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Increased pre-dispersal seed predation in sunflower crop-wild hybrids

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Abstract The fitness of crop-wild hybrids can influence gene flow between crop and wild populations. Seed predation levels in crop-wild hybrid plants can be an important factor in determining plant fitness, especially in large-seeded crops such as sunflower. To determine patterns of pre-dispersal seed predation, seeds were collected from wild sunflowers (*Helianthus annuus* L.) and wild×crop F₁ hybrids at three experimental field sites in eastern Kansas. Seed heads were dissected and each seed was counted and scored for categories of seed damage by lepidopteran and coleopteran larvae. Hybrid seed heads showed significantly higher levels of insect-damaged seeds. The average hybrid plant had 36.5% of its seeds (or 45.1 seeds per plant) eaten by insect larvae while the average wild plant lost only 1.8% (or 95 seeds) to seed predators. Hybrid populations had higher levels of total insect damage even when date of flowering, flower head diameter, and the number of open heads within the study site were accounted for. These results suggest that the reduced fecundity of F₁ crop-wild sunflower hybrids demonstrated in other studies may be augmented by the increased seed predation in hybrid flower heads. Fecundity estimates of crop-wild hybrid and wild plants that disregard differential seed predation levels may not accurately reflect the actual relative contributions of hybrid and wild plants to future generations.

Key words Crop-wild hybridization · Seed predation · Sunflower · *Helianthus annuus*

Introduction

Gene flow between crop plants and their wild relatives can be of significant evolutionary importance if it alters the genetic makeup of either population (Anderson 1949; Harland 1965; Ellstrand 1988). Interest in crop-wild hybridization was originally based on the concern that selected crop lines could be contaminated by wild genes (Haskell 1943; Bateman 1947; Nieuwhof 1963), and more recently has focused on the potential spread of transgenes from genetically engineered crop plants into wild populations (Snow and Moran-Palma 1997; Van Raamsdonk and Schouten 1997). Gene flow from crop to wild plants has been documented in many plants, including radish (Klinger et al. 1992), squash (Kirkpatrick and Wilson 1988) sorghum (Arriola and Ellstrand 1996), and sunflower (Arias and Rieseberg 1994).

For gene flow to be of evolutionary importance to the wild population, the crop genes must persist beyond the F₁ generation. The establishment of crop genes in wild populations could be delayed if crop-wild hybrids have very low fitness (Barton and Hewitt 1985). Relative fitness of crop-wild hybrid versus wild plants has been recognized as an important component in determining the risk of transgene escape (Gliddon 1994; Linder and Schmitt 1995; Arriola and Ellstrand 1996). Fitness differences have been examined through measurement of seed production and relative seed survival and germination (Alder et al. 1993; Linder and Schmitt 1995; Snow et al. 1998) but quantitative studies of relative levels of pre-dispersal seed predation are rare. In fact, although there has been some work on relative levels of disease resistance (e.g., sunflower crop-wild hybrids show increased resistance to rust fungus; Snow et al. 1998), interactions between wild-crop hybrids and natural enemies are largely unknown.

Insect damage to seeds may be a particularly important mechanism reducing the overall fitness of hybrids as it influences the total number of viable seeds dispersed by the plant (Louda 1982, 1989; Louda et al. 1990). It is reasonable to assume that there may be differences in the

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seed predation levels of crop-wild hybrid and wild plants, as there exists a rather extensive literature examining relative levels of parasitism, disease, and herbivory in hybrids (for reviews see Strauss 1994; Fritz 1999). Hybrid zones have been hypothesized to exhibit increased levels of herbivory and parasitism (Whitham 1989; Floate et al. 1993), creating further interest in the comparative levels of herbivory in crop-wild F_1 hybrids and wild parents. However, no general consensus has been reached regarding the overall abundance of herbivores and parasites in hybrid zones or the mechanisms behind the levels of attack of hybrids (Strauss 1994).

Knowledge of relative seed predation and herbivory levels of crop-wild hybrid plants versus wild plants is also necessary to evaluate the potential effect of selectively advantageous transgenes that are likely to be introduced into crops. Transgenes that confer insect and/or disease resistance traits were the focus of 75% of the crops involved in field tests in 1996 (see Table 1 in Snow and Moran-Palma 1997). Baseline knowledge of the levels of herbivory and disease in crop-wild hybrids versus wild plants will allow quantitative estimates of the increases in fitness that are expected to result from introducing resistance to these natural enemies. For example, transgenic crops that express a *Bacillus thuringiensis* (Bt) crystal protein gene may have resistance to a particular group of insects (Peferoen 1997). Knowledge of not only the relative levels of insect damage in crop-wild hybrid plants and wild plants, but additionally, the relative damage levels from various insect species would allow prediction of the selective advantage of these types of transgenes.

In this study we focus on insect damage in wild and crop-wild hybrid sunflowers. This species is relevant to study because wild and crop sunflowers co-occur and spontaneously hybridize, and crop markers can persist in wild populations for many generations (Whitton et al. 1997; Linder et al. 1998). In a recent paper, Snow et al. (1998) investigated several traits of F_1 crop-wild sunflower hybrids that could influence persistence of crop genes, including seed dormancy, flowering phenology, disease resistance, and fecundity, and found, in general, lowered fitness of F_1 hybrids relative to wild plants. Sunflowers are the hosts for a wide variety of insect pests (66 species in Kansas – Walker 1936; 159 infesting species in the United States – Hilgendorf and Goeden 1981), and provide ample opportunities to study levels of seed predation. Several traits make sunflower a good candidate for further study of the effect of seed predation as a mechanism contributing to lowered F_1 hybrid fitness. For example, sunflower seeds are large and pre-dispersal insect seed predators are known (Schulz 1978). Further, wild and crop-wild sunflowers have different flowering phenologies which may lead to differences in insect predation. Such a relationship is expected given that differences in flowering phenology have been shown to be correlated with levels of insect damage in a variety of plants (e.g., Evans et al. 1989; Mopper and Simberloff 1995) including sunflowers (D. Pilson, unpublished work).

In this study we collected seed heads from known wild and crop-wild sunflower F_1 hybrids from three experimental populations in eastern Kansas and quantified the type and amount of seed damage on individual heads. We asked three questions:

1. Are there differences in the levels of pre-dispersal insect damage in crop-wild hybrid and wild sunflower heads?
2. If so, are these differences consistent for seed damage caused by different insect seed predators?
3. Crop-wild hybrids and wild sunflowers have been previously shown to have differences in flowering phenology, numbers of heads flowering, and head diameter (Snow et al. 1998). Are changes in any of these factors associated with levels of pre-dispersal insect damage?

Materials and methods

Plant material and study site

Helianthus annuus L. is a common annual species, native to North America, that inhabits disturbed areas throughout much of the United States (Heiser 1954). *H. annuus* is also grown commercially throughout many temperate regions around the world. Crop breeding has commonly included intentional hybridization with wild sunflower to improve traits such as pest and drought resistance (Seiler 1992). Wild sunflowers (*H. annuus*) hybridize spontaneously with crop sunflowers in some populations (Linder et al. 1998).

Experimental plants were of two cross types: wild (from Kansas, collected in Douglas County) and crop-wild hybrids (USDA Cultivar 894 × wild Kansas plants) (see Snow et al. 1998 for further collection information). Crop-wild crosses were performed by hand in an insect-free greenhouse as described in Snow et al. (1998) with wild plants as maternal parents (pollen receptors) and USDA Cultivar 894 as pollen donors. Seedlings were grown in a greenhouse at Ohio State University and transported in mid-June 1997 to the Kansas Ecological Reserves of the University of Kansas, located 12 km northeast of Lawrence, Kansas (Douglas Co.). Upon arrival, seedlings were transplanted into greenhouse potting soil mix and maintained in a mesh-enclosed partly shaded outdoor area to acclimate them to outside conditions.

We used three widely spaced (approximately 0.5 km apart) sites to establish experimental populations (sites 1, 2, and 3). We chose the site locations to minimize gene flow between the populations, as this project is part of a larger study of the persistence of crop genes over multiple generations (A.A. Snow, unpublished work). The sites were 25 m by 55 m and were enclosed by a 3.05-m-high fence capable of excluding deer and other large vertebrate herbivores. We planted hybrid and wild plants in the three study sites on 30 June, 1 July, and July 8 (at sites 1, 2, and 3, respectively) in equal numbers (100 of each cross type per site). Within each site there was a gridwork of holes dug into the ground spaced 1.2 m apart and arranged in 15 rows and 40 columns for a total of 600 holes at each site. At each site 100 hybrid and 100 wild plants were planted in a random, mixed arrangement. To prevent extreme "clusters" of wild or hybrid plants, each site was divided into ten blocks of 15 rows and 4 columns, for a total of 60 holes per block. Within each block, 10 holes were randomly assigned to hybrid plants, 10 holes were randomly assigned to wild plants, and 40 holes were left empty (to be used in additional years of a larger genetic study). Each hole contained a plastic pot 14 cm in diameter and 14.5 cm deep filled with local field soil (we removed bottoms of pots to allow root growth). Throughout the study, wild sunflowers outside of the plots were removed in an area with a radius of approximately 0.5 km.

When plants began to flower, we took a census of new flower heads every 2 or 3 days and recorded date of first flowering (when at least one floret was open) and head diameter (measured by placing a ruler across the front of the head). The first date of flowering, 22 July, was designated as day 1. Flowering ended in early October. All hybrid heads and 30% of all wild heads were labeled to be collected for seed counts. During the flowering census we randomly designated three of every ten wild heads to be collected by tagging them with a colored label. During peak flowering of wild heads a rainy week created a lapse in the 2- to 3-day census procedure and backdating was done to account for this (i.e. some flower heads were assigned to slightly earlier dates). We calculated the total number of heads with open flowers on each date by adding the total number of hybrid heads with flowers opening on that date and 3.333 times the number of wild heads recorded as opening on that date (to account for the 70% of the wild heads on each date on which data were not taken). We did these calculations separately for each site.

Heads designated to be collected were covered with a bridal-veil mesh to prevent bird predation and early seed dispersal. This was done when the achenes had turned from green to brown, no florets were open, and all petals had been lost. We believed this was done late enough so as not to decrease the probability of head infestation by insects (D. Pilson, personal communication). Hybrid heads (late September until mid-October) and wild heads (late October until the 1st week of November) were collected when the bracts appeared completely brown and the head appeared dry. Heads were stored in individual paper bags at room temperature.

Seed damage

We examined seed damage in 600 heads: 100 heads for each cross type for each of the three sites. Each set of 100 heads was a random collection of the total heads of that cross type produced at a site over the season. Seed damage was assessed by examination of achenes (single-seeded indehiscent fruits of the Asteraceae, commonly referred to as "seeds"). We removed all achenes from each head and classified them into categories based on appearance and texture and counted the total number of achenes in each category. Achenes that were easily crushed when squeezed with forceps were classified as unfilled ("flat"). If signs of external damage were lacking and achenes were firm when squeezed with forceps they were classified as viable ("good").

Achenes that appeared to have insect damage were classified according to position and type of damage (Table 1). The sunflower moth (*Homeosoma electellum* Hultst: Pyralidae), banded sunflower moth (*Cochylis hospes* Walsingham: Cochylidae), sunflower "budworm" (*Sulemia helianthana* Riley: Tortricidae), the red sunflower seed weevil (*Smicronyx fulvus* LeConte: Curculionidae) and the gray seed weevil (*S. sordidus* LeConte) are head-infesting insects that have been observed to damage sunflower achenes (Schulz 1978) and have been observed in Kansas populations (Aslam and Wilde 1991). The red and gray sunflower weevils oviposit eggs singly between the achene wall and the seed coat and leave no visible sign of oviposition (Satterthwait and Swain 1946). Damage characteristics of the red sunflower weevil include an exit hole in the "top" (flattened, distal end) of the achene (Peng and Brewer 1995). The gray sunflower weevil is also believed to produce a similar type of damage (D. Pilson, personal communication). Achenes with a hole

in the top half were counted as having this type of damage ("top"). The banded sunflower moth and the sunflower moth do not oviposit directly into the achenes, but they penetrate into the achenes and feed on the developing kernels as later instars (Charlet et al. 1987; Charlet and Gross 1990). In commercial sunflower, each larva fed on an average of 2.5 seeds (Westdal 1949). The banded sunflower moth can produce long feeding cracks in the sides of the achene (Peng and Brewer 1995) or entrance/exit holes in the "bottom" of the seed (D. Pilson, personal communication). Achenes with a hole in the bottom half of the seed were classified as having this type of damage ("bottom"). Long feeding cracks as described by Peng and Brewer (1995) were not distinctly observed. Like seed weevils, sunflower moth larvae make an entrance/exit hole in the top of the achene, and these two causes of damage could not be distinguished (both were classified as "top"). Female sunflower moths lay 30 or more eggs singly or in groups of four or five within or among the corolla tubes of individual florets (Drake and Harris 1926).

Sunflower budworm eggs are laid singly on the leaves or at the base of sunflower heads (Ehart 1974). In Texas, three peaks of egg-laying activity were observed, in mid-June, early July, and mid- to late August (Phillips et al. 1973). Larvae from second-generation infestations enter receptacles through disk flowers and developing seeds and feed on seeds (Heinrich 1921; Phillips et al. 1973). Sunflower budworm damage is indicated when large pieces of the bottom of the seed are removed (D. Pilson, personal communication). Achenes that were missing at least 25% of the total area and appeared to have a portion removed from the bottom, rather than a hole drilled into them, were classified separately ("bite"). In our study, these three damage classifications are mutually exclusive; cases in which one achene had more than one type of damage were exceedingly rare (2 out of nearly 75,000 achenes observed).

Statistical analysis

We used a two-part approach for data analysis. We first examined whether the presence or absence of seed damage in heads differed between hybrid and wild sunflowers. We then examined whether the level of damage (proportion of damaged seeds) within damaged heads differed between hybrid and wild sunflowers. These analyses were conducted separately for biological reasons. For example, the initial infestation of a head and the subsequent extent of seed loss per head may be two separate processes. Additionally, this approach was statistically necessary, as preliminary analyses of level of damage using a data set of both damaged and nondamaged heads revealed strongly non-normal residuals.

All analyses were performed on SAS (SAS Institute 1989) or MINITAB 11 (Minitab 1996). We used multiway contingency tables (Proc CATMOD – SAS) to determine if presence or absence of damage differed among cross types and sites using the data from the 300 randomly chosen heads of each cross type (100 from each site). We analyzed continuous variables with general linear models, with cross types and sites as fixed effects; continuous variables included differences in date of flowering, diameter of the heads (square root transformed), and proportion (number of total damaged seeds divided by total number of seeds, excluding flat seeds) of damage types for heads with at least one damaged seed of that type. In some analyses, date of flowering, number of heads opening on the same day, and diameter of the head were used as covariates (in ANCOVA, analysis of covariance). In all analyses

Table 1 Insect larvae associated with damage classifications in sunflower

Damage	Characteristics	Likely insect predator(s)
"Top"	Small hole in the flattened, distal half of the achene	Red sunflower seed weevil (<i>Smicronyx fulvus</i>) Gray sunflower seed weevil (<i>S. sordidus</i>) Sunflower moth (<i>Homeosoma electellum</i>)
"Bottom"	Small hole in the pointed, proximal half of the achene	Banded sunflower moth (<i>Cochylis hospes</i>)
"Bite"	Achene missing at least 25% of total area from the proximal half	Sunflower budworm (<i>Sulemia helianthana</i>)

with proportion damage values, rank values were used in parametric general linear models (e.g., ANOVAS, ANCOVAS) instead of the raw data, to correct for heterogeneity of variances.

Results

Insect damage differences between cross types

We used multiway contingency tests ($2 \times 2 \times 3$) to explore whether or not the proportion of heads experiencing insect damage depended upon cross type or site (Table 2A, Fig. 1). Proportion of heads was defined as the number

of heads out of the 100 heads sampled for each cross type at each site with at least one damaged seed. On average across sites, significantly more hybrid heads (10%) experienced some form of insect damage compared to wild heads (Table 2A, Fig. 1A). When we analyzed individual damage types separately, heads that had at least one seed with bottom hole seed damage were at least twice as common in the hybrid versus wild head collections (Fig. 1C). There were no significant overall effects of site for either total damage or bottom hole damage. Although there were significant type \times site interactions for both analyses, hybrid heads experienced more damaged heads at each site. When we analyzed top and bite dam-

Fig. 1. Comparison of proportions of hybrid and wild flower heads in each of three sites containing at least one seed with: **A** any type of damage, **B** holes in the top of the seed, **C** holes in the bottom of the seed, **D** a large portion of the seed removed ("bite" damage). At each site, $n=100$ flower heads per cross type. One flower head may contain more than one type of damage

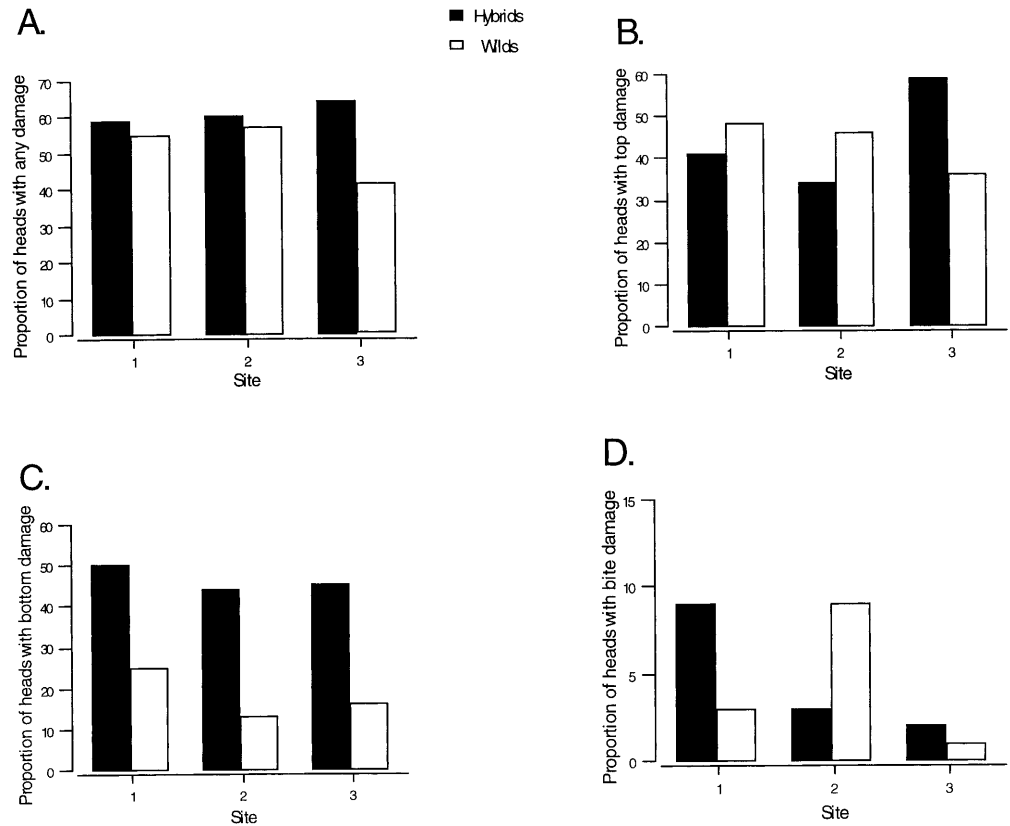
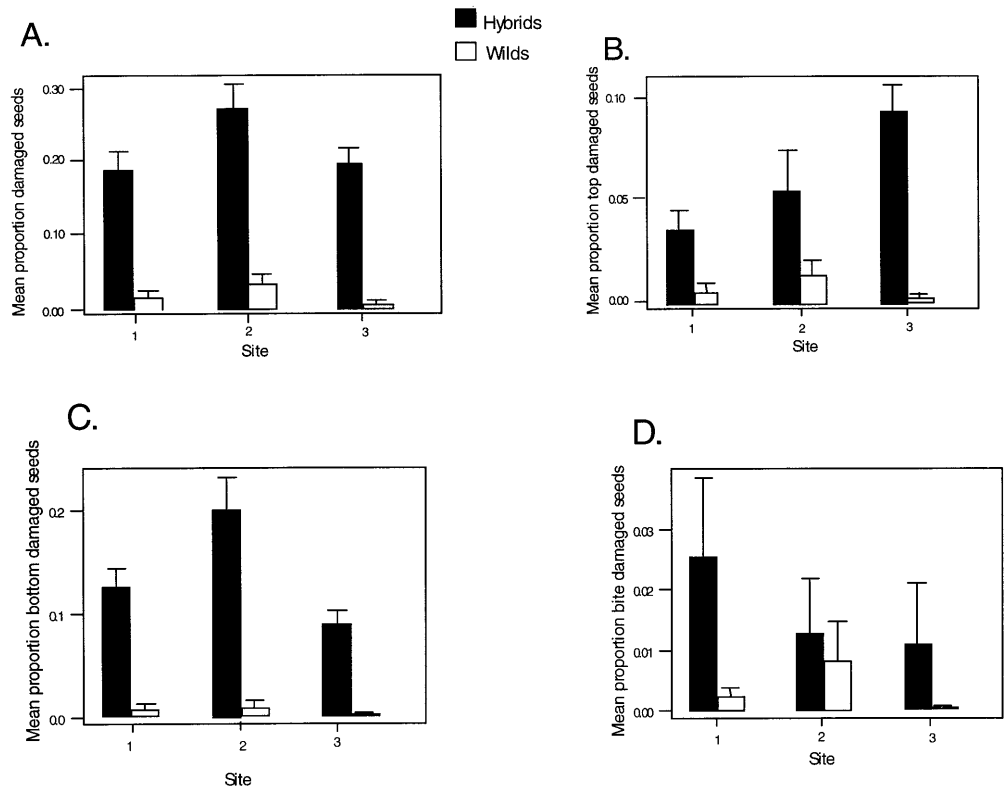


Table 2 Summary of site and type effects on **A** proportion of heads with or without damaged seeds, **B** proportion of damaged seeds within seed heads that sustained some type of damage, **C** flowering date, **D** flower head diameter. Results in **A** reflect χ^2 values from multi-way contingency table analyses. Results in **B-D** reflect F -values from two-way ANOVAs

Variable	Site	Cross-type	Site \times Cross-type
A Proportion of heads			
Total damage	$\chi^2_2=1.60$	$\chi^2_1=6.08^*$	$\chi^2_2=5.10$
Top damage	$\chi^2_2=2.25$	$\chi^2_1=0.09$	$\chi^2_2=14.31^{***}$
Bottom damage	$\chi^2_2=4.48$	$\chi^2_1=52.41^{***}$	$\chi^2_2=1.51$
Bite damage	$\chi^2_2=4.15$	$\chi^2_1=0.20$	$\chi^2_2=6.09^*$
B Proportion of seeds			
Total damage	$F_{2,330}=7.23^{***}$	$F_{1,330}=343.90^{***}$	$F_{2,330}=3.14^*$
Top damage	$F_{2,258}=1.19$	$F_{1,258}=109.55^{***}$	$F_{2,258}=1.21$
Bottom damage	$F_{2,187}=14.34^{***}$	$F_{1,187}=192.98^{***}$	$F_{2,187}=0.80$
Bite damage	$F_{2,21}=0.11$	$F_{1,21}=1.62$	$F_{2,21}=0.74$
C Flowering date	$F_{2,594}=9.92^{***}$	$F_{1,594}=1080.41^{***}$	$F_{2,594}=4.75^{**}$
D Flower head diameter	$F_{2,588}=85.48^{***}$	$F_{1,588}=4.99^{**}$	$F_{2,588}=5.79^{**}$

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Fig. 2. Comparison of the proportion of damaged seeds for hybrid and wild heads in each of three sites experiencing: **A** any damage, **B** holes in the tops of seeds, **C** holes in the bottom of seeds, **D** a large portion of the seed removed (“bite” damage). Only heads with the damage type specified were included in each graph. *Interval bars* represent 1 SE



age types, the effect of head cross-type depended on site as indicated by significant type×site interaction terms (Fig. 1B, D). In most cases there were a higher proportion of damaged hybrid heads in some sites, while for number of heads with top damage at sites 1 and 2 there were a higher proportion of damaged wild heads (Fig. 1B).

Additional analyses focused only on heads with at least one seed with the damage type and examined proportion of damaged seeds within these heads. Hybrid heads had significantly higher percentages of total insect damaged seeds, top hole damaged seeds, and bottom hole damaged seeds than did wild heads (Table 2B, Fig. 2). Hybrid heads did not have a significantly higher proportion of bite-damaged seeds than wild heads although the same trend was observed (Fig. 2D). There was a significant effect of site for proportion of total damaged and of bottom damaged seeds. Although there was a significant interaction term (type×site) for proportion of total damage, hybrid heads had more damage than wild heads in all sites. These analyses were repeated using numbers of damaged seeds instead of proportions. The results were nearly identical and we have chosen to present proportions only.

Our analyses focus on damage to individual heads, but we also wanted to compare damage levels on a per plant basis. To do this we first estimated the number of flower heads produced by hybrid and wild plants. The total number of flower heads produced at each site (for wild plants, data were taken from only 30% of the total heads, so total head production was estimated) was di-

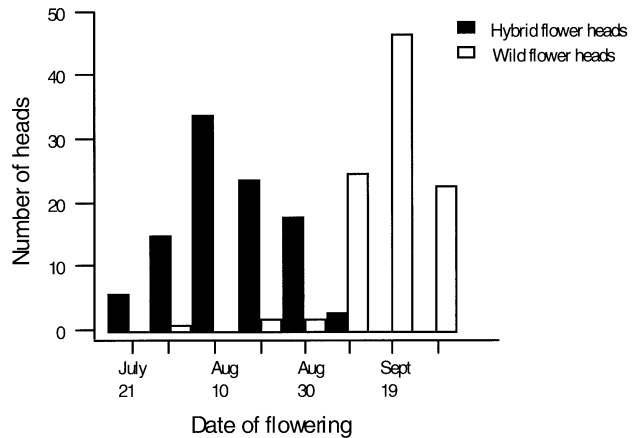


Fig. 3 Flowering phenology of 100 hybrid and 100 wild heads from site 1. Similar results were seen at sites 2 and 3

vided by the total number of flowering plants in that site to get an approximate value for the number of flower heads produced per hybrid and wild plant. The average seed production and average seed predation for hybrid and wild heads in each site was found by calculating the total number of viable seeds, and total number of damaged seeds, at each site for the 100 hybrid and 100 wild flower heads examined. Using the average flower head and seed production for hybrid and wild plants, and averaging values across all three sites, we calculated that the average hybrid plant had 36.5% of its seeds (or 45.1 seeds per plant) eaten by insect larvae while the average wild plant lost only 1.8% (or 95 seeds) to seed predators.

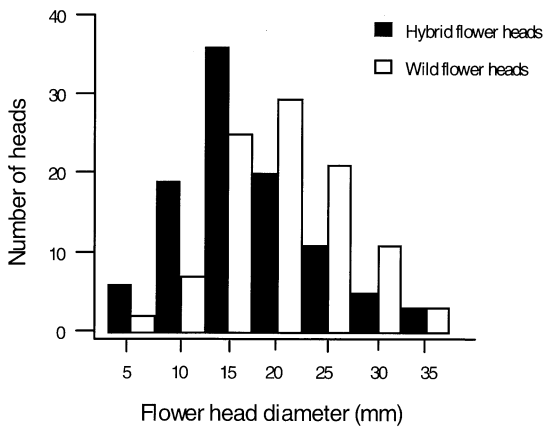


Fig. 4 Flower head diameter of 100 hybrid and 100 wild heads from site 1. Similar results were seen at sites 2 and 3

Flowering phenology and flower head diameter differences between cross types

Hybrids flowered earlier than wild plants (Table 2C, Fig. 3). Although there was a significant effect of site and a significant site \times type interaction, the average hybrid head flowered at least 20 days earlier than the average wild head in all sites. Hybrid heads were found to be significantly smaller than wild heads (Table 2D, Fig. 4).

Flowering phenology and head diameter effects on insect damage

Given the large differences in flowering phenology and head diameter between cross types, we explored the degree to which differences in proportion of top and bottom damaged seeds could be explained by these variables. To do this, date of flowering and head diameter were added as continuous covariates in a general linear model. Additionally, for each of the 600 heads, we used data collected on all plants at each site to calculate the number of heads with flowers that opened at the site on the same date. We did this to examine whether there was an increased attraction of insects to a site when a high density of heads was present or, conversely, if predator satiation at high flowering densities would result in lowered seed losses. This variable was also used as a continuous covariate. To aid with interpretation, each site was analyzed separately. Results are presented in Table 3.

Significant interaction terms made interpretation of the analyses difficult. All three sites showed significant main effects for type in proportion of top damaged seeds, even with date of flowering, diameter of the head, and number of heads flowering at the same time included in the analyses. None of the significant interaction terms indicate a situation in which wilds experienced more damaged seeds than hybrids (however, the significant four-way interaction at site 1 was difficult to interpret).

A significant main effect of date and/or a significant date \times type interaction is seen at all three sites when exam-

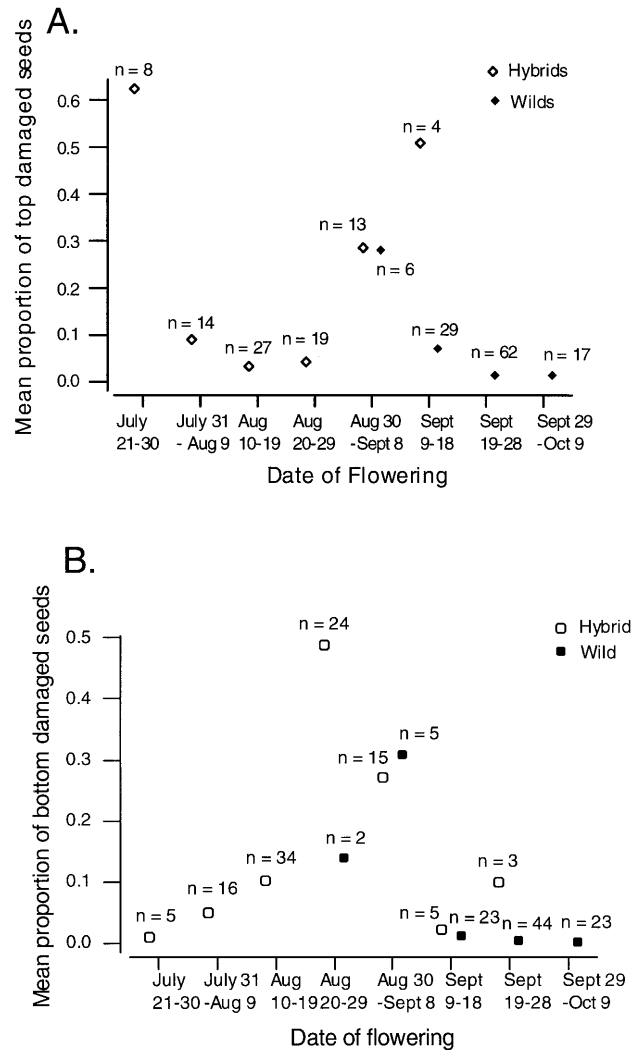


Fig. 5. Interactions of flower head type and flowering date for **A** top damage at site 2, **B** bottom damage at site 1. Mean proportions of damaged seeds are shown and the number of flower heads represented by each point are shown. Points representing one flower head only are not presented

ining top damage levels. However, when examining bottom damage levels there appears only to be a significant effect of date (and significant type \times date interaction) at site 1. Examples of patterns of damage levels in hybrid and wild flower heads with date are shown for sites with significant interaction terms in Fig. 5. In no cases did a significant interaction date \times type term indicate wilds showing overall higher average seed damage levels than hybrids. Although flower head diameter and number of open flower heads in the site at the time of flowering do appear to be significant in some sites, no consistent pattern is evident as to the effect of these factors.

Discussion

This study demonstrates a large difference in pre-dispersal seed predation levels between crop-wild hybrid and wild

Table 3 Analysis of variance for proportion of top and bottom damaged seeds in hybrid and wild flower heads with some insect damage. All analyses began with all terms and interactions tested; non-significant interaction terms were removed sequentially (n.s.). Final models are presented

Variable	df	Top damage			Bottom damage		
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Type	1	5.10*	5.95*	5.35*	2.70	7.00*	1.28
Date	1	4.14*	5.92*	3.72	5.29*	3.38	3.58
Diameter	1	1.98	3.08	0.06	9.02**	0.58	3.38
No. heads	1	1.45	0.32	6.27*	0.55	2.96	0.73
Type×Date	1	1.86	15.55***	9.07**	15.62***	n.s.	0.19
Type×Diameter	1	6.15*	n.s.	n.s.	n.s.	n.s.	3.33
Type×No. heads	1	9.70**	n.s.	n.s.	11.07**	4.08*	1.36
Date×Diameter	1	3.63	n.s.	n.s.	5.49*	n.s.	3.41
Date×No. heads	1	2.09	n.s.	9.29**	n.s.	n.s.	n.s.
Diameter×No. heads	1	2.34	n.s.	12.01***	2.79	n.s.	3.04
Type×Date×Diam	1	1.62	n.s.	n.s.	n.s.	n.s.	n.s.
Type×Date×No. heads	1	6.73*	n.s.	n.s.	n.s.	n.s.	n.s.
Type×Diam×No. heads	1	12.23*	n.s.	n.s.	n.s.	n.s.	3.88
Date×Diam×No. heads	1	3.14	n.s.	n.s.	n.s.	n.s.	n.s.
Type×Date×Diam×No. heads	1	8.56**	n.s.	n.s.	n.s.	n.s.	n.s.
Error df		73	74	87	66	51	50

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

seed heads. Seed predation differentially reduced seed production and can be considered to further reduce the relative contribution of crop-wild hybrid seeds to the next generation. The mechanisms for the increased seed predation in hybrid seed heads are not completely clear, but the high damage levels observed during periods when primarily hybrids were flowering is a likely contributing factor.

Damage levels in hybrid and wild heads

In our study, sunflower crop-wild hybrid heads had a significantly greater proportion of pre-dispersal seed loss due to head-infesting insects than wild sunflower heads in the same area at the same densities. It is important to note that this proportional reduction in seed loss for hybrids at the level of individual heads also translates to lower seed production per hybrid plant since hybrid plants produce fewer flower heads. Details of hybrid and wild seed production in these populations will be discussed elsewhere (A.A. Snow, unpublished work). Briefly, the average hybrid plant produced approximately 9.5 times fewer heads and 3 times fewer total seeds per flower head than did the average wild plant. The average hybrid plant had 36.5% of its seeds (or 45.1 seeds per plant) eaten by insect larvae while the average wild plant lost only 1.8% (or 95 seeds) to seed predators. Thus, higher levels of insect seed predation in hybrids act to exacerbate an already demonstrated low hybrid fitness as compared to wild plants (Snow et al. 1998).

Date of flowering and head diameter also differ between hybrid and wild plants. The earlier flowering of hybrid plants supports previous reports of differences between wild and wild-crop F_1 sunflower hybrids (Snow et al. 1998). However, the differences in flower head diameter contradict earlier evidence that hybrids had larger heads than wild plants (Snow et al. 1998). We explored whether date of flowering, diameter of heads, or number of heads opening on the same day accounted for the dif-

ference in seed damage between cross types. Although these factors, especially date of flowering, did influence herbivore damage, they did not remove the effect of cross type on seed damage levels: amount of damage was always higher in hybrid than in wild seed heads for all combinations of conditions.

Date of flowering had the strongest and most consistent effect on top damage levels. In sites with significant type×date interaction terms, damage levels in hybrid and wild flower heads showed different responses to date (Fig. 5). Overall, damage levels seem to peak during late August to early September. At site 2 (Fig. 5A) top damage levels in hybrid heads seem to increase with date and damage levels in wild heads seem to decrease with date (the same trend is seen at site 3 – the other site with a significant type×date interaction). Thus, flowering phenology could contribute to the differences in seed predation levels between the cross types because few wilds were flowering at peak damage times. One possible explanation for this late summer peak could be that the insect predators were most abundant and actively ovipositing during the late summer. Seasonal patterns of the activity of the sunflower moth (top damage) (Beckham and Tippins 1972; Satterwait and Swain 1946) and banded sunflower moth (bottom damage) (Westdal 1949; Westdal and Barret 1955) are reported to peak in late summer, supporting this hypothesis.

An alternative explanation for the late summer peak in damage levels could be that large numbers of open heads during the end of August created an increased attraction for ovipositing insects. Such a scenario is unlikely, given that the highest number of open heads occurred near the end of September, and that proportion of damage was either unaffected by, or generally decreasing with increasing numbers of open heads. If wild or crop sunflowers outside of our study area were at peak flowering during late summer, an increased attractiveness could have been operating at a larger scale. However, this scenario is also unlikely given that wild sunflowers

were removed around our experimental populations, few crop sunflowers are grown in the area, and the peak flowering of wild sunflowers in the area in 1997 was during the 2nd week of September. The peak flowering times of alternative host plants for the insects we studied are not known and any effect of insect abundances due to alternative host plants populations cannot be completely ruled out.

In contrast to flowering phenology, the number of open flower heads and flower head diameter did not seem to be able to consistently explain differences in damage levels between hybrid and wild flower heads. It is noteworthy that crop×wild Kansas F₁ hybrid sunflowers have been found to have larger seeds than plants resulting from wild×wild crosses (Snow et al. 1998), and were larger in our experiments (C.L. Cummings, unpublished work). Thus, we suggest that one possible explanation for the increased hybrid damage levels could be that larger seeds may have increased the survivorship of the insect larvae resulting in higher damage levels in hybrid flower heads.

Seed damage type patterns

One goal of this study was to determine whether different types of seed predators (Table 1) had similar effects on wild and hybrid flower heads. Dramatically different responses to hybrid versus parental taxa have been found even within the same guild of herbivores (Fritz et al. 1994). The proportion of infested heads was quite different between top (weevils, sunflower moth) and bottom damage (banded sunflower moth) types. Hybrids had significantly more heads affected by bottom damage but not by top damage, for which wild sunflowers had a higher proportion of affected heads in two of the three experimental populations. These results may indicate some preference of banded sunflower moth for hybrid heads, for which the cues are not entirely clear. For heads with some damage, hybrids had a greater proportion of insect damaged seeds for all damage types we examined, except for bite damage which was quite rare at our sites. There was no clear and consistent pattern of differences between damage types in the effects of flowering date, diameter of the head, or in the number of open heads.

Implications for “escape” of crop transgenes

The higher level of herbivory in the hybrid populations indicates that hybrids are more negatively affected by natural enemies than wild plants. We examined crop-wild hybrids with a specific interest in how herbivory differences contribute to the wild parent plant versus F₁ hybrid plant fitness differences and, therefore, the potential for introgression of alleles from crop populations into wild populations. We did not examine herbivory levels in crop parents. Relative fitnesses of crop and

crop-wild hybrid plants would be important in determining the rate of introgression of alleles from wild populations into crop populations. However this question is largely irrelevant, since new crop populations are planted each year.

Increased information pertaining to crop-wild hybrid fitness is necessary because gene flow from crop to wild plants is known to occur rather frequently and over great distances in sunflowers (Arias and Rieseberg 1994; Whitton et al. 1997; Linder et al. 1998) and in other plants (e.g., Klinger et al. 1992; Wilson and Manhart 1993; Darmency et al. 1998). There is ample opportunity and a demonstrated ability for crop-wild hybrids to occur in natural situations for many crop-wild complexes. Accurate assessment of the F₁ hybrid fitness relative to the wild is important when trying to examine the potential fate of alleles from crop populations into wild populations. It is clear in our study that F₁ crop-wild sunflowers do experience higher levels of seed predation than wild sunflowers and that previously demonstrated fitness differences will be exacerbated by including information on pre-dispersal seed predation.

Seed dormancy and germination levels have been recognized as important characteristics of crop-wild hybrid seeds (Alder et al. 1993; Hails et al. 1997) but information on relative seed predation levels in other crop-wild hybrid plants is rare. Predation, disease, and herbivory levels in crop-wild hybrids can be expected, a priori, to differ from those of parental taxa (Strauss 1994; Fritz et al. 1994; Fritz 1999). Seed predation levels are particularly relevant in systems involving crops in which the incorporation of Bt toxin is proposed. Insect seed herbivory should be decreased in the crop and in any crop-wild hybrids that incorporate this transgene. The relative fitness of the crop-wild hybrid compared to the wild plant could therefore increase upon incorporation of Bt transgenes. Investigation of specific damage types that are the result of various insect taxa is especially relevant since crop transgenes, like Bt genes, may act to reduce effects of specific insect orders. The relative selective advantage of this type of gene in crop-wild hybrid and wild plants due to the reduction of a specific insect seed predator can be calculated using the type of data we have provided. To make solid predictions of the probability of crop gene escape, information on F₁ hybrid fitness will thus need to be integrated not only with levels of crop to wild gene flow and hybrid versus wild seed production but also with seed predation levels and the relative selective advantages (e.g., insect resistance), if any, of the transgene in question.

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