PART 2
OF A 3-PART SERIES

# Safety Tests

## ON TRANSGENIC AMERICAN CHESTNUT

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## EVALUATING SAFETY TO WILDLIFE

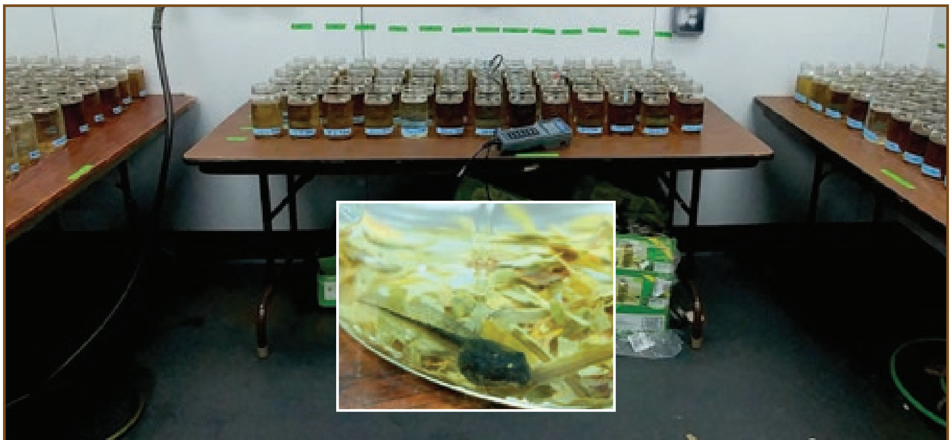
As described in the previous issue of *Chestnut*, the transgenic American chestnuts known as 'Darling 58' are being evaluated by regulators, have undergone many types of safety tests, and even more tests are underway. These trees contain a gene called oxalate oxidase, or OxO, which breaks down toxic oxalic acid produced by the blight fungus. OxO genes are ubiquitous in nature, as they are found in many types of plants, mosses, fungi, and bacteria. The previous article in this series described safety to people, in terms of chestnuts as a food product; this second installment will cover safety to wildlife.

As readers of this journal probably know, American chestnuts were incredibly important to a wide variety of wildlife: animals ranging from bees to bears benefited from these trees. It's well known that the productive nut crops provided a stable source of energy and nutrition for many birds and mammals, but other parts of the tree including prolific catkins, leaves, and large stems also provided sustenance and shelter for diverse groups of animals. Therefore, it is essential to consider interactions with wildlife for potential restoration efforts using blight-tolerant American chestnuts. Following are summaries of some of the experiments we have conducted involving wildlife interactions with transgenic American chestnuts.

Monarch butterfly near a flowering American chestnut tree.

Starting from the ground up, the first interaction we will look at involves vernal pools, which are temporary wetlands common in forests of the northeastern U.S. These pools get lined with deciduous leaves and form critical breeding habitat for unique forest inhabitants including insects, snails, and amphibians. We tested one interaction that takes place in vernal pools: wood frog tadpoles consume leaves and associated detritus in the pools, and we know they are sensitive to impacts like pollution or changes in leaf species. Our test (see photo on next page) involved 195 tadpoles, each in its own quart jar containing one type of leaves: transgenic American chestnut, non-transgenic American, hybrid, and Chinese chestnut, and other unrelated tree species controls. The most distinct difference we observed was with one of the controls: fewer tadpoles survived on American beech leaves compared to all other leaf types. There were no significant differences in survival or growth rates between transgenic and non-transgenic American chestnut leaves. In fact, in some conditions the tadpole's development rate was slightly faster with American chestnut leaves (whether or not they were transgenic) compared to all other leaf types.

The next interaction involves compounds called tannins: these chemicals are involved in plant pigmentation, and were used in the leather tanning industry until synthetic alternatives were developed. Tannins are relevant to wildlife because very high concentrations can impart a bitter flavor (this is why acorns don't taste good), but recent research has shown that moderate tannin concentrations can actually be beneficial in some animal diets. We know chestnuts in general have lower tannin content than relatives like acorns, but we wanted to look specifically at tannin levels in transgenic chestnuts compared to non-transgenic relatives. This test was also recommended by the



**Tadpole experiment setup at SUNY-ESF. Each quart jar contains 0.8 grams of dried leaves and one wood frog tadpole. Inset: healthy tadpole in a jar with crushed chestnut leaves.**

**Example microcolony containing five native bumble bees (*Bombus impatiens*) and pollen with OxO. Two connected chambers allow separate feeding and nesting areas.**

FDA for their regulatory analysis of the transgenic chestnut, since chestnuts can be used as livestock feed. We had tannins analyzed at an independent testing facility, and results showed substantial variation in tannin concentrations among different types of non-transgenic chestnuts – it's clear that growing conditions or ancestry make a difference. However, transgenic and related non-transgenic American chestnuts showed almost identical tannin concentrations.

Moving up the tree to the canopy level, there have been a few different types of experiments done to look at leaf herbivory by insects. Initial studies have shown that Chinese chestnut leaves may be less attractive to caterpillars than American chestnut leaves, but that transgenic American chestnut leaves aren't substantially different than non-transgenic relatives. However, to turn this around a bit, there are also several invasive forest pests that consume deciduous tree leaves, such as gypsy moth caterpillars. Forest managers use various types of treatments for gypsy moths, including natural biocontrol treatments. This results in a three-level interaction: biocontrols affect gypsy moths, which affect chestnut leaves. We looked in detail at these "tri-trophic" interactions: does chestnut leaf type change effectiveness of biocontrol treatments on invasive insect pests? As with the previously described tests, we observed differences among non-transgenic controls. In this case, Chinese chestnuts showed some differences in caterpillar mortality after biocontrol treatments compared to American chestnut lines. But again, the Darling 58 transgenic chestnut was not significantly different than its non-transgenic relative.

Finally, anyone who has been near a flowering chestnut tree in early summer can easily appreciate that catkins are numerous and very fragrant. Many insects take advantage of these chestnut flowers, including native pollinators like bumble bees, which are currently facing several environmental threats. Chestnut restoration could thus potentially benefit many types of insects that rely on pollen as a source of nutrients or hive-building material, and we know that insects contribute to successful pollination of chestnut trees. Since this is such an important interaction, we looked at potential effects of the OxO enzyme in pollen on native bumble bees. The bees were reared in a series of "microcolonies" made of take-out food containers (example photo at left), each containing five bees. This setup allowed bees to experience some natural social interactions, while allowing us to have enough replicated colonies for a good experiment. Each microcolony was supplied with chestnut pollen containing OxO, or a non-OxO control. We observed survival, body size, pollen use, and reproduction throughout the seven-week experiment, and saw no differences in any of these measures when bees were exposed to a field-realistic concentration of OxO in pollen.

All of these wildlife interaction tests show the same thing we have seen in other types of experiments: there may be variances between different chestnut species, hybrids, or even individuals of the same species, but any changes associated with the OxO transgene are insignificant by comparison. The next installment in this series will describe interactions with other plants and fungi, concluding our summaries of environmental interaction experiments. More detail on these tests and many others are described in our petition to the USDA for non-regulated status of the Darling 58 American chestnut, which should be publicly available soon.