

Ecological effects of pest resistance genes that disperse into weed populations.

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Abstract

Gene flow from transgenic crops to cultivated or free-living plants has been the subject of much recent research, but little is known about the ecological and evolutionary consequences of this process. Here we focus on the question of whether transgenes that confer resistance to herbivores or diseases are likely to affect the fitness and population dynamics of free-living plants. An important first step in this research is to determine whether natural populations that can hybridize with crops are exposed to the types of herbivores and diseases that would be thwarted by resistance genes from the crop. In many cases, the fitness consequences of particular resistance genes may be negligible in wild populations. Wild plants may be unaffected by the pest, or they may not be exposed to it. However, our studies of wild sunflowers (*Helianthus annuus*) show that a *Bt* gene for lepidopteran resistance can be associated with reduced herbivory and enhanced fecundity under natural levels of insect pressure. Moreover, we did not detect any fitness costs of this transgene. Once it is known that resistance genes can enhance the fitness of wild or weedy plants, further studies are needed to assess whether these populations could become more widespread or invasive. In general, little empirical information is available about the extent to which various herbivores and diseases limit populations of wild or weedy relatives of crop plants. Due to the difficult, long-term nature of research on plant population dynamics, we recommend fitness studies as a key element in assessing the ecological effects of pest resistance genes. From a regulatory standpoint, it is also useful to examine whether new transgenic constructs could have greater ecological effects than ongoing gene flow involving nontransgenic resistance traits.

Key words: crop-wild hybridization, gene flow, transgenic resistance, herbivory, disease, fitness effects, population-level effects, ecological consequences of gene flow, *Bacillus thuringiensis* (*Bt*)

Introduction

Worldwide, many cultivated plants hybridize spontaneously with wild or weedy relatives (Small 1984, Ellstrand et al. 1999). In the USA, for example, this occurs in more than twenty species, including sunflower, sorghum, squash, canola, rice, sugar beet, poplar, turf grasses, and forage grasses (NRC 2000). In addition, many crops can become naturalized and persist as feral weed populations (Ellstrand et al. 1999). Thus, transgenes conferring novel traits that enhance survival and reproduction may

inadvertently disperse from cultivated plants to wild or weedy populations that lack these traits. In the short term, the spread of transgenic herbicide resistance is likely to pose challenges for controlling weeds and unwanted “volunteer” crop plants (Snow et al. 1999, Hall et al. 2000). Over the longer term, we need to know whether the spread of transgenes coding for other fitness-related traits could exacerbate weed problems in agricultural settings and affect the population dynamics of wild relatives in unmanaged areas (Snow and Morán Palma 1997, NRC 2000, Snow 2002). This raises fundamental questions about the extent to which herbivores, diseases, and stressful abiotic conditions regulate populations of wild and weedy plants.

A transgenic trait that increases a crop plant’s survival or yield has the potential to enhance the fitness of free-living crop relatives. Crop species with transgenic resistance to certain insects and diseases have already been commercialized, so we focus on these types of resistance genes in this paper. With regard to herbivores, studies of both native and exotic species suggest that herbivores can have a dramatic impact on plant population dynamics (e.g., Crawley 1997, Rees and Paynter 1997, Marvier and Kareiva 1999). Also, several recent studies have reported negative impacts of viral or fungal diseases on the growth, survivorship, and reproduction of plants in natural populations (e.g., Friess and Maillet 1996, Packer and Clay 2000, Funayama et al. 2001, Power 2001). However, little is known about the extent to which herbivores and diseases affect the population dynamics of wild or weedy relatives that hybridize with cultivated plants.

Previously, little attention has been paid to the effects of nontransgenic resistance genes on populations of wild relatives. One reason for this may be related to the fact that useful agronomic traits have often been obtained from wild relatives in the first place, creating the impression that these taxa are already very well adapted to local conditions. In some cases, however, agricultural breeding has resulted in crops with single-gene resistance traits that are easily transferred to wild populations lacking these traits. For example, resistance to a fungal disease (*Puccinia* spp.) has been bred into cultivated sunflowers (*Helianthus annuus*) using wild germplasm (Seiler 1992). This trait is not ubiquitous in wild/weedy sunflower populations (also *H. annuus*) and can spread to wild populations via crop-to-wild gene flow (Snow et al. 1998). A field experiment with wild-crop hybrids showed that naturally infected plants produced ~20% fewer seeds per plant than plants that lacked *Puccinia* symptoms (Snow et al. 1998). In general, it is not known which fitness-related crop genes have spread to wild populations and persisted, so it is difficult to generalize about this process and its ecological consequences. Single-gene resistance traits, especially dominant ones, are expected to move from crops to wild populations more easily than polygenic, quantitatively inherited resistance traits.

Another reason that the fitness consequences of crop-to-wild gene flow have not been examined is that crop genes are often considered to be harmful to wild plants. Traits such as short flowering periods, lack of seed dormancy, lack of seed dispersal mechanisms, and a lack of secondary compounds that deter insects would likely be detrimental to wild plants, leading to the conclusion that many crop genes would have deleterious effects on the fitness of wild or weedy relatives (e.g., National Research Council 1989). Moreover, the very low fertility of some wild-crop hybrids could impede the flow of crop genes into wild populations. However, recombination and introgression can allow deleterious crop genes to be purged from wild populations,

while other crop genes that confer neutral or beneficial effects persist. This can occur even when the fertility of F₁ wild-crop hybrids is much lower than that of wild plants. Based on principles of population genetics, we expect that beneficial genes that are tightly linked to strongly deleterious crop genes will be lost from wild populations (e.g., Gressel 1999), while other beneficial crop genes will increase in frequency. It is these types of crop genes, including transgenes, that have the potential to have unintended and unwanted ecological consequences. Thus, it is useful to determine whether particular transgenes, and specific transgenic events, are associated with fitness costs or benefits in wild populations.

General Approaches to Fitness Studies

Fitness is typically defined as the product of survival and lifetime seed production of a given group of genotypes, such as transgenic wild plants, relative to the survival and reproduction of another group of genotypes, such as control plants lacking a particular transgene (e.g., Silvertown and Charlesworth 2001, Gurevitch et al. 2002). A rigorous way to examine fitness effects of a given transgene is to conduct field experiments involving two groups of wild plants that differ in the presence or absence of the transgene, but otherwise have the same genetic composition. This approach is described in the case study below, but resistance transgenes generally are not available to ecological researchers prior to commercialization of a crop, when risk assessments are carried out. Therefore, it is often necessary to begin with ecological studies of the prevalence of target pests in wild populations. In any event, it is useful to have a broad understanding of ecological factors that could affect the survival and reproduction of crop relatives when evaluating risks associated with novel crop genes.

A first step in this research is to determine whether pests that are the target of particular resistance (trans)genes occur in natural populations, and whether natural populations are susceptible to these pests. For example, wild cabbage (*Brassica oleracea*) in the United Kingdom is commonly infected by several viruses that reduce survival, growth, and reproduction, so transgenic resistance to these viruses could be beneficial (Maskell et al. 1999, Raybould et al. 1999). In contrast, wild carrot (*Daucus carota*), appears to be resistant to a common fungal disease, *Alternaria dauci*, in the Netherlands (Schouten et al. 2002), in which case wild plants are not expected to benefit from obtaining a transgene that confers resistance to this disease. Similar surveys can be carried out for insect groups (e.g., lepidoptera or coleoptera) that are the target pests of various Bt genes.

In many cases, quantifying the prevalence and effects of naturally occurring herbivores and diseases on plant populations is challenging. Few researchers study plant-pathogen interactions in wild and weedy plants, so little previous information is available. Moreover, site-to-site and year-to-year variation in pest populations can be considerable, making it difficult to generalize from short-term and small-scale field studies (NRC 2000, 2001). Assessing the importance of infrequent outbreaks and/or patchily distributed pest populations may be impractical within the time frame that is available for regulatory decision-making. A lack of empirical data on the prevalence of target insects or diseases in populations of crop relatives calls for a cautious approach to the deregulation of novel transgenic resistance traits (NRC 2000, 2001).

When pest populations are known to be fairly common, a further challenge is to determine the extent to which these herbivores and diseases affect the fitness and

population dynamics of wild plants. Sometimes it is possible to exclude plant pests in experimental field plots, for example by using insecticides (e.g., Louda and Potvin 1995), to quantify possible increases in seed production and seedling recruitment. But it is often difficult to mimic the effects of specific transgenes, such as constitutively produced Bt toxins or viral coat proteins, on the growth and reproduction of wild plants. To gain an understanding of how a given transgene or group of transgenes will affect the fitness of wild relatives, it is useful to carry out field and greenhouse experiments using transgenic wild plants. These experiments should be carried out under careful confinement procedures in much the same way as field tests of experimental transgenic crops are performed. Ideally, these two types of research should proceed simultaneously so that results pertaining to risk assessment can be made available to regulatory agencies and the public in a timely manner.

Bt Wild Sunflower: A Case Study

Note: A more detailed description of this case study can be found in a workshop contribution by Pilson et al. at http://www.biosci.ohio-state.edu/~lspencer/gene_flow.htm

Wild sunflower (*Helianthus annuus*) represents an excellent model system with which to address these questions. Wild sunflower is a native, self-incompatible, annual plant that is widespread throughout much of the USA, reaching its greatest abundance in midwestern states where most cultivated sunflower is grown. The process of crop-to-wild introgression has been well documented in sunflowers. Field experiments have shown that pollinators can transfer crop pollen to wild plants as far as 1,000 m away, with the frequency of hybrid seeds being greatest (up to 42%) at the crop margin (Arias and Rieseberg 1994, Whitton et al. 1997). Additional studies have shown that first generation wild-crop hybrids usually produce fewer seeds per plant than their wild counterparts, but the magnitude of this difference varies a great deal among plants, regions, and growing conditions (Snow et al. 1998). Under some field conditions, seed production of F₁ crop-wild hybrids is comparable to that of purely wild plants, and in several cases hybrids produce at least 50% as many seeds per plant as wild genotypes. Furthermore, selectively neutral crop markers have persisted for many generations in wild plants sampled in California, Kansas, North Dakota, and Canada (Whitton et al. 1997, Linder et al. 1998). These studies demonstrate that introgression of neutral or beneficial crop genes into wild gene pools can be an ongoing process wherever these taxa occur sympatrically. Clearly, both genetic and geographic barriers to gene flow from crop to wild sunflower are minimal.

Wild sunflower is a host for many insect herbivores (Pilson 2000), several of which are also pests of the crop. Wild sunflowers often are damaged by lepidopteran and coleopteran insects that feed inside the plant on seed heads, stems, and roots (Pilson 2000; Snow et al. 2002). The most damaging insect pests of cultivated sunflower are those that infest developing seed heads (weevil, moth, and midge larvae) and those that transmit disease (e.g., stem weevils that transmit phoma black stem; Schneiter 1997). Polygenic resistance to insects has been documented in other species of *Helianthus*, but efforts to introgress strong resistance into the crop have been unsuccessful (Seiler 1992). For these reasons, cultivated lines with transgenic resistance conferred by Bt

toxins are being developed by a number of seed companies, and several field trials have been approved by regulatory agencies (<http://www.isb.vt.edu>). Different Bt-toxins are specific to different groups of insects, including lepidoptera, coleoptera, and diptera. Bt-induced resistance to coleoptera was first field-tested in the US in 1996 and resistance to lepidoptera was approved for field-testing in 1999, although none have been commercialized to date. Additional field trials have taken place in the Netherlands and Argentina (<http://www.isb.vt.edu>, <http://siiap.sagyp.mecon.ar/http-hsi/english/conabia/liuk4.htm>). Broad-spectrum resistance involving multiple Bt genes and other genes for insect resistance may also be developed in the future.

We studied a crop-developed *Bacillus thuringiensis* (Bt) transgene, *cry1Ac*, in backcrossed wild sunflower populations (Snow et al., 2002). To simulate the effects of introgression of a Bt transgene from the crop, male-sterile wild plants from a population near the Cedar Point Biological Station in Nebraska were bred with transgenic cultivars to create BC₁ progeny that segregated for both the Bt transgene (Bt+ or Bt-) and for male-sterility (male-sterile or male-fertile). However, to prevent the accidental escape of the transgene we did not use Bt+/male-fertile plants in the field. BC₁ progeny were planted in the field in 1999 at the Cedar Point Biological Station in western Nebraska and in an agricultural field in eastern Colorado, near Burlington. The effect of the transgene was examined by comparing insect damage and fecundity between Bt+/male-sterile and Bt-/male-sterile plants. These experiments were carried out under USDA-APHIS Permits 99-096-01N and 99-095-07N. All wild and BC₁ seed heads were collected, and any sunflower seedlings that appeared at the sites after tilling in 2000 and 2001 were destroyed.

Transgenic resistance to lepidopterans appears to be a dominant trait because BC₁ plants that were hemizygous for this gene had very low levels of lepidopteran damage (50% of the BC₁ plants inherited the transgene, as expected). Lepidopteran damage on transgenic plants was strongly reduced relative to control plants at our two study sites, while damage by several weevil and fly species was unaffected. As a result of reduced herbivory, transgenic plants produced an average of 55% more seeds per plant relative to nontransgenic controls at the field site in Nebraska. A similar but non-significant trend was seen at the site in Colorado (14% more seeds per plant). At both sites, plants that were male-sterile had less lepidopteran damage and more seeds per plant than control plants that were male-fertile (based on comparisons between non-transgenic plants, with or without pollen). This could be due to the fact that lepidopterans also feed on sunflower pollen (Delisle et al. 1989, Korman and Oseto 1989) and may prefer pollen-producing plants. If this is the case, we may have underestimated the advantage of a Bt transgene because this estimate was based on comparisons between two groups of male-sterile plants, with or without the transgene.

In any study of a single transformation event, it is not clear whether phenotypic effects (e.g., greater fecundity) are caused by the transgenic construct or by other mechanisms, such as position effects, pleiotropy, or close physical linkage with other crop genes. Thus, it is useful to determine whether effects associated with the Bt transgene can occur in the absence of lepidopteran herbivores. We performed a greenhouse experiment using BC₁ plants to examine this possibility, while recognizing there are many biotic and abiotic differences between field and greenhouse conditions. The Bt transgene had no effect on the number of inflorescences or seeds per plant in the

greenhouse, regardless of whether the plants were grown under water-stressed, drought-stressed, or control conditions, and regardless of whether they were male-fertile or male-sterile. This suggests that the transgene was not associated with an inherent fitness cost or benefit. It would be preferable to employ a wider range of growing conditions and several transgenic events in this type of study, but our results suggest that the fecundity advantage of transgenic plants in the field was due to protection from lepidopteran herbivores.

To summarize, this study shows that selection favoring an increase in the frequency of a Bt transgene has the potential to be quite strong. Therefore, we expect that subsequent generations of Bt wild plants would produce more seeds per plant than non-transgenic individuals in many locations and growing seasons, depending on the abundance of lepidopteran herbivores. If so, the transgene is expected to increase in frequency. When this occurs, we expect that this very effective Bt transgene would be expressed in many wild plants and would kill susceptible, native lepidopterans that feed on these plants. Thus, proteins from pest resistance genes could potentially affect non-target organisms and ecological communities when these genes become common in wild sunflower populations. It is possible that specialist herbivores would eventually evolve resistance to transgenic Bt toxins, but this has not been reported yet in target pests of transgenic Bt cotton or corn (e.g., Carriere et al. 2001).

Prior to this experiment, we predicted that Bt wild sunflowers would gain a fecundity benefit of about 10-15% at the Nebraska field site, based on preliminary ecological surveys of lepidopteran damage within seed heads. Without access to a constitutively expressed Bt transgene, we would not have known that a much larger fecundity benefit could occur under field conditions. It would be very instructive to repeat this experiment in other years and locations, using advanced generations of Bt wild sunflowers, and following accepted methods for confinement of the transgene construct. However, the biotechnology companies that developed this transgene have not allowed us to continue our research. Thus, legal constraints associated with patented genes can hinder ecological studies involving non-commercialized transgenic crops.

Moving Beyond Small-Scale Fitness Studies

Once it is known that a given transgene has the potential to enhance the fitness of wild relatives, we need to know whether transgenic plants that produce more seeds per plant are likely to give rise to larger populations, more populations, and/or more extensive seed banks. Experimental manipulations of local population dynamics and models of metapopulation dynamics are needed to understand these processes. We are currently pursuing these approaches with wild sunflower. In addition, to gain a better understanding of how lepidopteran herbivores affect wild sunflower populations, experiments similar to those described above should be repeated over several study sites and seasons. Fitness studies are an essential first step in understanding the ecological and evolutionary effects of gene flow because it is important to know the magnitude of presumed fecundity effects of a given transgene. This knowledge, together with an evaluation of the ecological effects of transgenes, is critical for biosafety risk

assessments. Due to the difficult, long-term nature of research on the effects of naturally occurring herbivores and diseases on plant population dynamics, we recommend fitness studies as a key element in assessing the ecological effects of pest resistance genes. From a regulatory standpoint, it is also useful to examine whether new transgenic constructs could have greater ecological effects than ongoing gene flow involving nontransgenic resistance traits.

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