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CULTIVATED *HELIANTHUS ANNUUS* (ASTERACEAE) VOLUNTEERS AS A GENETIC "BRIDGE" TO WEEDY SUNFLOWER POPULATIONS IN NORTH AMERICA[1]

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In many crops, unharvested seeds can create populations of volunteer plants that increase opportunities for crop-to-wild gene flow. Pollen-mediated gene flow between cultivated and wild sunflower (both *Helianthus annuus*, Asteraceae) is well documented, but the role of seed dispersal and volunteers has not been investigated. We compared flowering times and other phenotypic traits of volunteers from both ''normal'' and ''off-type'' (multi-headed) crop plants with those of wild sunflowers. Normal and off-type volunteers typically had a maternally inherited, crop-specific DNA marker. Seedlings of wild plants, normal volunteers, and off-type volunteers from Colorado were cultivated in a greenhouse and at a field site in Ohio. We used a classification tree approach to differentiate the three plant types and identify phenotypic traits that can be used to recognize volunteers in the field in future surveys. In greenhouse and field experiments, we observed sufficient overlap in flowering times to allow gene flow among the three plant types. Volunteers from off-type crop plants were more likely to cross-pollinate with wild plants than volunteers from normal crop plants. Our results suggest that both types of crop volunteers have the potential to act as conduits for gene exchange between cultivated and wild sunflowers.

Key words: crop–wild gene flow; *Helianthus annuus*; seed dispersal; volunteers.

The development and wide-scale adoption of genetically engineered crops has raised concerns about the ability of engineered traits (or transgenes) to ''escape'' cultivation (Snow and Moran Palma, 1997). The escape of transgenes is likely because crops and their wild relatives form a larger species complex composed of ''crops, accompanying weeds, and wild-related species mutually influencing each other by means of introgression'' (Anderson, 1954). Interactions among members of crop-wild-hybrid (CWH) complexes provide multiple pathways for crop genes to enter wild populations (Fig. 1). These interactions include cross-pollination among cultivated plants, feral crop plants, and sexually compatible wild relatives, as well as gene movement via seeds. Most studies of gene flow within CWH complexes have focused on the dispersal of pollen rather than seeds (Ellstrand et al., 1999; Ellstrand, 2003), in part because of difficulties in separating the effects of pollen and seed dispersal and the prevailing view that pollen movement is mostly responsible for genetic subdivision among plant populations (Cain et al., 2000). As a result, only a few studies have examined the role of seed dispersal in determining gene flow rates in CWH complexes (Arnaud et al., 2003). However, if certain types of transgenic crops are designed to minimize pollen flow from the crop, for example by having male sterility or maternally inherited transgenes, it will also be important to gain a better understanding of seed-mediated gene flow (NRC, 2000).

Harvesting crops inevitably results in some unintentional loss of seeds. When these seeds germinate in subsequent years, they are called ''volunteers.'' Volunteers can be weeds in successive crops or along field margins and often present a management problem for farmers (e.g., Anderson and Soper, 2003; Brighenti et al., 2003; Gulden and Shirtliffe, 2003). The widespread adoption of transgenic herbicide-resistant crops can present a potentially high economic cost to farmers due to volunteer control issues and contamination of nontransgenic crops (e.g., Smyth et al., 2002). Transgenic contamination of pedigreed canola seed stocks is most likely due to the movement of crop seeds and gene flow involving volunteers (Friesen et al., 2003).

The significance of volunteers to gene flow within CWH complexes is that they can increase the temporal and spatial scales over which wild populations and crop-derived plants co-occur. In CWH complexes where the crop is harvested or mowed before flowering occurs (e.g., beet, radish, lettuce, turf grass), volunteers may be the only viable pathway for gene flow. This has been shown to be the case in sugar beet: a recent study of weed beet populations in France found that crop seed dispersal and backcrossing from volunteers was the primary pathway for crop-to-wild gene flow (Arnaud et al., 2003).

Opportunities for direct pollen-mediated gene flow are limited within many CWH complexes due to crop rotation schedules or other agricultural practices (mowing or applying herbicides). If volunteer seeds become incorporated into the seed bank and subsequently germinate and flower, wild populations may continue to be exposed to gene flow even in the absence of nearby cultivated fields. Also, the distance over which crop seeds can travel may be much greater than that of pollen, especially in crop species with low outcrossing rates (e.g., soybean) and/or those that are wind-pollinated (e.g., sorghum). In addition to natural means of dispersal, human movement of crop seeds can be extensive.

The overall goal of this study was to determine whether crop volunteers represent a possible conduit for gene flow from cultivated sunflowers (*Helianthus annuus* L.) to the common wild sunflower (also *H. annuus*), which frequently co-occurs with the crop in the Great Plains of North America. Pollen-mediated gene flow has been well documented between crop and wild sunflowers (Arias and Rieseberg, 1994; Whitton et al., 1997; Linder et al., 1998; Massinga et al., 2003), and sunflower is considered to be a high-risk crop for transgene

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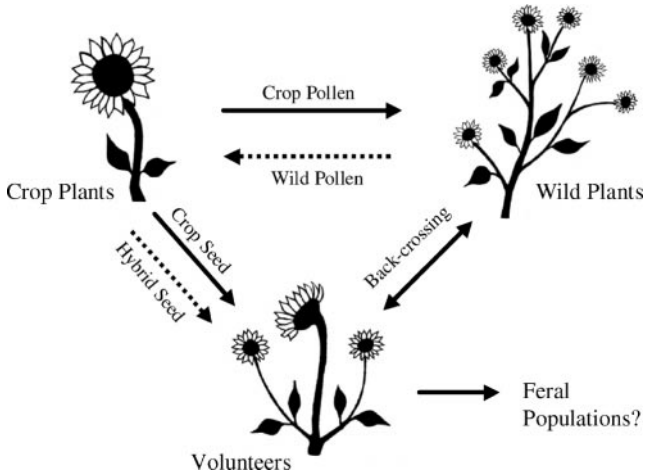

Fig. 1. Pathways of gene flow in crop–wild hybrid complexes. Most gene flow can occur by unidirectional pollen flow from the crop to wild plants (hybridization). Seed dispersal from crop plants results in volunteers that can backcross with wild plants. Pollen movement can occur in both directions and could result in the introgression of maternally inherited crop alleles. Volunteers may be able to create feral populations in the absence of wild plants.

escape. However, none of these previous studies investigated the role of volunteers in mediating crop–wild gene flow in sunflower. Cultivation practices in sunflower can produce many potential volunteers; on average, 3–5% of all seeds produced remain unharvested (Blamey et al., 1997). Putative volunteers are commonly observed in field margins, along highways, and as weeds in crop fields (M. Reagon and A. Snow, personal observation). Despite their ubiquity, sunflower volunteers have not been considered to be important in facilitating crop-to-wild gene flow or capable of founding new weed populations in the USA (e.g., Whitton et al., 1997), although studies on volunteers and gene flow have recently been initiated in Europe (Berville et al., 2005). The dispersal of sunflower crop seeds by farm vehicles may affect wild populations that are many kilometers away from cultivated fields and facilitate the introgression of maternally inherited crop genes. Volunteers or early generation hybrids may also colonize disturbed habitats in place of wild sunflowers, perhaps contributing to the homogenization of wild gene pools.

To our knowledge, this is the first study to examine morphological and phenological variation in volunteer populations from open-pollinated, cultivated sunflowers. Another unique aspect of this work is our inclusion of progeny from off-type crop plants. Sunflower fields often contain a few ''off-type'' crop plants in addition to ''normal'' crop plants. Off-types are taller, multibranched crop plants that are probably derived from wild pollen contamination during the production of hybrid crop seed that is sold to farmers (G. Cole, Pioneer Hi-Bred, personal communication). A few off-type crop plants are found in most cultivated sunflower fields (M. Reagon and A. Snow, personal observation) and were considered as a separate group within the general category of volunteer genotypes. We included progeny from off-type crop plants as they may impact gene flow rates more than progeny from normal crop plants due to longer flowering period.

In this study, we compared morphological and flowering characteristics of the two types of volunteer sunflowers—progeny from normal vs. off-type crop plants—with wild sunflowers. Throughout the text, we refer to offspring from off-type plants as ''off-type volunteers'' and offspring from normal crop plants as ''normal volunteers.'' The volunteer plants in this study represent typical first-generation volunteers, and they were obtained from the seeds of open-pollinated, cultivated plants. Seeds from the three sunflower parent groups—normal crop, off-type crop, and wild—were planted in a greenhouse and in a common-garden field plot to address the following questions: (1) What is the maternal origin of off-type volunteers, i.e., is their maternal parent a cultivated plant, as we assume, or could it be a wild plant? If the maternal parent of off-types is wild, then they are not true volunteers, and could not be a pathway for maternally inherited crop genes to introgress into wild populations. (2) How much overlap in flowering times and fecundity occurs among the three plant types? Data on phenology were used to examine the potential for cross-pollination among plant types, while lifetime fecundity provided an estimate of a major component of fitness for each plant type. (3) Do volunteer plants have morphological traits that can be used to distinguish them from wild-type plants in the field? Morphological traits that are reliably diagnostic of volunteers can be used to estimate the frequency of volunteers in future surveys of wild populations. Without this information, first generation volunteers with multiple flower heads, could easily be misidentified as wild plants, as we discuss later.

MATERIALS AND METHODS

Seed sources—To obtain first-generation volunteer plants for our experiments, we collected achenes from open-pollinated crop plants in Colorado. Achenes for the greenhouse experiment were collected in August 1999 from Kit Carson County, Colorado, a major production area for cultivated sunflower. We collected achenes from 25 normal crop plants and 40 off-type crop plants from several cultivated fields of oilseed sunflowers. For the wild experimental plants, we collected achenes from 60 wild plants located in a single wild population located near Burlington, Colorado, USA (Kit Carson County).

For the field experiment, we collected achenes from more populations in an effort to capture a greater amount of the phenotypic diversity present in both wild and crop-derived populations. In August of 2000, achenes for off-type volunteer plants were collected from off-type crop parents located in 10 oilseed sunflower fields in western Kansas and eastern Colorado. We collected achenes from 3–6 off-type crop plants per field (depending on availability), for a total of 65 individuals. We also collected achenes from 10 normal crop plants in each of five oilseed crop fields, for a total sample of 50 individuals. Fewer normal volunteer parents were considered necessary because crop plants typically self-pollinate, and within-field diversity is expected to be low. We collected achenes from five wild populations that were located from 1 m to 5 km away from cultivated fields, using 25 individuals in each population, for a total of 125 wild individuals. No achenes were collected from wild plants that appeared to be early generation crop-wild hybrids.

Greenhouse and field experiments—Achenes from different populations of each plant type were pooled, and a random sample of 80–100 seeds per type was germinated for each experiment. To break dormancy and ensure adequate germination, achenes were nicked with a single-edge razor, placed on wet filter paper, and refrigerated at 20°C for 3–5 d. After refrigeration, the achene coat was removed and seeds were transferred to soil-filled flats to germinate.

For the greenhouse experiment, 40 seedlings of each plant type were transplanted to 4-L pots after second true leaf emerged, and were randomly arranged in the greenhouse on 18 January 2000. Pots were attached to an automatic watering system, and 5 g of Osmocote (The Scotts Company, Marysville, OH, USA.) slow-release fertilizer were applied to the soil surface of each pot. To avoid position effects, pots were rotated several times during the first 2 months, after which the plants were too large to be conveniently moved. The experiment ran for 16 weeks and ended when the last plant died.

The field experiment was carried out at the Waterman Farm on the campus of Ohio State University in Columbus, Ohio. Ten days before seedlings were transplanted, the plot was tilled, and a pre-emergent herbicide (Prowl, BASF Ag Products, Research Triangle Park, North Carolina, USA.) was applied. Sixty seedlings of each type were transplanted 1 m apart in a randomized complete block design (six blocks with 10 individuals of each plant type per block) on 16–17 June 2001. Young plants were watered during the first week, and the plot was weeded several times during the first month to reduce competition from emerging weeds. The experiment ended on 30 September 2001, when the onset of cooler temperatures precluded further seed maturation.

Flowering, self-compatibility, and fecundity—The amount of overlap in flowering time among plant types was measured differently in the field and greenhouse experiments. In the greenhouse, we recorded the date that each plant began to flower. This gave a good approximation of overlap due to the small number of heads (inflorescences) produced by the normal volunteers (typically less than three). In the field, in addition to recording the date of first flower, we counted the daily number of heads produced by each plant for 45 d. This provided a good estimate of how daily flower production by each plant type changed over time.

In the greenhouse, we recorded the frequency of male fertile plants that produced seeds spontaneously (only four off-type and two normal volunteers were male sterile). No pollinators were present and plants were spaced sufficiently far apart to prevent pollination by contact; therefore, any seeds produced were the result of self-fertilization. Previous research has shown that wild plants are almost completely self-incompatible, whereas cultivars used in the United States are mostly self-compatible and self-pollinating (Fick and Miller, 1997). The genetic control of self-compatibility is complex and greatly influenced by the environment (Fick and Miller, 1997), but we expected both types of volunteers to be self-compatible.

To estimate differences in lifetime fecundity, we counted the total number of heads produced by each plant in both the field and greenhouse experiments. Although crop-derived plants were expected to produce larger heads, and more seeds per head than wild plants, previous studies of wild and crop-wild hybrids have shown that total head production provides a good measure of lifetime seed production (Snow et al., 1998; Pilson, 2000). In the field experiment, we stopped counting heads after 30 September 2001 because these flowers would not have produced seeds before being killed by frost.

Other traits of wild and crop-derived progeny—We measured two aspects of branching patterns in the two experiments. In the field experiment only, we counted the number of primary branches (branches emerging directly from main stem) on each plant. Volunteer plants grown in the greenhouse produced few primary branches, and these were not counted. In both experiments, we recorded the presence or absence of basal branching at the time of plant death. Plants that have basal branches only branch within ~5 cm of the base of the stem. Crop plants occasionally branch at the base of the stem, but this trait is not found in wild sunflowers. Branching in sunflowers is under complex genetic control and is influenced by both dominant and recessive genes (Fick and Miller, 1997). Crop plants typically do not branch, but many of the male restorer lines used in hybrid seed production branch profusely due to the effects of recessive genes, which may result in volunteers that branch (Fick and Miller, 1997). In wild sunflowers, branching is most often controlled by dominant genes, which cause branching over the entire length of the stem.

Total plant height was measured in the greenhouse, but due to problems with lodging (plants falling over) it was not measured in the field. Height is a plastic character in wild plants, which can range from 0.5 to >4 m tall in the field. Height is a more uniform trait in the crop, due to the effects of breeding and more uniform growing conditions. Off-types crop plants are usually taller than normal crop plants (M. Reagon and A. Snow, personal observation).

In both experiments, we measured the diameter of the first capitulum (head) produced on each plant after the ray flowers senesced. Larger head diameter is considered to be an indication of crop-derived genotypes in wild populations that occur near cultivated fields (Linder et al., 1998). Typically, the first head produced by wild sunflowers is slightly larger than later heads (Pilson, 2000). In the greenhouse, we observed that the first head produced by crop-derived plants was much larger than other heads produced. To test if size differences between first and subsequent heads could be used to distinguish volunteers from wild plants, we also measured the diameter of the next two heads a plant produced in the field experiment. The average size of these two heads was than subtracted from the size of the first head produced, and this difference score was used in our analysis.

We recorded the frequency of plants with bent-over seed heads in each plant type in both experiments. Mature flowers of wild sunflowers are inclined upward in most cases, whereas crop flowers are bent down. Crop breeders in the USA have selected for the bent-over position to decrease effects of bird predation on mature seeds (Fick and Miller, 1997). This trait is influenced by the interaction of several genes, but the bent-over head position appears to be partially dominant (Fick and Miller, 1997).

The frequency of plants with yellow disk flowers was recorded in both experiments. Disk flowers of wild plants are typically a dark reddish-brown to deep purple. Yellow-disked wild plants are rare (<1%, M. Reagon and A. Snow, personal observation), and Heiser (1954) found no wild sunflowers in Colorado with this trait. In contrast, many crop varieties have yellow disk flowers, and the trait may be useful to identify wild plants with crop parentage.

In the greenhouse experiment, we noticed that volunteer plants occasionally produced deformed or fused heads, which could indicate developmental instability. Developmental instability is often observed in studies of hybridization (Siikamaki, 1999) and may indicate plants of crop-wild hybrid origin (although this has not been examined in sunflower). In the field experiment, we recorded the frequency of plants with fused or deformed heads, which we refer to as ''head asymmetry.'' Deformed heads may also lead to a decrease in lifetime fecundity, as they produce fewer seeds (data not shown).

In the field experiment, we also compared the relative amount that plants ''shattered'' (dispersed seeds). At physiological maturity, an inflorescence from each plant was tapped on the back of the receptacle and ranked based on the number of seeds dispersed. Plants were ranked in three categories: (1) no seeds dispersed, (2) less than ~50% of seeds dispersed, and (3) most seeds dispersed. Wild plants shatter easily in the field, whereas seeds of cultivated plants remain attached to the receptacle.

Classification of phenotypic variation—One goal of our data analysis was to find morphological characters that could be used to differentiate wild and volunteer plants in the field. We used a classification tree approach after De'ath and Fabricius (2001) to identify morphological variables that could be predictive of plant type. Classification trees explain variation in a single response variable (plant type) by repeatedly splitting the data into more homogeneous groups using combinations of explanatory variables (phenotypic characteristics). Group (or node) homogeneity is defined by impurity, which is zero for completely homogeneous nodes and increases as homogeneity decreases. We determined node impurity by using an index that is identical to the Shannon–Weiner diversity index. Variables that give nodes with the lowest impurity are used to split the data until nodes contain too few entries (<5) or if impurity cannot be improved by further splitting. For classification trees, the overall tree misclassification rate can be used to summarize the impurity of terminal nodes and model quality. The combination of explanatory variables that resulted in the tree with the fewest nodes and lowest misclassification rate was used to determine morphological variables that would be useful for field identification. Data from the greenhouse and field experiments were analyzed independently using SAS Enterprise Miner release 4.3 (SAS Institute, Cary, North Carolina, USA).

Maternal origin of off-types—To confirm that maternal parents of off-type volunteers were cultivated sunflowers, rather than wild genotypes growing within the crop, we used a polymerase chain reaction-based marker system developed by Rieseberg et al. (1994) that identifies sunflower plants that have a gene for cytoplasmic male sterility (CMS89). CMS89 is found in the mitochondrial genome of nearly all male sterile lines used for hybrid seed production in the USA (Rieseberg et al., 1994; G. Seiler, ARS, Fargo, North Dakota, USA, personal communication). Almost all commercial sunflower produced in the USA is from hybrid seed, and we therefore expected volun-

TABLE 1. Frequency of qualitative phenotypic traits of wild, off-type, and crop volunteers in the greenhouse and field experiments (N = 40 and 59–60, respectively).

Trait	Experiment	Character trait frequency		
		Wild	Off-type volunteer	Normal volunteer
Crop head inclination	Greenhouse	0.03	0.73	1.00
	Field	0.15	0.87	1.00
Basal	Greenhouse	0.05	0.42	0.51
	Field	0.13	0.78	0.62
Yellow disk	Greenhouse	0.05	0.13	0.41
	Field	0.05	0.23	0.38
Head asymmetry	Field	0.05	0.28	0.10
	Greenhouse	0.00	0.12*	0.96

* Only three plants produced viable seeds (30 seeds from 8 individuals were tested). All of the tested crop seeds germinated (30 seeds from 10 individuals were tested).

teers to have the CMS89 marker. Reiseberg et al. (1994) found that CMS89 was strictly maternally inherited, making it an ideal marker for confirming the maternal parentage of off-type volunteers and detecting seed-mediated gene flow from the crop.

We screened 65 off-type volunteers (from the same collections as our off-type volunteer experimental plants) collected in crop fields in Kansas and Colorado in 1999 and 2000 for the CMS89 marker. To confirm that our reaction protocols worked, we also tested (1) a cultivar known to contain CMS89 (Triumph number 565: Triumph Seed Co., Ralls, Texas, USA), (2) 15 normal crop volunteer plants collected from the field (same generation as our normal volunteer experimental plants), and (3) 25 wild plants. Extractions of seedling tissue were carried out using Sigma's (St. Louis, Missouri, USA) Extract-N-Amp plant PCR kit. PCR amplifications were conducted as specified by Sigma's Extract-N-Amp using a Strategene Robocycler (La Jolla, California, USA) programmed for 1 min at 94°C, followed by 35 cycles of 1 min at 94°C (denaturation), 1 min at 60°C (annealing), and 2 min at 72°C (extension), and a final 7 min extension at 72°C. Amplification products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light.

RESULTS

Maternal origin of off-type plants—None of the wild plants tested positive for the marker, as expected. All 15 normal crop volunteers evaluated had the marker diagnostic of CMS89, which is in agreement with earlier studies by Rieseberg et al. (1994) and Linder et al. (1997). Ninety-one percent of the off-type volunteers that were examined had the crop-specific DNA marker, as would be expected if an off-type volunteer's maternal parent were a cultivated plant (N = 65 total plants). The few off-type volunteers that did not have the marker could be derived from cultivars without CMS89 or from wild maternal plants. Although we were careful to collect seeds for off-type volunteer plants from individuals growing within crop rows at regular intervals, and away from the edges of fields, it is possible that we inadvertently collected plants with wild maternal parents rather than crop plants. However, we assume that the off-type volunteers used in our experiments were derived from cultivated mothers because they all exhibited one or more morphological traits that were typical of crop plants (see next section and Tables 1 and 2).

Results of classification tree—The classification tree analysis was able to differentiate wild and crop derived plants in both the greenhouse and field experiment (Fig. 2). Data used for the tree analysis are summarized in Tables 1 and 2. The overall tree misclassification rate for the greenhouse data was 5% (6 of 120 individuals misclassified). No wild plants were classified with normal volunteers or vice versa. All normal volunteer plants were classified into a single terminal node, based on three morphological traits: head angle, head diameter, and plant height. Off-types were intermediate between normal volunteers and wild plants and were distributed among all of the terminal nodes. However, most off-type and normal volunteers were distinguishable based on plant height and head diameter, which is consistent with observations made in sunflower production fields. More off-types had basal branches (Table 1), and the model used this trait to distinguish off-types and wild plants. Yellow disk color was not useful in differentiating plant type and was rejected by the model. Date to first flower and total head number were not used in this analysis because these traits are likely to be either unknown in a field survey or too plastic to be used for field identification.

The overall tree misclassification rate for the field data was 14% (26 of 178 plants misclassified). Similar to the greenhouse, head angle was the most useful in differentiating wild and crop derived plants in the field. Using the difference in head diameter as the primary splitting criterion resulted in a similar tree, but this trait may not be as obvious as head angle for field identification. Using seed shattering as the primary

TABLE 2. Flowering and morphological characteristics of wild, off-type, and crop volunteer plants in the (A) greenhouse and (B) field experiments (mean ± 95% CI are shown).

A) Greenhouse (N = 40) Trait	Wild	Off-type volunteer	Normal volunteer
Days to first flower	94.5 ± 3.2	74.7 ± 5.0	65.1 ± 1.7
Total head number	16.7 ± 2.5	10.9 ± 2.5	2.1 ± 0.6
Plant height (cm)	195.0 ± 7.5	160.5 ± 9.2	117.1 ± 5.1
First head diameter (mm)	30.4 ± 1.5	46.0 ± 5.0	71.7 ± 2.7
B) Field			
Trait	**Wild (N = 59)**	**Off-type volunteer (N = 60)**	**Normal volunteer (N = 59)**
Days to first flower	49.6 ± 1.8	46.2 ± 1.7	46.9 ± 0.8
Total head number	267.5 ± 30.9	136.8 ± 35.4	20.1 ± 8.9
Total branch number	22.7 ± 1.6	16.3 ± 2.6	6.0 ± 1.7
First head diameter (mm)	46.0 ± 5.3	116.3 ± 18.5	191.2 ± 19.6
Difference score (mm)[a]	4.9 ± 3.8	44.6 ± 27.4[b]	98.2 ± 24.5[c]

[a] Calculated by subtracting size of first head from average of the second two heads.
[b] 57 (95%) of the off-type volunteers had more than one head.
[c] 39 (66%) of the normal volunteers had more than one head.

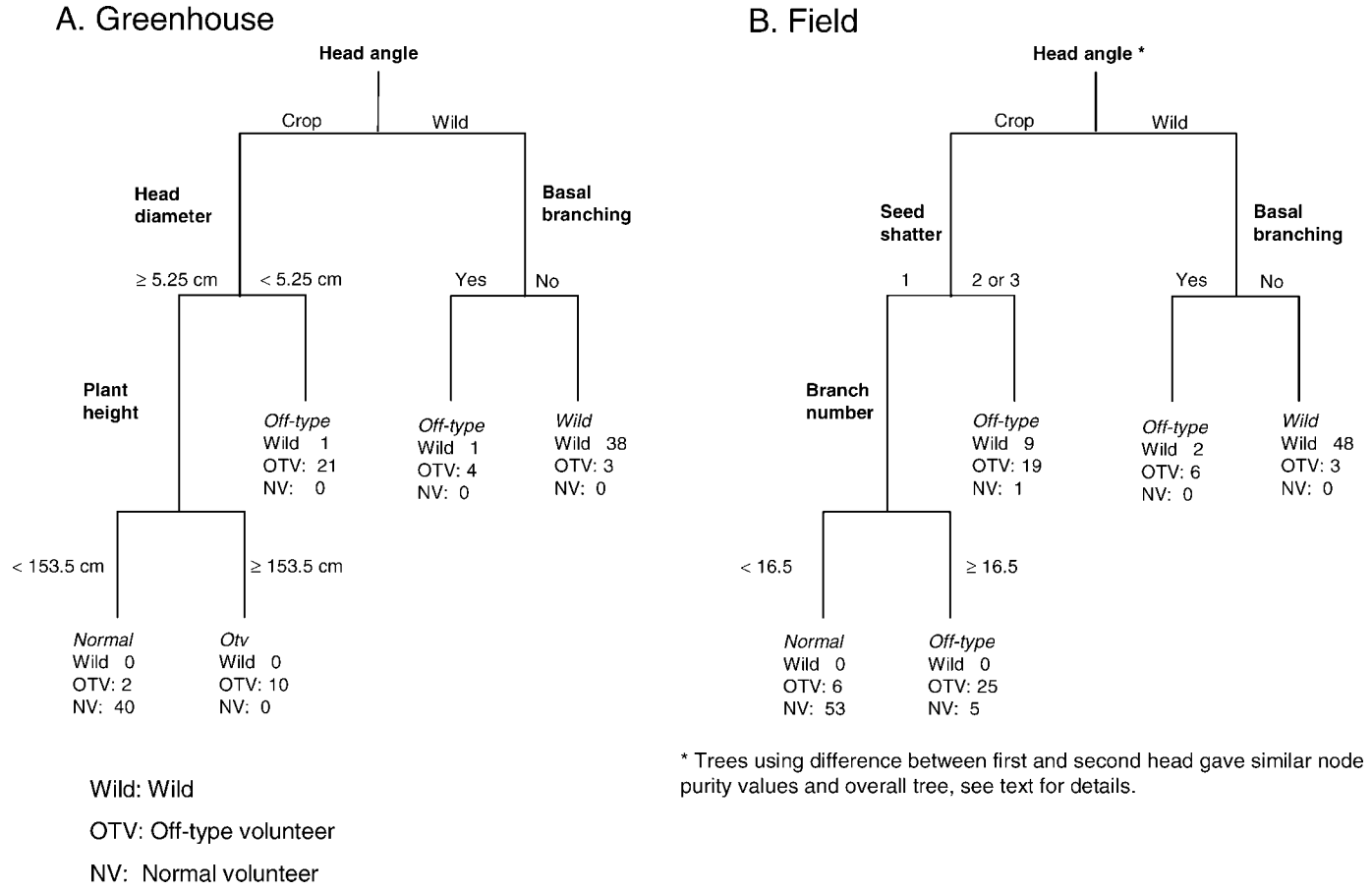

Fig. 2. Classification trees for identifying plant types based on phenotypic characters. The predicted plant type of each terminal node (or leaf) is labeled with italics. The distribution of individuals assigned by plant type to each leaf is also listed. (A) Tree for greenhouse data that had the lowest overall misclassification rate (5%). (B) Tree for the field data that had the lowest overall misclassification rate (14%). Each split (nonterminal node) is labeled with the variable in bold and the criterion that determined the split. Plants with ''crop'' head angle had heads that were angled down, whereas ''wild'' plants the heads were inclined up.

splitting criterion also resulted in a tree with only slightly higher misclassification rate, but contained more nodes (see Fig. 3 for seed shattering data). Normal and off-type volunteers grown in the field were more similar than in the greenhouse, which resulted in greater node impurity than the greenhouse tree. However, normal volunteers did form the most cohesive group, with 53 of 60 clustering in one node. Off-types were again intermediate between wild and normal volunteers, but more wild plants had characteristics typical of crop plants. This is likely due to the unintended inclusion of a few F_1 or other early generation hybrids in our wild experimental plants. Fused or deformed heads were uncommon in all plant types and this trait was rejected by our model.

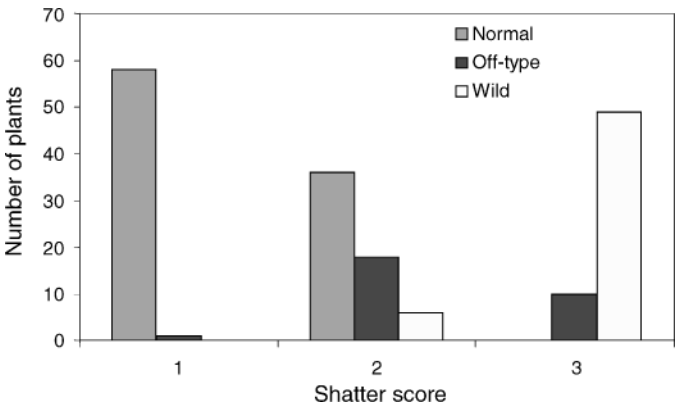

Fig. 3. Seed shattering scores for the three plant types from the field experiment. 1 = no shattering, 2 = <50% seeds shattered, 3 = nearly all seeds shattered.

Flowering, self-compatibility, and fecundity—Duration of flowering of the three plant types overlapped in both experiments, with normal volunteers flowering earliest, off-type volunteers being intermediate, and wild plants flowering latest and over the longest period of time (Figs. 4 and 5). In the greenhouse, where plants grew more slowly (possibly due to light quality or day-length), the number of days to flowering averaged 65 days for normal volunteer plants, 75 days for off-type volunteers, and 95 days for wild plants (Table 2). In contrast, all three plant types began flowering within 46 days of transplanting in the field experiment (Fig. 5, Table 2). The extent of concurrent flower production among groups was greater in the field experiment. This was due to more similar initial flowering dates and because field-grown plants produced more branches and therefore flowered over a longer period of time (total number of heads and branches are highly correlated) (Table 2).

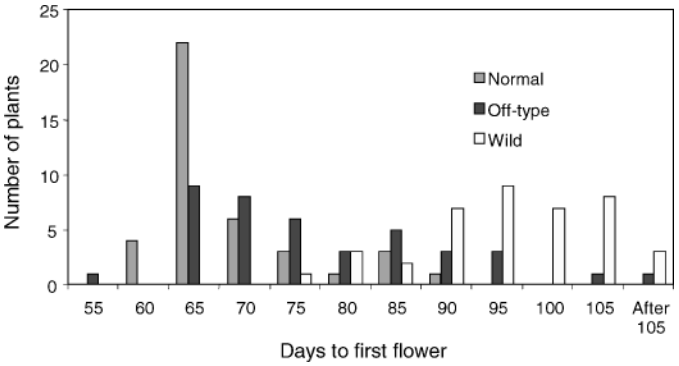

Fig. 4. Days to first flower of normal volunteer, off-type volunteer, and wild plants ($N = 40$) in greenhouse experiment. Date categories are number of days since planting.

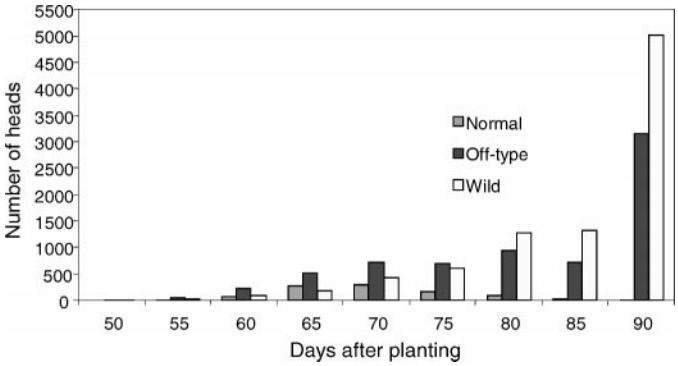

Fig. 5. Number of flower heads produced by normal volunteer, off-type volunteer, or wild plants during each 5-d interval in the field experiment. Data were pooled from 59–60 plants in each group. After day 45, wild plants produced an additional 8912 flowers, off-types produced 1554 flowers, and crop plants produced 241 flowers.

Although most wild inflorescences were produced after both normal and off-type crop volunteers finished flowering, variability within groups was evident, especially in the off-type volunteers (Figs. 4 and 5, Table 2). Off-type volunteers flowered for a much longer time period than normal volunteers in both experiments. Given that flowering times of the three plant types are likely to be much more variable under natural conditions, these results indicate that cross-pollination is likely to occur among all three plant types.

We found that normal volunteers were self-compatible and autogamous, like their cultivated parents, while the off-type volunteer plants were consistently self-incompatible (Table 1). Because the off-type volunteers cannot self-pollinate, the seeds of occasional off-type volunteers that occur in natural populations are more likely than normal volunteers or crop plants to be sired by nearby wild plants. Our data suggests that the primary direction of pollen movement may shift as the growing season progresses, increasing the likelihood that wild plants pollinate volunteers (Fig. 5). Therefore, the amount of wild pollination of crop-derived plants could be considerable in mixed crop–wild populations along field margins.

Lifetime fecundity was estimated by counting the numbers of heads per plant. In both experiments, wild plants had the greatest average fecundity, but there was considerable overlap among the plant types (Table 2, Fig. 6). The number of heads produced by off-type volunteers was intermediate between that of normal volunteers and wild plants. Although on average normal volunteers had much lower fecundity than either of these two groups, they were more similar to wild plants than their maternal parents (hybrid cultivars have one head).

DISCUSSION

In this study, we observed sufficient overlap in flowering times between volunteers and wild types in both the field and greenhouse experiments for cross-pollination to occur. In the field experiment, the potential for cross-pollination was considerable because many inflorescences were produced simultaneously by the three plant types (Fig. 5). The conditions in our field experiment were similar to those in sunflower populations along field margins in sunflower production areas, where normal volunteers, off-type volunteers and wild genotypes can co-occur. Off-type volunteers in the greenhouse ''bridged'' the gap between normal volunteers and wild plant

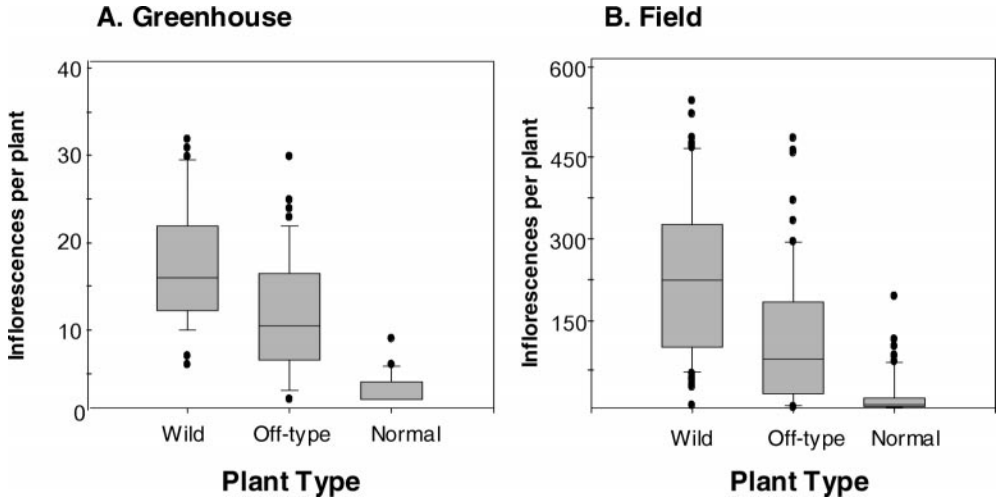

Fig. 6. Fecundity of wild, off-type, and normal volunteers in (A) the greenhouse and (B) the field experiment ($N = 40$ and 59–60, respectively). Box plots show the overall median and the median of the first quartiles; error bars indicate the median of the second quartiles, and dots indicate extreme values. See Table 2 for other statistics.

types and in the field flowered nearly as long as wild type plants. These results suggest that wild populations that co-occur with volunteers will continue to be exposed to crop gene flow even in the absence of nearby cultivated fields.

Although Burke et al. (2002) found that nearly all crop fields surveyed had adjacent wild populations, all wild populations are not adjacent to cultivated fields. In the area of Nebraska and Colorado where we work, fewer than 10% of wild populations occur within 1000 m of crop fields (M. Reagon and A. Snow, unpublished data). Depending on economic and environmental conditions, crop rotation schedules limit direct crop-wild plant contact to once every 3–5 years. Volunteers emerging from the seed-bank therefore may be an important mechanism for crop genes to reach wild populations adjacent and allopatric to cultivated fields. The classification tree analysis identified several morphological characters that are diagnostic of volunteers (head inclination, seed shattering, and head diameter) and could be used in conjunction with the CMS marker to survey wild populations for the presence volunteers. Quantifying the occurrence of volunteers in the field will be important to determining their overall impact on gene flow.

In conclusion, a major finding of this study is that sunflower volunteers often produce multiple heads and flower over an extended time that overlaps with wild plants. This is true for both normal volunteers and off-type volunteers. In addition, because off-type volunteers have many traits that are intermediate between normal crop volunteers and wild sunflowers, the progeny from natural crosses between these volunteers and wild plants may have relatively high fitness. We conclude that seed-mediated gene flow from cultivated sunflowers to wild sunflowers may be common, and the persistence of maternally inherited genes, including transgenes, is expected to be greatest when the crop parent is an off-type plant (which could have resulted from a wild paternal parent growing near seed production fields). Understanding the contributions of seed and pollen dispersal to gene flow within CWH complexes will be important for designing strategies to confine transgenes. Confinement techniques that rely on chloroplast transformation or male sterility may need to be reconsidered in sunflowers because volunteers are often common. Given the numerous pathways by which gene flow can occur in the sunflower CWH complex, these techniques may not be feasible to prevent the escape of transgenes.

LITERATURE CITED

ANDERSON, E. 1954. Plants, man and life. A. Melrose Press, London, UK.

ANDERSON, R. L., AND G. SOPER. 2003. Review of volunteer wheat (*Triticum aestivum*) seedling emergence and seed longevity in soil. *Weed Technology* 17: 620–626.

ARIAS, D. M., AND L. H. RIESEBERG. 1994. Gene flow between cultivated and wild sunflowers. *Theoretical and Applied Genetics* 89: 655–660.

ARNAUD, J. F., F. VIARD, M. DELESCLUSE, AND J. CUGUEN. 2003. Evidence for gene flow via seed dispersal from crop to wild relatives in *Beta vulgaris* (Chenopodiaceae): consequences for the release of genetically modified crop species with weedy lineages. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 270: 1565–1571.

BERVILLE, A., M.-H. MULLER, B. POINSO, AND H. SERIEYS. 2005. Ferality—risks of gene flow between sunflower and other *Helianthus*. *In* J. Gressel [ed.], Crop ferality and volunteerism, 209–230. CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA.

BLAMEY, F. P. C., R. K. ZOLLINGER, AND A. A. SCHNEITER. 1997. Sunflower production and culture. *In* A. A. Schneiter [ed.], Sunflower technology and production, 595–670. Soil Science of America, Madison, Wisconsin, USA.

BRIGHENTI, A. M., C. DE CASTRO, D. L. P. GAZZIERO, F. S. ADEGAS, AND E. VOLL. 2003. Phytosociological census of weeds in the sunflower crop. *Pesquisa Agropecuria Brasileira* 38: 651–657.

BURKE, J. M., K. A. GARDNER, AND L. H. RIESEBERG. 2002. The potential for gene flow between cultivated and wild sunflower (*Helianthus annuus*) in the United States. *American Journal of Botany* 89: 1550–1552.

CAIN, M. L., B. G. MILLIGAN, AND A. E. STRAND. 2000. Long-distance seed dispersal in plant populations. *American Journal of Botany* 87: 1217–1227.

DE'ATH, G., AND K. E. FABRICIUS. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* 81: 3178–3192.

ELLSTRAND, N. C. 2003. Current knowledge of gene flow in plants: implications for transgene flow. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* 358: 1163–1170.

ELLSTRAND, N. C., H. C. PRENTICE, AND J. F. HANCOCK. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30: 539–563.

FICK, G. N., AND J. F. MILLER. 1997. The genetics of sunflower. *In* A. A. Schneiter [ed.], Sunflower technology and production, 395–440. Soil Science of America, Madison, Wisconsin, USA.

FRIESEN, L. F., A. G. NELSON, AND R. C. VAN ACKER. 2003. Evidence of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. *Agronomy Journal* 95: 1342–1347.

GULDEN, R. H., AND S. J. SHIRTLIFFE. 2003. Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. *Weed Science* 51: 83–86.

HEISER, C. B. 1954. Variation and subspeciation in the common sunflower *Helianthus annuus*. *American Midland Naturalist* 51: 287–305.

LINDER, C. R., I. TAHA, G. J. SEILER, A. A. SNOW, AND L. H. RIESEBERG. 1998. Long-term introgression of crop genes into wild sunflower populations. *Theoretical and Applied Genetics* 96: 339–347.

MASSINGA, R. A., K. AL-KHATIB, P. ST. AMAND, AND J. F. MILLER. 2003. Gene flow from imidazolinone-resistant domesticated sunflower to wild relatives. *Weed Science* 51: 854–862.

NATIONAL RESEARCH COUNCIL. 2000. Genetically modified pest-protected plants: science and regulation. National Academy Press, Washington, D.C., USA.

PILSON, D. 2000. Herbivory and natural selection on flowering phenology in wild sunflower, *Helianthus annuus*. *Oecologia* 122: 72–82.

RIESEBERG, L. H., C. VAN FOSSEN, D. ARIAS, AND R. L. CARTER. 1994. Cytoplasmic male-sterility in sunflower: origin, inheritance, and frequency in natural populations. *Journal of Heredity* 85: 233–238.

SIIKAMÄKI, P. 1999. Developmental instability in hybrids between *Lychnis viscaria* and *Lychnis alpina* (Caryophyllaceae). *American Journal of Botany* 86: 1683–1686.

SMYTH, S., G. KHACHATORIANS, AND P. W. B. PHILLIPS. 2002. Liabilities and economics of transgenic crops. *Nature Biotechnology* 20: 537–541.

SNOW, A. A., AND P. MORAN-PALMA. 1997. Commercialization of transgenic plants: potential ecological risks. *Bioscience* 47: 86–89.

SNOW, A. A., P. MORAN-PALMA, L. H. RIESEBERG, A. WSZELAKI, AND G. J. SEILER. 1998. Fecundity, phenology, and seed dormancy of F_1 wild-crop hybrids in sunflowers (*Helianthus annuus*, Asteraceae). *American Journal of Botany* 85: 794–801.

WHITTON, J., D. E. WOLF, D. M. ARIAS, A. A. SNOW, AND L. H. RIESEBERG. 1997. The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization. *Theoretical and Applied Genetics* 95: 33–40.