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NEWS AND NOTES

National Research Initiative: Animal Protection Funding Opportunity

The USDA CSREES National Research Initiative (NRI) announces a funding opportunity as part of their competitive grants program. The FY 2006 NRI RFA provides funding opportunities for 32 programs, organized in the RFA within the following 5 Program Clusters: **Agricultural Genomics**; **Agricultural Biosecurity**; **Agricultural Production and Value Added Processing**; **Nutrition, Obesity, Food Safety, and Quality**; and **Agroecosystems**. The priorities for research projects and/or integrated research, education, and extension activities are listed within each program description. The maximum award size for research projects or integrated research, education, and extension activities varies for each program. Please read the RFA for additional details: **http://www.csrees.usda.gov/funding/rfas/nri_rfa.html.** Research and integrated proposals must be received by 5:00 p.m. Eastern time on **December 15, 2005**.

The Animal Well-Being section of the Animal Protection Program (44.0) invites research and integrated research, education, and extension activities to: (a) develop science-based criteria to improve measurements of well-being, including pain, stress, fear, and behavioral needs; and the assessment of how these conditions impact animal well-being; (b) determine the impact of alternative management practices on animal well-being and food quality, including housing, handling, transportation and harvest; and (c) **assess the behavior and well-being of genetically modified food animals**. Questions may be directed to Dr. Peter Brayton, National Program Leader (pbrayton@csrees.usda.gov).

RISK ASSESSMENT NEWS

Found-and-Lost: Transgenic Maize in Oaxaca, Mexico

Kelly M. Paulson

Since the advent of transgenic maize (*Zea mays* L.) in the United States and Canada in 1996, gene flow to other, non-transgenic varieties of corn has been of concern to scientists, farmers, and the public. However, the concern about gene flow to landraces of maize in Mexico intersects with a suite of cultural, historical, and spiritual connotations.[1] The extraordinary significance of maize in Mexico is at least part of the reason that the country enacted a *de facto* moratorium on the commercial planting of genetically engineered corn varieties in 1998,[2] in spite of permitting transgenic varieties of other crops like soya and

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cotton. Perhaps this is why Quist and Chapela's November 2001[2] revelation that gene flow from transgenic corn had occurred in Oaxaca, Mexico, came as such a surprise. The controversy that was sparked by this paper continues to simmer, but most scientists agree[1] with the major conclusion of Quist and Chapela's research[2,3]: transgenes were present in native landraces of maize in Oaxaca. However, the Quist and Chapela study was not designed to detect frequency, only presence. The most recent contribution to this story, by Ortiz-García et al.,[4] represents the first published attempt to quantify the *frequency* of transgenes in the same region (personal communication, Dr. Allison A. Snow (AAS), 07 October 2005).

Ortiz-García *et al*.[4] collected corn cobs and seeds from eighteen different villages ("locations") during November-December 2003 and 2004 (**Figure 1**). At each location, from one to eight different fields were sampled. In 2003, four to five maternal plants were selected haphazardly within each field meaning that they were distributed throughout the field and not growing close to one another (pers. comm., AAS, 07 October 2005). In 2004, five "normal" and five "stressed" maternal plants were selected from each field, on the basis of the assumption that early hybrids between transgenic and locally adapted maize plants might appear maladapted to the local field conditions. In both years, one cob was collected from each maternal plant, and kernels were taken from each cob for analysis (a range of 104-503 seeds per cob in 2003 (pers. comm., AAS, 10 October 2005)).

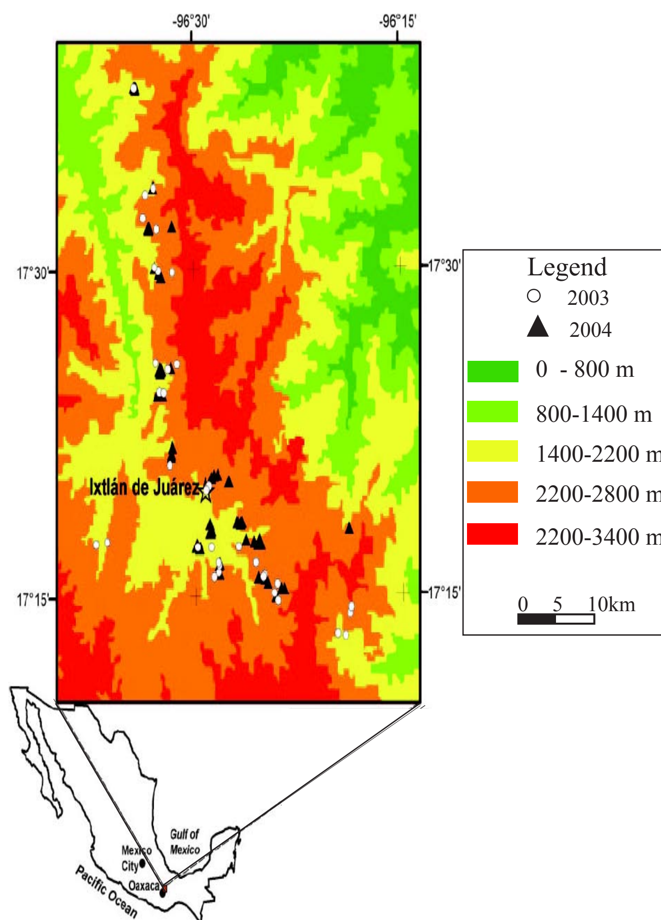

Figure 1. Nested sampling regime used by Ortiz-García *et al*. [Adapted from Figure 1 (p. 12340), Ortiz-García S, Ezcurra E, Schoel B, Acevedo F, Soberón J & Snow AA (2005) *PNAS* **102**, 12338-12343. Copyright 2005 National Academy of Sciences, U.S.A)

Seeds from 2003 were analyzed by Genetic ID (www.genetic-id.com) and seeds from 2004 were divided evenly between Genetic ID, GeneScan (www.gmotesting.com), and an archive in Mexico. Both of the labs are capable of detecting transgenes at a frequency of 0.0001 (i.e., one transgenic seed in a homogenized sample of 10,000 seeds) with nearly 100% accuracy. In 2003 the researchers chose the conservative strategy of delivering ground samples representing ≤503[a] seeds each, while in 2004 the sample size was 810 to 5,630 seeds per ground sample. Both laboratories used two markers to probe for transgenic DNA in the samples: the CaMV 35S (cauliflower mosaic virus) promoter and the NOS (nopaline synthase, from *Agrobacterium tumefaciens*) terminator sequence. The CaMV 35S sequence is present in all varieties of commercialized transgenic corn, with the exception of the GA21 Roundup-Ready® event. The NOS terminator sequence, however, is present in the GA21 corn and in several other varieties of transgenic corn. In addition, the *adh1* gene, which is native to maize, was amplified as a positive control. Using a combination of quantitative and qualitative PCR techniques, all samples were scored negative for both transgenic markers.[4] The possibility that transgenic seeds were sampled but were undetected by the PCR analysis is unlikely because both companies use proper controls designed to avoid false negatives as well as false positives, in compliance with international seed-testing standards.[4]

2003			2004		
Total number of fields	Total number of maternal plants sampled	Total number of seeds analyzed	Total number of fields	Total number of maternal plants sampled	Total number of seeds analyzed
43	164	50,126	81	706	103,620[b]

Table 1. Sample sizes of fields, maternal plants (i.e., cobs), and kernels in 2003 and 2004 [Adapted from Table 1 (p. 12341), Ortiz-García S, Ezcurra E, Schoel B, Acevedo F, Soberón J & Snow AA (2005) *PNAS* **102**, 12338-12343. Copyright 2005 National Academy of Sciences, U.S.A)

Given the previous reports of the discovery of transgenes in this same region of Mexico (e.g.,[2, 1]), one might wonder how likely it is that these researchers simply "missed" the transgenic kernels. Handily, Ortiz-García *et al*.[4] address this very question in two different ways: using the kernel, then the cob, as the unit of observation. Understanding the reasoning for these two alternative analyses requires a brief lesson on corn reproduction: a single cob on a maternal maize plant can contain several hundred seeds. Theoretically, each kernel on a plant could have a different paternity, subject to the diversity of pollen donors growing nearby.[5] While corn is able to self-pollinate, most of the kernels on a cob are the result of outcrossing.[6] In other words, it is unlikely that all the kernels on a cob have the same paternity, but it is also unlikely that no two kernels are full siblings, either because they are derived from self-pollination or from the same paternal plant's pollen. The proportion of kernels on a maternal plant in a field situation that are full sibs does not seem to have been investigated.

First, the authors calculated the binomial probability that they missed the transgenic elements, assuming that transgenes *did* exist at a frequency q of 0.0001. The use of such a low frequency for these analyses was a conservative strategy, especially considering the 2003 genetic analysis was designed for the possibility that transgenes were in excess of 5% in some of the fields.[4] Using the hypothetical underlying frequency, the joint probability of missing all transgenic seeds in the sample from all locations in a given year can be calculated as $P_{overall}$ (0 inclusions|q = 0.0001). When each kernel was considered as an independent observation, $P_{overall}$ is equal to 0.00003 in 2004. If the maternal cob is considered as the unit of observation, however, the binomial probability of detecting no transgenes in 2004 increases to 0.932.[c] In the latter case, failing to detect any transgenic seeds, if they were actually present at the underlying frequency of 0.0001, becomes unsurprising. The range between these two estimates, from 0.003% to 93.2%, certainly makes interpretation difficult. A second calculation estimated the transgene frequency at which at least one seed ought to have been sampled with 95% certainty ($q_{0.95}$) across all locations. Ortiz-García *et al*.[4] estimated that in 2004, if kernels are the unit of observation, they could be 95% certain that transgenes were present at <0.003%. However, when the same analysis was based on cobs, they could only be 95% sure that transgenes were present at less than 0.43%. Because the real sample size is somewhere between the kernel and the cob as experimental unit, the authors conclude that transgenes are "absent or extremely rare" in the sampled fields, and that 0.01% might be a realistic mid-point estimate based on the second set of analyses, and considering data from both years.[4]

Two questions relevant to biosafety might be raised by this study. First, could one have predicted these results? Second,

how could the Ortiz-García *et al*.[4] methodology inform future experiments to monitor for transgene escape?

In January 2004, Information Systems for Biotechnology sponsored a workshop to discuss the application of the net fitness model[7] to gene flow from crop plants.[8] The net fitness model was originally developed by Muir and Howard to predict gene flow from a group of transgenic fish into a population of wild-type conspecifics. To predict population size and transgene frequency over a number of generations, the model uses six quantitative life history measurements or "net fitness traits": juvenile viability, age to sexual maturity, mating success, female fecundity, male fertility, and adult viability. Workshop participants discussed the potential for making these measurements suitable to the life history of plant species. While *Brassica spp*. and cotton were suggested as useful test cases for the model's application to crop species,[8] thinking about transgenic corn varieties through the lens of the model may provide a useful indicator of what data is lacking. For example, Ortiz-García *et al*.[4] assumed that transgenic-landrace hybrids would appear "stressed" in the Mexican fields they sampled. While this assumption was based on sound reasoning, a study to characterize relevant performance traits of landraces and commercial cultivars in the environment of interest would be useful for future studies such as this one. Further, more information on the fitness of F_1 and advanced-generation hybrids between transgenic and local varieties could give scientists a better understanding of the likelihood that transgenes would persist, or spread, in the years following a hybridization event. If there were particular agronomic traits that distinguished hybrid from landrace plants, small-scale farmers could select or deselect them from their fields (pers. comm., AAS, 07 October 2005). If the challenge posed by measuring life history traits on corn plants is not enough, imagine modeling human behavior as a component of the potential for transgene flow and dispersal.

In any large-scale environmental release of a transgenic plant or animal, it is important to have a monitoring plan. This study raises several issues relevant to the design of monitoring experiments: (1) site selection; (2) sample size determination; (3) sampling methodology; and (4) detection of transgenic elements. What decisions to make regarding these issues will vary depending on the investigator's specific objectives. First, while a few sites in this study were selected in the same villages as the original discovery by Quist and Chapela,[2] it is likely that different farmers' fields were sampled (pers. comm., Dr. Sol Ortiz-García, 10 October 2005). In the case of corn in which most pollen settles within 100 m of the source plant,[5] it may be important to design studies where site selection is informed by the location of previous discoveries. However, crop rotation regimes and seed exchange will make site selection difficult in managed cropping systems. Next, Ortiz-García *et al*.[4] provided two analyses using the cob and the kernel as the unit of observation. Given the current understanding of relatedness of kernels on a single cob, their strategy of providing an upper and lower bound for n is warranted. However, using their 2004 data for illustration, we know that neither $n = 706$ (cobs) nor $n = 103{,}620$ (kernels) is *true*. Pending further studies of the paternity of kernels on a cob, one sampling strategy is to collect fewer kernels per cob to minimize the chance that two kernels are full siblings (pers. comm., AAS, 07 October 2005) and to collect samples from a larger number of maternal plants. Of course, this entails quite a bit more fieldwork and cooperation from farmers. Related to site selection, one can also decide how to sample within the locations and fields. In this study, plants were chosen haphazardly in that they were scattered throughout the field, and some plants that appeared "stressed" were also chosen on the assumption that they would be more likely to carry a transgene. One might choose instead to select plants purely randomly, or to select individual cobs or kernels at random after they have been harvested to remove any possibility of sampling bias. On the contrary, one could intentionally sample plants on the edges of fields; for example, fields or ditches bordering roads where corn kernels in transit could have bounced free of a truck and appeared as volunteer plants. Finally, there is a need for independent, empirical testing of the limits of transgene detection at the commercial labs that perform such services (pers. comm., AAS, 07 October 2005).

Clearly, developing monitoring strategies that are scientifically robust and cost-effective will continue to challenge biosafety science as more transgenic products are released around the world. We have an opportunity to meet this challenge by building on previous research, identifying gaps in existing science, and refining methodologies in an iterative process.

Thanks to Drs. Allison Snow and Sol Ortiz-García for their input on a draft of this review.

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(Endnotes)

[a] This appeared in the original paper as 300; this correction is in press.

[b] This figure is correct in Table 1 as it was published in Ortiz-García et al.[4], but there is an error in the text where the number is written as 103,020. The larger total is correct and a correction is in press.

[c] These calculations are not reported in Ortiz-García et al.[4] These values were calculated using P(k out of n) = $[n!/k!(n-k)!](p^k)(q^{n-k})$ where p = 0.0001, q = 1-p, k = 0, and n = 706 (2004). (http://faculty.vassar.edu/lowry/VassarStats.html). This result can also be reproduced using the *pbinom* function in R (http://www.r-project.org/): i.e., pbinom(.95,706,.0001).

Employing a Composite Gene Flow Index to Numerically Quantify a Crop's Potential for Gene Flow

Ewen Mullins

Guidelines to ensure the efficient coexistence of genetically modified (GM) and non-GM crops are currently being considered across the European Union. Curtailing pollen/seed-mediated gene flow between GM and non-GM crops is central to effective coexistence. While models have been designed for specific crops,[1] traditional commentary associated with a crop's potential for gene flow would typically rank the crop as a high, medium, or low risk. By its qualitative nature, this approach does not provide the detail required to highlight those aspects of a crop's biology that will serve to challenge coexistence management. The substitution of this classification system with a numerical gene flow index would permit a background level of gene flow, specific for a particular crop, to be calculated. In turn, this would underscore those crops that require additional measures when genetically modified, in order to minimize gene flow in accordance with anticipated coexistence guidelines.

The concept of a gene flow index or botanical file is not new[2] and their potential as tools to assist risk assessment strategies has already been suggested.[3] However, present systems fall short by not encompassing all modes of gene flow that are of relevance to coexistence. Here we present a gene flow index (GFI)[4] model that we have applied to seven conventional crops in Ireland. By combining four strands of gene flow—crop pollen-to-wild relative (CPW); crop pollen-to-crop (CPC); crop seed-to-volunteer (CSV); and crop seed-to-feral (CSF)—we have established a baseline data set that describes the potential of Ireland's primary arable crops for both pollen- and seed-mediated gene flow.

Approach

Information inputted into the model was collated from a broad literature base, and only information that pertained to systems comparable to the Irish agricultural and geographical environment was used. The calculated GFI value pertains to the propensity of each crop to form viable hybrid/volunteer/feral individuals. A clear distinction was made between the volunteer and feral niches by differentiating between the ability of a plant to grow within/outside a managed crop system, respectively. Responsive to regional parameters, we applied the model to sugar beet, oilseed rape, potato, perennial ryegrass, maize, wheat, and barley. Alternative pathways for gene flow (e.g., wild, volunteer, or feral originating pollen to a related crop) were not considered.

For all four strands the decisive factor for successful gene flow was deemed to be the establishment of a viable, reproducing hybrid/volunteer/feral individual, without which the introgression/gene spread exposure element of any future GM crop risk assessment could not occur. By restricting the analysis to just the dispersal and preliminary stage of establishing a viable individual/population, it is accepted that the model excludes the issue of hybrid/feral competitive ability. It does, however, provide an initial data set that will quantify the propensity of a conventional crop to spread its genetic material.

Retaining a simple format (**Table 1**), each of the four strands (CPW, CPC, CSV, CSF) contains several sequential questions, with each question designed to provide a 'yes/no' answer, which in turn equates to a relevant score.[4] By following this linked progression, when a question incurs an answer with a zero value, that strand automatically records a total value of zero, as no gene flow can take place for the specified crop under the selected criterion. The adoption of this worst-case scenario approach was intentional and maintains the practicality of the model by encompassing real-life factors that, while not desired, will occur all the same—for example the occurrence of bolters in a sugar beet crop.

Table 1. Gene Flow Index (GFI) model describing the propensity for successful pollen and/or seed-mediated gene flow through strand CPW, CPC, CSV and CSF.

Strand	Question	Score
CPW	**Propensity for successful pollen-mediated gene flow between the crop and wild relatives**	
CPW1	Do interfertile wild relatives of this crop exist in Ireland?	0/1
CPW2	Is there a probability that the crop will flower and produce viable pollen during its cultivation?	0/1
CPW3	Upon flowering, is 95% of the crop pollen deposited within 1m (1), 10m (2), 50m (3), 100m (4), 250m (5) or 500m (6)?	1/2/3/4/5/6
CPW4	If flowering does occur is the wild relative in question rated as an obligate inbreeder (0), a partial inbreeder/outbreeder (1) or an obligate outbreeder (2)?	0/1/2
CPW5	If fertilization is achieved by the deposited pollen, will a viable F_1 hybrid individual establish itself?	0/1

Strand	Question	Score
CPC	**Propensity for successful pollen-mediated gene flow between the crop and related commercial varieties**	
CPC1	Is there a probability that the crop will flower and produce viable pollen during its cultivation?	0/1
CPC2	Upon flowering, is 95% of the crop pollen deposited within 1m (1), 10m (2), 50m (3), 100m (4), 250m (5) or 500m (6)?	1/2/3/4/5/6
CPC3	If flowering does occur is the receptive crop rated as an obligate inbreeder (0), a partial inbreeder/outbreeder (1) or an obligate outbreeder (2)?	0/1/2
CPC4	If fertilization is achieved by the deposited pollen, will a viable F_1 individual establish itself from the hybrid seed in the absence of mechanical/chemical control?	0/1

Strand	Question	Score
CSV	**Propensity for successful seed-mediated* gene flow from commercial crop to volunteer**	
CSV1	Does the crop produce seed during its cultivation?	0/1
CSV2	Post-harvest, will the seed survive and germinate within the confines of a managed field?	0/1
CSV3	Will the volunteer develop into a viable individual?	0/1

Strand	Question	Score
CSF	**Propensity for successful seed-mediated* gene flow from commercial crop to feral**	
CSF1	Does the crop produce seed during its cultivation?	0/1
CSF2	Following transfer from the site of cultivation will wayward seed survive and germinate?	0/1
CSF3	Will the resulting individuals establish into a viable feral population?	0/1

* 'Seed-mediated' encompasses both flower originating seed and root derived tubers

Outcome

A composite GFI value for each crop was calculated from which the gene flow potential of both ryegrass and sugar beet (**Fig. 1**) was clear (GFI=25/27). The justification for such a value is supported by the fact that both species co-exist in Ireland with inter-fertile wild relatives, both can disperse their pollen over large distances, and the initiation of feral populations from each species is a reality. Importantly, the high GFI value for conventional sugar beet does not necessarily advocate the non-cultivation of GM sugar beet in Ireland. Conversely, it underlines the importance of bolter control in the effective coexistence of GM and non-GM sugar beet. This point is clear when a readjusted model utilizes the sugar beet data for a management system that assumes stringent bolter control. In this scenario, GFI = 6 where the potential for gene-flow is minimized to the establishment of volunteer and feral populations from harvested tuber fragments. The potential for pollen and seed-mediated gene flow in potato (GFI = 11/27) is related to combined tuber and true potato seed (TPS) production. When recalculated for districts where potato production is strictly for tuber production, the GFI = 6/27. Both wheat and barley recorded low indices (GFI = 8/27 for each), in contrast to oilseed rape, which confirmed its ability to disperse its genetic material with a GFI = 19 (**Fig. 1**). Cultivation of maize in Ireland is solely for animal forage. Coupled with an absence of wild relatives, the gene flow potential (GFI = 9/27) for maize is limited to pollen-mediated crop-to-crop and seed-mediated crop-to-volunteer (**Fig. 1**).

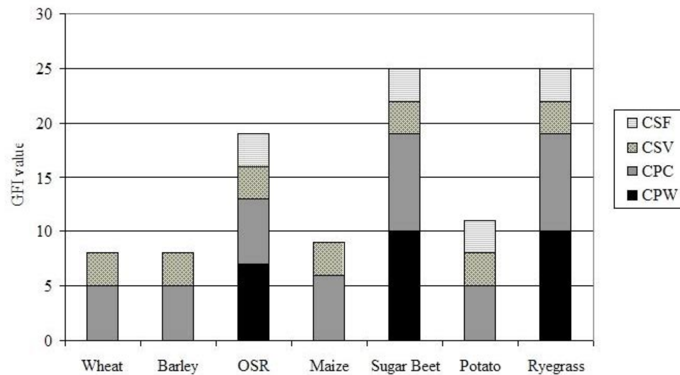

Figure 1. Graphical representation of combined pollen and seed-mediated gene flow for wheat, barley, oilseed rape (OSR), maize, sugar beets, potato, and ryegrass. GFI values attained from strands CSF, CSV, CPC, and CPW (see text).

Discussion

Ecologically, the consequence of gene flow from a GM crop is wholly dependent upon the physiological impact of the transgene and must be addressed on a case-by-case basis. In contrast, the potential for gene flow is primarily reliant upon the reproductive biology of the crop (be it GM or non-GM) and this can be addressed by calculating a crop's GFI value. In this research, several conventional crops (oilseed rape, ryegrass, and sugar beet) attained a high GFI value. Importantly, it must not be implied from this result that these crops are not suitable for GM development. Similarly, for the crops that scored low GFI values, this does not imply gene flow will not occur. Rather a high GFI score implies that a specific crop/variety possesses a higher propensity for gene flow and thus requires greater management precautions if efficient coexistence is to be attained. Conversely, a low GFI value indicates a crop which should not pose a significant challenge to the implementation of a coexistence strategy.

Notably, our work has highlighted several coexistence-based questions that require further research and which should be addressed prior to the commercialization of GM crops in Ireland. Specifically, the potential for seed-mediated gene flow requires attention, for, due to a scarcity of research data, we were limited in the number of questions we could ask in regard to the efficacy of seed-mediated gene flow for each crop. This contrasts with pollen-mediated gene flow (strand CPW and CPC) for which a substantial research data set is available. The role of volunteers as potential 'genetic bridges', facilitating the transfer of genetic material from crop-to-wild/crop-to-crop/wild-to-crop, is also of particular concern, especially as it would be naive to assume that total volunteer control will be achieved in a coexistence-based management system.

From a non-scientific perspective, it is hoped that the GFI ranking scheme will increase the public's understanding of 'gene flow', an issue central to the GM debate. Within the scientific community, it is hoped that the described index will revive discussion on the merits of gene flow indices; specifically in regard to the feasibility of establishing a collective GM crop risk index that encompasses not only a crop's propensity for gene flow but also the elements that contribute to invasiveness, changes in genetic diversity, and broader ecological disturbance.

Acknowledgements
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Plant Research News

Tailoring Chemically-induced Gene Switches for Research and Agriculture

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Transgenic technologies enable the generation of primary, protein, or secondary, metabolite or trait, gene products. When expressed constitutively some gene products are deleterious to general plant growth whilst others are unstable. These problems could be alleviated, with associated improvements in yield, by the use of gene switches. Conditional gene expression systems allow a given gene to be turned on or off at will and, in the research lab, have become important tools for analyzing gene function. Outside the lab, gene switches also have great potential. Plant growth habit or developmental stage could be controlled—for instance, if flowering or fruit ripening was subject to direct agronomic control, the range of many crops could be increased. One of the factors

limiting the development of this technology is the nature of the chemicals used as part of the switch. These chemicals can be directly toxic to humans or could have undesirable ecological effects. Others are very expensive. The *alc* gene switch seems to be an exception, being regulated with low amounts of a cheap, non-toxic, biodegradable chemical inducer, namely ethanol.

Originally derived from *Aspergillus nidulans*, the ethanol switch (*alc* system) is a two-component, chemically inducible gene expression system, consisting of the ALCR transcription factor and the *alcA* promoter. In *Aspergillus* the alcR protein coordinates the expression of a number of genes concerned with the metabolism of ethanol. Two simple adaptations rendered the *alc* system useful in plants. First, the *alcR* gene was driven by the cauliflower mosaic virus (*CaMV*) *35S* promoter, and second, a minimal *CaMV 35S* transcriptional element was added to the *alcA* promoter.[1]

The ethanol switch has since been successfully used for research purposes in a wide range of crop plant species, including tobacco,[1] oilseed rape,[2] potato,[2] tomato,[3] and poplar (wwwdata.forestry.oregonstate.edu/tgbb/sfiles/AlcMSdraft1.pdf). It has also been used widely in the non-crop model species *Arabidopsis*.[4] Its versatility is indicated by the diverse set of biological processes that have been placed under ethanol control: flowering,[5] cell division, and metabolic[1] and hormone manipulations[6] in plant development. Inducible transgene expression can often avoid secondary, and often undesirable, traits that are associated with continuous expression. For example, constitutive overexpression of the "green revolution" dominant negative *gai* mutant gene, a putative transcriptional regulator, resulted in both dwarfism (desired) and lowered seed yield (not desired). However, modulating the expression of *gai* in *Arabidopsis* with the ethanol switch produced an 83% reduction of plant height with no associated loss of seed yield.[6]

Recently, modifications have been made to the original plant-adapted *alc* system that has increased its versatility. The first improvement addressed whether the spatial expression of the *alc* system-controlled genes could be tailored to specific tissues/organs. Using the original *alc* system, it has been shown that gene expression can be limited to single leaves by restricting the application of ethanol using polyethylene bags[7]. There are many potential applications where it might be desirable to spatially restrict gene expression—for example, directing the production of defensive compounds to defined tissues and so reduce the impact on non-target organisms—but agronomic practice usually entails treatment of the entire crop. By the simple expedient of replacing the viral 35S promoter with a suitable tissue-specific promoter, the alcR transcription factor can be restricted to almost any particular tissue, enabling gene expression to be also restricted. This phenomenon has been reported in lab-based experiments using *Arabidopsis* shoot and floral intra-meristem domain-specific promoters, in conjunction with the *alc* system, to express genes that alter shoot and floral architecture.[8,9] More closely relevant to crops, carbon metabolism-manipulating experiments in potato tuber demonstrated the efficacy of the B33 patatin promoter-driven *alc* system.[10]

A slightly different approach has opened up a large range of spatially restricted gene expressers in *Arabidopsis* for use with the *alc* system. In this newly modified *alc* system, the advantages of a two-component gene trap reporter system have been coupled to the *alc* system. This was achieved by again replacing the alcR promoter, this time with the same promoter as utilized by the gene trap reporter. In our hands, this system works very well, even for restricted gene expression in the endosperm (Sakvarelidze, Roberts, Doonan and Rawsthorne; unpublished).

One possible drawback of the original switch is that many organisms, under certain conditions, can produce inductive compounds that lead to spontaneous expression. Water logging, a common occurrence in nature, is particularly effective in this regard, and added ethanol is not required for full and continuous gene expression. In addition, the practice of tissue culture, which is often used in plant transformation and cell suspension culture experiments, can also lead to spontaneous induction. This characteristic limits the utility of the *alc* system in the lab and may also impose limits in the field. However, the chemical specificity of the switch is easily altered. Primarily for research purposes, we have fused the GR domain from the glucocorticoid receptor to the C-terminus of the alcR protein and thus added dexamethasone dependency onto the switch.[11] This modified switch (the *alc-GR* system) has been tested successfully in driving regulated expression in tobacco Bright Yellow 2 (BY2) tissue culture, where previously the old *alc* system could only unconditionally express genes. Tissue cultures are particularly useful for the analysis of cell cycle activities. The conditional expression of an *Antirrhinum* Cyclin D1 in BY2 suspension cells, using the *alc-GR* system, exploited the BY2's cell cycle assay strengths.[12] These experiments revealed that Cyclin D1 accelerates the cell cycle by, unexpectedly, enhancing many cell cycle phases. Thus, the

alc-GR system is a useful addition to the existing plant *alc* systems because it allows the effects of transgene expression to be quickly tested in tissue culture before or in parallel to the generation of equivalent whole plant transgenics.

It is unlikely that many, if any, of the current chemically-inducible gene switches employ chemically-inducible transcription factors that exclusively target the transgene target promoter. An extreme consequence of this is the manifestation of gene switch-specific deleterious plant traits[13]—whereby, presumably, the activated gene switch transcription factor interferes with the host's gene expression, in addition to performing its (desirable) gene switch function. In the longer term, this may be alleviated by replacing the current gene switch transcription factor DNA binding domains with DNA binding domains of proteins displaying greater fidelity towards their particular targets. We have demonstrated that the chemical specificity of the alcR protein can be easily altered, and one can foresee the further development of novel modified switches with particular chemical requirements and greater target specificity.

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ENVIRONMENTAL NEWS

Biotechnology as a Key Driver for Sustainable Bioenergy Production

James McLaren

The idea that bioenergy will become the white knight of the 21st century is intuitively attractive, and receives much press, across a broad range of political and social agendas. However, on a detailed development level it remains unclear how bioenergy will allow a sustainable platform for continued world economic growth. Einstein said that "problems cannot be solved by the same level of thinking that created them"—solutions to the energy crisis will require different ways of thinking. Shifting from a petro-driven economic base to a bio-based foundation is a significant challenge and success will require more than just "substitution" strategies. There is a need to clearly understand the magnitude of the problem, to accept that new breakthroughs in technology applications are required for any chance of success, and to acknowledge that acceptance of dramatic change is probably required before we can begin to build a more sustainable future.

The problem is straightforward and can be quantified with reasonable accuracy. The world currently utilizes 420 quads/year (quad = 10^{15} Btu) and the conservative case projection is that within 30 years the world requirement will be 650 quads/year, largely due to economic development in India and China. While energy demand is growing rapidly, fossil fuel reserves are finite. In addition, if the current global temperature elevation is even partly related to anthropogenic gas emissions then what will happen during the projected massive increase in the use of fossil fuels? A conceptually attractive feature of bioenergy is that carbon dioxide release will be at least neutral due to carbon recycling on a relatively short time-scale.

Currently, bioenergy and bio-based inputs account for less than 5% of all basic inputs to the existing Western economy. While several government-industry initiatives[1] have highlighted the issues and challenges, and some companies have also taken steps to embrace the emerging bio-industry, the pace of change may be too slow. Moving from 5% of inputs to >50% of inputs in less than 20 years is a "moon-shot" type of challenge.

Current Situation

First, it is important to define what "biomass" really means—while there are several meanings being associated with this word, for the purposes of this article biomass is taken as

any output from primary production (i.e., plant materials). Traditional biomass can make a useful contribution to bioenergy production and, in recent years, biofuels have been on the leading edge of developments. For example, in 2004, approximately 3.4 billion gallons of ethanol fuel were produced in the US for blending as an oxygenate in gasoline. In this commercial case, the biomass used was largely maize starch (~95%), sorghum starch (~4%), and a small amount of other crop inputs. The application of new production technologies, conventional plant breeding, and early-stage biotechnology traits, have resulted in significant yield increases (at the same level of inputs) in maize. Hence, an increasing volume of grain has been made available for conversion into ethanol with no negative impact on the feed/food segments of the market.[2]

Lignocellulose biomass has been considered as a potential feedstock for biofuels and other bioenergy[3] (e.g., gasification and the generation of electricity as well as steam). Lignocellulose is an abundant material created from solar energy in primary production. Theoretical calculations of conversion to ethanol indicate high potential to generate 25 to 50 billion gallons of ethanol per year. However, lignocellulose is a complex material (lignin, cellulose, pectin) and is not easily converted into biofuel in an economically viable manner. Consequently, progress over more than 20 years of research into conversion technologies has been disappointing in terms of creating an overall viable process for lignocellulose to ethanol.

The current use of biomass (for biofuels) is heavily focused on the development of complex conversion technologies, typically involving a fermentation step. It is only very recently that the first indications of change in the feedstock have appeared. For example, the major maize seed companies have screened their germplasm for hybrids that produce a higher fermentation yield in the dry-mill process.[4] The results indicate that genetic components for higher ethanol do exist, but these have never been specifically targeted in the past. Major crop plants have been bred (genetically altered via recombination and recurrent selection) primarily for food or feed production, and when was there ever selection pressure to optimize for industrial biofuel traits in wild plants? It would seem there is a huge opportunity to optimize plants for use in bioenergy strategies.

Applications of biotechnology

Biotechnology is a tool that provides an opportunity to design and optimize the feedstock materials, not just the microbial bioconversions in the process. For example, for corn-based ethanol, the particular traits now being explored for improved ethanol production include overall starch production (yield per unit impacts efficiency), starch types (amylose:amylopectin ratios), and compositional interactions. For lignocelluose, much progress has been made on enzymatic conversion of cellulose to ethanol, but the lignin and other components inhibit the overall process.[3] Several research groups are now exploring the outcome when lignin biosynthesis is down-regulated—potentially a major breakthrough in moving lignocellulose into the commercial biofuel market.

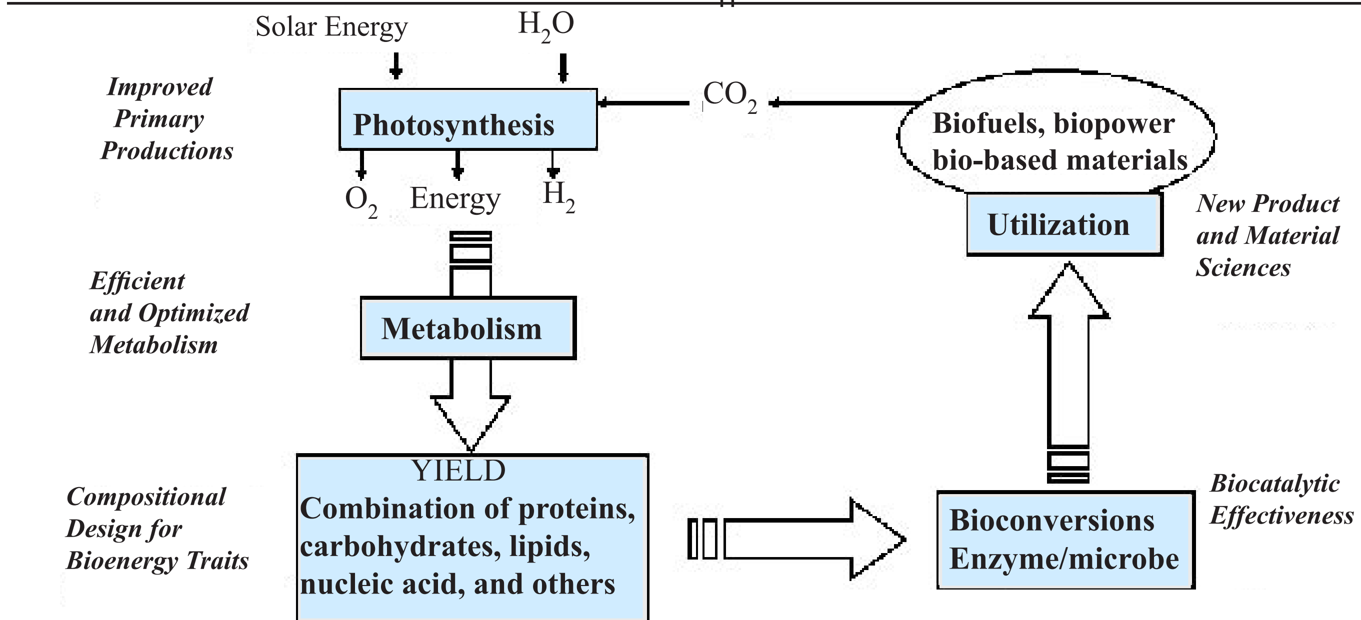
The preceding comments are focused on ethanol only because it is currently the major biofuel. A very analogous situation exists for biodiesel (methyl esters of plant fatty acids, although recycled cooking oil and animal fats can be used) where the market potential is high but limited by the current overall economics. Strategies that focus on stacking industrial traits, for example, in specifically-designed non-feed soybeans, could open the door to directed design for improvements in subsequent bioenergy use.

There is much written about the future potential of a "hydrogen economy." Nevertheless, it is widely recognized that some inherent technical hurdles may take 10 – 15 years to resolve. Assuming success with those, there remains a need to have an energy source (hydrogen is an energy carrier, not a source) to drive the hydrogen economy. In schools of thought, the current assumption is that fossil fuels (reformulated natural gas) will be the main source, which seems to be a self-defeating achievement. Nuclear power appears to be a more logical choice. However, why would biomass not be a high priority, at least to be explored as a major energy source for a future hydrogen-based system. Research is ongoing into the use of ethanol to power bio-fuels cells.[5] Biotechnology could also be a valuable tool to explore the possibilities of improved solar energy capture via plants with biosynthesis of material that facilitate energy transfer to hydrogen.

Currently, a number of bio-based products are made from various parts of different crops. The classic example is pulp/paper from lignocellulosic biomass. Others include specialty fibers, adhesives, boards, veggie-candles, crayons, and additives. However, to-date, and with the exception of paper, most have been small niche products due to difficulties in processing and/or product performance issues. Biotechnology really opens several new doors to creating "natural" bio-based products that are viable in contributing to a more sustainable future. For example, 1,3-propandiol (to be used for a polymer that replaces petro-derived polyester) can now be generated from a microbial bioconversion of starch-derived glucose, a process that required 18 genetic-

driven changes in the biosynthetic pathway.[6] A large number of exciting opportunities exist to utilize natural polymers, rather than petro-polymers, for future needs with functional as well as resource advantages. The current well-known example is the spider silk protein that is very light but is stronger than steel. Since it is difficult to harvest spider webs, biotechnology is being used to express the protein in situations where high levels can be produced and harvested with relative ease.

has focused on decreasing the temperature of the process to save energy. Biomass has potential as a feedstock and biotechnology has the potential to remove the decades-old hurdles[7], but we need a unified strategy if a white knight is to appear. An integrated cross-discipline strategy will be vital to making large enough technical and economic breakthroughs for biomass utilization to contribute to any future sustainable energy platform.



Biotechnology can make a significant difference to the success of a sustainable bio-system for the future, via specifically-designed improvements in several high impact areas (*shown in italics in the above diagram*):

In this short review, the point has been made that biomass R&D must move beyond enhancing conversion technologies alone (analogous to petro-based chemical fractionations) and, for example, use biotechnology tools to re-design the feedstock for specific products. In addition, biotechnology opens the door for future success by being useful in an integrated product design strategy—for example, where feedstock and bioconversion can both be designed to allow optimal interaction in the system. Currently, such integrated approaches, requiring broad scientific coordination, managed teamwork, and complex intellectual property agreements, are not being given high enough priority for R&D support funding. Even in conventional starch to ethanol processes we see contradictory strategies: e.g., particular research to develop thermophilic enzymes, knowing that this requires more heat energy in the process, while practical research

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