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Effect of Synthetic Auxin Herbicides on Seed Development and Viability in Genetically Engineered Glyphosate-Resistant Alfalfa

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Feral populations of cultivated crops have the potential to function as bridges and reservoirs that contribute to the unwanted movement of novel genetically engineered (GE) traits. Recognizing that feral alfalfa has the potential to lower genetic purity in alfalfa seed production fields when it is growing in the vicinity of foraging pollinators in alfalfa seed fields, industry has established production standards to control feral plants. However, with the commercialization of GE glyphosate-resistant (GR) alfalfa and the need to support the coexistence of both GE and conventional production, effective methods to control transgenic feral alfalfa need to be developed. Therefore, a study was conducted in 2012, 2013, and 2014 to determine the effect of several synthetic auxin herbicides on seed development in GR alfalfa. GR alfalfa, var. Genuity (R44BD16), was treated with dicamba, 2,4-D, triclopyr, and aminopyralid when alfalfa plants contained green seed pods. Two weeks after herbicide application, plants were harvested, air dried, and seed yield, seed germination, and seedling emergence from the soil were determined. In 2013, dicamba, triclopyr, and 2,4-D decreased alfalfa seed yield per plant compared wih nontreated plants, whereas in 2014, all four herbicides decreased alfalfa seed yield per plant 24 to 49% (by weight) compared with nontreated plants. The same trend was evident in 2012, but seed yield was variable and was not significantly different among treatments. Seed germination averaged 43, 50, and 72% in 2012, 2013, and 2014, respectively, and was not affected by the four herbicides applied at early pod-fill stage. However, seeds harvested from plants treated with dicamba, 2,4-D, and triclopyr often produced deformed and abnormal seedlings, and when planted in soil, frequently failed to emerge. The combined effects of dicamba, 2,4-D, and triclopyr in reducing seed yield, seedling emergence, and seedling growth could contribute to managing feral alfalfa populations.

Nomenclature: Aminopyralid; 2,4-D; dicamba; triclopyr; alfalfa, *Medicago sativa* subsp. *sativa* L.
Key words: Adventitious presence, feral, gene flow, genetically engineered, glyphosate resistant, synthetic auxin herbicides.

Poblaciones ferales de cultivos tienen el potencial de funcionar como puentes y reservorios que contribuyan al movimiento indeseable de nuevas características producto de la ingeniería genética (GE). Al reconocer que la alfalfa feral tiene el potencial de reducir la pureza genética en los campos de producción de semilla de alfalfa cuando crece en la vecindad de polinizadores de campos de semilla de alfalfa, la industria ha establecido estándares de producción y de control de plantas ferales. Sin embargo, con la comercialización de alfalfa GE resistente a glyphosate (GR) y la necesidad de apoyar la coexistencia de producción GE y convencional, se necesita desarrollar métodos efectivos de control de alfalfa feral transgénica. Por esta razón, se realizó un estudio en 2012, 2013, y 2014 para determinar el efecto de varios herbicidas del grupo auxinas sintéticas sobre el desarrollo de la semilla en alfalfa GR. Alfalfa GR, var. Genuity (R44BD16) fue tratada con dicamba, 2,4-D, triclopyr, y aminopyralid cuando las plantas de alfalfa tenían vainas verdes. Dos semanas después de la aplicación del herbicida, las plantas fueron cosechadas, secadas al aire, y se determinó el rendimiento de semilla, la germinación de la semilla, y la emergencia de plántulas del suelo. En 2013, dicamba, triclopyr, y 2,4-D disminuyeron el rendimiento de semilla de alfalfa por planta en comparación con plantas sin tratamiento, mientras que en 2014, todos los cuatro herbicidas disminuyeron el rendimiento de la semilla de alfalfa por planta 24 a 49% (por peso) al compararse con plantas sin tratamiento. La misma tendencia fue evidente en 2012, pero el rendimiento de semilla fue variable y no fue significativamente diferente entre los tratamientos. La germinación de la semilla promedió 43, 50, y 72% en 2012, 2013, y 2014, respectivamente, y no fue afectada por ninguno de los cuatro herbicidas aplicados temprano durante el estadio de llenado de la vaina. Sin embargo, las semillas cosechadas a partir de plantas tratadas con dicamba, 2,4-D, y triclopyr frecuentemente produjeron plántulas deformadas y anormales, y cuando estas fueron sembradas en el suelo, frecuentemente fallaron en emerger. Los efectos combinados de dicamba,

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2,4-D, y triclopyr sobre la reducción del rendimiento de la semilla, la emergencia de las plántulas, y el crecimiento de plántulas podría contribuir a manejar poblaciones ferales de alfalfa.

Alfalfa is the fourth-most widely grown crop in the United States after corn (*Zea mays* L.), wheat (*Triticum* spp.), and soybean (*Glycine max* L. Merr.). The U.S. alfalfa export market is a multimillion dollar industry, with hay exports doubling within the last decade (NAFA 2014). It is grown for multiple purposes including animal forage in the form of hay or hay products or as a pasture component and for seed (including human consumption), as a green manure, and as a soil-stabilizing crop as permitted in specific Natural Resources Conservation Services plans. Alfalfa is a perennial, cross-pollinated plant that has the potential to form feral populations (Greene et al. 2015). Escaped alfalfa populations are commonly observed in roadsides and other unmanaged habitats in alfalfa-growing regions (Bagavathiannan et al. 2006; Fitzpatrick et al. 2003; Greene et al. 2015; Jenczewski et al. 1999a; Kendrick et al. 2005; Prosperi et al. 2006). The existence of roadside alfalfa populations has been documented in Europe (Jenczewski et al. 1999a) and in North America (Kendrick et al. 2005; Greene et al. 2015). Kendrick et al. (2005) surveyed 940 roadside sites in 47 counties in California, Idaho, Pennsylvania, South Dakota, and Wisconsin, and reported that approximately 22% of the sites had feral alfalfa populations within 2 km of cultivated alfalfa. Intentional roadside plantings also play a significant role in the occurrence of roadside feral populations (CAST 2008; Putnam 2006). Roadside surveys carried out in California, Idaho, and Washington in 2011 and 2012 found that 4.5% of surveyed sites had feral plants, and that plants tended to be clustered in alfalfa seed and hay production areas (Greene et al. 2015). Alfalfa has found its way to roadside habitats through seed escape during transport and farming activities (Greene et al. 2015), intentional planting to stabilize roadsides, and in some cases, planting by farmers for haying.

Cultivated and feral alfalfa share ploidy level, pollinators, and synchronized flowering periods in the landscape (Prosperi et al. 2006). As such, feral alfalfa populations can contribute to transgene movement by means of gene flow. According to Gressel (2005a), with the introduction of genetically engineered (GE) crops, there was a realization that feral plants could hinder the coexistence of GE and non-GE crop production, since feral plants may serve as bridges to convey GE traits into conventional fields. Van Acker (2007) expressed similar concerns. Feral crops may also act as repositories for GE traits (Ellstrand 2006; Knispel et al. 2008). As such, the persistence and spread of GE genes may be facilitated by feral crop populations in natural habitats (Wolfenbarger and Phifer 2000). In the United States, GE glyphosate-resistant (GR) alfalfa was available to farmers briefly from 2005 to 2007 (APHIS 2005a). An injunction was passed in March of 2007 barring further planting of GR alfalfa (USDC 2007a,b). In 2011, GR alfalfa received final approval for commercial production. In 2014, GE low-lignin alfalfa was deregulated and will be available commercially in the near future (APHIS 2014).

Little is known of the distances and patterns of pollen dispersal by insects from and to feral alfalfa populations. St. Amand et al. (2000) investigated glutamine synthetase gene movement among widely dispersed, individual feral plants on roadsides, and confirmed pollen movement at a distance of 230 m, with an outcrossing frequency of 92%. Similarly, a study in southern Manitoba reported levels of outcrossing between feral and cultivated alfalfa populations. Estimated levels of outcrossing varied between 62 and 85% within a maximum distance of 15 m (Bagavathiannan and Van Acker 2009). These studies suggest that feral alfalfa plants can act as bridges for GE trait movement out of cultivated fields. Greene et al. (2015) found that transgenic plants were found in 32.7, 21.4, and 8.3% of feral plant sites surveyed in Fresno County, CA, Canyon County, ID, and Walla Walla County, WA, respectively. They also reported spatial correlation between feral populations to be 190, 70, and 82 m in Fresno, Canyon, and Walla Walla counties, respectively, which suggested that feral plants may influence the spread of neighboring populations.

Of particular concern to the alfalfa seed and hay industry is the occurrence of GE feral alfalfa growing in areas where alfalfa seed and hay are produced for the export market that is sensitive to

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the adventitious presence of transgenic traits. Recognizing the potential risk gene flow has upon seed purity, the alfalfa industry proactively developed best management practices to support the coexistence of GE and conventional alfalfa production. Eradication of feral alfalfa is a major component of their coexistence strategy. The Alfalfa Seed Stewardship Program, developed to provide more stringent standards for seed lots bound for sensitive export markets, recommends removing feral alfalfa within a 274-m buffer around fields. This is a labor-intensive task, and developing cost-effective strategies to limit seed production of feral populations would be beneficial, since a key trait of feral plants is establishment and persistence along long stretches of roadside (Claessen et al. 2005a). Because flowering times can vary among feral plants growing in natural and unmanaged sites with diverse conditions, management of pollen-mediated gene flow in these unmanaged sites requires diligent efforts to continually identify and remove potential donors and receptors of pollen. This is particularly true for feral alfalfa. Flower buds begin to form on stems approximately 4 to 6 wk after field mowing during long-day photoperiods and warm weather. However, flowering is not activated by short days or cool weather (CAST 2008). Once flowering commences, alfalfa continues to flower indeterminately, with duration dependent on moisture, temperature, light, and several other factors (McGregor 1976). Alfalfa requires a pollinator to trip the flower and transfer pollen to the stigma. Although flowers wilt within 4 h after they are tripped (Vansell and Todd 1946), they can remain viable for 5 to 7 d if not pollinated (Carlson 1928; Free 1993).

Feral alfalfa populations can be managed by mowing, application of PRE herbicides, and application of POST translocated herbicides (Bagavathiannan et al. 2011; Kesoju et al. 2016). However, seedling alfalfa is difficult to detect along roadsides, especially if growing in a stand of mixed species early in the season. Feral alfalfa plants are often easier to detect once the plants are larger and have begun to flower. As a result, feral alfalfa plants that escape early-season control methods are often treated with herbicides in later developmental stages when plants are easier to locate and identify. Various herbicides are often used for roadside weed control and can be useful for killing or suppressing feral alfalfa. Several herbicides that are helpful in removing old stands of alfalfa include glyphosate and the synthetic auxin herbicides such as 2,4-D and dicamba (Buhler and Mercurio 1988; Bullied et al. 1999; Glen and Meyers 2006; Moomaw and Martin 1976; Swanton et al. 1998). Swanton et al. (1998) reported that herbicides applied in the spring control alfalfa much more than when applied in the fall because during fall application plants may have been entering a dormant phase and plant reserves are at a maximum, thereby resisting herbicide injury. Spring-applied treatments with dicamba alone followed by dicamba with glyphosate provided greatest control of alfalfa. Tank mixture of glyphosate and 2,4-D applied in spring also gave consistent control of alfalfa (Swanton et al. 1998). Conversely, Smith et al. (1992) reported that glyphosate applied in the fall gave greater control. Two other synthetic auxin herbicides, aminopyralid and triclopyr, have also been shown to be phytotoxic to alfalfa, and may be useful for managing feral populations in noncrop areas (Mikkelson and Lym 2011; Renz 2010).

Controlling the spread of transgenic roadside alfalfa plants by applying herbicides that negatively affect seed production and progeny seedling establishment would complement management practices carried out to support coexistence. This study was conducted to determine the effect of synthetic auxin herbicides dicamba, 2,4-D, triclopyr, and aminopyralid on seed development in GR alfalfa when applied to plants in the late bloom to the early seed development stage (green seed pods).

Materials and Methods

GR alfalfa, var. Genuity (R44BD16), was planted near Touchet, WA in August of 2011. Dicamba (Clarity, BASF, containing 480 g ae L^{-1} of diglycolamine salt of 3,6-dichloro-*o*-anisic acid) at 0.8 kg ae ha^{-1}, 2,4-D (Weedar 64, Nufarm, containing 456 g ae L^{-1} of 2,4-dichlorophenoxyacetic acid, dimethylamine salt) at 1.1 kg ae ha^{-1}, triclopyr (Garlon, Dow AgroSciences containing 480 g ae L^{-1} of 3,5,6-trichloro-2-pyridinyloxyacetic acid, butoxyethyl ester) at 0.8 kg ae ha^{-1}, and aminopyralid (Milestone, Dow AgroSciences, containing 240 g ae L^{-1} of tri-isopropanolammonium salt of 2-pyridine carboxylic acid, 4-amino-3,6-dihloro) at 0.09 kg ae ha^{-1} were applied July 25, 2012, July 10, 2013, and July 11, 2014, when alfalfa plants contained green seed pods and 5% or less tan-colored (mature) seed pods. In 2012, 5 to 10% of seed pods were beginning to turn tan color, whereas in 2013 and 2014, less than 2% of seed pods were turning tan. Nontreated control plots were included for comparison. Rates of each herbicide were selected on the basis of standard use rates labeled for control of perennial legumes. Treatments were arranged in a randomized complete block design, replicated six times, and plots were 1 by 1.3 m with a 0.5-m nontreated buffer between plots.

Herbicides were applied with a two-nozzle backpack sprayer equipped with 8002XR flat-fan nozzles calibrated to deliver 190 L ha^{-1}. Six nontreated control plants (nontreated early) were also harvested at the time of herbicide application to determine the amount of mature seed present at the time of herbicide application. Additionally, six nontreated control plants were harvested at the time when herbicide-treated plants were harvested. Two weeks after herbicide application, one plant from each plot (a total of six plants per treatment) was harvested, air dried at 20 C, and the seed yield determined. After harvest, 300 seeds from each plant were placed in petri dishes with moistened filter paper at 20 C for 3 wk and tested for germination. Germinated seeds were counted and removed weekly, and seedling growth abnormalities were recorded in 2013 and 2014.

The ability of seedlings to emerge and establish from planted seed was tested by planting 50 seed from each plant 5 mm deep into 15-cm-diam containers filled with potting soil and placed in the greenhouse. Greenhouse temperatures averaged 23 C and were maintained between 19 and 27 C, and day length was extended to 13 h with metal halide lamps delivering 250 μmol m^{-2} s^{-1} photosynthetic photon flux density. The numbers of emerged seedlings were recorded at 9 and 19 d after planting. The numbers of live seedlings and aboveground plant dry weight per container were recorded at 4 wk after planting (WAP).

Statistical Analysis. Data were subjected to ANOVA using the PROC GLM procedure in SAS (Statistical Analysis Systems®, version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513). Mean separation was conducted using Fisher's protected LSD at $P < 0.05$. Year-by-treatment interactions were significant for all variables except germination (%); therefore, the data were presented by year.

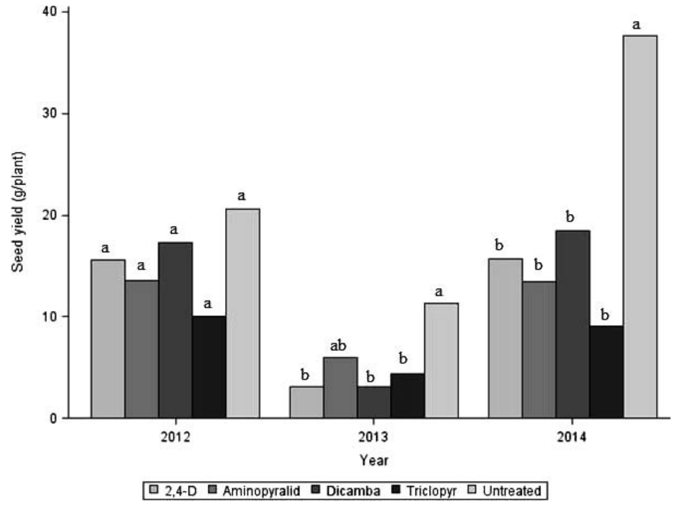

Figure 1. Alfalfa seed yield from plants treated with four herbicides applied during the green pod stage in 2012–2014 near Touchet, WA. Bars with the same letter within each year are not significantly different ($P \leq 0.05$).

Results and Discussion

Alfalfa seed yield of nontreated control plots harvested at 2 wk after herbicide applications averaged 21, 11, and 38 g of seed $plant^{-1}$ in 2012, 2013, and 2014, respectively (Figure 1). Nontreated control plants (nontreated early) harvested at the time when the herbicides were applied produced 66% of the seed produced by the nontreated control plants harvested at the same time as the herbicide-treated plants in 2012 (data not shown). A similar effect was evident in 2013 and 2014 with the early-harvested controls, averaging 41 and 17% of the seed yield of late-harvested controls. In 2013, dicamba, triclopyr, and 2,4-D, applied during early pod-fill period, decreased alfalfa seed yield per plant compared with nontreated plants, whereas in 2014, all four herbicides (dicamba, 2,4-D, triclopyr, and aminopyralid) decreased alfalfa seed yield per plant 24 to 49% (by weight) compared with the nontreated plants (Figure 1). A similar effect was evident in 2012, but seed yield variability was high and was not different among treatments (Figure 1).

In studies by Dawson (1992), alfalfa treated with sublethal rates (150 g ae ha^{-1}) of glyphosate at the

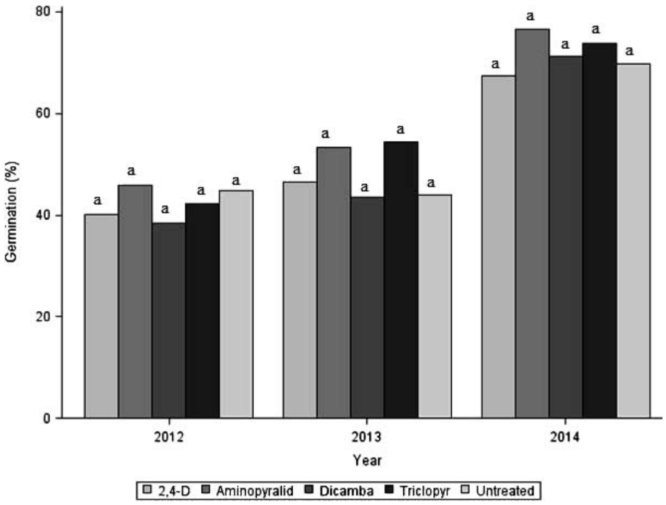

Figure 2. Percent germination of alfalfa seed collected from plants treated with four herbicides applied during the green pod stage in 2012–2014 near Touchet, WA. Bars with the same letter within each year are not significantly different ($P \leq 0.05$).

early seed-set and advanced seed-set stages reduced seed yield by 44 to 56 and 34%, respectively. Similarly, application of glyphosate at early flowering to early seed-set stage reduced up to 99% of seed production in sicklepod (*Senna obtusifolia* (L.) Irwin & Barneby), velvetleaf (*Abutilon theophrasti* Medik.), pitted morningglory (*Ipomoea lacunosa* L.), and prickly sida (*Sida spinosa* L.) (Biniak and Aldrich 1986; Clay and Griffin 2000; Thomas et al. 2005; Walker and Oliver 2008). Dicamba (0.280 kg ha^{-1}) and 2,4-D (1.06 kg ha^{-1}) applied at the first visible sign of inflorescence reduced seed production of Palmer amaranth (*Amarathus palmeri* S. Wats.) plants by 75 and 84%, respectively (Jha and Norsworthy 2012). Similar results were found in the curly dock (*Rumex crispus* L.) by Maun and Cavers (1969), who reported that 2,4-D applied before anthesis almost completely prevented seed production, and the proportion of heavy seeds increased with the age of the plant at the time of herbicide treatment. When sprayed after anthesis, 91% of the curly dock seeds contained embryos (Maun and Cavers 1969). Kelley et al. (2005) reported greater soybean yield reductions from V3 (prebloom) applications of dicamba, 2,4-D, and clopyralid compared with R2 (full bloom) applications of those herbicides.

Seed germination averaged 26, 27, and 55% from control plants that were harvested at the time of herbicide application (nontreated early), whereas later-harvested plants (nontreated late controls and herbicide-treated plants) averaged 43, 50, and 72% germination in 2012, 2013, and 2014, respectively. Seed germination was not significantly affected by treating with the four herbicides during early pod-fill stage compared with nontreated late-harvested plants (Figure 2). This is in contrast to results reported by Dawson (1992) in which alfalfa treated with glyphosate at the early seed-set and advanced seed-set stages slightly increased the percentage of seed that germinated and reduced the number of hard seeds.

Although these herbicides did not affect seed germination, seedlings grown from seed harvested from dicamba-, 2,4-D-, and triclopyr-treated plants were often deformed and appeared abnormal. Figure 3a illustrates normal seedlings (long radicle) grown from seed collected from nontreated control plants, whereas Figure 3b illustrates abnormal (short and swollen [3- to 5-mm] radicle) seedlings grown from seed collected from herbicide-treated plants. In 2012, swollen and abnormal seedlings were evident from dicamba-, 2,4-D-, and triclopyr-treated plants, but were not enumerated. Only seedlings grown from seed harvested from nontreated control plants and plants treated with aminopyralid appeared normal and were free of deformed and abnormal seedlings in all 3 yr (Figure 4). In 2013, seedling deformation averaged 16 and 11% from dicamba- and triclopyr-treated plants, respectively, whereas seedling deformity averaged 8% from 2,4-D-treated plants (Figure 4). Seedlings grown from seeds harvested from dicamba-treated plants had the greatest percent deformation (37%), followed by triclopyr (28%) and 2,4-D (15%) in 2014. Similarly, soybean seedlings from plants treated with 0.03 and 0.22 kg ha^{-1} of dicamba at the pod-filling stage germinated normally, but many of the seedlings were abnormal with pronounced swelling (3 to 5 mm) of the radicle (Thompson and Egli 1973). In 2013 and 2014, dicamba tended to cause the greatest percentage of deformed seedlings, followed by triclopyr and 2,4-D.

The number of alfalfa seedlings emerged per container at 4 WAP averaged 22, 23, and 36 for the nontreated controls in 2012, 2013, and 2014, respectively (Figure 5). All herbicide treatments reduced alfalfa seedling emergence compared with the nontreated control in 2012, with aminopyralid having the least effect (Figure 5). Seedling emer-

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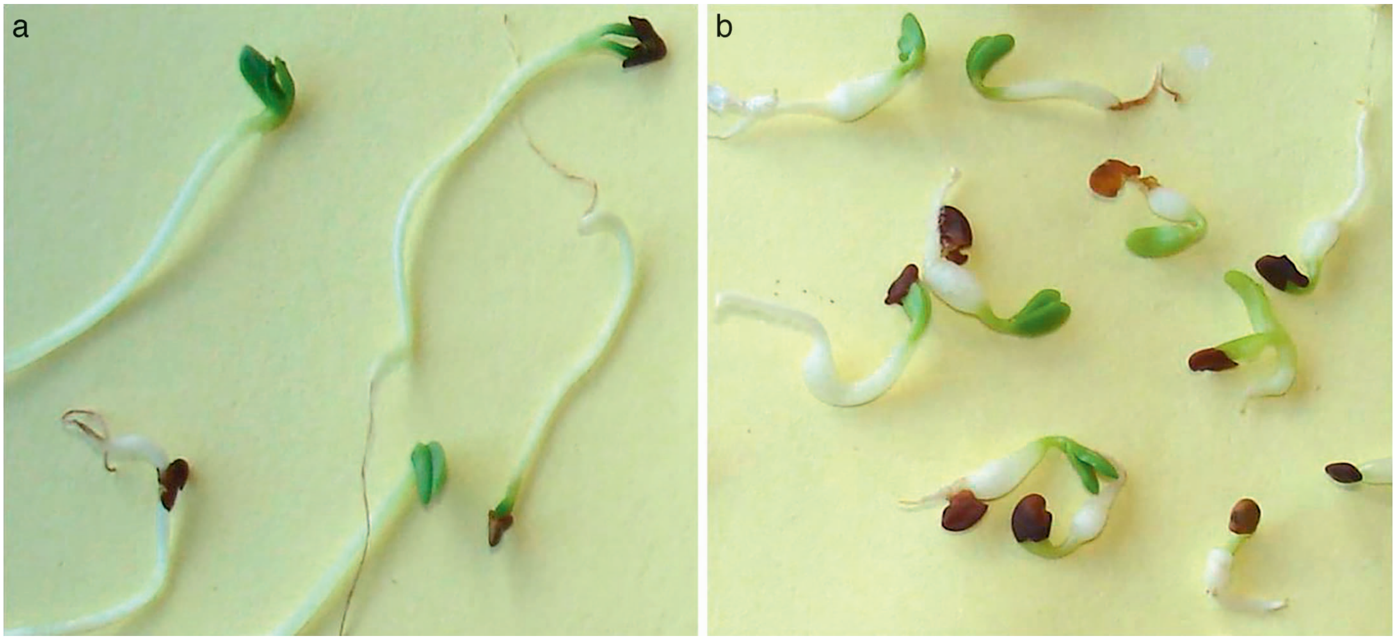

Figure 3. (a) Normal seedlings from alfalfa seed collected from untreated control plants. (b) Swollen and abnormal seedlings from alfalfa seed collected from plants treated with triclopyr applied during the green pod stage near Touchet, WA.

gence was not reduced by aminopyralid treatment compared with the nontreated control in 2013 and 2014. In 2013, only dicamba significantly reduced seedling emergence (by 50%) compared with nontreated controls, averaging only 11 seedlings per container. In 2014, seedling emergence was reduced by 75% from dicamba, 69% from triclopyr, and 45% from 2,4-D compared with nontreated controls (Figure 5). Dry weight of seedlings grown from alfalfa seed collected from plants treated with the four herbicides was significantly reduced in 2012 and 2014 compared with the nontreated controls (Figure 6). Reductions in seedling dry weight from herbicide treatments were

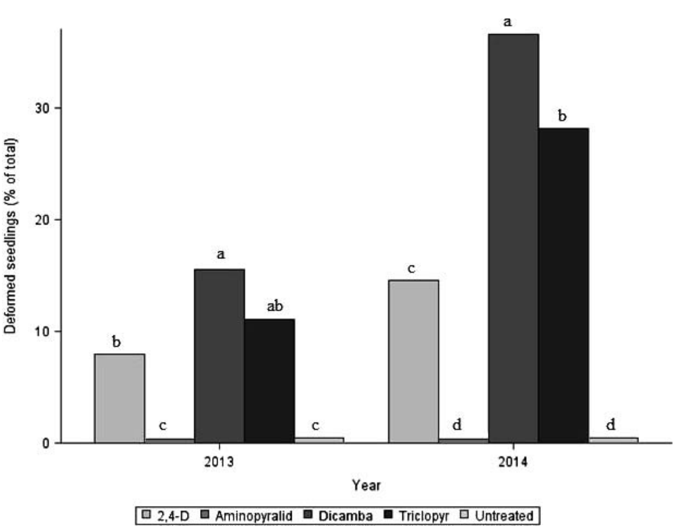

Figure 4. Percent deformed seedlings from alfalfa seed collected from plants treated with four herbicides applied during the green pod stage in 2013–2014 near Touchet, WA. Bars with the same letter within each year are not significantly different ($P \leq 0.05$).

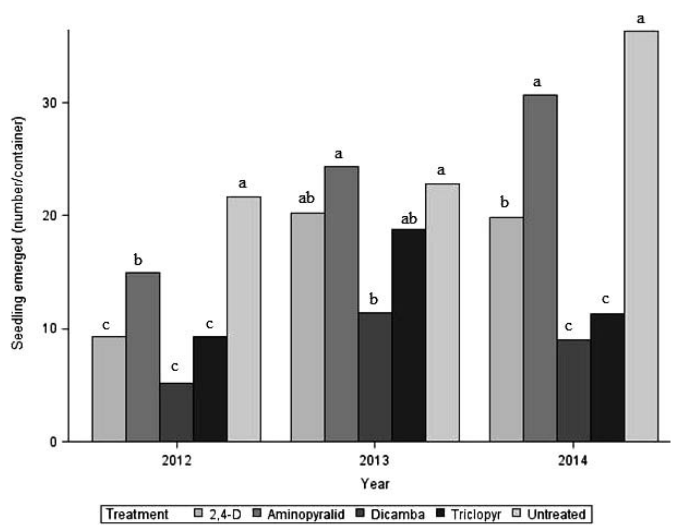

Figure 5. Seedlings emerged per container 4 wk. after planting from plants treated with four herbicides applied during the green pod stage in 2012–2014 near Touchet, WA. Fifty seed were planted per container. Bars with the same letter within each year are not significantly different ($P \leq 0.05$).

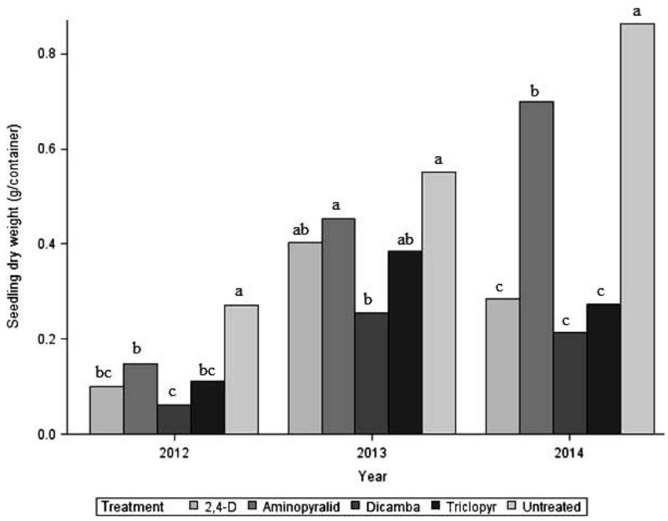

Figure 6. Seedling dry weight from alfalfa seed collected from plants treated with four herbicides applied during the green pod stage in 2012–2014 near Touchet, WA. Bars with the same letter within each year are not significantly different ($P \leq 0.05$).

consistent with the reductions in seedling emergence, with dicamba reducing the seedling dry weight to 22 to 55% of the nontreated control. In 2013, only dicamba reduced seedling dry weight (0.25 g container^{-1}) significantly compared with the nontreated control (0.55 g container^{-1}) (Figure 6). The effect of dicamba, triclopyr, and 2,4-D on causing seedling deformities appears to restrict the ability of many seedlings to emerge and produce vigorous, healthy plants.

Research Implications. Feral alfalfa in unmanaged habitats can contribute to pollen contamination and potentially lower purity in nearby alfalfa seed production fields. Transgenic feral alfalfa can be problematic in the production of adventitious presence-sensitive alfalfa seed. Therefore, controlling feral alfalfa in the vicinity of seed production fields is an important management practice to minimize movement of GE traits into either conventional or organic seed production areas (Kesoju et al. 2016). The four herbicides tested significantly reduced seed yield in 2 of 3 yr when applied during early pod development stage. Germination of seed collected from herbicide-treated plants was not reduced, but dicamba, 2,4-D, and triclopyr caused malformed seedlings and reduced the number and dry weight of seedlings that emerged from the soil. The combined effects of reduced seed yield and lower percent seedling emergence from dicamba-, 2,4-D-, and triclopyr-treated-plants could impair the ability of feral alfalfa plants to reproduce and colonize feral habitats, reducing their contribution to gene flow in the environment. These three herbicides applied to feral alfalfa in the early seed production stage could be useful components of an integrated management program for feral alfalfa. Although not the focus of this research, the detrimental effects of these four herbicides on the survival and regrowth of the treated mother plant would also contribute to the management of feral alfalfa populations. Bullied et al. (1999) reduced alfalfa biomass by 82 to 89% with spring applications of dicamba at 0.6 kg ae ha^{-1}. Bagavathiannan et al. (2011) reported good control of feral alfalfa with a tank mix of 2,4-D and dicamba applied in July. Similarly, Buhler and Mercurio (1988) controlled alfalfa better with a combination of 2,4-D and dicamba than either applied alone. In addition, the longevity of aminopyralid in soil may impede the establishment of feral alfalfa from seed. Aminopyralid reduced spring-planted alfalfa stands when applied 8 mo earlier at 60 to 240 g ha^{-1} in studies by Mikkelson and Lym (2011) and reduced spring-planted alfalfa stands when applied 7 mo earlier at 122 g ha^{-1} in Wisconsin (Renz 2010).

Ideally, a feral alfalfa management program would include both fall applications of translocated herbicides and early-season applications of herbicides or mowing to prevent flowering and new seed production on feral plants. All the herbicides tested in these studies if applied at earlier or later growth stages may contribute to the control of feral alfalfa or the establishment of alfalfa from seed, but the degree of control that is obtainable would require additional studies. Gene flow from GR feral alfalfa is affected by feral plant density and crop competitiveness of the surrounding vegetation in the feral environment, as well as by effectiveness of control methods utilized (mowing, herbicide applications, etc.). Best management practices for minimizing gene flow should include sanitizing farm equipment, mechanical removal of feral plants (where possible and feasible), utilizing separate sealed bins for conventional, organic, and GE seed, and timely application of effective herbicides such as dicamba, 2,4-D, and triclopyr.

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