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## ISSUES – FIGURE SET

# What Are the Ecological Impacts of Plant Biotechnology?

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**Figure Set 1: Evidence for Brazil Nut Allergen in Transgenic Soybeans**

**Purpose:** To show that allergens can be transferred from one plant to another through crop biotechnology and, thus, may pose a food safety issue.
**Teaching Approach:** pairs share
**Cognitive Skills:** (see Bloom's Taxonomy) — comprehension, analysis, evaluation
**Student Assessment:** minute paper

**CITATION:**

Zycherman, D., and J. Taylor. August 2004, posting date. What Are the Ecological Impacts of Plant Biotechnology? Teaching Issues and Experiments in Ecology, Vol. 2: Issues Figure Set #1 [online]. http://tiee.ecoed.net/vol/v2/issues/figure_sets/biotech/abstract.html

## BACKGROUND

This TIEE Issues Figure Set explores the safety of biotech crops for the case of transgenic soybeans. The presence of GMO's in our diet has caused some concern in the field of plant biotechnology because the safety of these organisms is not always well studied. Scientists are currently researching the potential health effects of biotech crops and caution that genetic manipulation can produce unintended side-effects such as increasing the levels of natural plant toxins in food or even creating or adding new toxins. These can occur in unexpected ways such as by switching on genes that produce toxins or by switching off genes that suppress them.

In addition, more subtle effects can occur due to inadvertent food allergy transmission during transgenic modification. A gene transferred from one plant into another could cause an allergic reaction in the consumer who is allergic to products of genes that were transferred. Thus, plant biotechnology may not only be transferring traits that are beneficial to humans, but also those that may be harmful to some people as well. Much research may still need to be conducted to fully understand how genetic manipulation of plants affects the quality and safety of biotech food products.

In the United States, three federal agencies share responsibility for food and environmental safety issues for genetically engineered crops. According to FDA Commissioner Dr. J. E. Henney, from an interview for the FDA publication FDA Consumer published in 2000: "FDA is responsible for the safety and labeling of all foods and animal feeds derived from crops, including biotech plants. EPA regulates pesticides, so the BT used to keep caterpillars from eating the corn would fall under its jurisdiction. USDA's Animal and Plant Health Inspection Service oversees the agricultural environmental safety of planting and field testing genetically engineered plants." (Thompson 2000).

Interestingly, under US Law (Food, Drug, and Cosmetic Act - US Code, Title 21, Chapter 9, {available at www.access.gpo.gov/uscode/title21/chapter9_.html}) transgenic foods are not covered under the more stringent regulations for food additives (food colors, sweeteners, preservatives, etc.) because, according to FDA Commissioner Henney, "we are talking about adding some DNA to the plant that directs the production of a specific protein. DNA already is present in all foods and is presumed to be GRAS [generally recognized as safe]... adding an extra bit of DNA does not raise any food safety issues." (Thompson 2000). Thus, under US Law, because it is only DNA that is being added and all plants have DNA, GMO crops are generally recognized as safe (GRAS status). In contrast, if the segments of biotechnologically inserted genetic material and the gene products they create were to be considered "additives," the US FDA would require an "intensive review" for which scientific data collection is mandated to assess if the plants with the added DNA and novel gene products are non-toxic, non-allergenic, and otherwise safe. However, this does not apply to most GMO crops in the US - and for many, this policy designation lies at the heart of the controversy.

Specifically regarding food allergies, although FDA Commissioner Henney does not dismiss the possibility that biotech foods can cause allergies, she contends that "we have no scientific evidence to indicate that any of the new proteins introduced into food by biotechnology will cause allergies" (Thompson 2000).

In spite of the FDA's stance, some contend that since consumers have never eaten many of the foreign proteins and other gene products now in GMO foods, stringent credible pre-market safety-testing is essential to protect public health. There is still public fear about GMO's and without appropriate labeling, consumers do not have a choice, especially if they are very sensitive to specific foods.

This problem was exemplified in a study that is the focus of this TIEE Issue. Nordlee et al. (1996), in the New England Journal of Medicine, identified a Brazil nut allergen in transgenic soybeans. To improve the nutritional quality of soybeans as feed for poultry, methionine, an essential amino acid, had been added through modern biotechnology. This was accomplished by inserting into the soybean genome the gene for 2S albumin, a protein high in methionine, from the Brazil nut.

Unfortunately, many people are allergic to Brazil and other nuts. For these people, an overreaction of their immune system to a specific allergen in Brazil nuts can produce severe allergic reactions such as rashes, shortness of breath, vomiting, shock, or even death. The human immunoglobulin IgE (one of the five types of human immunoglobulins) is often the key to allergic sensitivity. Therefore in this study, the allergenicity of the transgenic soybeans was determined by assessing the binding affinity of the 2S albumin protein to IgE from people who are allergic to Brazil nuts.

Lastly, it is worth noting that the research reported in Nordlee et al. (1996) was funded by the Dupont subsidiary Pioneer Hi-Bred International, which originally developed the transgenic soybeans of study. The research was conducted at the University of Nebraska-Lincoln. This is a nice example of a corporate-University partnership leading to a ground breaking case study of allergen transfer through biotechnology. In fact, as a direct result of the research by Nordlee et al., Pioneer decided to discontinue its research program on transgenic soybeans using 2S albumin from Brazil nuts long before any products reached the commercial market (Pioneer 2004).

**Literature cited:**

Nordlee, J. A., S. L. Taylor, J. A. Townsend, L. A. Thomas, R. K. Bush. 1996. Identification of a Brazil-nut allergen in transgenic soybeans. *The New England Journal of Medicine* 334: 688-692.

Pioneer Hi-Bred International (Dupont). 2004. Press Room: Biotechnology - Biotech Soybeans and Brazil Nut Protein. www.pioneer.com/biotech/brazil_nut

Thompson, L. 2000. Are bioengineered foods safe? *FDA Consumer* 34: No. 1, January-February 2000: 1-6. {available online at www.fda.gov/fdac/features/2000/100_bio.html}

## STUDENT INSTRUCTIONS

Soybeans are nutritional superstars by providing essential dietary amino and fatty acids. In addition, consumption of soy products have been shown to reduce cholesterol levels, and reduce risks of kidney and heart disease, osteoporosis, and possibly some cancers. However, soybeans are not a "complete" protein source for people and animals since soybeans lack the essential amino acid methionine ("essential" here means that animals cannot create methionine themselves). To rectify this deficiency, plant biotechnologists used methods of recombinant-DNA technology to insert a gene into a strain of soybeans to enable them to synthesize this missing amino acid.

The problem with this approach was that the original source of the gene for methionine, "2S albumin," came from Brazil nuts (*Bertholletia excelsa*). Many people are allergic to Brazil nuts and their reaction can range from severe rashes to anaphylactic shock. The study we focus on here by Nordlee et al. (1966) addresses this issue.

As a reminder of basic human immuno-biology, allergic or immediate hypersensitivity reactions are caused by binding of the antibody Immunoglobulin E (IgE) to the allergen (in this case, the methionine rich 2S albumin). This causes a series of reactions including the release of substances such as histamine, prostaglandins, and other compounds that produce the symptoms of an allergic reaction listed above. A glossary below lists some terms you may not be familiar with.

The purpose of the study by Nordlee et al. (1996) was to determine the extent to which the transgenic soybeans, containing the Brazil nut 2S albumin gene, caused allergic reactions similar to Brazil nuts.

Figure 1 is based on data from a radioallergosorbent test (RAST) in which a sample of blood from a potentially allergic person is checked for allergic sensitivity to specific substances. The approach used by the RAST is to determine whether specific IgE antibodies in serum drawn from sensitized subjects are able to recognize the protein of interest, in this case the 2S albumin from Brazil nuts.

In the standard radioallergosorbent test (RAST), the allergen, in this case 2S albumin extracted from Brazil nuts, is bound to an inert solid support, such as a tiny glass bead or some other microscopic solid. This is called the "solid" phase. Next, solutions of the "solid phase" of the allergen are incubated with blood serum from people who were allergic to Brazil nuts, but not to normal soybeans. During this incubation, serum IgE specific to the allergen (2S albumin) will bind to it. Following this, excess serum and non-allergen specific IgE are then washed away leaving behind only the serum IgE specifc for, and bound to, the "solid phase" allergens. Next, a radio-labeled anti-IgE antibody protein is added that will bind to the IgE molecules currently bound to the "solid phase" allergens creating a kind of molecular sandwich. A subsequent wash then removes all unbound radiolabeled anti-IgE proteins. Thus, by

measuring the amount of radioactivity in the remaining sample, one can estimate the amount of allergen specific IgE in the original serum sample, and the degree of IgE binding affinity (i.e. the allergenicity) of the allergen.

Nordlee et al. (1996) used a slightly more elaborate procedure involving multiple RAST assays in sepatare trials on serial dilutions of the liquid protein extracts from Brazil nuts, transgenic and non-transgenic soybeans. Each of these are referred to as "inhibitor proteins" because they compete for the binding sites of IgE's that are already bound to the solid phase produced as described above. In each RAST assay, if the "inhibitor proteins" (either protein extracts from Brazil nuts, transgenic and non-transgenic soybeans) have a high affinity for the serum IgE, relative to the affinity between IgE and the solid phase Brazil nut 2S albumin, then there will a high rate of binding between the "inhibitor proteins" and the IgE. This will inhibit and/or displace the binding of the IgE to the solid phase Brazil nut 2S albumin - thus the "inhibitor" competes with the solid phase Brazil nut 2S albumin. When this happens, IgE will remain in solution and will be washed out before the label anti-IgE is added. This will decrease the amount of radioactivity in the remaining sample, and will result in a lower radiation count in the final step of the RAST assay (again, because everything not attached to the solid phase will be washed away). Since the counts are a relative measure of affinity, the authors calculated an index of "Inhibition of IgE binding (%)" as:

$$\frac{\text{(counts without inhibitor protein - counts with inhibitor protein)} * 100\%}{\text{(counts without inhibitor protein)}}$$

**Discuss the Following:**

Turn to your neighbor and take 5 minutes to interpret the figure.

Take a moment to absorb the equation above. Run it through in your mind replacing the words "inhibitor protein" with "Brazil nut protein." With very little added Brazil nut protein, the counts should be as high as they can get since all of the solid phase should get labeled and counted. The index of "Inhibition of IgE binding (%)" (y-axis) should be low in this case because the numerator should be near zero.

However, with a lot of added Brazil nut protein, a lot of the IgE will be displaced from the solid phase (because the added Brazil nut protein would compete for the IgE with the solid phase Brazil nut protein) and washed away, and the counts should plummet. The index of "Inhibition of IgE binding (%)" (y-axis) should be quite high in this case because the numerator should be large. Note that with more added Brazil nut protein, the counts should plummet to zero as all of the IgE is displaced from the solid phase and washed away. This would result in a value of 100% for the index of "Inhibition of IgE binding (%)." Do you and your neighbor understand why?

After understanding exactly what the axes mean in Figure 1, now ask and discuss answers to the following:

1. What were the main research questions the scientists were asking?

2. What was the benefit of conducting this test to determine the allergenicity of trangenic soybeans?

3. Contrast the slopes of the 3 treatments; what conclusions can you draw about the relative allergenicity of the transgenic soybean?

4. Did Pioneer Hi-Bred International (Dupont) make the right decision to abandon this research program? What were some alternatives?

5. The ethical question of whether transgenic foods should be labeled is hotly debated. Can you assess the value or importance of this experiment within the context of bioethics? Should governments require labeling of genetically engineered foods?

6. According to statements made by FDA Commissioner Dr. J. D. Henney (Thompson 2000, see Background to Figure Set 1) "we have no scientific evidence to indicate that any of the new proteins introduced into food by biotechnology will cause allergies." Based on your understanding of Nordlee et al. (1996), is Dr. Henney correct? Explain your response.

**Glossary of Terms**

Allergy- overreaction of the immune system to specific substances called allergens (such as pollen or bee stings) that in most people result no symptoms. Allergies often involve IgE (one of the 5 types of immunoglobulins produced by humans) antibodies.

Allergen- an antigen that produces allergic reactions by inducing formation of IgE.

Antibody- proteins, produced by the immune system, that recognize a foreign substance and starts a process of removal of the foreign material from the body.

Antigen- a substance that stimulates the production of an antibody (see Allergen)

Immune system- a system in mammals that recognizes and then eliminates or neutralizes foreign substances.

Immunoglobulin- a group of proteins active in the immune system that serve as antibodies. They work by binding to foreign antigens.

The Health On the Net Foundation: Allergy Glossary (http://hon.ch/Library/Theme/Allergy/Glossary/a.html)

**FIGURES**

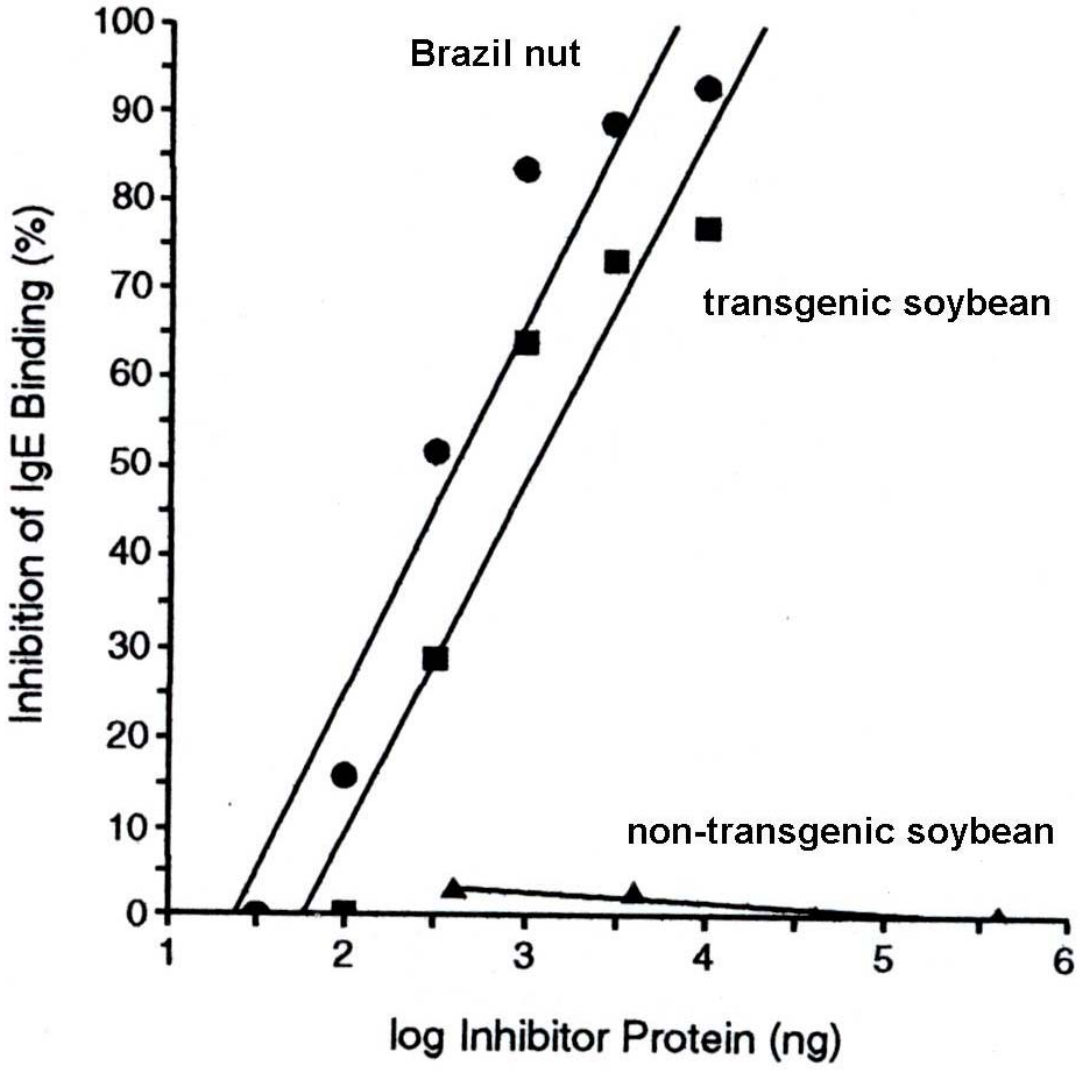


Figure 1. Results of Radioallergosorbent Assays with Extracts of Non-transgenic Soybean (triangle), Transgenic Soybean (square), and Brazil Nut (circle). Concentration of 2S albumin (in nanograms, log scale) in each dilution of extract in relation to percent inhibition of IgE binding (see Student Instructions for further description of axes) (from Nordlee et al. 1996. Identification of a Brazil-nut allergen in transgenic soybeans. The New England Journal of Medicine 334: 688-692).

## NOTES TO FACULTY

The purpose and principal conclusion of Nordlee et al.'s (1996) paper is well articulated by the authors with:

> "It is prudent to assess the allergenicity of proteins in transgenic foods if those proteins have been derived from sources that are commonly allergenic. The use of currently available animal models alone to predict the allergenicity in humans does not produce accurate results. Techniques such as radioallergosorbent tests... can identify IgE-binding proteins that are probable allergens in transgenic foods derived from sources known to be allergenic." (p. 691)

> "Our findings demonstrate the transfer of a major food allergen during the development of improved crop varieties through genetic engineering." (p. 691)

Despite the clarity and simplicity of the above statements, the purpose, procedure, and output of the radioallergosorbent assay test, RAST (Figure 1) can be quite challenging for many students, as well as for faculty who have not recently taken an immunology course.

We suggest that you some time to explain the RAST assay. The take home message is that the RAST assay is an allergy test performed on a sample of blood that checks for allergic sensitivity to specific substances by measuring binding rates between the putative allergen and IgE. Recall that proteins that trigger the production of IgE (allergen specific immunoglobulin) are likely to be allergens. An excellent description of RAST tests is provided by the US Food and Drug Administration Center for Devices and Radiological Health.

In Nordlee et al. (1996), multiple RAST assays are used to determine the relative allergenicity of Brazil nuts, transgenic and non-transgenic soybeans by testing the ability of liquid protein extracts to bind to IgE from people who are allergic to Brazil nuts. In Figure 1, the "inhibitor proteins" are the protein extracts from Brazil nuts, transgenic and non-transgenic soybeans that compete with the Brazil nut 2S albumin protein (in solid phase) for binding to the IgE from allergic people. The labeled antibody (IgE) is allowed to bind to 2S albumin and the unbound antibody that remains is washed away. The degree of binding of the antibody (indicated using an index of relative binding affinity on the y-axis of this figure) is measured by the radioactivity still remaining.

In addition, we suggest that you discuss your students' understanding of the term bioethics. In this case, this term refers to ethical problems that may or may not arise from biological research. There are many web resources for bioethics and food labeling.

You will have to decide how much time your students will need to interpret the figure. Then, ask each question aloud, giving each question between 1-3 minutes, depending on difficulty, so students pace themselves.

After discussing the figure, provide a brief lecture, or reading assignment, that encompasses the importance of scientific-based concerns about biotechnology. If you wish to emphasize the ethical element in your discussion, you could focus on the reasons for GM food labeling. Also, you should emphasize that scientists disagree about crop biotechnology and the value of risk assessment.

The intention of the Student Assessment question is for students to use scientific evidence and information to support their point of view. You will probably need to explain this and perhaps give an example of the kinds of evidence you are looking for. It is second nature for scientists to use scientific evidence and reasoning in support of arguments, but this is a sophisticated skills that students need to be shown how to do - even after you have spend a long time discussing the "science."

There are many web resources for bioethics and food labeling including:

Bioethics and the NIH: Biotechnology Industry Organization: http://www.bio.org/

IFT Expert Report on Biotechnology and Foods:
http://www.ift.org/publications/docshop/ft_shop/09-00/09_00_pdfs/09-00-bio-label.pdf

**Student Assessment:**

Address the following question in writing: What is your opinion of food labeling for GM foods? Support your opinion with scientific information from our discussion. 300-500 words.