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Genetically Engineered Crops: Environmental Impacts 1996-2009

Graham Brookes

Introduction

Although the first commercial genetically engineered (GE) crops were planted in 1994 (tomatoes), 1996 was the first year in which a significant area of crops containing GE traits were planted (1.66 million hectares). Since then there has been a dramatic increase in plantings, and by 2010/11 the global planted area reached over 139 million hectares. This rapid rate of adoption of GE crop technology is probably without precedent in terms of speed of adoption for a new technology. It largely reflects the significant benefits that farmers using the technology have derived¹. A considerable body of literature exists that consistently reports the economic benefits derived from use of GE technology and the reasons why farmers have adopted the technology. However, the literature examining impacts of GE technology adoption in agriculture on the environment is less prolific.

This article summarizes the findings of research into the global environmental impact of biotech crops since their commercial introduction in 1996. It is largely drawn from analysis by Brookes and Barfoot that annually assesses the global economic and environmental impact of GE crops, the latest of which can be found in the peer reviewed journal *GM Crops* 2:1, 1-16, January–March 2011.

The environmental impact analysis focuses on the impacts associated with changes in the amount of insecticides and herbicides applied to the GE crops relative to conventionally grown alternatives. The analysis also examines the contribution of GE crops towards reducing global greenhouse gas (GHG) emissions.

Environmental impacts of insecticide and herbicide use changes

GE traits have contributed to a significant reduction in the environmental impact associated with insecticide and herbicide use in areas devoted to GE crops (**Table 1**). Since 1996, the use of pesticides on GE crops has decreased by 393 million kg of active ingredient (8.7% reduction), and the environmental impact associated with herbicide and insecticide use on these crops, as measured by the EIQ indicator², fell by 17.1%.

1 The cumulative global farm income benefit derived by farmers from using GE traits between 1996 and 2009 was \$64.7 billion (source: Brookes G and Barfoot P (2011)).

2 The environmental impact quotient (EIQ), developed by Kovach et al., effectively integrates the various environmental impacts of individual pesticides into a single 'field value per hectare'. The EIQ value is multiplied by the amount of pesticide active ingredient (ai) used per hectare to produce a field EIQ value. For example, the EIQ rating for glyphosate is 15.33. By using this rating multiplied by the amount of glyphosate used per hectare (e.g., a hypothetical example of 1.1 kg applied per ha), the field EIQ value for glyphosate would be equivalent to 16.86/ha. The EIQ indicator provides an improved assessment of the impact of GE crops on the environment when compared to only examining changes in volume of active ingredient applied, because it draws on some of the key toxicity and environmental exposure data related to individual products, as applicable to impacts on farm workers, consumers and ecology.

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In absolute terms, the largest environmental gain has been associated with the adoption of GE insect resistant (IR) cotton and reflects the significant reduction in insecticide use that the technology has allowed, in what has traditionally been an intensive user of insecticides.

The volume of herbicides used in GE soybean crops also decreased by 41 million kg (1996–2009), a 2.2% reduction, whilst the overall environmental impact associated with herbicide use on these crops decreased by a significantly larger 16%. These data reflect the switch in herbicides used with most GE herbicide tolerant (HT) crops to active ingredients with a more environmentally benign profile than the ones generally used on conventional crops.

Important environmental gains have also been made in the maize and canola sectors. In the maize sector, herbicide and insecticide use decreased by 176.7 million kg, and the associated environmental impact of pesticide use on this crop area decreased due to a combination of reduced insecticide use (34.8%) and a switch to more environmentally benign herbicides (10.5%). In the canola sector, farmers reduced herbicide use by 14 million kg (a 16.2% reduction) and the associated environmental impact of herbicide use on this crop area fell by 23.2% (due to a switch to more environmentally benign herbicides).

In terms of the division of the environmental benefits associated with less insecticide and herbicide use for farmers in developing countries relative to farmers in developed countries, **Table 2** shows a 54%:46% split of the environmental benefits (1996–2009), respectively, in developed (54%) and developing countries (46%). Over three-quarters of the environmental gains in developing countries have resulted from the use of GE IR cotton.

Table 1: Impact of changes in the use of herbicides and insecticides from growing GE crops globally 1996-2009

Trait	Change in volume of active ingredient used (million kg)	Change in field EIQ impact (in terms of million field EIQ/ha units)	% change in ai use on GE crops	% change in environmental impact associated with herbicide & insecticide use on GE crops	Area GE traits 2009 (million ha)
GE herbicide tolerant soybeans	-40.85	-5,632.0	-2.2	-16.0	67.9
GE herbicide tolerant maize	-140.26	-3,435.4	-9.22	-10.49	25.2
GE herbicide tolerant canola	-13.98	-455.8	-16.2	-23.2	6.03
GE herbicide tolerant cotton	-8.87	-281.5	-4.0	-6.9	3.0
GE insect resistant maize	-36.46	-1,292.3	-40.6	-34.8	29.6
GE insect resistant cotton	-152.66	-7,088.0	-21.8	-24.7	13.4
GE herbicide tolerant sugar beet	+0.35	-1.0	+18.0	-2.0	0.45
Totals	-392.73	-18,186.0	-8.7	-17.1	145.58

Table 2: GE crop environmental benefits from lower insecticide and herbicide use 1996-2009: developing versus developed countries

	Change in field EIQ impact (in terms of million field EIQ/ha units): developed countries	Change in field EIQ impact (in terms of million field EIQ/ha units): developing countries
GE HT soybeans	4,053.9	1,578.1
GE HT maize	3,354.3	81.1
GE HT cotton	236.7	44.8
GE HT canola	455.8	0
GE IR corn	1,124.7	167.7
GE IR cotton	515.6	6,572.4
GE HT sugar beet	1.0	0
Total	9,742.0	8,444.1

It should be noted, however, that in some regions where GE HT crops have been widely grown, some farmers have relied too much on the use of single herbicides like glyphosate to manage weeds in GE HT crops, which has contributed to the development of weed resistance. Worldwide, there were 21 weed species that were resistant to glyphosate in 2010³ compared to, for example, 68 weed species resistant to triazine herbicides such as atrazine, and several of the confirmed glyphosate resistant weed species have been found in areas where GE HT crops have been grown (e.g., marehail (*Conyza Canadensis*) and palmer pigweed (*Amaranthus Palmeri*) are reasonably widespread in the US). Where this has occurred, farmers have had to adopt reactive weed management strategies incorporating the use of a mix of herbicides.

In recent years, there has also been a growing consensus among weed scientists of a need for changes in the weed management programs in GE HT crops because of the evolution of these weed populations that are resistant to glyphosate. While the overall level of weed resistance in areas planted to GE HT crops is still low, growers of GE HT crops are increasingly being advised to be more proactive and include other herbicides in combination with glyphosate in their weed management systems, even where instances of weed resistance to glyphosate have not been found. This is because proactive weed management programs generally require less herbicide and are more economical than reactive weed management programs. At the macro level, the adoption of both reactive and proactive weed management programs in GE HT crops has already begun to influence the mix, total amount, and overall environmental profile of herbicides applied to GE HT soybeans, cotton, maize, and canola and, where relevant, this is reflected in the data presented in this paper for the most recent years.

Impact on greenhouse gas (GHG) emissions

GE crops are contributing to lower levels of GHG emissions by two principle means:

- Reduced fuel use from less frequent herbicide or insecticide applications and a reduction in the energy used for soil cultivation. The fuel savings associated with making fewer spray runs (relative to conventional crops) and the switch to conservation, reduced, and no-till farming systems have resulted in permanent savings in carbon dioxide emissions. In 2009 this amounted to about 1,409 million kg (arising from reduced fuel use of 512 million litres). Over the period from 1996 to 2009, the cumulative permanent reduction in fuel use is estimated at 9,947 million kg of carbon dioxide (arising from reduced fuel use of 3,616 million litres);
- The use of 'no-till' and 'reduced-till'⁴ farming systems. These production systems have increased significantly with the adoption of GE HT crops because the GE HT technology has improved growers' ability to control competing weeds, reducing the need to rely on soil cultivation and seed-bed preparation

³ www.weedscience.org

⁴ No-till farming means that the ground is not plowed at all, while reduced tillage means that the ground is disturbed less than it would be with traditional tillage systems. For example, under a no-till farming system, soybean seeds are planted through the organic material that is left over from a previous crop such as maize, cotton or wheat

as a means to getting good weed control. As a result, tractor fuel use for tillage is reduced, soil quality is enhanced, and levels of soil erosion are reduced. In turn, more carbon remains in the soil, which leads to lower GHG emissions. Based on savings from the rapid adoption of no till/reduced tillage farming systems in North and South America, an extra 4,430 million kg of soil carbon is estimated to have been sequestered in 2009 (equivalent to 16,261 million tons of carbon dioxide that has not been released into the global atmosphere). Cumulatively, the amount of carbon sequestered may be higher due to year-on-year benefits to soil quality. However, with only an estimated 15%–25% of the crop area in continuous no-till systems, it is currently not possible to confidently estimate cumulative soil sequestration gains.

Placing these carbon sequestration benefits within the context of the carbon emissions from cars, **Table 3**, shows that:

- In 2009, the permanent carbon dioxide savings from reduced fuel use were the equivalent of removing 0.626 million cars from the road;
- The additional probable soil carbon sequestration gains in 2009 were equivalent to removing 7.227 million cars;
- In total, in 2009 the combined GE crop-related carbon dioxide emission savings from reduced fuel use and additional soil carbon sequestration were equal to the removal from the roads of 7.853 million cars, equivalent to about 27.6% of all registered cars in the UK;
- It is not possible to confidently estimate the probable soil carbon sequestration gains since 1996 (see above). If the entire GE crop using reduced or no tillage agriculture during the last 15 years had remained in permanent reduced/no tillage, then this would have resulted in a carbon dioxide saving of 115,178 million kg, equivalent to taking 51.19 million cars off the road. This is, however, a maximum possibility and the actual levels of carbon dioxide reduction are likely to be lower.

Table 3: Context of carbon sequestration impact 2009: car equivalents

Crop/trait/country	Permanent carbon dioxide savings arising from reduced fuel use (million kg of carbon dioxide)	Permanent fuel savings: as average family car equivalents removed from the road for a year	Potential additional soil carbon sequestration savings (million kg of carbon dioxide)	Potential soil carbon sequestration savings: as average family car equivalents removed from the road for a year
US: GE HT soybeans	291	130	4,711	2,094
Argentina: GE HT soybeans	695	309	7,018	3,119
Other countries: GE HT soybeans	102	45	1,507	670
Canada: GE HT canola	244	108	3,025	1,344
Global: GE IR cotton	33	15	0	0
Brazil: GE IR corn	43	19	0	0
Total	1,408	626	16,261	7,227

Notes: Assumption: an average family car produces 150 grams of carbon dioxide per km. A car does an average of 15,000 km/year and therefore produces 2,250 kg of carbon dioxide/year

Concluding comments

GE crop technology has, to date, delivered several specific agronomic traits that have overcome a number of production constraints for many farmers. This has resulted in improved productivity and profitability for the 15.4 million adopting farmers who have applied the technology in 2010. During the last 15 years, this technology has delivered important positive environmental contributions through a combination of inherent technical advances and the role of the technology in the facilitation and evolution of environmentally friendly farming practices. More specifically:

- The environmental gains from the GE IR traits have mostly been delivered directly from the technology in the

form of decreased use of insecticides;

- The gains from GE HT traits have come from a combination of effects. In terms of the environmental impact associated with herbicide use, important changes have occurred in the profile of herbicides used (in favor of more environmentally benign products). Secondly, GE HT technology has facilitated changes in farming systems. Thus, GE HT technology (especially in soybeans) has allowed farmers to capitalize on the availability of a low cost, broad-spectrum herbicide (glyphosate), and in turn, facilitated the move away from conventional to low/no-tillage production systems in both North and South America. This change in production system has delivered important environmental benefits, notably reduced levels of GHG emissions (from reduced tractor fuel use and additional soil carbon sequestration).

Use of GE HT crops, however, has led to an overreliance on glyphosate by some farmers in some regions, which has contributed to the development of weed resistance. As a result, farmers are increasingly adopting a mix of reactive and proactive weed management strategies incorporating a mix of herbicides. Despite this, the overall environmental gains arising from the use of GE crops have been, and continue to be, substantial.

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Scientific Opinion Releases from European Food Safety Authority

Scientific Opinion on application EFSA-GMO-RX-MON531 for renewal of the authorisation for continued marketing of existing cottonseed oil, food additives, feed materials and feed additives produced from MON 531 cotton that were notified under Articles 8(1)(a), 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003 from Monsanto

EFSA Panel on Genetically Modified Organisms (GMO) European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion evaluates the risk assessment for the authorisation for continued marketing of genetically modified insect resistant cotton MON531 for food and feed produced from it. MON531 was transformed via *Agrobacterium*. It contains single copies of functional Cry1Ac and NPTII expression cassettes; two fragments of the Cry1Ac cassette and *aadA* as non-functional elements.

Stability of the inserted DNA was confirmed over several generations. Bioinformatic analyses and the levels of recombinant proteins did not reveal safety concerns. Analysis of compositional, phenotypic and agronomic characteristics indicated that MON531 is not different from its conventional counterpart and is compositionally within the range observed among conventional cotton varieties, except for Cry1Ac and NPTII. Safety assessment of Cry1Ac and NPTII proteins and cotton MON531 identified no concerns regarding potential toxicity and allergenicity.

Products from MON531 do not contain viable plant parts. The *aadA* and *oriV* sequences in MON531 may facilitate the stabilisation of *nptII* through double homologous recombination in plasmid sequences in the environment. However, considering the expected low frequency of gene transfer from MON531 to bacteria compared to that between bacteria, and the very low exposure to MON531 DNA, the GMO Panel concludes that gene transfer from MON531 to bacteria does not indicate a risk to human or animal health or to the environment in the context of its intended uses.

The exposure of potentially sensitive non-target organisms to Cry1Ac protein is likely to be low and of no biological relevance. A PMEM plan is not required. The EFSA GMO Panel considers that information available for cotton MON531 addresses the questions raised by the Member States and that cotton MON531, as described in this application, is as safe as its conventional counterpart and is unlikely to have adverse effects on human and animal health and the environment in the context of its intended uses.

Source

EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application EFSA-GMORX-MON531 for renewal of the authorisation for continued marketing of existing cottonseed oil, food additives, feed materials and feed additives produced from MON 531 cotton that were notified under Articles 8(1)(a), 8(1)(b) and 20(1)(b) of Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2011;9(9):2373. [1-30] doi:10.2903/j.efsa.2011.2373. Available online: www.efsa.europa.eu/efsajournal © European Food Safety Authority, 2011

Scientific Opinion on application (EFSA-GMO-NL-2008-52) for the placing on the market of herbicide tolerant genetically modified soybean A5547-127 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience

EFSA Panel on Genetically Modified Organisms (GMO), European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion is an evaluation of a risk assessment for the genetically modified herbicide tolerant soybean A5547-127 for food and feed uses, import and processing. Soybean A5547-127 was developed through particle bombardment. It contains a single insertion site consisting of a copy of the intact pat expression cassette, encoding the PAT protein that confers tolerance to glufosinateammonium containing herbicides. Other inserted sequences include two truncated parts of the betalactamase (bla) gene from the transformation vector on each side of the pat expression cassette.

The stability of the inserted DNA was confirmed over multiple generations. The results of the bioinformatic analyses of the insert and the flanking regions, and the levels of newly expressed protein did not raise a safety concern. The comparative analysis of compositional, phenotypic and agronomic characteristics indicated that soybean A5547-127 is not different from its conventional counterpart (A5547), except for the newly expressed protein (PAT). The safety assessment of the PAT protein and the soybean A5547-127 identified no concerns regarding potential toxicity and allergenicity. A feeding study on broiler chickens confirmed that seeds of soybean A5547-127 are as nutritional as seeds of the conventional counterpart.

There are no indications of an increased likelihood of establishment and spread of feral soybean plants, except in the presence of the glufosinate-ammonium containing herbicides. The risk caused by a possible transfer of the recombinant gene from soybean A5547-127 to environmental micro-organisms is regarded to be negligible due to the lack of any advantage that would be conferred in the context of its intended uses. The potential interactions of the GM plant with target organisms, non-target organisms and the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

The monitoring plan and reporting intervals are in line with the intended uses of soybean A5547-127. The EFSA GMO Panel considers that the information available for soybean A5547-127 addresses the scientific comments raised by the Member States and that the soybean A5547-127, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses.

Source

EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on application (EFSAGMO-NL-2008-52) for the placing on the market of herbicide tolerant genetically modified soybean A5547-127 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience. The EFSA Journal (2011); 9(5):2147, 1-28. [27 pp.] doi:10.2903/j.efsa.2011.2147. Available online: www.efsa.europa.eu/efsajournal

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Bioplastic Production by Transgenic Poplar

David A. Dalton, Cathleen Ma, Ganti S. Murthy, Steven H. Strauss

Plants have long been the source of biopolymers of great economic importance to humans. Starch and cellulose – both biopolymers – have obvious roles as major sources of food and fiber, respectively. It is increasingly likely that biotechnology can develop plant-based methods for the production of non-traditional biopolymers with profound benefit to humans. Bioplastics such as polyhydroxybutyrate (PHB) are particularly intriguing in this regard. Although plants do not produce PHB, many bacteria do, and plants can be engineered to produce PHB by transfer of the relevant bacterial genes. The ability of transgenic plants to produce PHB was first demonstrated about 20 years ago, and since that time at least 11 additional species of plants have been successfully engineered to do so^{1,2}. PHB can potentially partially replace petroleum-based plastics and it has other compelling advantages, including biodegradability and possibly a neutral carbon footprint.

PHB is a polymer of 3-hydroxy butyrate that forms a linear polyester typically 10^3 to 10^4 units in length (Fig. 1). Just as with starch, PHB is osmotically and metabolically inert so it can be stored without hazard to the host. Its biosynthesis is a 3-step process starting with acetyl CoA¹. In the first reaction, two molecules of acetyl CoA condense to form acetoacetyl CoA. In the second reaction the keto group on the number 3 carbon atom is oxidized to a hydroxyl group to form 3-hydroxyl butyryl-CoA. The third and final step involves polymerization via the enzyme PHB synthase.

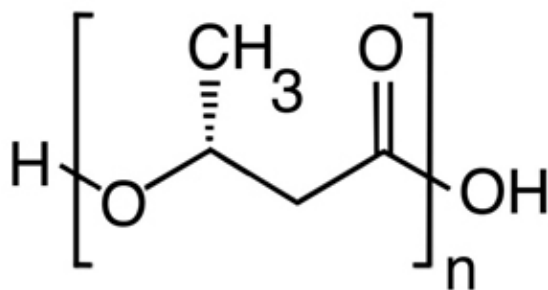


Figure 1. The chemical structure of PHB, which is a repeating polymer of 3-OH butyrate monomers

The precursor for PHB – acetyl CoA – is abundant in plants, which suggests that plants might be good platforms for making bioplastic, once the proper genes are introduced. The flux of acetyl CoA is particularly high in chloroplasts,

because this organelle is the site of fatty acid synthesis, which also requires acetyl CoA as the precursor. For several years, the general strategy for creating plants capable of making PHB has used a strong constitutive promoter (usually the cauliflower mosaic virus 35S promoter) along with plastid targeting sequences for each of the three genes of PHB biosynthesis (*phbABC*) derived from *Ralstonia eutropha* (formerly *Alcaligenes eutrophus*). While this approach has created plants capable of producing PHB, it has not yet resulted in plant systems that show commercial feasibility for production of PHB. The major underlying constraint seems to be that the diversion of resources away from growth and towards the production of PHB reduces plant vigor and yield. For example, in the most extreme example of this problem, Bohmert *et al.* (2000) reported yields as high as 40% (dry weight) in *Arabidopsis*, but the plants were extremely stunted and chlorotic³. Even fairly modest yields of PHB (< 3%) can have profound impacts on overall plant productivity.

New approaches for bioplastics production in plants

Recent work in our labs has focused on expanding the range of PHB-producing plants to include poplar (*Populus*)². Poplar is a well-developed model for genetic studies; its high biomass and perennial growth make poplar attractive as a biofactory for products such as PHB, especially if PHB is considered a secondary co-product in addition to fiber or biofuel. A further advantage – mostly speculative – is that it might be eventually possible to target bioplastic production directly to woody tissue (i.e., xylem cell walls), thus creating an entirely new class of material with a novel structural or industrial applications.

We have attempted to minimize the deleterious effects of PHB production on plant vigor by engineering plants in which the genes for biosynthesis of PHB remain silent until expression is induced by addition of a chemical agent. Thus the plants are allowed to reach a size and stage of development at which the diversion of resources to PHB production is potentially less damaging. The control of gene expression through inducing chemicals uses appropriate promoters upstream of one or more of the structural genes for PHB biosynthesis. We chose to use an ecdysone-based system that achieves PHB yields of up to 14.3% in *Arabidopsis*⁴. The inducing agent, known generically as methoxyfenozide, is available commercially from Dow

AgroSciences under the brand name Intrepid®. Among the advantages of this compound is that it is already licensed for field use (as an insecticide) and has limited toxicity to non-target organisms.

The genetic construct for PHB production in transgenic poplar includes a kanamycin resistance marker and all three structural genes for *phb* biosynthesis (*phbABC*) under control of the ecdysone-based promoter (Fig. 2). A plastid-targeting sequence is included for each gene. This construct was introduced into poplar (*Populus tremula* x *alba* clone 717-1B4) using *Agrobacterium*-mediated transformation that is routine for our labs. Following tissue culture, we eventually recovered plants from 49 events in which PCR confirmed the presence of the transgenes for PHB biosynthesis. Of these 49 events, 18 contained detectable levels of PHB (Fig. 3) with the highest level (~2%) in events nos. 34 and 397. We also used fluorescence dyes to detect the presence of PHB granules in induced leaves (Fig. 4). Control (non-transformed) plants lacked these fluorescent granules. For quantification, PHB was first converted to the butyl ester form of 3-OH butyrate monomers and then quantified by gas chromatography. Typical chromatograms are also shown in Fig. 4.

Metabolic tradeoff studies

We selected the two strongest events (nos. 34 and 397) for detailed studies to determine the metabolic expense of PHB production. These studies also addressed the possible direct phytotoxicity effect of Intrepid. We determined that a concentration of 0.5 to 1 mM Intrepid is sufficient to obtain maximum induction. Statistical analyses of growth parameters revealed that there is no direct toxicity from Intrepid unless the concentrations exceeded 20 mM – a concentration far above that required for induction.

Production of PHB is a metabolic expense that causes reduced growth. This expense most likely explains our inability to recover viable plants when we used the constitutive 35S promoter to drive expression of the *phb* genes (unpublished data). This negative impact of PHB production is typified by a decline in volume

index (diameter² x height), a frequently used means of representing cumulative growth in trees (Fig. 5). We also observed negative effects of PHB production on plant mass (dry weight) and on chlorophyll fluorescence (an indicator of plant stress). Plants that produced more than 1% PHB (dry weight in leaves) grew 20 to 30% less than those that produced less than 1% PHB.

Extraction of PHB

Several extraction methods for PHB recovery have been developed. These involve centrifugation, filtration, extraction with organic solvents (chloroform and methanol), treatment with sodium hypochlorite, and digestion with enzymes. These methods are targeted for extraction of PHB from bacterial cells, as historically most of the research has been focused on microbial PHB production, which is already a commercial success. Extraction from plant leaves is more problematic due to the lower concentrations of PHB and the presence of many interfering substances, such as chlorophyll. Recently we used a modified solvent sequential extraction method to extract the PHB from genetically modified hybrid poplar leaves⁵. The process involved grinding leaves to a fine mesh, extracting with ethanol to remove chlorophyll, and then extracting the leaf residue with chloroform to recover the PHB. In addition, it is possible to quantify extracted PHB with a spectrophotometric method based on the conversion of PHB into crotonic acid by H₂SO₄. This method is quicker and less technically demanding than other methods based on gas chromatography that have been previously used in plant studies.

Although the technical feasibility of PHB production and extraction have been demonstrated, economic viability, process scalability, and safety of the process are equally critical for commercial success. There is a need to develop additional extraction methods that are specifically optimized for PHB extraction from plant biomass.

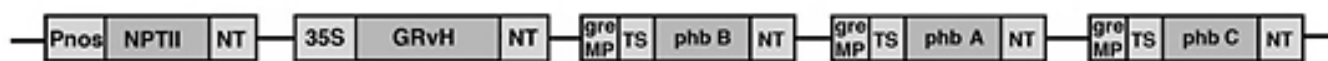


Figure 2. T-DNA region of PHB expression cassette used to transform poplar. Pnos, promoter for nopaline synthase; NPTII, kanamycin resistance gene; NT, terminator for nopaline synthase gene; 35S, promoter for 35S cauliflower mosaic virus; GRvH, glucocorticoid response element; greMP, minimal promoter with a glucocorticoid response element binding site; TS, plastid targeting sequence; *phbABC*, genes for biosynthesis of PHB.

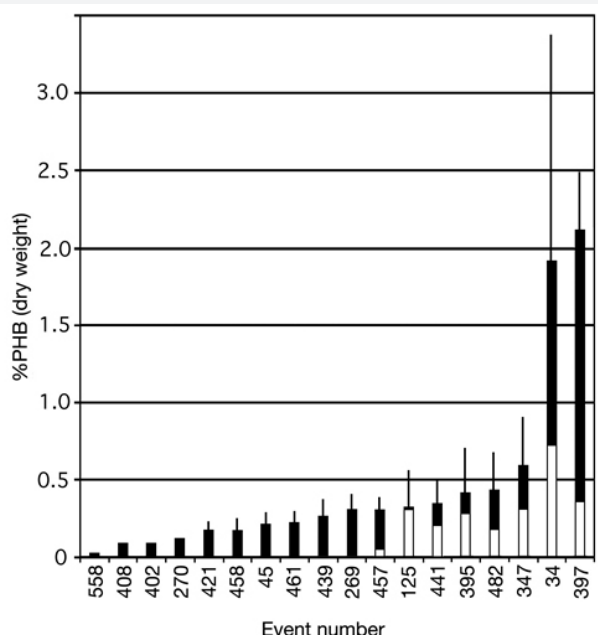


Figure 3. Concentration of PHB in poplar leaves after 7–8 weeks of induction with 10 mM Intrepid. Open bars represent the levels in noninduced (no Intrepid) plants. Noninduced levels in weak events (nos. 558–269 from left to right) were not determined. Each value is the mean of 4–6 replicates +1 standard error of the mean (reproduced from Dalton *et al.*, *Plant Biotechnol J* 9:1-9, 2011)

Future directions

While yields of 1–2% are encouraging, these levels fall considerably short of commercial goals of 12.5%, which may be necessary if PHB production in poplar is to be commercially feasible as a stand-alone product⁵. We are engaged in further studies aimed to increase yield by using native promoters associated with senescence to see if postponement of PHB biosynthesis until leaf senescence begins might enable high levels of production with acceptable effects on plant growth and health.

Transcriptome studies in our laboratory, extending the work by Andersson *et al.*⁶, have identified dozens of genes with very strong upregulation during the early stages of senescence, when leaves still appear green. These might be useful for triggering senescence-associated biosynthesis. However, many questions still remain about the feasibility of this system. Will the senescing chloroplasts still be able to support PHB biosynthesis? Will the PHB remain intact as general cellular contents are degraded and readied for transport into stems and leaves? Will there be adequate levels of PHB produced? And can economic systems for leaf collection and processing,

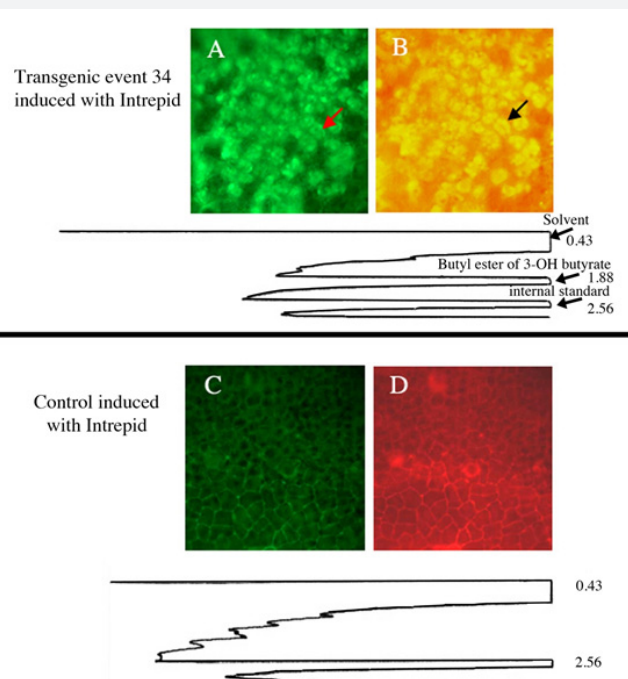


Figure 4. Demonstration of PHB in poplar leaves. Microphotographs are epifluorescence micrographs of poplar leaves stained with Nile Blue A. A and C; excitation wavelength of 450–490 nm. B and D; excitation wavelength of 510–560nm. PHB granules are evident as fluorescence granules (arrows) within the chloroplasts of the transgenic event. The chromatogram beneath each photo confirms the presence of the derivatized form of PHB in the transgenic event and its absence in the control. Numbers on the chromatograms indicate retention times in minutes.

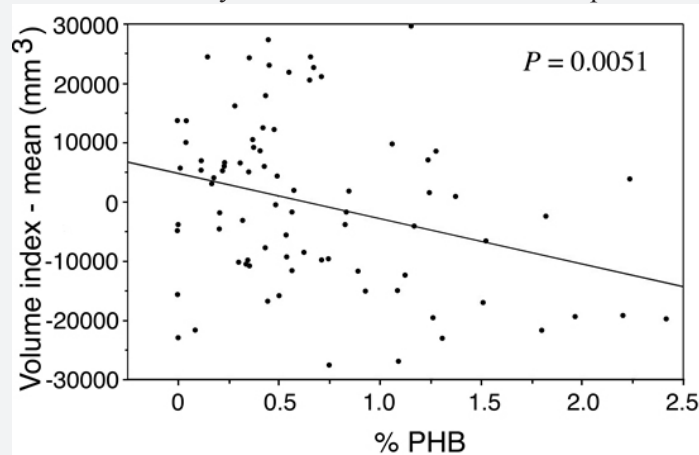


Figure 5. Negative correlation between PHB content of leaves and volume index (diameter² x height) following 6 weeks of induction with four different concentrations of Intrepid. Each point is a single observation from an individual plant corrected for event (group effect) by subtracting the mean value for that event. (reproduced from Dalton *et al.*, *Plant Biotechnol J* 9:1-9, 2011)

perhaps as part of coppice biomass harvest, be developed? As a first step, we have begun to test candidate promoter strength and specificity. If yields can be increased as hoped, then we will address the remaining issues related to harvest and extraction.

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