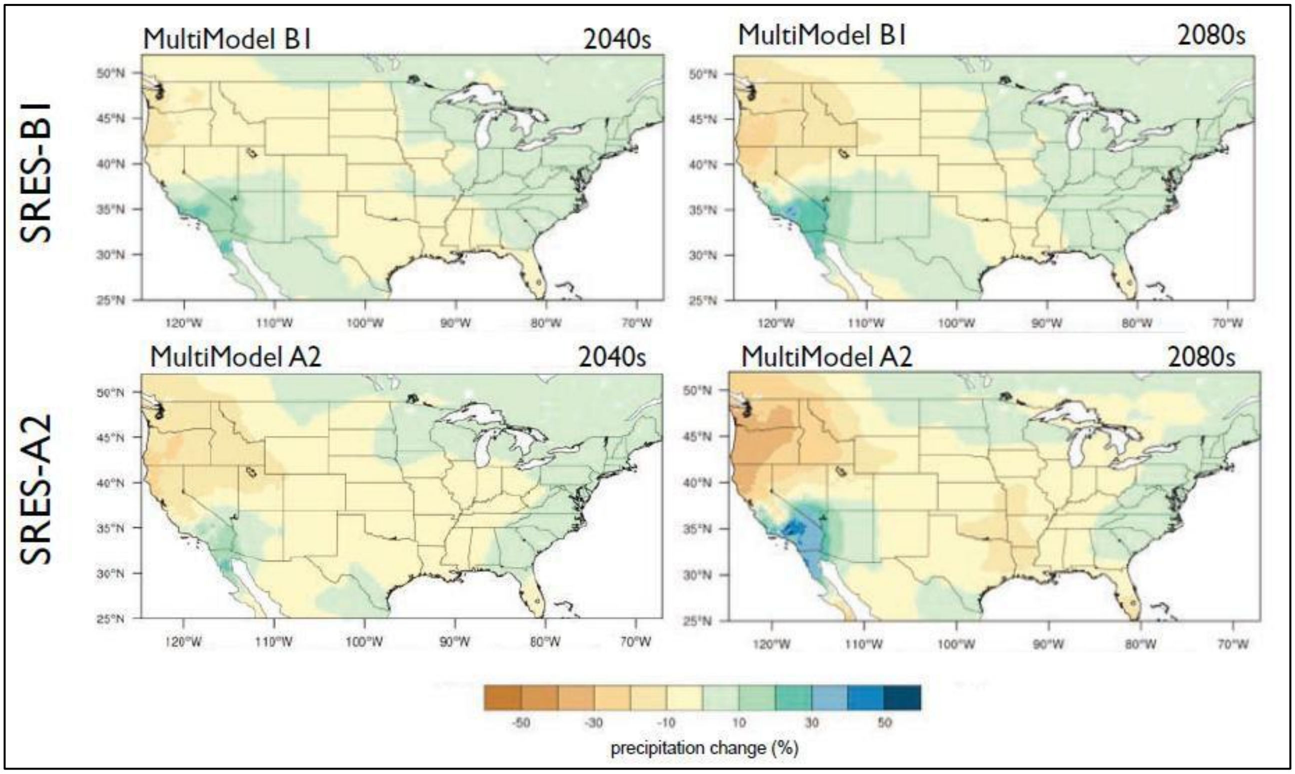

Figure 6. Summer precipitation projections for a low-emissions scenario (Special Report on Emissions Scenario-B1, upper panels) and a high-emissions scenario (Special Report on Emissions Scenario-A2, lower panels) relative to 1970–1999. Source: Walthall 2013.

Commercializing Mutualists—The Biological Challenges and Opportunities

There are numerous, potential challenges to commercialization of mutualists. Examples include lack of sufficient, repeated field trials demonstrating success in biofeedstocks—in part due to lack of biofeedstocks in the field, insufficient temporal data about the stability of symbionts in terms of yields, insufficient data about symbiotum response to climate change phenomena, and lack of cost analyses of various conversion methods reflective of diverse crops. Symbioses in the field are responsive to resource availability, causing uncertainty in terms of mutualistic outcome (Bever 2002, Furseth *et al.* 2011, Furseth *et al.* 2012, Fellbaum *et al.* 2012, Hart *et al.* in press). As mentioned previously, some of this antagonism or lack of mutualistic interaction may be the result of working in tilled fields or agronomic fields, where both tillage and previous fungicide and nutrient applications would disrupt the soil microbial community composition, as well as the ability of plants to interact successfully with microbes (Johnson 1993, Thiele-Bruhn *et al.* 2012, Bissett *et al.* 2013).

Persistence of mutualists and microbial community composition impacts, either in soil or in the plant host (especially perennials and woody crops), is another issue (as reviewed in Verbruggen *et al.* 2012). The question is: after soil inoculation, does the resulting community of mycorrhizal, rhizobial, or other microbial mutualists persist, colonize, and contribute to increased plant performance? According to Pellegrino *et al.* (2012), agricultural soil inoculation with arbuscular mycorrhizal fungi, regardless of strain composition, leads to significant increases in plant biomass correlated with increased root colonization. According to Furseth *et al.* (2011), additions of rhizobial inoculants do not increase soybean production due to naturalized populations already existing from decades of previous soybean production. Examples of the diversity of interactions and plethora of potential responses in complex field systems have been explored in literature and will be briefly addressed here. First, genotypic interaction and genotype by genotype by environmental interactions can shift the symbiosis to unpredictable positions on the continuum of symbiotic interactions. West *et al.* (2012) report strain-dependent and strain-independent host plant responses to a bacterial symbiont, resulting in both costs and benefits to host biomass production. In contrast, Kiers *et al.* (2003 and 2011) demonstrated that the benefit of soil microbial associations varied based on species or strain, though both remained beneficial. The degree of mutualism appears to be, at least in part, a result of the host exerting influence on the interacting species via sanctions (Kiers *et al.* 2003 and 2011). Phenomena such as partner choice and sanctioning are evolutionarily defensible because they ultimately lead to the continued existence of mutualistic symbiotum participants (West *et al.* 2002). In summary, strain-specific bacterial symbionts can more effectively increase productivity compared to naturalized strains. Overcoming mediocre responses between symbiota via the production of microbial genetic strains, which are prolific, amenable to host colonization, and resilient to both management and climate change variability (comm. pers. AJ Franzluebbers).

Additional issues with commercialization of mutualists include market and behavioral limits to adoption of novel products, especially if efficacy is negatively impacted by previous agricultural management practices. For example, multiple benefits originating from mutualistic endophytes in Brazilian sugarcane production have been identified (as reviewed in Muthukumarasamy *et al.* 2002, Saravanan *et al.* 2008, Taule *et al.* 2012, Chauhan *et al.* 2013). Yet, commercialization of nutritional and defensive microbial symbionts affiliated with sugarcane production appears to be nonexistent. Is this because, as with many rhizobial species, it is not necessary (Furseth *et al.* 2011)? Perhaps natural field conditions consistently provide sufficient inoculums? Or, perhaps methods of inoculating plants or soil with microbial mutualist are cost prohibitive? Do producers fear using microbes in their applications? These are questions to be addressed by the research community specific to biofeedstock production.

Challenges to commercial application include the unintended consequences of introducing mutualists either lacking high host specificity initially, or which lose host specificity. Common mycorrhizal networks (CMNs) are an example. This is where multiple hosts will share a mycorrhizal network and some plants may invest lower C resources while acting as a nutrient sink for mycorrhizal resource contributions to the detriment of focal plant hosts (Walder *et al.* 2012). Basically, one plant is cheating at the expense of another. However, polyculture agroecosystems (e.g., the three sisters) often used in Asia and Africa are demonstrably more efficient and potentially more productive than conventional monocropping systems due to CMNs (Perfecto & Vandermeer 2010, Hinsinger *et al.* 2011). Thus, rather than a cheater system in which non-specificity of symbiosis leads to target crop decline, facilitation through complex signaling between mycorrhizae and multiple plant hosts, as well as associated opportunities for sanctioning of cheating hosts, could lead to complementary resource use, facilitation, and mutualistic interactions for all involved.

What have individuals and companies done well when commercializing microbial symbiont products, such as mycorrhizae and rhizobia? This is difficult to assess due to the proprietary nature of research produced by private companies. All companies contacted for this conference report have made significant strides in terms of field-scale studies due to increased symbiotum productivity (biomass and or yields). For example, Symbiogenics identifies more than 60 field trials and BioWorks has 75 field trials. Their experiments include multiple plant and fungal taxa exposed to multiple stressors. Symbiogenics has patented species/strains of *Curvularia* and *Fusarium*, BioWorks has patented *Trichoderma* strains, and the former have experiments involving biofeedstock species (Appendix B). In 2008, BioWorks had zero sales; in 2012, it can report millions of acres of *Trichoderma* in use in 11 countries, with 12 countries pending (though the company was not willing to report current sales). This commercialized product increases corn, tomato, and other crop yields on nutrient-poor land in third-world countries without access to irrigation systems. BioWorks has ensured success by developing multiple application methods to account for unique crop requirements, developing multiple strains to provide mixed strains in single applications (hedging bets), and ensuring mutualistic outcomes dominate. The company has also provided education and resources to farmers in developing countries to allow for production of the *Trichoderma* strains locally, thereby increasing adoption and decreasing producer costs.

However, because previously reported field trials are primarily on food crops, the impacts to biofeedstocks at large spatial and temporal scales require experimentation. This will help address questions of biomass production and plant resistance and robustness in response to stress, as well as the consistency of symbiotum performance following application. To address these concerns, research has and is being funded by a variety of entities, including the U.S. Department of Energy's Office of Science and the U.S. Department of Agriculture (Tuskan & Walsh 2001, Lammers *et al.* 2004, Hong *et al.* 2012, Kim *et al.* 2012, Weston *et al.* 2012).

Summary

During the SBC, participants described general issues of biofeedstock performance and grower adoption of microbial products aimed at increasing plant yield. Biofeedstocks are typified by low resource requirements and relatively high annual production, which is good for a variety of reasons, including reducing production barriers by reducing the cost of inputs required to maintain yields (McLaughlin & Kszos 2005, Tilman *et al.* 2006). To facilitate grower adoption and optimize yields, ideal cultivars will be able to produce high seedling germination percentages (Vassey *et al.* 1985, Ghimire *et al.* 2009), withstand pressures of disease and herbivory, maintain average yields despite the vicissitudes of weather and climate—and thus be resilient in the face of drought, extreme heat, and other abiotic pressures. Cultivation and genetic modification are effective means of maintaining average yields by addressing this list of challenges (Gould 1988, Palloix *et al.* 2009, Guan *et al.* 2010, Spiertz 2010).

Cultivation and genetic engineering are not the only solutions, and they come with a suite of long-term issues (Matson *et al.* 1997, Whitham & Slobodchikoff 1981, Pink 2002), including negative public perception of genetically modified organisms, which leads to legislative prohibition from the European Union (ec.europa.eu/food/food/biotechnology/index_en.htm) and public rejection in the United States (Frewer *et al.* 2013). Breeding cultivars for specific phenotypes is limited by the number of phenotypes that can be simultaneously expressed, as well as the potential tradeoffs in expression of one phenotype at the expense of another.

There is abundant research spanning several decades documenting the benefits of numerous types of microbes to plants (as reviewed in Rodriguez *et al.* 2009 and Mei & Flynn 2010). These include—but are not limited to—N-fixing bacteria, mycorrhizal uptake of P, herbivore resistance via microbial alkaloid production, and increased resistance to a plethora of stresses through increased oxidative stress tolerance (Bush *et al.* 1997, Lazarovits *et al.* 1997, Compant *et al.* 2005, Newman *et al.* 2005, Rodriguez *et al.* 2008, Tanaka *et al.* 2008, Kiers *et al.* 2011, as reviewed by Hamilton *et al.* 2012). Inclusion of microbial symbionts in precision agriculture strategies to address abiotic stress is supported by decades of research and new private companies coming online. Provided the species/strain of microbe is not a genetically modified organism, these microbial symbionts are ubiquitous organisms with a global distribution and no regulatory limits. What is clear from the conference discussions is that no panacea exists, and all technologies come with potential costs. A combination of research exploring the impact of microbial products currently available with biofeedstocks bred for increased yield is warranted. Exploration of combined effects of employing commercial microbial products with classic breeding warrants research to determine how biofeedstock yields will respond to identified mutualists. Utilization of the abundant literature on mutualistic symbiotum in various crop and turf crops is recommended to inform and facilitate production of commercial products specific to biofeedstock production, both now and in a future potentially dominated by extreme climatic events.

Appendix A: Definitions

Word	Definition	Example
Abiotic	Non-biological	Soil density, type of rock, ambient temperature
Biofeedstock	A terrestrial, bioenergy crop	Short-rotation woody species, switchgrass
Biotic	Biological	Plants, pollinators, microbes
Dicot	Broad taxonomic group of plants producing two leaves at seedling stage and representative of ancient divergence	Deciduous trees, flowering perennials
Marginal Land	Land that cannot produce food due to poor-quality soils, toxic soils, and inaccessibility	Tailing piles from mining activities
Monocots	Broad taxonomic group of plants producing a single leave upon germination	Grasses, palm trees
Genotype	The genetic information distinct to an individual resulting from sexual or asexual recombination or pseudo-recombination of DNA (inherited)	A single organism's set of instructions
Phenotype	The visible manifestation of the genotype	Expressed characters, such as growth rate, disease resistance, morphology, metabolic rate, etc.
Plant Performance	Including, but not limited to, biomass production, seed production, stress resistance, and stress tolerance	Amount of dry weight produced in a given time t.
Taxon (Taxa)	Grouping of organism that can be broad or narrow	Plants are a taxonomic group as are flower plants, plants producing seeds, and plants in the Rose family
Symbiotum	An interaction involving 2+ organisms resulting in changes to one or all organisms involved both in terms of genotype and phenotype	Pollinators and their plant hosts, fungal pathogens and plant/animal hosts, gut microbes
Mutualisms	An interaction between a symbiotic organism mutually beneficial to both organisms	Pollinators and plants; gut microbes and mammals

Appendix B: Relevant Patents

The following table contains example patents, modified from Mei & Flinn (2010).

Patent Number	Title	Inventor(s)	Assignee	Year of Patent
US 4,996,157	Biological Control of *Phytophthora* by *Trichoderma*	Smith, V.L.; Wilcox, W.F.; Harman, G.E.	Cornell Research Foundation, Inc.	February 26, 1991
US 5,165,928	Biological Control of *Phytophthora* by Gliocladium	Smith, V.L.; Wilcox, W.F.; Harman, G.E.	Cornell Research Foundation, Inc., a corporation of NY	November 24, 1992
US 5,173,419	Purified Chitinases and Use Thereof	Harman, G.E.; Broadway, R.M.; Tronsmo, A.; Lorito, M.	Cornell Research Foundation, Inc. a corporation of NY	December 22, 1992
US 5,326,561	Antifungal Synergistic Combination of Enzyme Fungicide and Non- enzymatic Fungicide and Use Thereof	Harman, G.E.; Lorito, M. Di Pietro, A.; Hayes, C.K.	Cornell Research Foundation, Inc.	July 5, 1994
US 5,360,608	Fungicidal Compositions Comprising Chitinase and Enterobacter Cloacae, and a Method for Stimulation Proliferation of E. Cloacase	Harman, G.E.; Lorito, M.; Hayes, C.K.	Cornell Research Foundation, Inc.	November 1, 1994
US 5,378,821	Gene Encoding for Endochitinase	Harman, G.E.; Tronsmo, A.; Hayes, C.K.; Lorito, M.	Cornell Research Foundation, Inc.	January 3, 1995
US 5,474,926	N-acetyl-b-glucoaminidase Isolated from *Trichoderma Harzianum*	Harman, G.E.; Lorito, M.; DI Pietro, A.; Hayes, C.K.	Cornell Research Foundation, Inc.	December 12, 1995
US 5,723,720	Process for the Development of Endophyte-Infected Plants	Brede, A.D.; Sun, S.	J.R. Simplot Company	March 3, 1998
US 5,774,442	A Disk Transferring Device with a Single Motor for Moving a Disk Along First and Second Position and for Separating the Disk Guides When the Disk is in the Second Position	Nakamichi, N.; Harman, G.E.; Hayes, C.K.	Nakamichi Corporation; Cornell Research Foundation, Inc.	June 30, 1998
US 5,880,343	Grass and Method of Introducing Endophytic Fungi into a Grass	Hiruma, N.; Shinozaki, S.	Mayekawa Manufacturing Co. Ltd.	March 9, 1999
US 5,914,107	Method of Introducing an Endophytic Fungus into Rough Bluegrass Belonging to *Poa Trivialis* and *Poa Compressa*	Hiruma, N.; Shinozaki, S.	Mayekawa Manufacturing Co. Ltd.	June 22, 1999
US 5,994,117	Use of *Bacillus subtilis* as an Endophyte for the Control of Disease Caused by Fungi	Bacon, C.W.; Hinton, D.M.	The USA as represented by the Department of Agriculture, Washington,	November 30, 1999

US 6,020,540	Gene Encoding Endochitinase	Harman, G.E.; Tronsmo, A.; Hayes, C.K.; Lorito, M.; Klemsdal. S.	Cornell Research Foundation, Inc.	February 1, 2000
US 6,069,299	Fungus and Insect Control with Chitinolytic Enzymes	Broadway, R.M.; Harman, G.E.	Cornell Research Foundation, Inc.	May 30, 2000
US 6,072,107	Ryegrass Endophytes	Latch, G.C.M.; Christensen, M.J.; Tapper, B.A.; Easton, H.S.; Hume, D.E.; Fletcher, L.R.	New Zealand Pastoral Agriculture Research Institute Ltd.	June 6, 2000
US 6,111,170	Tall Fescue Endophytes	Latch, G.C.M.; Christensen, M.J.; Tapper, B.A.; Easton, H.S.; Hume, D.E.; Fletcher, L.R.	New Zealand Pastoral Agriculture Research Institute Ltd.	August 29, 2000
US 6,180,855 B1	Method of Introducing Endophytic Fungi into a Grass	Hiruma, N.; Shinozaki, S.	Mayekawa Manufacturing Co. Ltd.	January 30, 2001
US 6,251,390	Purified Chitinases and Use Thereof	Harman, G.E.; Broadway, R.M.; Tronsmo, A.; Lorito, M.; Hayes, C.K.; et al.	Cornell Research Foundation, Inc.	June 26, 2001
US 6,306,390 B1	Root Endophyte Having Soil Disease Inhibitory Activity, Process for Preparing Said Root Endophyte, and Method for Inhibiting soil Disease	Narisawa, K.	Ibaraki Prefecture	October 30, 2001
US 6,512,166	Combinations of Fungal Cell Wall Degrading Enzyme and Fungal Cell Membrane Affecting Compound	Harman, G.E.; Lorito, M.; Di Pietro, A.; Hayes, C.K.; Scala, F.; et al.	Cornell Research Foundation, Inc.	January 28, 2003
US 6,524,998 B1	Biological Compositions and Methods for Enhancing Plant Growth and Health and Producing Disease-Suppressive Plants	Kloepper, J.W.; Rodriguez-Kabana, R.; Kenney, D. S.	Auburn University, AL (US); Gustafson, LLC, TX (US)	February 25, 2003
US 6,548,745 B2	Italian Rye Grass and a Method of Introducing Endophytic Fungi into an Italian Rye Grass	Hiruma, N.; Shinozaki, S.	Corporate Judicial Person, Japan Grassland Farming and Forage Seed Association	April 15, 2003
US 6,748,045	Measurement of Wood/Plant Cell or Composite Material Attributes with Computer Assisted Tomography	West, D.C.; Paulus, M.J.; Tuskan, G.A.; Wimmer, R.	U.S. Department of Energy, Washington, D.C.	June, 8 2004
US 6,815,591 B1	Enhancing Endophyte in Grass	Hignight, K.W.; Rush, D.L.	Advanta Seeds B.V.	November 9, 2004
US 6,896,883 B2	Biocontrol for Plants Using *Bacillus subtilis, Pseudomonas putida, and Sporobolomyces roseus*	da Luz, W.C.; Bergstrom, G.C.	Cornell Research Foundation, Inc., Ithaca, NY (US); Embrapa Trigo (BR)	May 24, 2005
US 7,037,879 B2	Pest Control Method for Grass Family Plants Using Endophytic Bacteria, Pest Control Material, and	Imada, T.; Hiruma, N.; Isawa, T.; Noda, M.; Kurihara, Y.; Kon, M.	Society for Techno-Innovation of Agriculture, Forestry and Fisheries; Mayekawa Mfg. Co.,	May 2, 2006

	Seed Bound to the Pest Control Material		Ltd., Tokyo (JP)	
US 7,084,331 B2	Rice Containing Endophytic Bacteria and Method of Producing It	Isawa, T.; Hiruma, N.; Imada, T.; Noda, M.; Kurihara, Y.; Kon, M.	Society for Techno-Innovation of Agriculture, Forestry and Fisheries; Mayekawa Mfg. Co., Ltd., Tokyo (JP)	August 1, 2006
US 7,205,450	The DMI1 Gene Encodes a Protein That is Required for the Early Steps of Bacterial and Fungal Symbioses	Cook, D.R.; Penmetsa, R.V.; Kiss, G.B.; Ane, J-M; Denarie, J.	Institut National De La Recherche Agronomique (INR); United States Department of Energy, Washington, DC	December 17, 2003
US 7,232,565	Use of Endophytic Fungi to Treat Plants	Henson, J.M.; Sheehan, K.B.; Rodriguez, R.J.; Redman, R.S.	Montana State University; Government of the United States of America Represented by the Secretary of the Department of the Interior	June 19, 2007
US 7,259,004 B1	Endophytic Streptomycetes from Higher Plants with Biological Activity	Strobel, G.A.; Castillo, U.F.	Montana State University, Bozeman, MT (US)	August 21, 2007
US 7,465,855 B2	Non-Toxic Endophytes, Plants Injected Therewith and Methods for Injecting Plants	West, C.P.; Piper, E.L.	The Board of Trustees of the University of Arkansas	December 16, 2008
US 7,470,427 B2	Systemic Non-Nodular Endosymbiotic Nitrogen Fixation Plants	Cocking, E.	The University of Nottingham, Nottingham (GB)	December 30, 2008
US 7,498,173	Sulfur-Oxidizing Plant Growth Promoting Rhizobacteria for Enhanced Canola Performance	Banerjee, M.R.; Yesmin, L.	Brettyoung Seeds Limited	March 3, 2009
US 7,883,625	Removal of Oils from Solid Surfaces and Water with a Substance Having a High Humate Level	Harman, G.E.; Spittler, T.D.; Nielsen, S.F.; Thomas, B.P.	OSM Environmental, INC. (previously held by Terrenew, LLC)	February 8, 2011
US 7,906,313	*Curvularia* Strains and Their Use to Confer Stress Tolerance and/or Growth Enhancement in Plants	Henson, J.M.; Sheehan, K.B.; Rodriguez, R.J.; Redman, R.S.	Montana State University; Government of the United States of America Represented by the Secretary of the Department of the Interior	March 15, 2011
US 8,524,224	Methods of Using *Curvularia* Strains to Confer Stress Tolerance and/or Growth Enhancement in Plants	Henson, J.M.; Sheehan, K.B.; Redman, R.S.; Rodriguez, R.J.	Montana State University; Government of the United States of America Represented by the Secretary of the Department of the Interior	September 3, 2013
US 20,020,103,083 (publication)	Promoting Deeper Root Development, Reducing Nitrogen Fertilizer Usage, Imparting Drought Resistance, and Increasing Tolerance to Adverse Soil Conditions in Plants	Harman, G.E.	Cornell Research Foundation, Inc.	August 10, 2001 (filing date)
US 20,040,261,578 (publication)	Stable self-organizing plant-organism systems for remediating polluted soils and waters	Harman, G.E.; Lynch, J.G.; Lorito, M.	University of Surrey; Cornell Research Foundation, Inc.	April 5, 2004 (filing date)

US 20,040,265,953 (publication)	Production and use of inducible enzymes from *Trichoderma* and bacteria for control of plant pests and for industrial processes	Harman, G.E.; Donzelli, B.; Deng, S.	Cornell Research Foundation, Inc.	August 10, 2001 (filing date)
US 20,080,277,351 (publication)	Remediation and Reclamation of Heavy Metals From Aqueous Liquid	Harman, G.E.; Spittler, T.D.	Cornell Research Foundation, Inc.	February 25, 2008 (filing date)
US 20,100,136,102 (publication)	Terpene-containing Compositions and Methods of Making and Using Them	Franklin, L.; Cloud, E.; Harman, G.E.; Knapp, L.; Ostroff, G.	Eden Research PLC; Cornell Center for Technology Enterprise & Commercialization ("CCTEC") - Cornell University	August 28, 2009 (filing date)
US 20,100,227,357 (publication)	Fungal Isolates and Their Use to Confer Salinity and Drought Tolerance in Plants	Redman, R.S.; Rodriguez, R.J.	Montana State University; Government of the United States of America Represented by the Secretary of the Department of the Interior	April 8, 2010 (filing date)
US 20,110,027,232 (publication)	Formulations of viable microorganisms and their methods of production and use	Harman, G.E.; Custis, D.B.	Advanced Biological Marketing Incorporated; Cornell Research Foundation, Inc.	September 7, 2006 (filing date)
US 20,110,197,640 (publication)	Regenerable Removal of Sulfur from Gaseous or Liquid Mixtures	Harman, G.E.	Cornell University	November 18, 2011 (filing date)
US 20,120,096,598 (publication)	*Trichoderma* Strains that Induce Resistance to Plant Diseases and/or Increase Plant Growth	Harman, G.E.	Cornell University	November 23, 2011 (filing date)
US 20,120,107,889 (publication)	Endophytic Yeast Strains, Methods for Ethanol and Xylitol Production, Methods for Biological Nitrogen Fixation, and a Genetic Source for Improvement of Industrial Strains	Doty, S.L.; Staley, J.; Su, M.; Vajzovic, A.; Bura, R.; Redman, R.S.; et al.	University of Washington through its Center for Commercialization	December 5, 2011 (filing date)
US 20,120,178,624 (publication)	Method for Increasing Plant Growth Using the Fungus *Trichoderma Harzianum*	Kaminskyj, S.G.W.; Basinger, J.F.; Redman, R.S.; et al.	University of Washington; University of Saskatchewan	March 13, 2012 (filing date)
US 20,130,055,635 (publication)	Plant Propagation Medium and Methods of Making and Using It	Harman, G.E.	Cornell University	November 13, 2012 (filing date)

Appendix C: Full Conference Agenda

Symbiosis Conference: Expanding Commercialization of Mutualistic Microbes to Increase Bioenergy Crop Production Agenda
Thursday, June 20–Friday, June 21, 2013
Cornell University, Ithaca, New York

Conference Thesis: Utilization of mutualistic symbionts in bioenergy crop production will decrease resource requirements, enhance economics, and address negative impacts of climate variability		
Thursday, June 20, 2013		
8:30 a.m.–9:00 a.m.	Breakfast	
Session I: Plenary Introduction, Biomass Production, and Symbiont Basics		
9:00 a.m.–9:15 a.m.	Conference and Plenary Introduction	Cyd Hamilton, *American Association Advanced Science Fellow*
9:15 a.m.– 9:50 a.m.	Biomass Production, Historical and Current	John Ferrell, *Department of Energy’s Bioenergy Technologies Office*
9:50 a.m.– 10:25 a.m.	Overview of Basic Research and Microbial Plant Symbioses	Catherine Ronning, *Department of Energy’s Office of Science*
10:25 a.m.– 10:45 a.m.	Break	
Session II: Models and Systems Biology—Stable Mutualisms ***Moderated by Dr. James White, Jr.***		
10:45 a.m.–11:20 a.m.	Defensive and Nutritional Effects of Microbiome	Dr. James White, Jr., *Rutgers University*
11:20 a.m.–11:35 a.m.	*Populus* Root-Associated Microbial Community Exploration	Dr. Dale Pelletier, *Oak Ridge National Laboratory*
11:35 a.m.–11:50 a.m.	Maximizing Mutualistic Symbioses	Jean-Michel Ané, *University of Wisconsin*
11:50 a.m.–12:05 p.m.	Introgression, Yield, Nutrient Use, and Resistance	Michael Grisham, *U.S. Department of Agriculture*
12:05 p.m.–1:30 p.m.	Lunch	
Session II: Models and Systems Biology—Stable Mutualisms (continued) ***Moderated by Dr. James White, Jr.***		
1:30 p.m.–1:45 p.m.	Nutrient Movement within Complex Symbiont Systems	Dan Moebus-Clune, *Cornell University*
1:45 p.m.–2:00 p.m.	Mycorrhizal Effects on Nutrient Efficiency and Yield	Heike Bücking, *South Dakota State University*
2:00 p.m.–2:15 p.m.	Improving Colonization by Mutualists	Gerald Tuskan, *Oak Ridge National Laboratory*
2:15 p.m.–2:30 p.m.	Plant Breeding and Disease Resistance	Hilary Mayton, *Rutgers University*
2:30 p.m.–3:00 p.m.	Break	
Session III: Agency Perspective–Policy, Regulation, and Sustainability ***Moderated by Dr. Cyd E. Hamilton***		
3:00 p.m.–3:35 p.m.	Biomass Feedstocks at Low Cost and High Quality	Bryce Stokes, *Department of Energy’s CNJV*

3:35 p.m.–3:50 p.m.	Low-Input Production Systems on Marginal Land	Mei Chuansheng, *IALR*
Session IV: Commercialization—Barriers and Strategies to Move from Greenhouse to Field Scale ***Moderated by Dr. Cyd E. Hamilton***		
3:50 p.m.–4:20 p.m.	Why and How to Make Endophytes Commercially Accessible	Gary Harman
4:20 p.m.–4:50 p.m.	Exploring the Plant Microbiome and Realizing its Potential	Geoffrey von Maltzahn, *Flagship Ventures*
Friday, June 21, 2013		
8:30 a.m.–9:00 a.m.	Breakfast	
Session V: Discussions and Brainstorming		
9:00 a.m.–9:15 a.m. *Moderated by Cyd E. Hamilton*	**Summary and Report Outline**	
9:15 a.m.–10:45 a.m. *Moderated by James White, Jr.*	**Plant Characteristics Integral to Bioenergy Crop Production and Management**	
How can incorporation of symbionts enhance bioenergy crop yields? Is there a way symbionts can alter management of energy crops to facilitate maintenance of high community biodiversity, reduce soil erosion, increase GHG sequestration, reduce GHG emission, etc.? Are there plant characteristics altered due to mutualistic symbiosis possibly of concern in terms of bioenergy crop characteristics e.g., ash production or water content?		
10:45 a.m.–12:15 p.m. *Moderated by James White, Jr.*	**Systems Biology, Genomics Tools... Integral to Ensuring Stable Mutualistic Outcome**	
What have we learned about stability of mutualisms? What about potential resiliency of symbiotum when exposed to climate variability? Does stability or resiliency of symbiotum yield rely on multiple or dominant microbial taxa? If we don't have these answers do we need them to increase scale of commercialization? What technologies, collaborations, approaches can be used to address issues in a timely manner?		
12:15 p.m.–1:15 p.m.	Lunch	
Session V: Discussion (continued)		
1:15 p.m.–2:45 p.m. *Moderated by Cyd E. Hamilton*	**Addressing Environmental Sustainability of Bioenergy Crop Production via the Employment of Microbial Mutualists**	
Overall, what are the primary concerns about utilizing mutualistic symbionts from an ecological sustainability perspective? What are the indices and criteria used by agencies (DOE, BETO) to define sustainable? Is this different from what we see in policy such as the RFS2 and if so is it a concern for bioenergy crop production? Questions of land use change (LUC), indirect land use change (ILUC), tillage, soil phenomena, trophic dynamics, etc. Can we increase GHG storage and decrease GHGe via mutualistic symbionts in bioenergy crops?		
2:45 p.m.–3:15 p.m.	Break	
Session V: Discussion (continued)		
3:15 p.m.–4:45 p.m. *Moderated by Gary Harman*	**Successes and Challenges to Large Scale Commercialization of Mutualistic Symbionts for Bioenergy Crop Production**	
What have individuals and companies done well when commercializing these or similar products? What were the failures and why? Based on what we learned yesterday and the knowledge we came with, what are novel challenges? What are the potential challenges for end users? Based on end-user needs, what can extension specialists provide in terms of education and connecting diverse stakeholders (information dissemination)? What collaborations are needed for commercial success? How are collaborations maintained to reach the commercial goal?		
4:45 p.m.–5:30 p.m.	**Prepare Report Outline and Solicit Names for Suggested Authors**	

Appendix D: Speaker and Attendee List

For more information about the speakers, including presentation titles and summaries, see the Speaker and Attendee List (http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_speaker_agenda.pdf).

First Name	Last Name	Organization
Speakers		
Jean-Michel	Ané	University of Wisconsin
Heike	Bücking	South Dakota State University
Mei	Chuansheng	Institute for Advanced Learning Research
John	Ferrell	U.S. Department of Energy, Bioenergy Technologies Office
Michael	Grisham	U.S. Department of Agriculture, Agricultural Research Service
Cyd	Hamilton	U.S. Department of Energy, AAAS Fellow
Gary	Harmon	(formerly) Cornell University
Hilary	Mayton	Rutgers University
Dan	Moebus-Clune	Cornell University
Dale	Pelletier	Oak Ridge National Laboratory
Catherine	Ronning	U.S. Department of Energy, Office of Science
Bryce	Stokes	CNJV, LLC
Gerald	Tuskan	Oak Ridge National Laboratory
Geoffrey	von Maltzahn	VentureLabs
James	White	Rutgers University
Attendees		
Ibrahim	Aikawa	University of Wolverhampton, UK
Vanessa	Bailey	Pacific Northwest National Laboratory
David	Benham	General Services Administration
David	Culley	Pacific Northwest National Laboratory
James	Hannasch	AZDEMA AZARNG CFMO
Steve	Lewis	Innovation, POET
Christine	McKiernan	Bioferm Energy Systems
Manan	Parikh	American Council on Renewables
Charlie	Tang	eGEN Technology Corporation, Taiwan
Reda	Wahb	Egypt
Peter	Woodbury	Department of Crop and Soil Sciences, Cornell University

Appendix E: Conference Presentations

- Symbiosis: Addressing Biomass Production Challenges and Climate Change, *Cyd Hamilton, Bioenergy Technologies Office*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_hamilton.pdf

- The Future of Bioenergy Feedstock Production, *John Ferrell, Bioenergy Technologies Office*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_ferrell.pdf

- Plant-Microbe Interactions: An Overview of Basic Research Supported by the DOE Office of Science, *Catherine Ronning*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_ronning.pdf

- Enhancing Plant Growth and Stress Tolerance through Use of Fungi and Bacteria that Comprise Plant Microbiomes, *Jim White*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_white.pdf

- Potential of Diazorphic, Endophytic Bacteria Associated with Sugarcane for Energycane Production, *Michael Grisham*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_grisham.pdf

- Arbuscular Myrcorrhizal Interactions—An Important Trait for Biomass Production of Bioenergy Crops, *Heike Bucking*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_bucking.pdf

- Fungal Diversity within the Populus Rhizosphere and Endosphere, *Gerald Tuskan*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_tuskan.pdf

- Importance of Biomass Production and Supply, *Bryce Stokes, Bioenergy Technologies Office*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_stokes.pdf

- Developing a Low Input and Sustainable Switchgrass Feedstock Production System Utilizing Beneficial Bacterial Endophytes, *Chuansheng Mei*
  http://www1.eere.energy.gov/bioenergy/pdfs/symbiosis_conference_mei.pdf

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