Genetically modified lignin below ground

David W. Hopkins, Elizabeth A. Webster, Wout Boerjan, Gilles G. Pilate, Claire Halpin

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To the editor:

Correspondence in *Nature Biotechnology*[1–3] in response to a News Feature on the biotechnological potential and environmental risks of releasing transgenic trees[4] has drawn attention to our earlier paper reporting the first field trials of trees with modified lignin[5]. The focus of this debate on the below-ground effects of genetically modified (GM) lignin is highly appropriate because lignin is one of the most abundant biopolymers on the planet, an important regulator of the decomposition of plant residues and the precursor of much of the stable organic matter in soils. The amount of carbon in soils outweighs that in vegetation and the atmosphere combined[6] and has the potential to moderate future climate change.

Talukder[3] raises concerns that a low-lignin phenotype may pose an environmental risk by promoting faster decomposition of litter and increased CO_2 emission because microbial enzymes will reach their target polysaccharides more easily when the physical barrier presented by the degradation-resistant lignin is reduced. This proposed influence of altered lignin on decomposition is not speculation, as we have shown in both poplar[5] and tobacco[7] that short-term (<100 days) decomposition of plant material with modified or, in the case of tobacco, low-lignin, is significantly faster than that of corresponding wild-type material and that this difference is largely explained by reduced protection from microbial attack afforded to labile components by the modified lignin[8]. But before this evidence is wildly extrapolated to global scenarios of climate change, however, we would urge consideration of the longer term—after all, the complete breakdown of plant residues can take decades or more.

A study from our group spanning 18 months found no significant differences in the extent of decomposition between field-grown wild-type and lignin-modified poplar wood; indeed, the variation between replicates of each genotype was greater than the variation between genotypes[9]. This suggests that changing environmental conditions during growth in the field have a greater influence on wood decomposition than the genetic modifications to lignin in these genotypes. Similar work we have carried out on lignin-modified tobacco grown and allowed to decompose under controlled laboratory conditions demonstrates that once the accessible polysaccharides have decayed, the decomposition of the remaining lignin-rich material is either no different or actually slightly slower for modified plants than for the wild type (**Fig. 1**).

There could be several explanations for this. Lignin-biodegrading fungi rely in part on the energetic supplement from polysaccharides because of the low-energy yield from lignin itself. When these polysaccharides are more rapidly depleted, as in the modified material, subsequent decay of the lignin could be retarded. Alternatively, the more condensed structure of the modified lignin may make it more difficult to degrade. Investigating these hypotheses fully will require long-term studies and will be challenging because of the difficulty, for example, of growing isotopically labeled trees to maturity. Following the same reasoning as Talukder, the consequence of modified lignin plants decomposing more slowly over the longer term would be that carbon would be held up in soils for a longer period during its passage around the biogeochemical carbon cycle, thereby potentially reducing the flux of CO_2 into the atmosphere.

Before advocating the growth of trees with modified lignin as part of an atmospheric CO_2 mitigation strategy, we should recognize that the effects of lignin modification on decomposition have been fairly subtle, short-lived and detected only in controlled experiments in the laboratory and field. In the natural or semi-natural environment of forest plantations, the environmental variability of soils, hydrology and climate, the species and genotype of trees grown and their age at harvest, and other 'natural' variables, will have a much greater effect on decomposition and soil properties. Indeed, for the four-year field experiment of lignin-modified poplars, we showed that the differences in soil organic carbon and microbial biomass between samples taken from the experimental plots and those from the surrounding grassland were larger than those between soil samples from beneath wild-type and modified trees[5]. This is not surprising as it has been repeatedly demonstrated that plant species differ in the composition of the microbial communities around their roots and support different abundances of soil microbes[10].

Thus, introducing any new crop or tree to a soil is likely to have some effect on the local soil ecosystem. If this is not a concern for conventionally bred plants, why should

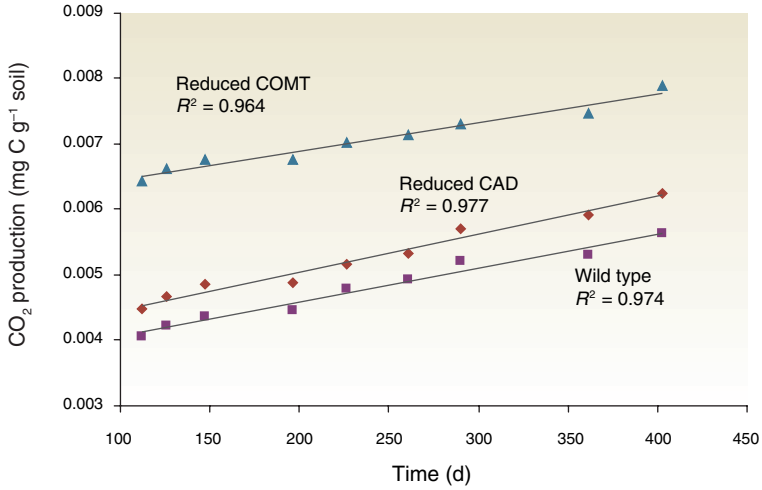

Figure 1 CO_2 production from soil amended with stem material from unmodified tobacco and tobacco plants with modifications that reduce expression of either cinnamyl alcohol dehydrogenase (CAD) or caffeic acid *O*-methyl transferase (COMT). Soil was amended with 1% (wt/wt) air-dried and chopped plant material and incubated at 20 °C. Over the period from 112–403 days, the accumulating CO_2 was measured by gas chromatography[7]. Regression analyses are shown and the means (s.d.) for the slopes (rates) are as follows: tobacco plants with unmodified lignin (■) = 0.00518 (0.00032) μg C g^{-1} soil day^{-1}; tobacco plants with reduced CAD (♦) = 0.00581 (0.00034) μg C g^{-1} soil day^{-1}; and tobacco plants with reduced COMT (▲) = 0.00441 (0.00032) μg C g^{-1} soil day^{-1}.

it be a concern for GM plants, unless the genetic modification causes greater and permanently detrimental effects relative to plants produced by conventional means? For lignin-modified GM trees, this is clearly not the case; the huge variability that exists between different varieties of plant (including naturally occurring mutants) in both lignin and decomposition is greater than the changes being introduced by genetic modification. Moreover, these trees potentially offer significant environmental benefits by reducing the amount of chemicals and energy consumed during papermaking and could potentially also provide improved lignocellulosic feedstocks for biofuel production. To reach a valid conclusion on the environmental impact of modified-lignin trees—whether they are bred conventionally or by recombinant DNA approaches—these benefits must be taken fully into account and offset against any potential risks.

With the recent publication of the poplar genome sequence, interest in the application of biotechnological approaches to tree improvement is set to increase[11].The wider conclusion from these observations is that biotechnological solutions to environmental problems need to be evaluated in the environment. In particular in relation to soils, we should not underestimate the resilience of soil biological, chemical and physical systems below ground when examining the ecological effects of plants with modifications to lignin.

COMPETING INTERESTS STATEMENT
The authors decare that they have no financial competing interests.

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Model for tuning GMO detection in seed and grain

To the editor:

A letter to your journal from Florian Weighardt (*Nat. Biotechnol.* **25**, 23–25, 2007) highlights the challenges facing scientists attempting to implement European Union (EU; Brussels) regulations for labeling food and feed products containing genetically modified organisms (GMOs). Demonstrably robust sampling, detection and decision procedures are required to comply with regulatory requirements both in the EU[1,2] and in Australia and New Zealand[3]. In the EU, robust procedures are also necessary to support the policy of coexistence[4,5] of authorized GMO products with non-GMO products. 'Demonstrably robust' means that detection methods must both reliably give positive results if small quantities of GMOs are present in large lots—in this context, a lot is a 'distinct and specified quantity of material'[6] from which samples are taken and which will be accepted or rejected on the basis of the analytical result(s)—and also keep under control the factors affecting the ability to detect GMOs. Measurements of GMOs in bulk grain lots[7] show that we cannot assume that large grain lots will be homogenous with respect to the distribution of small quantities of GMO grains. Thus, heterogeneity of bulk lots is an important factor that can affect the performance of detection methods.

To address some of the above challenges, we have produced a mathematical model that combines information on the performance of all stages of a GMO event detection: beginning with sampling from heterogeneous bulks, such as seed and grain lots, and DNA extraction, through to qualitative conventional PCR detection and rules used to interpret results. Input parameters include the following: sample increment mass, seed mass, number of increments, mass of laboratory sample, mass of analytical samples, concentration of DNA extracts, variation associated with concentration of DNA extract, haploid genome mass, copies per haploid genome, volume of PCR aliquot and number of gene copies necessary to give a 95% probability of detection in PCR. The model estimates the probability of detecting the presence of a quantity of GMOs in a bulk within which GMOs are heterogeneously distributed and thus such parameters as limit of detection (LOD).

LOD has not previously been estimated in this way, including every step of the analysis process, but our approach is necessary if the results of different sampling and analysis protocols are to be compared in regulation enforcement decisions (full details of our model are available in **Supplementary Notes** online; and in a UK Department for Environment, Food and Rural Affairs report (http://www2.defra.gov.uk/research/project_data/More.asp?I=CB02029#Docs); a spreadsheet implementation is available from http://www.csl.gov.uk/STAGED under the 'Scientific Papers' section).

As a test case, we have applied our model to examine the detection of unauthorized events in oilseed rape, *Brassica napus,* and in particular to explore how heterogeneity in the sampled lot affects the LOD, and how LOD values can be modified by choice of sampling plan, analytical replication scheme and critical level (the lowest response that reliably indicates, with a fit-for-purpose, false-positive rate, that an analyte is present)[8]. Simulation results are expressed in terms of percentage GMO DNA following an EU Commission recommendation[9]. Other ways of expressing the quantity of GMO material (e.g., mass of GMO-derived product as a proportion of total mass of product) can produce very different estimates of the quantity of material (as mentioned by Weighardt) and of parameters, such as LOD.

Using an analytical approach analogous to that of the UK Food Standards Agency in its 2005 survey of imported US maize products[10]—which analyzed each of ten