PART 1
OF A 3-PART SERIES

Safety Tests

ON TRANSGENIC AMERICAN CHESTNUT

By Andy Newhouse, SUNY's College of Environmental Science and Forestry

As part of TACF's 3BUR strategy, transgenic blight-tolerant American chestnuts are being produced and tested for potential use in forest restoration. These trees contain a gene called oxalate oxidase, or OxO, which breaks down toxic oxalic acid produced by the blight fungus. This article (and two more in subsequent issues) will highlight some of the many safety tests we've performed on OxO-containing transgenic American chestnuts.

This first installment describes tests for safety to people, in terms of chestnuts as a food product. While careful safety evaluations are prudent for any restoration efforts, results from these tests will be particularly informative to federal regulatory agencies, who will need to approve transgenic trees before they can be distributed or used for restoration. The primary question (for us as scientists and for the regulators) is whether there are substantial differences between the transgenic chestnut and a similar non-transgenic chestnut produced through traditional breeding.

One way we can evaluate an unfamiliar food product is by looking at the Nutrition Facts label, which essentially describes its composition. This generally tells us how nutritious it is, and lets us compare two food products to see how similar they are.

In order to create a Nutrition Facts label, most food manufacturers send samples to independent commercial testing labs, so that is exactly what we did with chestnuts. We sent samples of both transgenic and non-transgenic chestnuts, along with various unrelated non-transgenic samples, repeated over three growing seasons. The results (summarized in **Figure 1**) were not surprising to anyone familiar with growing chestnuts: the biggest variances were between different species of chestnuts (e.g. American and European), and then between unrelated American chestnuts harvested from different sites There were small differences between transgenic and non-transgenic nuts, but they were not consistent across multiple seasons, and they were minor compared to the species and site differences.

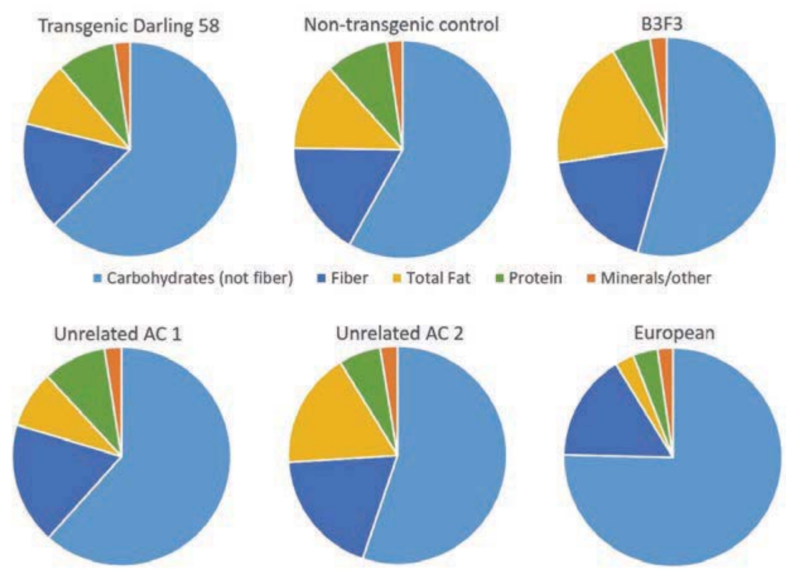
A more detailed look at one aspect of chestnut nutrition involves a "fatty acid analysis." This means identifying the specific types of fats that make up the total fat in a product, which can affect flavor and texture in addition to nutrition. Some fats are considered healthy (generally unsaturated fats), while others (generally saturated) are less healthy if consumed in large quantities. Our analyses (**Figure 2**) showed that most of the fat in American chestnuts is a monounsaturated type called Oleic acid, followed by the polyunsaturated Linoleic acid. (Interestingly, these relative proportions of Oleic and Linoleic acids are reversed in European chestnuts, which might explain some of the flavor differences between these species.) About 10-15% of fat in chestnuts is saturated Palmitic acid, followed by a few other types totaling less than 10%. Most American chestnut types and a B_3F_3 sample were quite similar to each other, but there were differences between chestnut species (namely the European as mentioned above), and one unrelated American chestnut type (AC 3 in figure 2), whose fatty acid ratios looked more like the European. In contrast to species differences, transgenic and non-transgenic samples were nearly identical, confirming that presence of the transgene does not affect the types of fats found in chestnuts.

Testing for potential allergenicity or toxicity is more complicated than direct nutritional analyses, but one relatively easy way to evaluate a new product or ingredient is to compare it to foods that are already consumed and considered safe. For example, rice is one of the most common foods in the world. Other grains such as amaranth and sorghum are commonly used in health-conscious gluten-free baked goods, while oats and corn are consumed daily by millions of people and not considered dangerous. Spinach, tomatoes, strawberries, and tea leaves are also considered part of a healthy diet. All of these foods contain OxO, the same gene inserted into blight-tolerant transgenic American chestnut trees. Since OxO is not a concern for people who eat these gluten-free, non-allergenic, non-toxic foods, there is no reason to assume it would be a concern when expressed in chestnuts. In order to further verify the safety of OxO, we used the amino acid sequence (the precise order of protein building blocks that make up the OxO molecule) to screen several databases of all known allergens and toxins. Even when we used very conservative thresholds (so any remotely similar matches would be found), there were no matches between OxO and any known allergens or toxins. Results were the same when searching databases of known gluten proteins. It is worth noting that people can be allergic to pollen or nuts from various trees including chestnuts, but the important fact while evaluating transgenic trees is that the presence of transgenes doesn't change or enhance these risks.

In contrast to the lack of matches between OxO and known toxins, oxalic acid (the same tree toxin produced by the blight fungus) is known to be toxic to people, and it showed clear matches in the toxin databases. Oxalic acid can be acutely poisonous in high concentrations, and even at low doses it can contribute to problems like kidney stones. Some researchers have actually been testing OxO for use as a medicine to treat oxalic acid poisoning: early tests show promise for successful treatment, with no noted side effects, even when OxO is supplied at very high concentrations. This provides further evidence that OxO will not be harmful to people at the relatively smaller concentrations found in transgenic chestnuts.

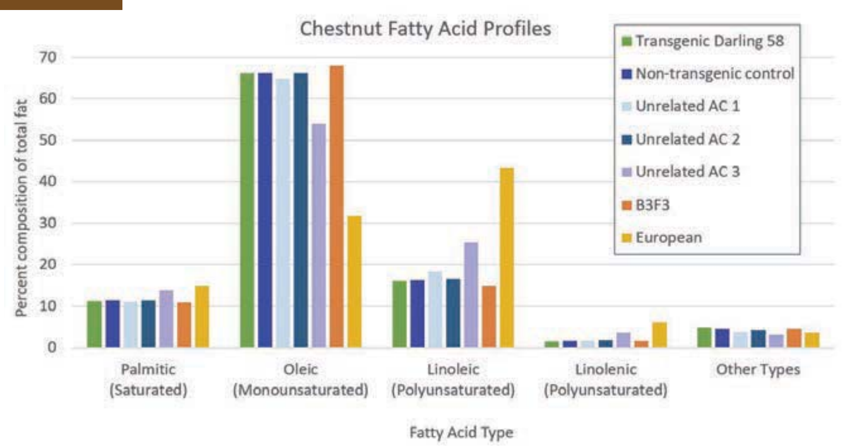
Each of these tests confirm that transgenic chestnuts do not present any enhanced safety risks to people. In conjunction with more than 100 years of research on OxO, which also has not shown any risks to people despite being found in many common foods, we are confident that transgenic chestnuts will be just as safe and nutritious as the chestnuts people have been eating and enjoying for centuries.

Figure 1



Percent composition of macronutrients in dried chestnut samples.

Figure 2



Fatty Acid Profiles, comparing relative proportions of different types of fat.