

## Assessment of gene flow and potential effects of genetically engineered sunflowers on wild relatives

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### Abstract

In the USA, cultivated sunflower (*Helianthus annuus*) is often sympatric with wild *H. annuus*, which is an agricultural weed that grows along roadsides and in other disturbed sites. We found that crop-to-wild gene flow was common (~5-40% hybrids) when wild plants occurred within <1,000 m of the crop, and crop-specific genetic markers persisted in wild populations for many generations. Crop-to-wild gene flow with other wild sunflower species is far less likely due to infertility barriers and non-overlapping ranges. Field experiments with *H. annuus* demonstrated that F<sub>1</sub> crop-wild hybrids typically produced fewer viable seeds than wild plants, but this disadvantage varied among plants, regions, and growing conditions, and diminished with further backcrossing. Thus, the F<sub>1</sub> generation is not a strong barrier to introgression of transgenes into wild populations. Little is known about how introgressed transgenes will affect the population dynamics of wild plants, but we suspect that release from insect damage and disease pressure will sometimes enhance the survivorship, competitive ability, and lifetime seed production of wild sunflowers, perhaps causing them to become more invasive. Our current research focuses on the ecological effects of insect seed predators and other pests in an effort to anticipate effects of transgenes for insect resistance in wild populations of *H. annuus* (transgenic sunflowers have not yet been marketed in the USA).

### Introduction

Crop-to-wild hybridization has the potential to influence the evolutionary ecology of related wild/weedy taxa, but little is known about the ecological effects of crop genes that enter wild populations via pollen movement (e.g., Rissler and Mellon, 1996; Snow and Morán-Palma, 1997). Examples of crops that hybridize spontaneously with wild/weedy populations include sunflower (Arias and Rieseberg, 1994), squash (Kirkpatrick and Wilson, 1988), radish (Klinger et al. 1992), rice (Langevin et al. 1990), foxtail millet (Till-Bottraud et al. 1992), sorghum (Arriola and Ellstrand, 1996), and canola (Crawley et al. 1993; Jørgensen and Andersen, 1995). In sunflower (*Helianthus annuus*), foraging bees carried crop-specific genetic markers to wild plants as far as 1,000 m away from small experimental stands of cultivated sunflower (Arias and Rieseberg, 1994). In addition, a 6.4 km isolation zone is recommended to protect commercial sunflower seed nurseries from unwanted wild sunflower pollen (e.g., Smith 1978). Thus, pollen from cultivated sunflower is certain to spread to adjacent wild populations due to the movements of foraging bees.

In the USA, cultivated sunflower is grown within the center of diversity of its ancestral species (primarily *H. annuus*) and often hybridizes with wild *H. annuus* (Whitton et al. 1997; Linder et al. 1998). Hybridization among several native *Helianthus* species has been demonstrated (e.g., Rieseberg et al. 1990; Rieseberg et al. 1999), so it is important to determine possible effects of “escaped” transgenes that may be beneficial to native species. Although cultivated sunflower is capable of hybridizing with other *Helianthus* species, especially *H. petiolaris*, most offspring from interspecific crosses are unsuccessful or yield infertile F<sub>1</sub> progeny (Rieseberg et al. 1999). Therefore, our research focuses on wild *H. annuus*. In particular, we need to know whether wild populations that acquire transgenes conferring resistance to herbicides, insects, or diseases are likely to become more invasive weeds. This requires controlled experiments in which the effects of specific transgenes can be quantified by comparing wild plants from the same genetic lines, with or without the transgene.

Wild *H. annuus* is a native, annual weed that occurs in disturbed sites and is widespread throughout much of the USA, reaching its greatest abundance in the midwestern states (Heiser 1954). Populations

are typically patchy and ephemeral, relying on the soil seed pool and long-distance dispersal for opportunities to become established in available clearings. In the absence of plowing or other types of disturbance, population size often declines due to competition from later successional species such as perennial grasses, herbs, and shrubs. In agricultural areas, however, repeated tilling allows wild sunflower populations to persist for many years, and this species is sometimes considered to be a noxious weed of corn, cultivated sunflower, and other crops (e.g., Burnside et al. 1996). Wild sunflower has the potential to become more troublesome due to the acquisition of beneficial transgenes, but we do not know how these transgenes are likely to affect its abundance and competitive ability in managed and unmanaged ecosystems.

The persistence of a long-lived seed bank makes it difficult to study the population dynamics of wild sunflower. Seed burial experiments show that ~30-40% of newly produced seeds remain dormant when exposed to optimal germination conditions in the spring (Teo-Sherrell 1996; Snow et al. 1998). Buried seeds sometimes remain dormant in the soil for several years and germinate following local disturbances such as plowing. Teo-Sherrell (1996) showed that seeds buried at depths of 5-20 cm exhibited annual dormancy cycles, with highest germination rates in the spring and intrinsic dormancy at other times of the year. The average longevity of buried seeds is not known, but at least 3% were still germinable after 17 years of burial in a Nebraska study (Burnside et al. 1996). Due to the difficulties of studying seed bank dynamics over a short time period, our research examines other life history stages of wild sunflower, namely, seedling establishment, survival, and lifetime seed production.

### **Spread of transgenes via pollen and seeds**

Gene flow occurs through the dispersal of both pollen and seeds. Because annual crops are harvested each year, it is likely that most hybridization with wild relatives occurs via the spread of pollen from the crop to the weed rather than *vice versa*. Wild sunflower is a self-incompatible species that requires visits from insect pollinators in order to set seed, and it easily crosses with cultivated sunflower. Like other outcrossing weeds, the extent of hybridization with a cultivated relative depends on the plants' proximity to the crop and the ratio of wild plants to cultivated plants. For example, in *Brassica rapa* (= *campestris*), rates of hybridization with oilseed rape (*B. napus*) were as high as 69% when the weed occurred as isolated individuals surrounded by rows of crop plants, and hence had little or no chance to cross intra-specifically (Landbo et al. 1996). In contrast, when the weed occurred in small populations adjacent to or within fields of oilseed rape, hybridization rates of 0-13% were detected (e.g. Landbo et al. 1996; Jørgensen et al. 1998; Scott and Wilkinson, 1998).

In sunflower, we found that the extent of pollen movement from the crop is greatest at the crop edge, diminishing to nearly zero at distances of 800-1,000 m. Research involving genetic markers showed that on wild plants within 3 m of crop plants, the frequency of F<sub>1</sub> crop-wild hybrid seeds averaged ~28% in one study (Arias and Rieseberg, 1994) and 42% in another (Whitton et al. 1997). Further away, frequencies of hybrid seeds were ~10-15% at 200 m, ~4-8% at 400 m, and 0-4% at 1,000 m. This leptokurtic pattern is typical of pollen dispersal by insects such as bees, which occasionally transport pollen to distances greater than 1,000 m from its source.

Once crop markers enter a wild population via spontaneous hybridization, they can spread further and faster by means of both pollen and seed dispersal. Long-distance seed dispersal is common in weedy species and is often facilitated by humans because seeds can be transported inadvertently by farm vehicles and as contaminants of hay, manure, topsoil, and seed lots. For sunflower and many other weeds, however, few attempts have been made to document rates of seed dispersal from a known source population. Ironically, studies of the combined effects of pollen and seed dispersal on gene flow may be easier to undertake in the future, when selectively neutral transgenes can be used as novel genetic markers in wild populations. In the meantime, efforts to produce useful mathematical models of the rate of transgene spread will be hampered by a lack of reliable data on seed dispersal.

### **Persistence of transgenes in weed populations**

#### **A. Fitness of F<sub>1</sub> hybrids and backcrossed generations**

Once a transgene has been introduced into a wild population, its frequency and long-term persistence depend on several factors, including the fitness of F<sub>1</sub> hybrids and whether the transgene confers a fitness

benefit that enhances the survival and/or lifetime seed production of these plants. Here we use the term “fitness” to describe the relative genetic contributions of different classes of genotypes, in this case wild-crop hybrids versus wild plants, to the next generation of plants in a natural population. We focus on two main components of fitness - survival and lifetime fecundity - as is standard in ecological studies of fitness-related traits. However, it is important to recognize that a genotype’s fitness can also be influenced by other life history stages, such as seed longevity and dormancy characteristics.

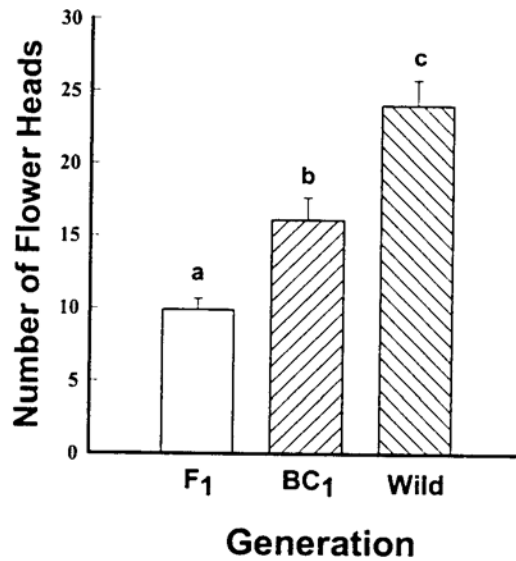
The F<sub>1</sub> generation may constitute a partial barrier to backcrossing and subsequent introgression of crop genes, particularly in the case of hybrids between different species. To test for lower survival and fecundity of F<sub>1</sub> wild-crop hybrids of sunflower (both *H. annuus*), we carried out field experiments in 1996 using wild sunflowers from North Dakota, Kansas, and Texas (Snow et al. 1998). F<sub>1</sub> hybrids were obtained using two varieties of cultivated, nontransgenic sunflower. Seed burial experiments in the region of origin showed that wild-crop hybrid seeds had no appreciable dormancy, although it is likely that deeply buried seeds can remain viable in the soil for several years if they are not exposed to appropriate germination cues (Snow et al. 1998). F<sub>1</sub> hybrid seeds germinated a few days earlier than wild seeds and exhibited high germination rates (>90%).

Common garden experiments carried out in Kansas and Ohio demonstrated that F<sub>1</sub> crop-wild hybrids grew vigorously and did not exhibit lower survivorship than wild plants (Snow et al. 1998). The hybrids produced hundreds of viable seeds per plant but on average they were inferior to wild plants in terms of their lifetime seed production. The number of flower heads per plant is correlated with total seed production, so we used this variable as an index of seed production. Wild plants branched more than F<sub>1</sub> hybrids and therefore produced more flower heads and more seeds per plant. The magnitude of this advantage varied considerably due to different growing conditions and the origins of the wild seeds (North Dakota, Kansas, or Texas). For example, when plants were fertilized and watered in large outdoor pots, wild plants from Kansas and Texas produced ~3-4 times more flower heads per plant than F<sub>1</sub> hybrids, and wild plants from North Dakota produced twice as many flower heads as their corresponding F<sub>1</sub> hybrids. This advantage diminished, however, when plants were grown with competitors (Morán-Palma 1998) and when they were grown in a weed-infested field in Kansas (Snow et al. 1998). At the latter site, wild plants produced more flower heads than some hybrid cross-types but not more than others, including the hybrids from North Dakota. These results show that the fecundity disadvantage of hybrids is quite variable and is sometimes insignificant. Under most conditions, however, we expect that hybrid plants contribute fewer seeds to the next generation than do wild plants.

The number of seeds a plant “sires” in a population by dispersing its pollen is also likely to be lower for hybrids because male reproductive success should be lower in plants that produce fewer flower heads. Also, in the case of wild plants from Kansas, we found that these plants flowered much later than F<sub>1</sub> hybrids, reaching their peak flowering when the hybrids nearly finished blooming (Snow et al. 1998; Snow et al., unpublished data). This difference in flowering time reduced their chances of mating with the more prolific wild plants and forced F<sub>1</sub> plants to cross mainly with each other. In contrast, flowering periods of wild and hybrid plants from North Dakota and Texas overlapped almost completely (Snow et al. 1998).

A further disadvantage of F<sub>1</sub> hybrids was that their seeds were more likely to be destroyed by insect seed predators than were those of wild plants (Cummings et al., unpublished data). In 1997 we compared the lifetime seed production of wild and F<sub>1</sub> crop-wild hybrids from Kansas at our Kansas field site (Cummings et al. unpublished data). Seed heads were collected at random throughout the growing season, and seeds were examined for damage from insect seed predators (300 heads per cross type). On average, 44% of the hybrid seeds were destroyed by seed predators, while only 6% of the wild seeds were killed. Reasons for this difference are unclear, but a preference for hybrid seeds may be related to the fact that their seeds tend to be larger than those of wild plants (Snow et al. 1998; Cummings et al., unpublished data). If these levels of damage are typical of other regions of sympatry between wild and cultivated sunflowers, seed predation could inhibit the spread of crop genes by reducing the fitness of F<sub>1</sub> hybrids.

Despite fitness disadvantages of  $F_1$  hybrids relative to wild plants, we expect that escaped transgenes will inevitably move into wild sunflower populations based on several lines of evidence. First, within each of the cross-types mentioned above the lifetime seed production of  $F_1$  hybrids was variable and a portion of the hybrids produced as many seeds as wild plants. Thus, reporting average values of lifetime fecundity can obscure the fact that some of the  $F_1$  plants were very successful relative to wild plants and were probably able to contribute comparable numbers of copies of their genes to subsequent generations. Second, we found that the fecundity of backcrossed plants ( $F_1 \times$  wild) was intermediate between that of  $F_1$  and wild plants (Figure 1; Morán-Palma 1998). Therefore, with each generation of further backcrossing, the fitness disadvantage associated with a particular transgene is expected to decrease. Finally, two studies involving genetic markers showed that 1) crop alleles persisted in wild populations for at least five generations after a known hybridization event (Whitton et al. 1997), and 2) crop alleles occurred at relatively high frequencies (31-38%) in wild populations located in areas where cultivated sunflower had been grown for ~20-40 years (Linder et al. 1998). Thus, even with low rates of hybridization and backcrossing, we expect that long-term cultivation of transgenic sunflowers will result in repeated opportunities for gene flow into nearby populations of wild plants.



**Figure 1:** Performance of backcrossed ( $BC_1$ ) plants relative to  $F_1$  crop-wild hybrids and wild sunflower. The average number of flower heads per plant in each genotype category is shown ( $N=20-23$  plants per genotype, superscripts show significant differences based on Tukey test,  $P<0.05$ ; error bars are 1SE). Plants were grown in a greenhouse for 22 weeks; wild plants were from Texas (see Snow et al. 1998 and Morán-Palma 1998).

The rate at which an introduced transgene spreads through wild populations can be enhanced if the transgene confers a fitness benefit, for example by protecting the plant from insect damage or disease. Most fitness-related transgenes are unlikely to be deleterious to wild plants because crop breeders will choose transgenic lines that perform well before being released commercially. In general, we expect the fitness effects of particular transgenes to be neutral or beneficial. Sunflower varieties with transgenic resistance to herbicides, insects, and various diseases are likely to be developed for marketing, although none has been deregulated yet. Some transgenes such as those conferring herbicide resistance may spread quickly because exposure to the herbicide will constitute strong selection for resistant genotypes. Other transgenic traits are less likely to confer a strong fitness advantage, as described further below, but this assumption needs to be tested empirically.

## Effects of transgenes on wild populations

Finding that a specific transgene enhances the fitness of wild genotypes and becomes common in wild populations does not necessarily mean that it will result in more invasive weeds. Even if transgenic progeny produce more offspring than nontransgenic plants and the transgene eventually becomes "fixed" in the population, the number of weed populations and the number of plants within each population (= population size) may not increase. For example, the number of plant populations could be determined by the number of sites with appropriate biotic or abiotic conditions for establishment. Similarly, at a single site, greater production of seeds will not necessarily lead to a greater number of plants at that site, especially if there are a limited number of locations for seedling establishment (e.g., Bergelson 1994). Even if seedling establishment does increase, other factors such as intraspecific competition or herbivore damage may limit the final abundance of plants at the site. For these reasons, it may be wrong to assume that greater fecundity due to the effects of transgenes will affect plant population dynamics.

Despite these caveats, however, there are reasons to expect that weedy species could become more abundant due to effects of escaped transgenes. In general, weeds have broad ecological requirements, and the size and numbers of weed populations may primarily reflect the availability of disturbed soil needed for seedling establishment. Given the prevalence of human-induced disturbances in and around agricultural fields, and thus opportunities for large populations (including dormant seeds in the soil), it seems likely that effects of transgenes on individual fitness could lead to population consequences. Greater survival and fecundity of individual plants could potentially result in larger weed populations and more effective dispersal of seeds to found new populations.

Transgenes that protect wild plants from insect herbivory and disease are of particular interest because wild plants are susceptible to many of the same pests as cultivated plants (and probably served as the original hosts prior to domestication). A recent sunflower production manual for the northern Great Plains region of the USA lists several moth, weevil, beetle, and midge larvae that frequently cause economic damage (Berglund 1994). To reduce damage from head-feeding insects that are difficult to control, VanderHave has developed transgenic varieties with resistance to coleoptera (<http://www.nbiap.vt.edu/>). With regard to diseases, the most important pathogens of commercial sunflower include *Sclerotinia* wilt, rust, and downy mildew. Transgenic resistance to *Sclerotinia* has been field-tested by both VanderHave and Pioneer Hi-Bred (<http://www.nbiap.vt.edu/>). Eventually, we expect that biotechnology companies will develop a variety of highly effective transgenic constructs that will protect cultivated sunflowers from many economically important pests.

At present, little is known about the effects of insects or disease on the population dynamics of wild sunflower, so it is difficult to assess the ecological effects of beneficial transgenes. With regard to insect seed predators, preliminary data from wild plants in Kansas suggest that seed damage is relatively low (about 6% in 1997; Cummings et al., unpublished data), but insect damage to seed heads of wild plants in Nebraska can be substantial (D. Pilson 1999; E. Sundvall and D. Pilson, unpublished data). Further research is needed to determine the overall benefits of transgenic, plant-produced toxins on plant fitness. Effects of *Bt* transgenes (derived from *Bacillus thuringiensis*) are uncertain because at present these toxins are only effective against specific insect taxa (e.g., lepidoptera or coleoptera), and it is not known whether other insect pests will become more abundant once a competing group of insects has been controlled. Another consideration is whether insects and pathogens that are unable to use transgenic host plants will rapidly evolve resistance to novel plant-produced toxins. Further studies are needed on the natural occurrences of insect herbivores and diseases in wild sunflower populations, and experimental studies are needed to evaluate the possible ecological impact of transgenic sunflowers on wild populations over the next few decades.

## Conclusions

Transgenes from cultivated sunflower will inevitably spread to wild populations via crop-to-wild gene flow. The rate at which transgenes will disperse among populations is difficult to predict because little is known about rates of seed dispersal, especially human-mediated dispersal. However, continued cultivation of transgenic sunflowers in regions where wild sunflowers also occur will allow beneficial transgenes to accumulate in wild populations. Transgenes conferring resistance to herbicides, insects, and diseases are likely to be beneficial to wild plants, perhaps enhancing survival, competitive ability, and

lifetime seed production, but their individual and combined effects on the population dynamics of wild sunflower are unknown (we are currently investigating these questions). Wild sunflower is an agricultural weed that can be adequately controlled in most agricultural systems, but it also occurs in unmanaged areas throughout much of the midwestern USA. Although this species is not considered to be an invasive species at present, release from insect damage and disease pressure could potentially cause wild sunflower to become more problematic in both managed and unmanaged areas.

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