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REPORT OF THE SUNFLOWER WORKING GROUP

Allison Snow
Ohio State University

Helen Alexander
University of Kansas

Joe Caroline
Mycogen Seeds

Svata M. Louda
University of Nebraska - Lincoln, slouda1@unl.edu

Diana Pilson
University of Nebraska - Lincoln, dpilson1@unl.edu

See next page for additional authors

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Authors

Allison Snow, Helen Alexander, Joe Caroline, Svata M. Louda, Diana Pilson, Craig Roseland, Gerald Seiler, Diane Shanahan, and Jeff Wolt

REPORT OF THE SUNFLOWER WORKING GROUP¹

Allison Snow

Ohio State University

Group Members

Helen Alexander, University of Kansas, *ecology, epidemiology, population genetics*

Joe Caroline, Mycogen Seeds, *breeding, entomology*

Svata Louda, University of Nebraska-Lincoln, *ecology, weed science*

Diana Pilson, University of Nebraska-Lincoln, *ecology, population genetics, plant evolution*

Craig Roseland, USDA-APHIS-PPQ, *entomology*

Gerald Seiler, USDA-ARS, *breeding, ecology, population genetics*

Diane Shanahan, Mycogen Corporation, *regulatory, botany/plant science*

Allison Snow (Group Leader), Ohio State University, *ecology, population genetics, weed science*

Jeff Wolt, Dow AgroSciences, *environmental science, weed science*

INTRODUCTION

Cultivated sunflower (*Helianthus annuus*) is grown in many temperate, semi-dry regions of the world, often in rotation with small grain cereals such as wheat. The largest areas of sunflower cultivation in the US are in the northern plains (North and South Dakota) and southern, high plains (western Nebraska and Kansas, plus areas of Colorado and Texas) where the growing season is often too dry and/or too short for profitable soybean and corn production. Most commercial sunflower is the oilseed type; in addition, the crop is grown for confectionery seed and is common as an ornamental in home gardens throughout the US.

The US is the center of diversity of the ancestral species of cultivated sunflower (Heiser 1954). The crop is capable of hybridizing with its wild progenitor, wild *H. annuus*, but most crosses with other *Helianthus* species such as *H. petiolaris* are unsuccessful or yield infertile F₁ progeny (Rieseberg *et al.* 1999). Cultivated sunflower also occurs as a volunteer weed. Although volunteer domesticated plants can represent a significant portion of the weeds infesting subsequent crops (Auwarter and Nalewaja 1976; Gillespie and Miller 1984), they do not persist for more than one or two years under most cropping systems and are not known to spread. For these reasons, the working group

focused on the consequences of gene flow to wild *H. annuus*.

Wild *H. annuus* is an outcrossing annual that occurs in disturbed sites and is widespread throughout much of the US, reaching its greatest abundance in midwestern states (Heiser 1954). Wild sunflower occurs at elevations ranging from sea level to 3,000 meters and in a variety of habitats that include roadsides, agricultural fields, abandoned fields, construction sites, and rangeland. Populations are typically patchy and ephemeral, relying on the soil seed bank and long-distance dispersal for opportunities to become established in available clearings. This species occurs as a common but manageable weed of wheat, cultivated sunflower, corn, soybean, sugarbeet, sorghum, safflower, and other crops (Al-Khatib *et al.* 1998; Geir *et al.* 1996; Irons and Burnside 1982; Schweitzer and Bridge 1982; Teo-Sherrell 1996).

Pollen from cultivated sunflower is certain to spread to adjacent wild populations by the movements of foraging insects, especially bees. Commercial sunflower seed companies are required to have 1.6-2.4 km of isolation between hybrid seed production fields and wild sunflower and/or other cultivated sunflower to prevent contamination by “foreign” pollen (e.g., Smith 1978; Schneiter 1997). The extent of pollen movement from the crop to wild sunflowers is greatest at the crop edge, where up to 42% of

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seeds can be crop-wild hybrids, diminishing to nearly zero at distances of 800-1,000 m (Arias and Rieseberg 1995; Whitton *et al.* 1997). F₁ crop-wild hybrids are fertile and capable of backcrossing with nearby wild plants, but they typically produce fewer flower heads per plant than purely wild genotypes (Snow *et al.* 1998). Once crop genes enter wild populations, they can spread farther by both pollen and seed dispersal. Seeds can be transported inadvertently by farm equipment and as contaminants of hay, manure, topsoil, and seed lots. Whitton *et al.* (1997) and Linder *et al.* (1998) have documented long-term persistence of crop genes in populations of wild sunflower.

PEST RESISTANCE GENES IN CULTIVATED AND WILD SUNFLOWERS

Common pests of cultivated sunflower are listed in Table 1 below and described further in Seiler (1992) and Schneiter (1997). Cultivated sunflower is susceptible to several economically important fungal diseases, and genes that confer disease resistance have been obtained through both conventional and transgenic breeding programs. Conventional breeding has produced commercial sunflower hybrids that are resistant to several races of rust and downy mildew. Resistance to other important diseases such as *Sclerotinia* (wilt, stalk rot, and head rot) has not been achieved, but transgenic expression of oxalate oxidase shows promise for enhancing resistance to *Sclerotinia* (Lu *et al.* 1998; transgenes were obtained from wheat).

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). In wild relatives of *H. annuus*, insect resistance is either absent or polygenic, and efforts to introgress strong resistance into the crop have been unsuccessful (Seiler 1992). A high priority for transgenic commercial hybrids is resistance conferred by Bt toxins, which are specific to different groups of insects such as Lepidoptera (moths, butterflies), Coleoptera (weevils, beetles), or Diptera (flies, midges). 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 (<http://www.isb.vt.edu>; note that VanderHave sunflower trials now take place primarily in the Netherlands). Broad-spectrum resistance involving multiple Bt genes and other genes for insect resistance (e.g., Stewart 1999) could also be developed.

POSSIBLE IMPACTS OF PEST RESISTANCE GENES ON WILD POPULATIONS

No studies have been conducted to determine whether gene flow from conventionally bred sunflowers has caused wild populations to become more abundant, although we suspect that traditional genes have had little impact on wild populations. Pest resistance genes have probably spread to sexually compatible wild relatives in the past, but in several cases these traits were derived from wild relatives in the first place (Seiler 1992; Snow *et al.* 1998, 1999). It is interesting to note that the frequency of rust resistance genes varies both within and among wild sunflower populations (Seiler 1992; Snow *et al.* 1998 and references therein).

Transgenic sunflower has not yet been released commercially, but several pest resistance traits may be introduced in the near future. Resistance to *Sclerotinia* is currently under development, prompting us to ask whether this trait could benefit wild genotypes, which are also susceptible to *Sclerotinia*. Dr. Gerald Seiler (USDA) has surveyed hundreds of wild sunflower populations without detecting *Sclerotinia* symptoms in mature plants. If this is true for most populations and for earlier life stages, we suspect that *Sclerotinia* is unlikely to regulate or limit the abundance of wild genotypes in the field. In contrast, transgenic resistance to insect seed predators might be beneficial to wild plants, which sometimes lose as many as 20-30% of their seeds to these insects (Pilson 1999 and unpublished data). Since transgenic insect resistance is now under development, this trait is the focus of our recommendations for further research.

Table: The most common pests of cultivated sunflower in the US¹

<u>Diseases</u>	<u>Insects</u>
****Wilt, middle stalk rot, and head rot (mainly <i>Sclerotinia sclerotiorum</i>)	****Sunflower moth (<i>Homeosoma electellum</i>)
Downy mildew (<i>Plasmopara halstedii</i>)	*Banded sunflower moth (<i>Cochylis hospes</i>)
***Stem canker (<i>Phomopsis helianthi</i> = <i>Diaporthe helianthe</i>)	***Red sunflower seed weevil (<i>Smicronyx fulvis</i>)
***Rust (<i>Puccinia helianthi</i>)	***Sunflower beetle (<i>Zygogramma exclamationis</i>)
***Verticillium wilt (<i>Verticillium dahliae</i>)	***Sunflower midge (<i>Contarinia schulzi</i>)
Head rots (<i>Rhizopus arrhizus</i> , <i>R. stolonifera</i> , <i>Botrytis cinerea</i>)	*Sunflower stem weevil (<i>Cylindrocopturus adspersus</i>)
**Phoma black stem (<i>Phoma macdonaldii</i>)	*Cutworms (<i>Euxoa messoria</i> , <i>E. ochrogaster</i> , <i>F. jaculifera</i>)
*Alternaria leaf and stem spot (<i>Alternaria helianthi</i> or <i>A. zinniae</i>)	*Gray sunflower seed weevil (<i>Smicronyx sordidus</i>)
*Septoria leaf spot (<i>Septoria helianthi</i>)	Sunflower bud moth (<i>Suleima heliantha</i>) ²
*Charcoal rot (<i>Macrophominia phasiolina</i>)	Sunflower head-clipping weevil (<i>Haplorthynchites aenes</i>) ²
*Bacterial Infections	
*Powdery Mildew (<i>Erysiphe cichoracearum</i>)	

¹**** Designates most important economically, * Designates least important, based on recommendations of our discussion group and Schneider 1997.

²Species that occur on wild sunflower but are not economically important to the crop.

RESEARCH RECOMMENDATIONS

The working group outlined a series of questions that should be addressed for each new type of transgene that confers resistance to insects or disease (volunteer sunflowers are not discussed because they are not known to persist as free-living populations). If the answer to any of these questions is “no” based on adequate empirical evidence, it is logical to conclude that the risk associated with a given type of transgene is minimal. This “decision tree” approach is similar to those described previously in Tiedje *et al.* (1989) and Rissler and Mellon (1996). We consider several scales that should be studied, including individual plants, local populations, and regional metapopulations.

Is the transgene inherited as a stable, Mendelian trait when it is artificially crossed into wild plants?

Beginning at the scale of individual wild plants, we need to know whether a particular

introgressed transgene is inherited as a dominant Mendelian trait. In addition, it will be essential to determine whether the transgene is expressed under a wide range of environmental conditions, and whether the anticipated phenotype (e.g., resistance to Coleoptera) is realized. Presumably, previous screening by crop breeders will ensure that a particular transgene is stable and predictable, but this should be confirmed in experiments involving backcrossed wild plants.

Do insects or diseases that are targeted by the transgene occur in populations of wild sunflower and, if so, how common are they?

By targeted species, we refer to organisms that would be killed or deterred by the effects of the transgene, including species that occur on wild plants but are not considered to be serious pests of the crop. Surprisingly little is known about the prevalence of insect pests and diseases in weed populations. Multi-year, multi-region surveys are

needed to determine the frequencies of insect and disease damage in wild sunflowers. Surveys that focus on mature plants could miss mortality or damage from insects or diseases that affect seeds, seedlings, or young plants, as is the case with many soil-borne pathogens. Likewise, if a disease is sporadic yet severe, it may kill the host population and escape being detected. Despite these problems, it is better to have quantitative baseline data from surveys than to evaluate risks based solely on anecdotal evidence.

When the transgene has introgressed into wild plants, will these plants exhibit greater survival or fecundity than their nontransgenic counterparts?

This question could be approached in two ways. To test for effects of insects or diseases on wild plants, these pests could be removed with insecticides or fungicides in field experiments. Examples of pesticide application experiments with wild plants can be found in Waloff and Richards (1977), Louda (1982), Simms and Rausher (1989), Louda and Potvin (1995), Louda and Rodman (1996), and Guretzky and Louda (1997). If broad-spectrum pesticides do not benefit wild plants (and are not harmful to plant growth), then further experiments to test for impacts of specific groups of insects are not necessary.

Alternatively, plant breeders could artificially introgress the transgene into wild genotypes to study characteristics of the backcrossed generations in the field (pending approval from APHIS). We recommend that APHIS encourage such projects if appropriate precautions will be taken. Field experiments can be used to quantify the ecological consequences of the transgene in backcrossed progeny that segregate for the presence or absence of the transgene. Survival and lifetime seed production could be compared to test for fitness differences between transgenic and nontransgenic plants. These experiments should be carried out at several sites where pest populations are known to occur. The level of insect damage seen in nontransgenic plants should be compared to natural levels that have been documented in baseline surveys from other regions and years in order to evaluate whether the experimental conditions were representative of commonly occurring field conditions.

If the transgene leads to greater survival or fecundity, will this cause wild populations to become more troublesome weeds?

This is a difficult question that will require a combination of field experiments and modeling. Field experiments can be used to determine whether populations are “seed-limited” on a local scale. In other words, we need to know how the addition of seeds affects seedling recruitment and population size (this very basic question has rarely been studied empirically). Carefully designed seed addition experiments should be carried out at a variety of sites for multiple years. Using these results, models could be used to examine the larger scale consequences of an increased seed production, taking into account the numbers of “unoccupied” sites in a region, the rate at which seeds disperse to and colonize these sites, and the rate at which sunflowers are killed by weed management practices or displaced by other species. In wild sunflower, recruitment from the seed pool in the soil may be delayed for many years. This aspect of their population dynamics is very important, as most populations are ephemeral and are out-competed by other species. Eventually, however, tilling or other soil disturbance in an area allows recruitment from dormant seeds. Scattered, temporary populations in a region are often referred to as constituting a metapopulation, the dynamics of which can be explored using mathematical models.

A good introduction to this approach can be found in a paper by Rees and Paynter (1997) titled “Biological control of Scotch broom: Modeling the determinants of abundance and the potential impact of introduced insect herbivores.” Models of metapopulation dynamics can be very instructive, especially when good empirical data are available to use as the main parameters of the model. Modeling efforts are needed to extend our understanding of population dynamics beyond the context of small-scale experiments to include regional changes in the abundance of wild sunflower. At the very least, models can help identify the specific conditions necessary for wild sunflowers to become more invasive in both managed and unmanaged ecosystems. This approach can be used to make informed decisions about the possible effects of genes for

pest resistance and is more reliable than decisions based on intuition and opinion.

OTHER ISSUES

Further research could include efforts to model the rate at which transgenes with different fitness benefits are expected to spread among populations and persist in seed banks. It will also be important to consider how quickly target insect pests will evolve resistance to Bt toxins and other transgenic types of pest resistance. Wild sunflowers could provide a refuge for Bt-targeted pests, at least initially (before the transgene has spread), and this might delay the evolution of resistance to Bt in insects.

Although not thoroughly discussed by our group, the potential impact of transgenic herbicide tolerance is as important as transgenic insect or disease control. Resistance to herbicides can evolve spontaneously in wild sunflower populations (e.g., Al-Khatib *et al.* 1998), or it can be acquired via crop-to-wild hybridization, including hybridization with transgenic sunflower. Here we present some of the issues that should be part of future dialogue on this topic.

Herbicide tolerance has the potential of being introduced into the crop as the transgenic trait of interest or it may be incorporated indirectly as the selectable marker for the transformation “cassette.” In the latter case, the herbicide tolerance is intended to serve as a tool for plant breeders to identify the absence or presence of the closely linked transgene of interest. Even though the herbicide tolerance is not the primary trait, it is still present and has the potential to move to the wild species via pollen flow. This issue can be minimized by using selectable markers that are not herbicides or by developing transformation systems that do not utilize selectable markers.

As with herbicide tolerance in other crops, the tolerance is specific to a given herbicide and does not confer resistance to all herbicides. Therefore if the tolerance genes are expressed in wild species, it should still be possible to control wild sunflowers possessing the transgene with other herbicides. Presently there is a wide array

of herbicides available to control sunflower in rangeland and cropland systems. Transgenic insect or disease tolerance typically will not impact other crops in a farming system, since the insects and diseases are specific to sunflower, but a unique feature of transgenic herbicide tolerance in sunflower is its impact on other crops in a farm rotation with the same herbicide tolerance gene. This situation may be problematic for farmers, but may be managed by 1) selecting crops with different herbicide tolerant genes to avoid the increase of herbicide tolerant wild sunflower, 2) tank mixing two herbicides, or 3) choosing not to grow herbicide tolerant sunflower (however this option does not consider the impact of neighboring farms which may be using herbicide tolerant sunflower).

CONCLUSIONS

In the short-term, the first types of transgenic sunflowers to be released may pose few environmental risks. To be confident of this, we recommend that risks associated with pest resistance transgenes be evaluated as outlined above. At present, the most urgent need for further research is an evaluation of how transgenes for insect resistance could affect the abundance of wild populations. A worst case scenario would be that transgenic wild plants would produce 20-30% more seeds per plant, perhaps leading to larger pools of dormant seeds in the soil and more successful colonization of disturbed sites in natural and agricultural areas, thereby exacerbating existing weed problems. Alternatively, empirical studies may show that effects of transgenic pest-resistance traits are negligible, especially in the case of narrow-spectrum Bt transgenes. We recommend a combination of baseline surveys, field experiments, and modeling of metapopulation dynamics to permit informed assessments of the risks associated with novel transgenes.

Taking a longer-term view, we expect that commercial sunflower hybrids with strong resistance to herbivores, diseases, herbicides, and even drought- or frost-induced stress (see Kasuga *et al.* 1999) could be developed for commercialization. Multiple transgenes could be “stacked” within the same cultivar, perhaps as tightly linked traits that would be transferred

together or by simply entering wild populations as separate transformation events. The combined effects of multiple fitness-related transgenes on wild/weedy populations should be carefully considered prior to their commercial release to avoid undesirable increases in the abundance of weedy sunflowers.

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