PART 3
OF A 3-PART SERIES

Safety Tests

ON TRANSGENIC AMERICAN CHESTNUT

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Previous articles in this series have described safety of transgenic American chestnuts to people and wildlife; this final installment will cover safety to other plants and fungi. Studying these interactions is uniquely important for transgenic trees – if the transgenic American chestnut is approved for distribution, it will be the first time a transgenic plant is intentionally grown in the wild, so interactions with wild plants and fungi are being carefully examined.

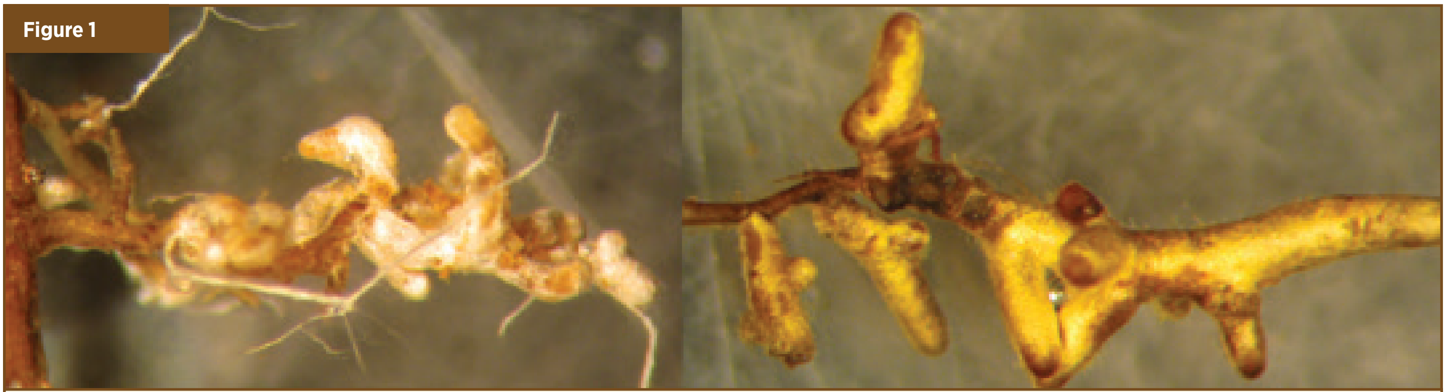
Since genetically engineered crop plants are common and highly regulated, safety tests on transgenic food products and animal interactions are common and relatively well-understood. However, evaluations involving environmental interactions in a forest ecosystem are relatively new, so we need to be both careful and creative in how we perform these tests. And since the purpose of the transgene is to provide tolerance to a fungal pathogen, it is especially important to understand how it could affect beneficial fungi.

The first step in looking at transgene interactions with fungi involves understanding how the transgene works. The oxalate oxidase (OxO) enzyme in 'Darling 58', which is found in wheat and a variety of other plants, does not kill or repel fungi – instead, it breaks down a toxin (oxalic acid) that is produced by the blight fungus. This suggests that OxO should not be directly harmful, even to the blight fungus itself, or to other fungi or life forms. In fact, oxalic acid is toxic to both people and other plants, so OxO can actually be considered an anti-toxin. (See Part 1 of this series in the 2020 winter issue, volume 34.)

Going beyond the logic arguments about the mechanism of OxO, we have conducted several types of experiments to look at chestnut roots and mycorrhizal fungi. Mycorrhizae are relationships between soil fungi and plant roots (**Figure 1**). These are typically "mutualistic" relationships, since they are beneficial to both organisms. If a new type of tree were unable to form mycorrhizae, we probably would not want to use it for wild restoration. But repeated tests[1, 2, 3] on OxO-expressing American chestnuts have shown that transgenic tree roots form mycorrhizae which are just as prevalent and diverse as those found on wild-type trees. These tests have taken place in both greenhouse and natural environments with similar results: in the most recent test, for example, more than 95% of the root tips we observed formed healthy mycorrhizae on both Darling 58 and non-transgenic controls.

In order to study a different environmental interaction that occurs in the soil[2], we started with seeds from other wild plants that are commonly found in American chestnut habitats. These included grasses, wildflowers,

Figure 1



Chestnut root tips encased in two types of mycorrhizal fungi. These fungi can help the tree access water and nutrients, in exchange for energy in the form of sugar from photosynthesis.

shrubs, and trees. We germinated these seeds in potting soil containing crushed chestnut leaves of various types: transgenic American chestnut; a related non-transgenic control; backcross; Chinese; F_1 hybrid; an unrelated American chestnut; and a no-leaf control. We counted seedling germination in each type of chestnut leaves (**Figure 2**), and recorded the total mass of all seedlings grown in each leaf type. There were only two statistically significant differences among leaf types in the whole study: first was dry mass of one wildflower type, which was slightly lower in B_3F_3 leaves than Darling 58 leaves. (Note – while this difference was statistically significant, we shouldn't assume that B_3F_3 leaves inhibit plant growth. Normal growth of this flower in F_1 hybrid and Chinese leaves would suggest we're just seeing a range of flower growth, rather than a real effect due to the B_3F_3's genetic background.) The other significant difference was in the number of pine seedlings that germinated in the unrelated wild-type American chestnut leaves compared to the no-leaf control treatment, but pine germination was not unusual in either Darling 58 or B_3F_3 leaves. An unrelated experiment in three of our field plots also looked at plant growth near various types of chestnuts: again, there were no differences in plant growth based on the type of chestnut that was growing nearby.

The final interaction to be described in this article also involves chestnut leaves after they fall from the tree. If you can picture a deciduous forest in autumn, you probably understand the ecological importance of leaf decomposition! One question about transgene safety involves how long the transgene product (the OxO enzyme, in this case) remains biologically active. Of course, OxO must be active while it's in the tree; that is what breaks down the toxin and imparts blight tolerance. But what happens in leaves or branches once they fall from a transgenic tree? We used a simple enzyme activity assay (see article by Thomas Klak on page 30) to test enzyme activity in the fall while leaves were still attached to trees, and then at a series of time-points after they had been removed. We determined that it is possible to preserve OxO activity in leaves for several months, but only if the leaves are carefully packed in plastic bags and immediately stored in a freezer. In natural conditions, where leaves are exposed to temperature fluctuations and dry relatively quickly, OxO activity ceased as soon as the leaf dried or turned brown (**Figure 3**). This occurred after about a week during normal outdoor conditions in our tests. This suggests that the presence of OxO in leaves should not substantially affect decomposition and associated nutrient cycling processes.

More detail on these tests and many others are described in our petition to the USDA for nonregulated status of the Darling 58 American chestnut, which should be publicly available soon, though not at the time this issue was in production. Watch for email and website updates (www.acf.org) from The American Chestnut Foundation!

Figure 2

Roots of a wildflower growing through a chestnut leaf.

Figure 3

Chestnut leaves at various stages of decomposition. The OxO enzyme is no longer active once the leaf dries or turns brown (middle leaf).

FOOTNOTES

[1] https://doi.org/10.1128/AEM.02169-14.

[2] https://doi.org/10.3389/fpls.2018.01046.

[3] https://pqdtopen.proquest.com/doc/1418032936.html?FMT=ABS.