

# Fitness Effects of Transgenic Disease Resistance in Sunflowers

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Fears about transgene escape have focused attention on the potential for hybridization between crops and their wild relatives. Although transgenes will often escape from cultivation (1), their rate of spread will be mainly governed by their fitness effects, not the migration rate (2). Thus, only highly advantageous transgenes will spread rapidly enough to have a substantial ecological impact. Therefore, research on the risks associated with transgene escape should focus on the fitness effects of the gene(s) in question.

Here, we examined the fitness effects of a transgene conferring resistance to white mold (*Sclerotinia sclerotiorum*) in sunflower (*Helianthus annuus*). Unfortunately, attempts to breed white mold resistance have met with little success, and chemical control methods are costly and often ineffective. Therefore, attention has turned to genetic modification. An oxalate oxidase (OxOx) transgene has now been used to enhance white mold resistance in cultivated sunflower [supporting online material (SOM) text], presumably by degrading oxalic acid, which contributes to white mold pathogenicity (3).

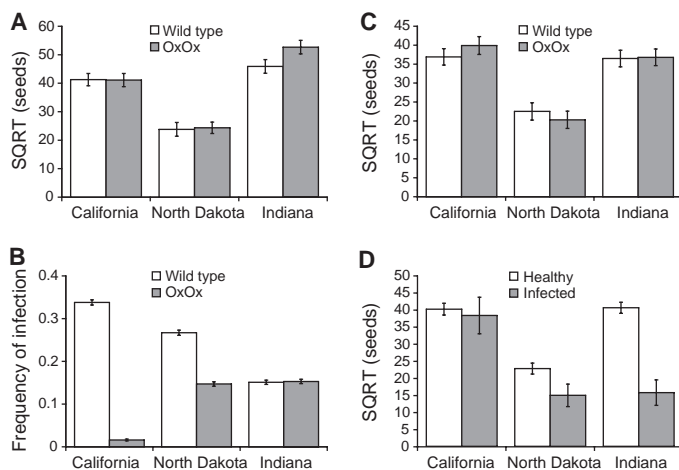
Most of the sunflower acreage in the United States occurs within the range of wild sunflower, and many fields flower coincidentally with neighboring wild populations (4). Where they come into contact, cultivated and wild sunflower often hybridize (5), making transgene escape a virtual certainty. Combined with the efficacy of the OxOx transgene, this raises the possibility of hybridization giving rise to a more invasive wild sunflower.

To simulate the early stages of escape, we backcrossed the OxOx transgene into wild sunflower and grew the resulting plants in containment cages at field sites in Indiana, North Dakota, and California (6). Just before flowering, we inoculated half of all the plants with white mold and

then monitored them for symptoms of infection.

Presence or absence of the OxOx transgene had no effect on seed output ( $P = 0.25$ ), indicating that there was no cost of resistance in the absence of a pathogen challenge (Fig. 1A). In terms of infection rates, the OxOx transgene did provide protection against white mold ( $P = 0.002$ ) (Fig. 1B). The transgene did not, however, have any effect on seed output after inoculation ( $P = 0.84$ ) (Fig. 1C). Though the transgene provided protection against white mold infection, it had no effect on reproductive output. This result has a simple explanation: Variation in the likelihood of infection was offset by variation in the severity of infection (compare Fig. 1, B and D). In California, where the transgene provided the most protection against infection, disease onset had no effect on seed output. In contrast, white mold infection caused a severe decline in seed output in Indiana, but infection rates were unaffected by the transgene. Thus, the transgene had a significant effect on the likelihood of infection, and infection had a negative effect on seed output ( $P \leq 0.0001$ ) (Fig. 1D), but the disease effect varied across locations ( $P = 0.001$ ), nullifying any advantage of the transgene.

Our results suggest that the OxOx transgene will do little more than diffuse neutrally after its escape. This is especially true because our experiment simulated the worst-case scenario, in which early generation hybrids faced a severe pathogen challenge.



**Fig. 1.** Effects of the OxOx transgene and white mold infection on crop × wild sunflower hybrids. (A) Seed output of control individuals. (B) Frequency of infection after inoculation. (C) Seed output after inoculation. (D) Effect of infection on seed output after controlling for presence or absence of the transgene. All values are expressed as least-squares means ± one standard error.

These results also illustrate the importance of quantifying fitness directly, rather than using a presumptive correlate such as disease incidence; had we relied solely on infection rates, our conclusions would have been quite different. Although the mechanism(s) responsible for the decoupling of infection rate and disease severity are unclear, one possibility is that conditions favoring white mold infection differ from those favoring development of the disease once it has been acquired. The hotter, drier climate of California might, for example, make plants more susceptible to infection but hinder development of the disease. Regardless, it appears that, by giving the OxOx transgene to wild sunflower, we gave it something that it already had: some degree of white mold resistance.

One caveat is that this work was performed within a single season and on a single genetic background. Therefore, our results may not be generalizable. Another caveat is that plants in this experiment were not subjected to environmental stresses such as drought; plants grown under stress may be less able to protect themselves from disease, and thus derive a greater benefit from transgenic disease resistance. Of course, stressful conditions could also reveal a cost of resistance not otherwise observed.

Future studies assessing the environmental impact of transgenes should not only be replicated over space and time, but should also examine the effects of genetic background and environmental stresses. Regardless of the form of future research, an informed judgment of the risks and benefits of genetic modification on a case-by-case basis is preferable to either the dismissal of transgenic approaches entirely, or the introduction of transgenic crops in the absence of appropriate scientific scrutiny.

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6. Materials and methods are available as supporting material on Science Online.
7. We thank C. Alarcon, G. Cole, J. Durphy, R. Essner, E. Haro, E. Hoefl, J. Holte, C. Scelonge, and L. Wang. This work was supported by grants from Pioneer Hi-Bred, Intl. (to L.H.R.) and the United States Department of Agriculture (to J.M.B.).

## Supporting Online Material

www.sciencemag.org/cgi/content/full/300/5623/1250/DC1  
SOM Text  
Materials and Methods

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pathogen or by mass fruiting episodes).

In summary, the chances of capturing carbon release with similar accuracy as carbon uptake are minute, preventing meaningful budgeting, irrespective of the precision of the equipment used. Reliable carbon budgeting beyond the plot level will thus mostly remain the domain of forest inventories and remote sensing. At larger (hemispheric or global) scales, we depend on atmospheric signals.

Plot-based carbon flux measurements in connection with tree and soil studies are and

will remain a powerful tool for understanding carbon fluxes in forests and for testing models (4, 6, 10, 12). But it is important to remember that they cannot produce a realistic picture of a landscape's contribution to carbon sequestration. The same holds for growth responses of forests to elevated CO<sub>2</sub>. When forest dynamics are accounted for, a stimulation of growth rate and acceleration of development may reduce the residence time of carbon per unit land area, and thus may even diminish long-term carbon storage (11, 13).

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## PLANT SCIENCES

# Super Sunflowers— Stopping the Rot?

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The creation of a “superweed” is an enduring image used by environmental lobbyists opposed to the introduction of genetically modified plants. On page 1250 of this issue, Burke and Rieseberg (1)

highlight an important point concerning the potential escape of genes inserted into

genetically modified crops. They examine the transgene that confers resistance to the white rot fungus (*Sclerotinia sclerotiorum*) in sunflowers (*Helianthus annuus* L). These authors demonstrate that it is not the transfer of genes per se that is important, but rather their fitness to persist in a new host plant (1).

Among the first generation of transgenic plants introduced into agriculture were crops rendered resistant to herbicides or made tolerant to insect attack. These traits presented clear benefits to farmers who wished to minimize crop loss and to simplify agricultural practices. It is easy to envisage the genes that encode these traits escaping from the crop plant and entering conventionally bred plants or related species in the wild. In Europe, a series of major food and feed scares have sensitized the public to “green” issues, perhaps to a much greater extent than elsewhere. The largest such problem has been the emergence of bovine spongiform encephalopathy and its link to variant Creutzfeldt-Jakob disease in humans. The recent epidemic of foot-and-mouth disease among farm animals in the United Kingdom has refocused public attention on agricultural practices,

at least in Europe. This perhaps explains the high level of skepticism or hostility toward the introduction of genetically modified plants in agriculture in Europe, and probably also contributes to Europe having the most thorough regulation of genetically modified crops.

The European Union has recently reviewed legislation regarding the deliberate release of genetically modified organisms into the environment. This resulted in the publication of European Directive, 2001/18/EC on the deliberate release into the environment of genetically modified organisms (2). This legislation recognizes that “...the effects of such releases [of live genetically modified organisms] on the environment may be irreversible.” The European Commission has recently published a guidance document (3) to aid risk assessors intending to apply for permission

to release genetically modified plants for commercial purposes. This document includes a discussion of the environmental risk-assessment process, including consideration of the potential impact of gene flow to unmodified plants and to wild relatives. This document does not make direct reference to an estimation of the “fitness” of the inserted transgene in a genetically modified plant. It recognizes, however, that genes will escape from genetically modified plants and that this is a significant factor in a proper risk assessment. This recognition is central to the argument made by Burke and Rieseberg (1).

Farmers grow sunflowers commercially for their oil, and sunflower seed cake, a by-product of oil production, is an important feed commodity for ruminants. Sunflower crops are particularly important in temperate climates, where the growing conditions are too dry for other oilseed crops, although in Europe sunflowers are less important as a crop than are soyabeans and canola (rape-seed) (4). Sunflower plants are, however, prone to attack by the white rot fungus *S. sclerotiorum* (see the figure). This fungus is a highly successful plant pathogen that attacks a range of economically important plants, including those involved in oilseed

production. It has a broad ecological and geographical distribution, often being found in temperate climes. Fungal mycelia may persist in soil to cause infection, but aerial spores are also responsible for dissemination of this plant pathogen. Head rot disease of sunflowers results from aerial spread of the fungal spores. Disease in sunflowers may also manifest as wilt and middle stem rot. *S. sclerotiorum* is one of the most economically significant pathogens of sunflowers. Despite its widespread geographical distribution and its broad host range, its hosts appear not to be naturally resistant to attack by *S. sclerotiorum*. Oxalic acid is an important viru-



**Sunflowers afflicted with white rot.** Stem rot in a sunflower plant caused by the white rot fungus *S. sclerotiorum*. The sunflower plant has an extensive infection that has caused it to wilt as evidenced by the leaves, which are drooping down the stem. The fungal mycelia and fruiting bodies can be seen erupting from the stem of the plant.

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## PERSPECTIVES

lence determinant for this fungus (5). Certain cereal plants are resistant to attack by *S. sclerotiorum* through the production of oxalate oxidase (OxOx), an enzyme that breaks down oxalate, producing carbon dioxide and hydrogen peroxide. The introduction into sunflowers of a gene that codes for oxalate oxidase results in plants that resist white rot attack (6).

Burke and Rieseberg measure the impact on the relative fitness of sunflowers of the transgene conferring resistance to the white rot fungus. Even in the presence of a direct challenge from the pathogen, the transgene has no direct effect on sunflower fitness. With this demonstration, the authors show that the effect of transgene spread is more important than considering the likelihood that the spread may happen. They conclude that, for this particular construct and for the three locations chosen for

their study, the transgene provides protection against white rot infection. Despite this finding, reproductive output did not improve in hybrid plants containing the OxOx transgene compared with wild-type plants. Antagonists of the use of genetically modified plants in agriculture may argue that this is a single case and that results derive from a single growing season. It would be unwise to extrapolate from this particular set of observations to other constructs in different conditions, but the authors themselves highlight this limitation. The strength of this paper does not lie in its demonstration that not all genetically modified plants will form “superweeds.” Rather, it shows that, when considering the persistence of transgenic material in the environment, it is important to ask the right questions. Only when we ask the correct questions will we reach an informed judgment

on the risks, and benefits, of genetically modified plants.

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## MATERIALS SCIENCE

# Nanocrystals Get Twins

Jørgen B. Bilde-Sørensen and Jakob Schiøtz

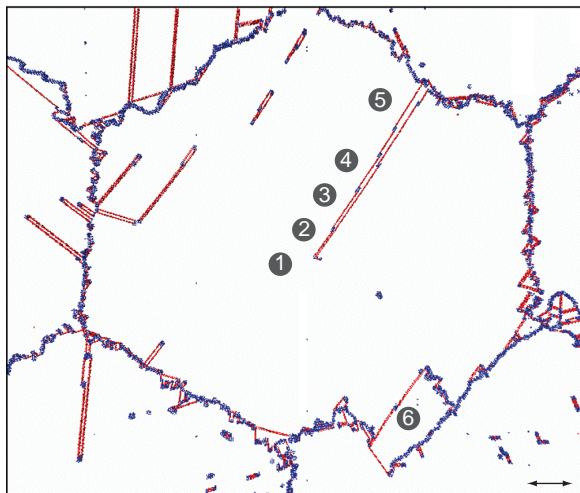
In the messy world of materials science, experimental observations often precede theoretical attempts to find a suitable explanation. But on page 1275 of this issue, Chen *et al.* (1) report the reverse scenario: an experiment that corroborates recent molecular dynamics simulations (2–4).

The authors study the plastic deformation of nanocrystalline aluminum. Textbooks tell us that deformation twinning has never been observed in coarse-grained aluminum, but in the simulations, deformation twinning occurred in aluminum with a sufficiently small grain size. And that is exactly what Chen *et al.* now show experimentally.

Plastic deformation of crystalline materials has long been known to occur by the movement of line defects called dislocations. A perfect dislocation moves one part of the crystal by a complete lattice vector with respect to the other, such that it leaves a perfect lattice. A partial dislocation, however, moves one part by less than a complete lattice vector, leaving a faulted layer in the crystal. If this occurs on consecutive planes in a face-centered cubic (fcc) crystal, it results in the formation of a twin, which has a lattice that is a mirror of the host lattice. Deformation twinning is well known from some fcc systems, such as brass, but is neither observed

nor expected in coarse-grained aluminum owing to the high energy of the planar faults.

Nanocrystalline materials have attracted much interest in recent years because of their extraordinary properties. The strength and hardness of materials increase with decreasing grain size until, below a certain grain size, the material starts to become softer again (5, 6). As the grain size decreases to very small values, the fraction of the atoms that resides in or near a grain boundary increases drastically, and it is therefore to be expected that grain-boundary effects will start to dominate at a cer-



**Emergence of twins in a simulation of nanocrystalline aluminum.** (1) to (5) mark one emerging twin; (6) marks another twin formed by a slightly different mechanism.

tain grain size. This has also been found by several atomistic simulations (7, 8).

Individual dislocation may interact with other dislocations and other lattice defects, such as grain boundaries. The generation, propagation, and annihilation of dislocations during deformation are therefore rather complex processes. Atomistic simulation is an obvious tool to apply to these processes. However, even with modern computers this is a challenging task, because a very large number of atoms has to be handled during the computation, and the geometry of the simulated volume has to be chosen carefully.

The first indication that deformation twinning could occur in aluminum under certain circumstances was reported by Tadmor *et al.* (2). The authors had used the so-called quasi-continuum method to simulate what happens when a nanometer-scale, rigid object is pressed into the surface of a thin aluminum film. However, the geometry was unusual, creating essentially a two-dimensional state of deformation. Furthermore, the deformation twins created during the indentation disappeared again on unloading.

Further support was provided by Yamakov *et al.* (3, 4), who observed several deformation twins in molecular dynamics simulations of nanocrystalline aluminum under tensile deformation (see the figure). These simulations still have some obvious limitations. For example,

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