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# GENETICALLY ENGINEERED ALFALFA AND FERAL ALFALFA PLANTS: WHAT SHOULD GROWERS KNOW?

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# Genetically Engineered Alfalfa and Feral Alfalfa Plants: What Should Growers Know?

## Abstract

Alfalfa (*Medicago sativa* subsp. *sativa* L.) is the world's most important forage crop. The western United States is the most important production area for both alfalfa forage and alfalfa seed. Unfortunately, feral alfalfa is commonly observed along roadsides, irrigation ditches, and unmanaged habitats in alfalfa growing regions. These plants can potentially lower the genetic purity of alfalfa seed when foraging bees transport pollen from feral alfalfa to alfalfa seed fields. The situation has become more complicated since the advent of genetically engineered (GE) alfalfa.

Alfalfa was the first major perennial GE crop when the trait for glyphosate resistance (GR) was available to farmers briefly from 2005 to 2007. Regulatory issues prevented the availability of GR alfalfa until 2011 when it received final approval for commercial production in the US. In 2014, another GE trait, low-lignin alfalfa, was deregulated and will be available commercially in the near future.

Because many markets have a low tolerance for GE traits, GR feral alfalfa growth in areas where conventional alfalfa seed and hay is produced for the export or organic market is of particular concern to the alfalfa industry. In response to this situation, the alfalfa industry has developed best management practices to support the coexistence of GE and conventional alfalfa production. An important management practice is to control feral alfalfa around both seed and hay fields to minimize the movement of GE traits into conventional alfalfa products.

The presence of feral alfalfa plants can be reduced by effectively eliminating old alfalfa fields with tillage, transporting seed in spill-proof containers, mowing roadsides regularly, applying specific growth regulator herbicides (such as aminopyralid, dicamba, clopyralid, and 2,4-D), and integrating these weed management strategies (such as applying herbicides to alfalfa prior to tillage or mowing). Glyphosate should not be used to control feral alfalfa unless it is mixed with other herbicides known to control GR alfalfa. Also avoid using alfalfa in revegetation seed mixes, especially in areas where GE-sensitive alfalfa seed is produced.

## Introduction

Alfalfa is one of the most important forage crops in the world. In North America, it consistently ranks among the top crops in terms of acreage grown. Most alfalfa seed is produced in the western US because the climate is suited for high quality and yields. California, Idaho, Oregon, Nevada, and Washington are the major alfalfa seed producing states (Mueller 1995; CAST 2008).

GE crops have been commercially grown worldwide since the late 1990s. Alfalfa became the first major perennial GE crop when glyphosate-resistant (GR) alfalfa was deregulated in the US in 2011 allowing for its commercialization as Roundup Ready<sup>®</sup>. In 2014, a GE low-lignin alfalfa was similarly deregulated, with the new trait stacked with GR in alfalfa. Alfalfa has characteristics that make confining GE traits to intentionally planted fields difficult. These characteristics include perennial growth, quick regrowth potential, symbiotic nitrogen fixation, deep taproot systems, drought and cold tolerance, and seed dormancy and longevity (Bagavathiannan et al. 2009; 2010).

Alfalfa is also a cross-pollinated species, which makes confinement even more difficult. Alfalfa is usually pollinated by managed bee species, such as alfalfa leaf cutting bees (*Megachile rotundata* F.), alkali bees (*Nomia melanderi* C.), and honey bees (*Apis mellifera* L.), but it is also pollinated by feral populations of these bees and by a number of wild, solitary, and social bees (CAST 2008; Brunet and Stewart 2010). These foraging bees can travel distances as great as seven miles in search of nectar (Eckert 1933). Alfalfa seed and forage crops have the potential to form feral populations, and escaped alfalfa populations have been commonly observed along roadsides and in unmanaged habitats in alfalfa growing regions (Kendrick et al. 2005; Bagavathiannan et al. 2010; Greene et al. 2015).

Conventional and organic alfalfa growers in the US have concerns regarding the AP of GE traits in their crops. Coexistence strategies developed by the National Alfalfa and Forage Alliance were developed to address these concerns (NAFA 2014). An important tactic in coexistence strategies is to eliminate feral alfalfa since GR feral alfalfa found outside of cultivated fields increases the potential for transgenic dispersal, which may negatively impact conventional and organic hay and seed grower fields.

This bulletin summarizes the occurrence of feral alfalfa in the western US, discusses how GE traits can become dispersed into these plants, and suggests stewardship practices to reduce the occurrence of feral plants and minimize dispersal of GE traits into non-cultivated areas.

**Genetically engineered (GE):** Modification of an organism’s genes by inserting, deleting, or rearranging specific genes using recombinant DNA technology.

**Adventitious presence (AP):** Unintentional or accidental presence of trace amounts of GE-derived traits in seed, grain, or food product.

**Feral:** Crop plants that grow and reproduce outside of cultivation.

**Coexistence:** The concurrent cultivation of crops produced through diverse agricultural systems, including traditionally produced, organic, identity preserved, and genetically engineered crops.

**Transgene:** A gene or genetic material that is taken from the genome of one organism and put into the genome of another organism by genetic engineering.

## Feral alfalfa

Feral alfalfa plants have been commonly observed on roadsides, irrigation ditches, and unmanaged habitats in the western US (Figures 1a, 1b, 2, 3a, and 3b). Where do these plants come from? Alfalfa seed can readily escape from fields, combines, planters, seed bins, and sometimes from transported hay.

Alfalfa is sometimes included in seed mixes used to revegetate roadsides and rangeland (Greene et al. 2015). Volunteer alfalfa plants may persist along the edge of fields if care is not taken to remove the entire stand.

As noted earlier, alfalfa seed has a high level of dormancy, which provides year-to-year germination from soil seedbanks. In a survey of 940 roadside sites in 47 counties in California, Idaho, Pennsylvania, South Dakota, and Wisconsin, approximately 22% of the sites had feral alfalfa populations within 1.25 miles of cultivated alfalfa (Kendrick et al. 2005). St. Amand et al. (2000) confirmed pollen transmission for distances up to 820 ft between populations of feral alfalfa and suggested that feral alfalfa plants may contribute to medium- and long-distance transgene flow.



Figure 2. Feral alfalfa plants growing on a ditch bank.



Figure 1A and B. Feral alfalfa plants growing along roadsides.

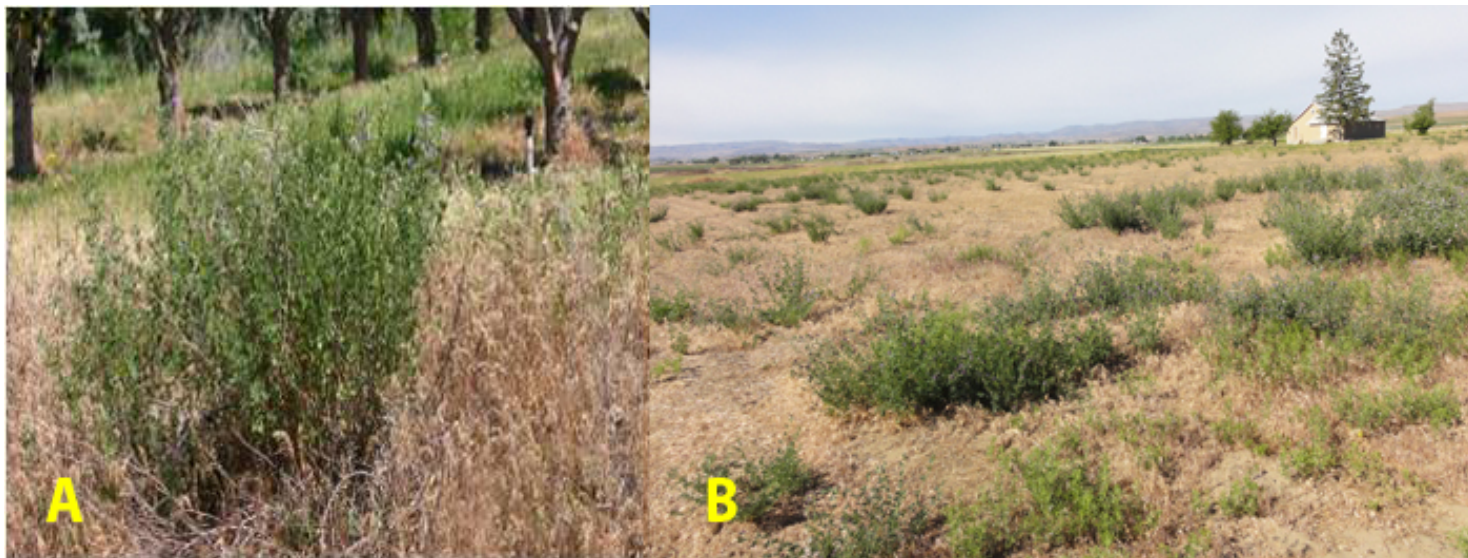


Figure 3A and B. Feral alfalfa plants growing in unmanaged areas.

A survey in southern Manitoba, Canada, concluded that feral plant occurrence was great enough to warrant management in order to effectively confine transgene movement (Bagavathiannan et al. 2010). Roadside surveys carried out in California, Idaho, and Washington in 2011 and 2012 found 4.5% of surveyed sites had feral plants, and they tended to be clustered in alfalfa seed and hay production areas (Greene et al. 2015). Forty-eight percent of these feral populations consisted of mixed age classes, suggesting that populations are self-sustaining. Also, transgenic feral alfalfa plants were found in Fresno, Canyon, and Walla Walla Counties, representing 32.7%, 21.47%, and 8.3% respectively, of feral populations surveyed (Greene et al. 2015).

## Gene flow and feral plants

According to Slatkin (1985), “gene flow is a collective term that includes all mechanisms resulting in the movement of genes from one population to another.” Management of gene flow requires recognition of the various routes of gene flow from cultivated fields. In alfalfa, there are two types of gene flow: pollen-mediated and seed-mediated.

Gene flow occurs more readily from seed production fields since fields are allowed to bloom and commercial bees are introduced, resulting in substantial pollen drift and plenty of pollinators. Seed is then harvested and transported to conditioning plants, which provides opportunities for seed escape. Hay fields are less likely to be a source of escape since growers typically cut hay at the pre- or early-bloom stage to optimize nutrition, so pollen drift is much lower. Figure 4 illustrates different means of gene flow of GE traits from cultivated GE fields into feral alfalfa.

## Pollen-mediated gene flow

Pollen-mediated gene flow occurs when pollen is transferred from one flower to another. The pollinated flower forms a viable seed that germinates into a plant. In the case of alfalfa, pollen is moved by a foraging bee. When a bee visits a GE alfalfa flower, it collects transgenic pollen. When the bee that collected the transgenic pollen visits flowers in a conventional alfalfa seed field and when pollination is successful, a transgenic seed will be produced on conventional plants.

When enough plants are pollinated with transgenic pollen, the level of AP in the harvested seed may increase. When the percentage of GE seed in the affected seed lot is too large, it may negatively impact the sale of seed to international or organic markets. If transgenic pollen is moved to blooming flowers in a conventional hay field, AP in harvested hay is not likely to increase since pollen is tiny and will not likely result in seed before hay is cut. When transgenic seed does occur in a conventional hay field, establishment of transgenic plants is not likely due to autotoxicity. If bee carrying transgenic pollen visits a feral plant, transgenic seed is likely to form and result in transgenic seedlings and plants that will be difficult to control with glyphosate.

Over time, transgenic plant numbers can increase, contributing to greater transgenic pollen drift. Growers that use glyphosate may inadvertently increase the number of transgenic feral plants by killing off non-GR plants. Even low-level AP (0.1%) of GE material in conventional alfalfa seed lots can result in GE alfalfa plants in the seeded field, which may result in hay from that field being rejected by buyers for export markets. Because of this situation, feral plants can become transgene reservoirs or bridges.

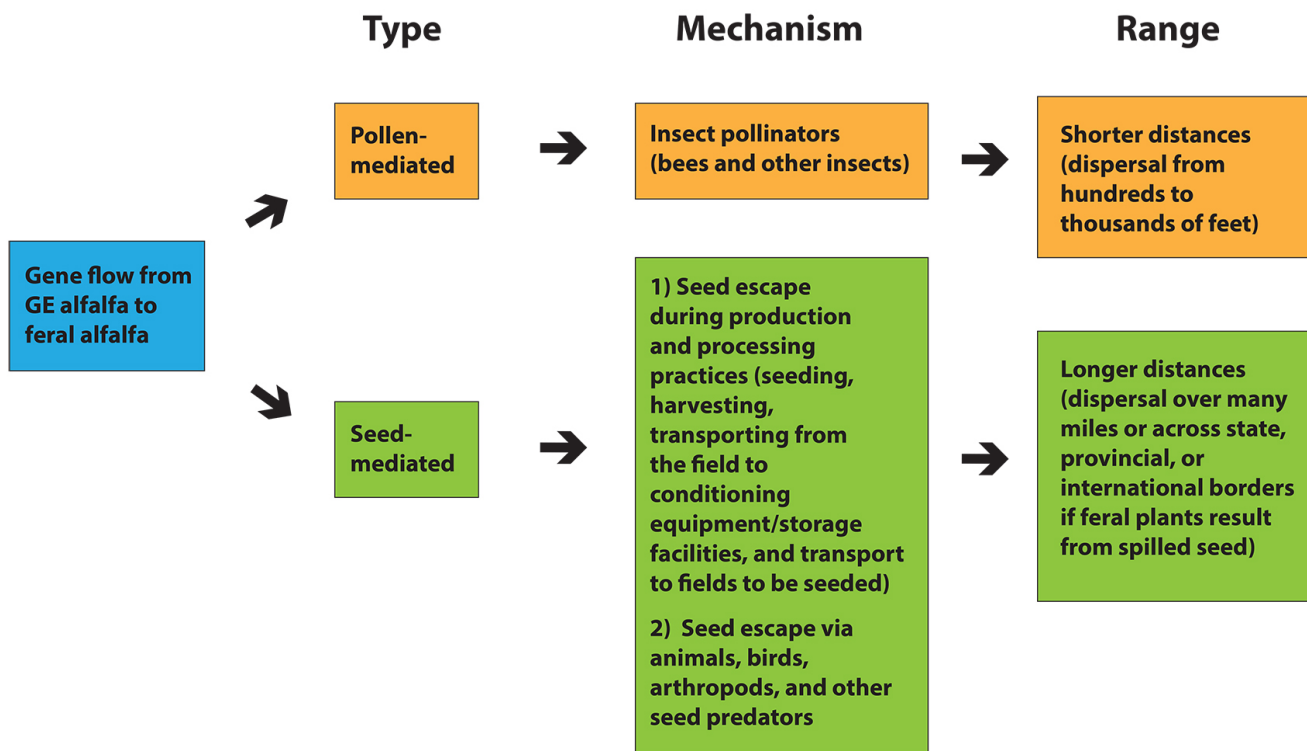


Figure 4. A generalized illustration of potential routes of transgene escape from GE alfalfa.

The amount of pollen-mediated gene flow occurring between feral alfalfa populations depends on the distance between feral plants, pollinator presence, synchrony of flowering, gene frequency, and damage to developing seeds or flowers caused by insect pests and local abiotic stress (CAST 2008; Mallory-Smith and Zapiola 2008). Spatial correlation has been observed between escaped feral plants and their feral neighbors (Greene et al. 2015). In Fresno County, neighboring feral populations influenced each other to a distance of 623 ft, while the distance was 230 ft and 269 ft in Canyon and Walla Walla Counties, respectively. This suggested that feral populations may contribute to the establishment of new populations but only up to a distance of about 650 ft.

It is important to note that feral plants in this study were distributed along roadways at distances that a pollinator bee could travel from one feral population to the next. The average nearest neighbor distance between feral plants was 0.8 miles in Canyon County and 0.5 miles in Fresno and Walla Walla Counties, which was within pollinator foraging range of 900 ft, 1 mile, and 3 miles for alfalfa leaf cutting, alkali, and honey bees, respectively (NAFA 2014).

### Seed-mediated gene flow

Gene flow initiated by movement of seed from one source to another source of reproduction by humans, animals, wind, or water is referred to as seed-mediated gene flow and often results in long-distance dispersal (Squire 2005).

It has been determined that spillage during seed production and transport is a significant factor in the occurrence of transgenic feral alfalfa populations (Greene et al. 2015). Transgenic feral alfalfa plants were consistently found at locations where the probability of seed escape was high (e.g., adjacent to original GR seed fields or along roadsides where GR seed was transported to conditioning plants).

Feral plants were found in clusters whether the location was in an alfalfa seed or hay production area, suggesting that seed spillage during hay production can contribute to transgenic feral plants (Greene et al. 2015). Dispersal of alfalfa seed by rodents and insects is possible both from alfalfa seed fields and from feral populations (Bagavathiannan et al. 2009; 2010). Remember, too, that since alfalfa seed has a high level of dormancy, seed-mediated gene flow occurs over time because only a portion of seed in the soil bank overcomes dormancy and germinates in any given year (Bagavathiannan et al. 2009).

### Stewardship practices to minimize transgene dispersal

Recognizing the need to support all facets of the market, the alfalfa industry has developed several coexistence programs to control feral alfalfa around seed production fields and to minimize pollen gene flow. Best management practices for GR seed production have been advanced by the National Alfalfa and Forage Alliance (NAFA 2015), and the Association of Official Seed Certifying Agencies has developed the Alfalfa Seed Stewardship Program (AOSCA 2010).

These programs, coupled with Grower Opportunity Zones (NAFA 2015) and alfalfa seed production systems aimed at maintaining field isolation (e.g., the field pinning program managed by the California Crop Improvement Association), provide tools to manage coexistence. Best management practices help alfalfa seed companies and growers successfully establish and maintain alfalfa production systems that preeminently serve their operations and their current and future markets (Figure 5). Links to these programs and tools are given below.

<https://www.alfalfa.org/CSCoexistenceDocs.html>

<http://www.aosca.org/>

<http://ccia.ucdavis.edu/>

- **Eliminate feral plants.** Growers can help minimize the dispersal of GE traits into the environment by eliminating feral alfalfa plants on their property, controlling and removing old alfalfa stands and volunteer plants as they appear
- **Monitor seed mixes.** In areas where AP-sensitive seed production occurs, revegetation seed mixes should not contain alfalfa or contain a different species of forage legume.

- **Maintain field edges.** Since alfalfa has a high tendency for outcrossing, growers should be watchful for potential transfer of transgenes from their GR hay or seed field to feral alfalfa plants via pollen movement. Hay field edges should be cut before flowering, and borders should be kept free of plants (Figure 6).
- **Transport securely.** Transport alfalfa seed in spill-proof containers.
- **Combine strategies.** Mechanically remove feral alfalfa by tillage or shovel. Remember that alfalfa is a deep-rooted perennial, so aggressive and repeated tillage is necessary to destroy crowns and bring roots and crowns to the soil surface where they can desiccate.

Repeated and timely mowing can prevent flowering to reduce both pollen spread and seed formation, but it will not eliminate existing feral alfalfa plants.

The most effective herbicides for control of feral alfalfa, whether GR or not, are the growth regulator (or synthetic auxin) herbicides, including aminopyralid, dicamba, clopyralid, and 2,4-D. Because herbicides are less effective on drought-stressed plants and feral alfalfa often occurs in locations where soil moisture is limited, try to make herbicide applications when plants are at least a foot tall and actively growing.

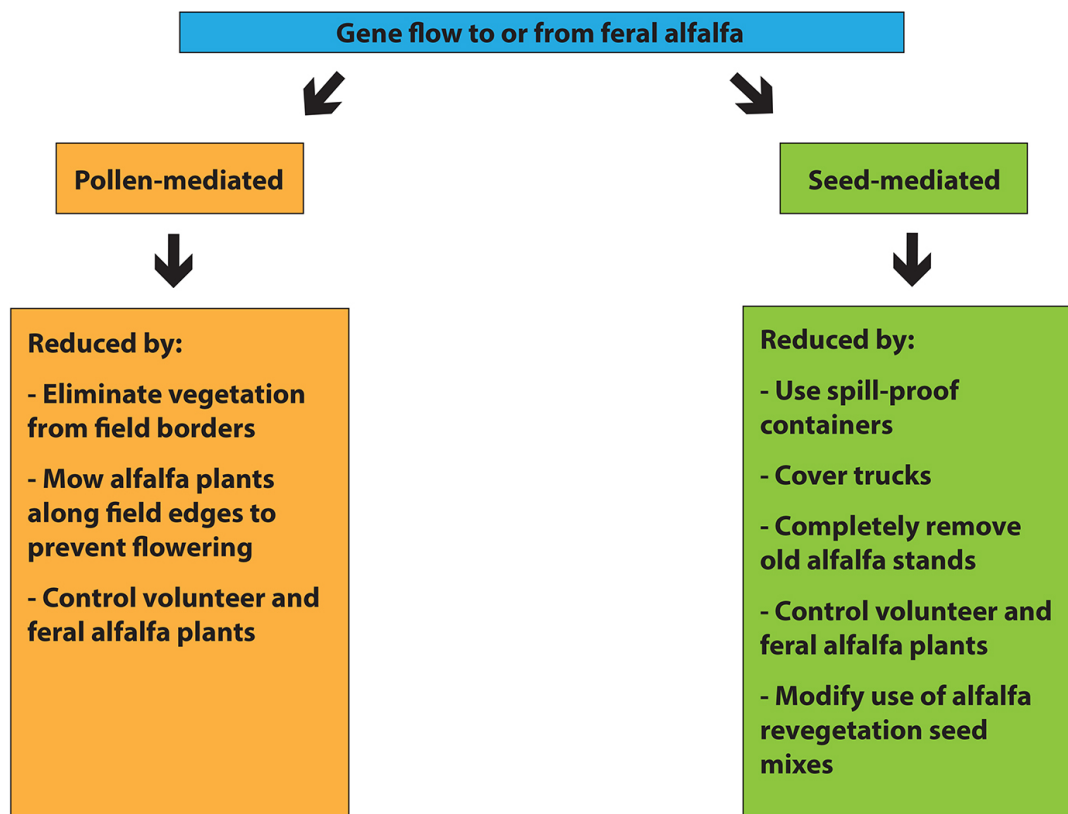


Figure 5. Factors that affect gene flow to or from feral alfalfa via pollen and seed. Adapted from Mallory-Smith and Zapiola (2008).



Figure 6. Flowering feral alfalfa plants along a hay field border.

Fall applications of systemic herbicides are generally more effective than spring applications. Also, be aware of the plant-back restrictions for any herbicide you use to control feral alfalfa on cropland, as soil residuals might persist to negatively affect rotationally grown crops.

An integrated weed management strategy is to follow herbicide application by tillage, which often provides better feral alfalfa control than either technique used alone. Another strategy would be to mow established feral alfalfa plants to remove the previous year's stems, followed by herbicide application after the new foliage is about a foot tall. This allows for more herbicide interception on fresh leaf tissues, resulting in improved control.

Application of glyphosate alone should be avoided since GR feral alfalfa plants will not be killed by the herbicide. Keep in mind, however, that glyphosate mixed with growth regulator herbicides may enhance overall control of mixed stands of weeds, especially when grasses are present.

Successful coexistence starts with conversations! Talk to your neighbors—let them know if your alfalfa is GE for hay or seed, certified organic, or bound for export. Work together to control feral alfalfa on the farm and along roadsides. Although county and state roadsides are maintained, efforts may not be sufficient to control feral plants. For example, in California, the least toxic herbicides are required by law, so glyphosate is often used to control roadside weeds. This has little impact on GR feral plants. Collaboration between Departments of Transportation, growers, county agents, and extension specialists will often be needed to facilitate the successful management of feral alfalfa populations.

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