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Research Article

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Abstract

The prolific seed production and polyploidy of annual bluegrass allow for the rapid development of herbicide resistance. Ethofumesate-resistant annual bluegrass plants were identified in the 1990s in grass seed production in Oregon, but their prevalence and distribution are not well documented. Therefore a dose–response experiment was initiated to determine the potential level of ethofumesate resistance in seed production systems. Seeds from 55 annual bluegrass populations were obtained from three sources: seed production fields (31 populations), the seed cleaning process (6 populations), and seed testing lots prior to retail distribution (18 populations). Additionally, two populations, one with known ethofumesate resistance and one with known susceptibility, were identified in preliminary testing and used as controls in this experiment. Seed from each collected population was increased. Individual seedlings were then transplanted into separate cone-tainers, grown to a size of 2 to 3 tillers in the greenhouse, and then sprayed using a compressed air track spray chamber with 10 doses of ethofumesate at 0, 0.56, 1.1, 2.8, 5.6, 8.4, 11.2, 16.8, 22.4, and 44.8 kg ai ha^{-1}, with 0.84 to 2.2 kg ha^{-1} as the label application rate for perennial ryegrass. The resistant to susceptible ratio of populations across all sources ranged from 0.5 to 5.5. The most resistant populations found in production fields, seed cleaning, and seed testing lots had the effective dose necessary to kill 50% of the population (ED_{50}) of 12.1, 9.4, and 13.1 kg ha^{-1}, respectively. Furthermore, 68% of the populations found in production fields had ED_{50} higher than 6 kg ha^{-1}, indicating common annual bluegrass resistance in grass seed production. As such, growers should implement integrated weed management strategies, as herbicides alone will likely be ineffective at controlling annual bluegrass.

Introduction

Ethofumesate (chemical family benzofurans) is a pre- and postemergence herbicide used in grass seed, sod production, and managed turfgrasses for the reduction of annual grasses and broadleaf weeds, most commonly annual bluegrass (Steinke and Stier 2002). Ethofumesate is also applied pre- or postplant incorporated in other crops, such as sugarbeet (*Beta vulgaris* L.) (Shaner 2014), where annual bluegrass can be one of the predominant weed species (Schittenhelm 1999).

Susceptible weeds are killed by ethofumesate through the inhibition of very-long-chain fatty-acid (VLCFA) synthesis (Weed Science Society of America [WSSA] Group 15) (Shaner 2014). Symptoms of Group 15 herbicide toxicity include stunting of growth, epinasty, swelling of the crown, darker green color, organ fusion, and wrinkled and deformed leaves (Kohler and Branham 2002; Yang et al. 2021). Ethofumesate also affects the development of leaf primordia, resulting in a swollen shoot base, senescence, and death of susceptible annual bluegrass (Jukes and Goode 1981). Translocation of ethofumesate in annual bluegrass is minimal (<3%), suggesting that ethofumesate will not likely enter the phloem (Kohler and Branham 2002).

Ethofumesate is often used for controlling annual bluegrass in perennial ryegrass because perennial ryegrass exhibits high levels of tolerance to this herbicide, withstanding rates of 5 to 10 kg ai ha^{-1} (Jukes and Goode 1981). The high level of annual bluegrass susceptibility to ethofumesate is partially due to higher herbicide absorption than other grass species (20% more than perennial ryegrass and creeping bentgrass [*Agrostis stolonifera* L.]) (Kohler and

Branham 2002). Kentucky bluegrass (*Poa pratensis* L.) and fine fescue (*Festuca* spp.) are much less tolerant to ethofumesate (Dernoeden 2000; Shearman 1986).

The maximum single labeled application rate of ethofumesate is 2.2 kg ha^{-1} when used on perennial ryegrass turf. However, the label states that one to two applications in the fall and a sequential application in spring are required for annual bluegrass control at this application rate in turfgrass systems (Anonymous 2019). In grass seed production, 1.68 kg ha^{-1} is the maximum single application rate of ethofumesate allowed either before or after weed emergence (Anonymous 2023).

Repeated applications of the same herbicide or herbicides with the same mode of action are the greatest factor in the development of herbicide resistance (Norsworthy et al. 2012). Initially, rare individual plants in a population possess genes enabling survival and reproduction despite the application of herbicides that are considered effective for that weed species. By repeated applications of herbicides with the same mode of action, all weeds in a given area are exposed to constant selection pressure, allowing the survival mostly of plants with resistant traits (Gaines et al. 2020). Additionally, cross-pollination allows for accumulating those resistance genes in a population, and resistant plants can multiply (Powles and Yu 2010). Furthermore, these plants may create large soil seedbanks that represent a source of new resistant plants (Haring and Flessner 2018).

Annual bluegrass is a weed prevalent in grass seed production fields, and ethofumesate is frequently used for control (Hebblethwaite 1980; Lee 1981; Peachey 2018). Resistance to ethofumesate was reported in 20 annual bluegrass populations from Oregon as early as 1994 (Heap 1995). These populations were also suspected to be resistant to herbicides in the triazine (WSSA Group 5) and urea (WSSA Group 7) families (Heap 1997). Mengistu et al. (2000a) documented tremendous genetic diversity in annual bluegrass populations from Oregon grass production fields. Weed populations with high genetic diversity are considered more capable of adapting to new areas and management practices. Furthermore, high genetic diversity and gene flow of a given weed species increase the risk of herbicide resistance development (Chauhan et al. 2022). While annual bluegrass primarily self-pollinates (Warwick 1979), up to 29% outcrossing occurs in annual bluegrass when grown in the high densities of grass seed production fields (Mengistu 1999; Mengistu et al. 2000b).

Currently no available literature addresses the magnitude of annual bluegrass resistance to ethofumesate in production fields. Despite initial dose–response research on ethofumesate resistance in annual bluegrass in the 1990s, data (Gamroth 1997) were lost and are no longer available. Furthermore, it is unknown if herbicide-resistant annual bluegrass plants are being harvested along with crops or if herbicide-resistant annual bluegrass plants may be present in turfgrass seed lots, which would potentially contribute to the spread of resistance throughout the United States.

The objective of this study was to determine the level of ethofumesate resistance in U.S. populations of annual bluegrass found (1) in seed production fields, (2) in seed removed during cleaning, and (3) as a contaminant in seed lots.

Materials and Methods

Seed from 55 annual bluegrass populations was tested to assess the potential for herbicide resistance development in U.S. seed production. Populations were obtained from three sources (Figure 1). The first source was annual bluegrass plants collected from seed production fields in Minnesota, Oregon, and Washington in 2019 and 2020. A total of 31 populations were collected from different production fields in the spring following fall herbicide applications. The second source included six annual bluegrass populations collected from seed cleaners (as weed seed removed from the grass seed lots after crop harvest but before seed lot testing) in 2019 or 2020. The third source of annual bluegrass was seed lots tested prior to retail distribution (i.e., either noncertified or certified seed) in 2019 or 2020. A total of 18 populations were isolated from these seed lots. Some seed lots had only 1 annual bluegrass seed, and others had up to 20 seeds found in a 1-g sample of annual ryegrass (*Lolium multiflorum* Lam.), hard fescue (*Festuca brevipila* Tracey), Kentucky bluegrass, perennial ryegrass, or tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.]. Additionally, two populations, one with known resistance (CTRLR) and one with known susceptibility (CTRLS), were identified in preliminary testing and used as controls in this experiment. To protect the identity of the farms and growers where these annual bluegrass populations were collected, no additional identifying information is provided.

Seed inventory was increased for plant populations harvested from the field and also for populations found in testing sources prior to the initiation of the experiment. To accomplish this, the seed was first germinated in 100 × 15 mm (diameter × height) petri dishes in a germination chamber (25/15 C day/night, 8-h photoperiod). Multiple seedlings from each population were then transplanted to a single 7 × 9 cm (width × height) square pot filled with potting mix (BM8, Berger, Saint-Modeste, QC, Canada) and grown in the greenhouse (at 27/21 C day/night, 14-h photoperiod). The plants were cultured in the greenhouse for several weeks, and seed was collected every 2 wk until a sufficient amount was obtained for testing. When multiple seeds were found in the same seed lot, they were treated as a single population. Seed increases from each population were pooled for testing. To reduce the potential for outcrossing, plants were grouped by population in clusters on greenhouse benches.

Next, a series of greenhouse experiments was initiated in February 2022 at the Purdue University Horticulture Plant Growth Facility (West Lafayette, IN; 40.421°N, 68.914°W) to assess potential ethofumesate resistance in these populations. Populations were tested in four separate cycles (batches) of the experiment due to greenhouse space restrictions and labor involved with harvests. All populations were tested once in one of the four cycles, with the known resistant and susceptible populations included for comparison in each cycle.

The start dates for the four cycles were February 14, April 12, May 18, and June 24, 2022. In each cycle, seeds were placed in 100 × 15 mm (diameter × height) petri dishes in a germination chamber (I-30BLL, Geneva Scientific, Fontana, WI, USA) set at 25/15 C (day/night) with an 8-h photoperiod. When seedlings reached 1.5 cm in height, a single plant was transplanted into a 2.5-cm-diameter cone-tainer (Ray Leach SC4U Pine Cell Cone-tainers, Stuewe and Sons, Tangent, OR, USA). Cone-tainers were filled with a mixture of Whitaker silt loam soil (fine-loamy, mixed, active, mesic Aeric Endoaqualf with a pH of 6.8 and organic matter content of 3.1%), germination potting mix (BM2, Berger), and medium textured sand at a 1:3:1 ratio. After transplanting, annual bluegrass plants were grown in a greenhouse with the same conditions as previously mentioned. Plants were irrigated daily with regular water and fertilized with 20-3-19 (Masterblend International–Tyler Enterprises, Morris, IL, USA) at 2,000 ppm every 2 wk until the end of the experiment. Plants were grown for 20 d in the cone-tainers, until they reached 2 to 3 tillers in size, then

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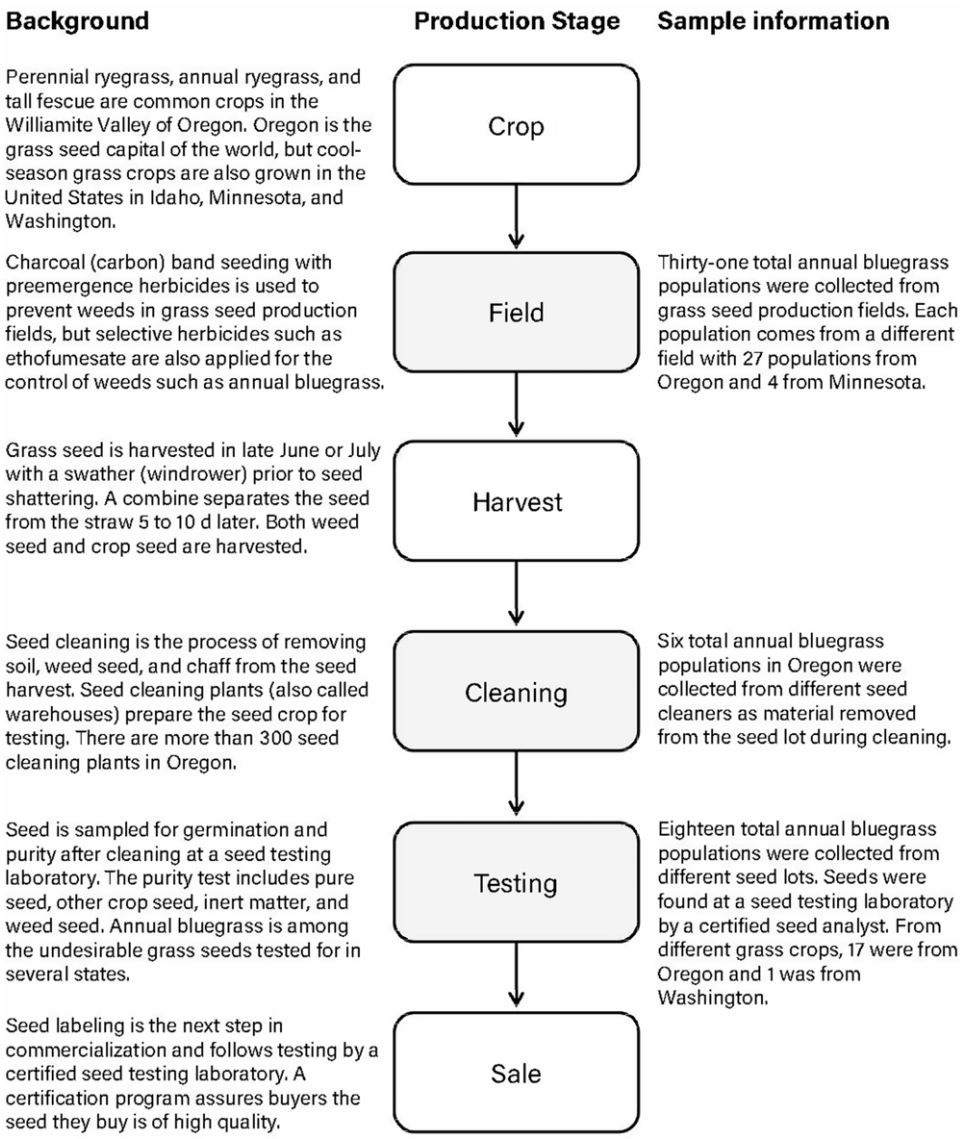

Figure 1. Seed of 55 annual bluegrass populations (50 from Oregon, 4 from Minnesota, and 1 from Washington) was obtained from three sources within the grass seed production system.

treatments were initiated. The experimental design before and after spraying was a randomized complete block within a population, with four blocks of each population.

To evaluate ethofumesate resistance in populations, each was tested for its response to 10 different doses of ethofumesate (Prograss® 1.5EC, Bayer Environmental Science, Research Triangle Park, NC, USA): 0, 0.56, 1.1, 2.8, 5.6, 8.4, 11.2, 16.8, 22.4, and 44.8 kg ha^{-1}. The rates were determined based on preliminary testing to ensure application rates that ranged from little to no injury to complete death. Herbicide applications were made using a compressed air track spray chamber (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN, USA) with an 8004 even flat-fan nozzle (TeeJet® Technologies, Wheaton, IL, USA). The nozzle was positioned approximately 43 cm above the plant canopy, and the spray chamber was calibrated to deliver 814 L ha^{-1} at 206 kPa.

Plants were returned to the greenhouse after application and not irrigated for 24 h to allow herbicide absorption. All data were collected 28 d after treatment (DAT). Herbicide control was visually estimated on a scale of 0% to 100% (where 0% was no visible injury and 100% represented complete plant death). Inflorescences present on each plant were counted. Images (1.44 megapixels at 180 dpi resolution) of individual annual bluegrass plants were collected using a digital camera (Canon PowerShot SX260 HS, Canon USA, Melville, NY, USA) connected to a lightbox (Ghali et al. 2012) with manual settings (f/3.5, 1/30-s exposure, ISO speed equal to 100). The percentage of green pixels in each image was calculated using ImageJ (v.1.48v, National Institutes of Health, Bethesda, MD 20892) (Schneider et al. 2012). An additional photo of a green calibration disk with a known area (58.7 cm^2) was taken so that green pixels could be converted to plant area in square centimeters. Green, living plant biomass was harvested at the soil surface 28 DAT immediately following image collection. The biomass of each plant was measured after 3 d of drying in a forced-air dryer at 60 C.

All data were analyzed using GraphPad Prism (Version 9.4; GraphPad Software, Boston, MA, USA). Data were fit to nonlinear regression using a sigmoidal dose–response curve. The dose

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required to reduce plant biomass was calculated using a four-parameter sigmoidal regression model:

$$y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{\left[1 + \left(\frac{ED_{50}}{x}\right)^{\text{Hillslope}}\right]}$$

where y is percent biomass relative to the nontreated plants, Bottom is the y value at the estimated bottom plateau, Top is the y value at the estimated top plateau, ED_{50} is the x value when the response is halfway between bottom and top ED_{50}, and Hillslope (no units) describes the slope of the curve at ED_{50}. The top was constrained to equal 100% in our regression analysis. The effective doses necessary to kill 50% of the population (ED_{50}) were calculated from the regression curves. A resistant to susceptible (R/S) ratio was calculated for each population using ED_{50} values with the known susceptible (CTRLS) population as the denominator. Annual bluegrass populations were screened for resistance based on Herbicide Resistance Action Committee confirmation criteria (Heap 2016).

Results and Discussion

Ethofumesate-resistant annual bluegrass plants were found in all three sources—grass seed production fields, seed cleaning, and seed testing lots prior to the retail distribution of commercial grass seed (Tables 1 and 2). Visual percent control ratings and digital image analysis of the percent green tissue were closely correlated with plant biomass ($r = -0.67$, $r = 0.93$, respectively). As such, ED_{50} values were calculated and presented using only plant biomass.

Regression analysis revealed that ED_{50} of the susceptible population (CTRLS) was 2.4 kg ha^{-1} (Table 1). In contrast, higher rates were needed to control the known resistant population (CTRLR). The ED_{50} for the CTRLR population was 12.2 kg ha^{-1}, almost 6-fold the recommended rate for use in perennial ryegrass turf (Anonymous 2019). The mean ED_{50} across all 31 populations from the grass seed production fields was 7.9 kg ha^{-1}. All tested populations from this source had ED_{50} values $\geq$ 3.6 kg ha^{-1}. Furthermore, 32% of the tested populations from the production fields had ED_{50} values up to 6 kg ha^{-1}, 45% had ED_{50} between 6 and 10 kg ha^{-1}, and 23% had ED_{50} above 10 kg ha^{-1} (Table 2). The population with the lowest ED_{50} from this source was FLD1, at 3.6 kg ha^{-1}, whereas the most resistant population within this source was FLD31, with ED_{50} of 12.1 kg ha^{-1}. The R/S for the FLD31 population was 5.1, which indicates that it was necessary to use approximately five times more herbicide to control at least 50% of the plants from this population compared to the susceptible (Table 1).

For populations from seed cleaning sources, the ED_{50} ranged from 5.5 to 9.4 kg ha^{-1}. The most resistant population was CL6, with an R/S ratio of 3.9, and the mean R/S ratio for all six populations from this source was 3.0 (Table 2). This indicates that all populations from the cleaning sources had higher ED_{50} than CTRLS, which is also above the highest recommended rate for annual bluegrass control in perennial ryegrass turf (2.2 kg ha^{-1}) (Table 1).

The mean ED_{50} across all tested populations from the seed testing sources was 6.2 kg ha^{-1}, again greater than the CTRLS population but lower than the CTRLR population. Two (TEST1 and TEST2) out of 18 tested populations from this source had ED_{50} values < 2 kg ha^{-1}, with three testing source populations within the confidence interval (CI) of the susceptible control's (CTRLS's) ED_{50} value of 2.4 kg ha^{-1} (CI = 1.1 to 3.7). The most resistant populations, TEST17 and TEST18, had an ED_{50} of 12.3 and 13.1 kg ha^{-1}, respectively. However, most of the populations from this source had an ED_{50} value between 3 and 6 kg ha^{-1}. Six populations had ED_{50} values greater than 6 kg ha^{-1} (Table 1).

Dose–response curves for the annual bluegrass populations with the highest ED_{50} values from each source are presented in Figure 2 and compared to the CTRLS and CTRLR populations. Examples of plants from these sources are presented in Figure 3. Resistance varied by population (Table 1), but the means across populations within seed source (Table 2) revealed that ethofumesate resistance in annual bluegrass is similar regardless of the seed source. The ED_{50} of 96% of the tested populations in this experiment was higher than the maximum recommended dose for annual bluegrass control in perennial ryegrass turf (2.2 kg ha^{-1}). Furthermore, the 95% CI around the ED_{50} for the known susceptible population (CTRLS) also indicates that a single application of ethofumesate to 2- to 3-tiller plants may not effectively control annual bluegrass. Previous research by Johnson (1983) reported that a single application of ethofumesate at 1.1 or 2.2 kg ha^{-1} in perennial ryegrass resulted in poor and inconsistent annual bluegrass control. However, Park et al. (2019) reported that annual bluegrass control improved with sequential applications of ethofumesate to greater than 90%, with three sequential applications of ethofumesate at 2.2 kg ha^{-1} each (Dernoeden and Turner 1988; Park et al. 2019). Thus the label recommends two sequential autumn applications followed by an optional spring treatment (Anonymous 2019). Despite the lack of sequential application in our treatment structure, our results align with the anecdotal reports of the failure to control annual bluegrass in the crop production fields sampled for this study. In addition, the ethofumesate label for seed production encourages application to plants no larger than 4 leaves (Anonymous 2023), whereas our plants were larger (2 to 3 tillers).

The ED_{50} value from the susceptible population (2.4 kg ha^{-1}) was slightly higher than the label application rate. This could also relate to the mode of action for this herbicide and the environmental conditions of the greenhouse experiment. As previously mentioned, ethofumesate inhibits elongase enzymes and prevents the formation of VLCFA, which are necessary components of epicuticular waxes (Cobb 2022). A well-developed epicuticular wax layer helps protect plant leaves from water loss and frost damage (Jenks and Ashworth 1999). Autumn applications of ethofumesate resulted in the best control of annual bluegrass (Park et al. 2019), which is partly due to the lack of protection by epicuticular wax at the young stage of development. In this experiment, plants were not exposed to cold temperature stress, which possibly allowed for better plant survival. Additionally, ethofumesate is more effective at controlling annual bluegrass when a competing grass crop is present (Shearman 1986), such as in grass seed production systems or turf systems.

Annual bluegrass plants produced inflorescences (panicles) in some cases, even when high rates of ethofumesate were applied. Plants were approximately 60 d old (from seeding to destructive harvest) when panicle counts were collected. Populations from the seed cleaning source (CL6) and from production fields (FLD31) were able to produce panicles at 11.2 and 8.4 kg ha^{-1}, respectively (data not shown). The most resistant population from the seed testing source, TEST18, did not flower before the destructive harvest in this experiment (Table 1). The percentage of populations that could produce panicles at a rate of 2.8 kg ha^{-1} or higher was

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Table 1. Effective ethofumesate doses necessary to kill 50% of 55 annual bluegrass populations from grass seed production systems, the magnitude of resistance, and the panicle production of treated plants.[a]

Population	Source	R^2	ED_{50}	CI	R/S ratio[b]	Panicle production at ≥2.8 kg ai ha^{-1}
			kg ai ha^{-1}			
CTRLS	known susceptible	0.54	2.4	1.1–3.7	1.00	yes
CTRLR	known resistant	0.33	12.2	8.1–16.2	5.10	yes
FLD1	production field	0.92	3.6	2.9–4.4	1.52	no
FLD2	production field	0.83	3.9	2.9–5.0	1.65	yes
FLD3	production field	0.90	4.1	3.2–5.1	1.73	no
FLD4	production field	0.90	4.7	3.8–5.7	1.99[c]	no
FLD5	production field	0.91	5.0	4.3–5.7	2.09[c]	yes
FLD6	production field	0.89	5.2	4.6–5.9	2.19[c]	no
FLD7	production field	0.83	5.3	4.5–6.0	2.21[c]	no
FLD8	production field	0.81	5.7	4.7–6.8	2.40[c]	yes
FLD9	production field	0.90	5.8	4.9–6.8	2.44[c]	no
FLD10	production field	0.91	5.9	5.2–6.6	2.46[c]	no
FLD11	production field	0.89	6.3	5.5–7.0	2.62[c]	yes
FLD12	production field	0.87	6.9	4.9–8.9	2.88[c]	yes
FLD13	production field	0.88	7.1	6.3–7.9	2.96[c]	no
FLD14	production field	0.90	8.1	7.1–9.2	3.40[c]	no
FLD15	production field	0.89	8.4	7.4–9.4	3.52[c]	no
FLD16	production field	0.86	8.7	7.6–9.7	3.63[c]	no
FLD17	production field	0.90	8.8	7.5–10.0	3.67[c]	no
FLD18	production field	0.84	8.8	7.3–10.4	3.69[c]	yes
FLD19	production field	0.76	9.0	7.5–10.6	3.78[c]	no
FLD20	production field	0.87	9.0	7.2–10.9	3.78[c]	yes
FLD21	production field	0.90	9.1	7.7–10.4	3.79[c]	no
FLD22	production field	0.85	9.3	7.7–10.9	3.87	no
FLD23	production field	0.85	9.7	7.9–11.5	4.06[c]	yes
FLD24	production field	0.80	9.9	8.1–11.6	4.13[c]	no
FLD25	production field	0.83	10.1	7.7–12.5	4.23[c]	no
FLD26	production field	0.85	10.3	8.7–11.9	4.32[c]	no
FLD27	production field	0.86	10.4	8.1–12.6	4.34[c]	yes
FLD28	production field	0.85	10.4	8.6–12.3	4.37[c]	yes
FLD29	production field	0.83	10.9	8.9–13.0	4.58[c]	no
FLD30	production field	0.88	11.2	9.8–12.7	4.70[c]	no
FLD31	production field	0.76	12.1	8.8–15.4	5.07[c]	yes
CL1	seed cleaning	0.88	5.5	4.3–6.8	2.31[c]	no
CL2	seed cleaning	0.87	5.7	4.9–6.5	2.38[c]	no
CL3	seed cleaning	0.89	6.2	5.0–7.4	2.60[c]	yes
CL4	seed cleaning	0.85	8.1	7.1–9.0	3.38[c]	yes
CL5	seed cleaning	0.79	8.3	7.2–9.5	3.49[c]	yes
CL6	seed cleaning	0.88	9.4	8.4–10.5	3.94[c]	yes
TEST1	seed testing	0.79	1.1	0.4–1.9	0.48	yes
TEST2	seed testing	0.85	1.3	0.8–1.8	0.54	no
TEST3	seed testing	0.90	3.6	2.9–4.4	1.51	no
TEST4	seed testing	0.82	4.8	3.0–6.5	2.00	no
TEST5	seed testing	0.94	4.8	4.3–5.3	2.01[c]	yes
TEST6	seed testing	0.90	5.0	4.4–5.6	2.09[c]	no
TEST7	seed testing	0.79	5.1	3.3–6.8	2.12	no
TEST8	seed testing	0.87	5.3	4.2–6.5	2.23[c]	no
TEST9	seed testing	0.87	5.4	4.6–6.2	2.27[c]	yes
TEST10	seed testing	0.91	5.6	4.7–6.6	2.35	no
TEST11	seed testing	0.91	5.7	4.6–6.8	2.37[c]	no
TEST12	seed testing	0.89	6.0	0.8–1.8	2.52	no
TEST13	seed testing	0.86	6.4	5.3–7.4	2.67[c]	yes
TEST14	seed testing	0.84	7.1	6.0–8.3	2.98[c]	no
TEST15	seed testing	0.77	9.4	6.0–12.8	3.94[c]	no
TEST16	seed testing	0.78	10.4	7.7–13.1	4.35[c]	no
TEST17	seed testing	0.75	12.3	9.0–15.5	5.14[c]	no
TEST18	seed testing	0.73	13.1	7.9–18.3	5.48[c]	no

[a]Abbreviations: CI, 95% confidence interval; ED_{50}, dose necessary to kill 50% of the population; R/S, resistant to susceptible ratio.
[b]R/S of the resistant population ED_{50} and the susceptible population ED_{50} that quantifies magnitude of resistance to the herbicide.
[c]Population has a higher ED_{50} than the known susceptible (CTRLS) as determined by the CI.

35%, 67%, and 22% from field production, cleaning, and testing sources, respectively (Table 2). For comparison, CTRLS flowered at ethofumesate rates up to 5.6 kg ha^{-1}, and CTRLR flowered at rates up to 44.8 kg ha^{-1} (data not shown). This confirms that populations with rapid fecundity will lead to the further selection and survival of resistant annual bluegrass biotypes (Vila-Aiub 2019).

Even though annual bluegrass is considered to predominantly self-pollinate (Ellis 1973), it was reported that populations

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Table 2. Summary of the annual bluegrass resistance to ethofumesate by the source of the weed seed removed during the grass seed production.[a,b]

Source	n[c]	ED_{50}		R/S		Panicle production at ≥2.8 kg ai ha^{-1} [h]
		Mean[d]	Max.[e]	Mean[f]	Max.[g]	
		kg ai ha^{-1}				%
Field[i]	31	7.9	12.1	3.3	5.0	35
Cleaning[j]	6	7.2	9.4	3.0	3.9	67
Testing[k]	18	6.2	13.1	2.6	5.5	22
P-value		0.1316		0.1292		0.1434

[a]Fifty populations were collected in Oregon, four in Minnesota, and one in Washington.
[b]Abbreviations: ED_{50}, dose necessary to kill 50% of the population; R/S, resistant to susceptible ratio.
[c]Number of populations screened for a given source.
[d]Mean ED_{50} across all populations for a given source.
[e]Maximum ED_{50} found across all tested populations for a given source.
[f]R/S showing the magnitude of resistance calculated by dividing ED_{50} of any given population by ED_{50} of the known susceptible population, which was 2.4 kg ai ha^{-1} in this experiment.
[g]Maximum R/S found across all tested populations for a given source.
[h]Mean frequency of inflorescence production of annual bluegrass populations treated with 2.8 kg ethofumesate ha^{-1} when counted 28 d after treatment.
[i]Populations collected in the grass seed production fields.
[j]Populations collected after cleaning harvested seed when numerous impurities, including weed seed, are removed.
[k]Annual bluegrass seed removed from samples taken from bags that are prepared for retail distribution.

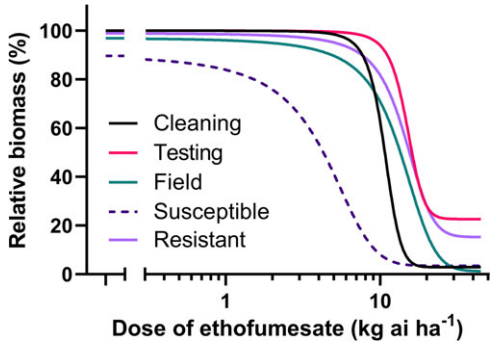

Figure 2. Dose–response curves for the most resistant annual bluegrass populations obtained from grass seed production fields, weed seed removed during cleaning relative to the desirable grass seed, and seed testing prior to retail distribution. Data are expressed as percent biomass of the nontreated control plants within each population. The susceptible and resistant control populations are shown for comparison.

originating from grass seed production fields in Oregon have an outcrossing rate of up to 29% (Mengistu 1999; Mengistu et al. 2000b). Gene flow between outcrossing resistant weeds leads to the facilitated accumulation of resistance. Additionally, such selection can lead to cross-resistance to other herbicide mechanisms of action (Vila-Aiub 2019). Cross-resistance has already been documented in annual bluegrass populations from Oregon grass seed production fields when plants resistant to diuron (mode of action: inhibition of photosynthesis at photosystem II) survived 5-fold applications of norflurazon (mode of action: inhibition of phytoene desaturase) (Hanson and Mallory-Smith 2000). Neither norflurazon nor any other active ingredient that inhibits phytoene desaturase herbicide had ever been applied to or registered for use in grass seed production at that time (Hanson and Mallory-Smith 2000). This fact and the level of resistance reported in this study indicate that the mechanism of resistance could be non–target site. Future research should examine potential cross-resistance of the ethofumesate-resistant populations found in this study as well as the mechanism of resistance in these populations.

The tendency of annual bluegrass to develop resistance toward ethofumesate is a major concern because it is one of the few available options for postemergence control in cool-season grasses (Brosnan et al. 2020). An additional concern is that the seed of ethofumesate-resistant annual bluegrass has been found in seed lots being prepared for retail distribution (i.e., seed testing lots) (Figure 1), which could contribute to the spread of resistance. Furthermore, the problem could be worse than anticipated, as we know that similar weed species, such as roughstalk bluegrass (*Poa trivialis* L.), are found in higher quantities when a larger sample of seed than required is tested (Reicher et al. 2011). The potential spread of resistance could further complicate the already challenging prospect of controlling annual bluegrass in turf systems downstream of grass seed production, such as sod farms, golf courses (Allen et al. 2022), athletic fields (Frisvold et al. 2023), and lawns (Ervin et al. 2022), if planting seed with this undesirable grass as a contaminant. One positive is that seed lots known to be contaminated by annual bluegrass are rarely purchased by those managing high-value turf sites (Christians et al. 2017), who are most likely to use ethofumesate. These high-value sites often plant certified or sod-quality seed, which undergoes a more rigorous purity test (Christians et al. 2017). Furthermore, eight states require an undesirable grass seed test, which specifically looks for annual bluegrass (USDA-AMS 2023). As such, seed lots with annual bluegrass are more likely to be used for low-quality turf areas, such as roadsides, where high mowing heights, no irrigation, no fertilization, and no ethofumesate applications will limit the survival and spread of annual bluegrass and its resistance.

The increase of ethofumesate resistance could also be problematic in areas with warm-season grasses, such as seashore paspalum (*Paspalum vaginatum* Sw.) and St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze], where annual bluegrass appears as a weed (McCullough et al. 2012). In these settings, ethofumesate is the only available option for bermudagrass control (McCullough et al. 2016), and these applications could lead to further selection of resistant annual bluegrass biotypes.

Maximizing the herbicide dose within labeled parameters (Mithila and Godar 2013) and utilizing mixtures with compatible herbicides will help delay resistance development (Das 2014). Ethofumesate could also be applied in a tank mixture with a preemergence herbicide in autumn (Lee 1981) or amicarbazone in spring (Perry 2011). For example, metribuzin and pendimethalin are preemergence herbicides that could be used in mixtures with ethofumesate in the production of cool-season grass seed in

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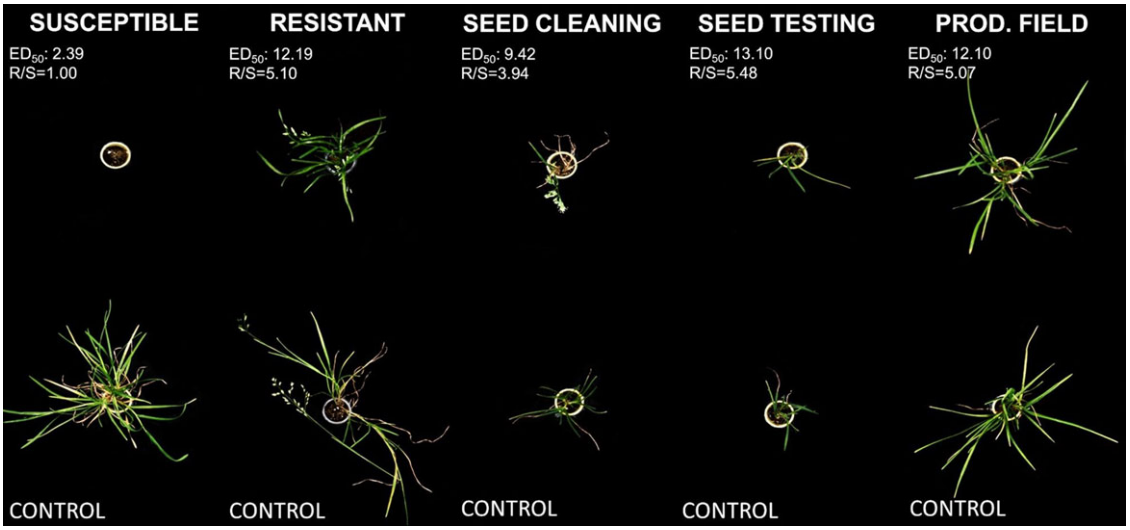

Figure 3. Annual bluegrass plants from the most resistant populations of each seed source, 28 d after application of 11.2 kg ethofumesate ha^{-1}. Treatment was applied at 2- to 3-tiller stage. ED_{50} values are expressed in kilograms ethofumesate per hectare. Data are from Table 1.

conjunction with carbon banding (Peachey 2018) to prevent establishment of seed; however, they are currently not registered for this use. Other herbicides that are labeled to provide control could be used for annual bluegrass suppression, including diuron (applied after planting but before rain or irrigation), flufenacet + metribuzin (before active growth of desirable crop), and nonselective herbicides for seedbed preparation, spot treatments, or interrow applications such as glyphosate and paraquat (Peachey 2018). Each location and cropping system should assess the potential presence of herbicide resistance to these other herbicides before incorporating them as part of a rotation or mixture with ethofumesate. For example, diuron-resistant annual bluegrass has been known to exist in seed production fields for many years (Hanson and Mallory-Smith 2000), so this is not likely to be a viable alternative on many farms.

Competition between plants, winter stress, and sequential applications of ethofumesate could lower the survival of these resistant plants. Nevertheless, integrated weed management (IWM) should be implemented by growers using both nonchemical and chemical control options (McCurdy et al. 2023) to combat these herbicide-resistant populations. Alternating modes of action for the chemical control of annual bluegrass in grass seed production present a challenge for seed producers due to the limited options available on the market. Resistance in annual bluegrass to all the active ingredients mentioned earlier, except flufenacet, has been reported in the United States (Brosnan et al. 2012; Eelen et al. 1999; Isgrigg et al. 2002; Mengistu et al. 2000b). Furthermore, current alternative programs (nonchemical) are often less effective and more expensive, which makes them impractical for growers (Norsworthy et al. 2012). Despite these higher costs, the wicked problem of annual bluegrass herbicide resistance (Allen et al. 2022) requires the utilization of techniques once thought insignificant or too labor intensive. For example, producers and contractors baling straw for export or as part of postharvest residue management (Hart et al. 2012) can reduce the spread of resistance from neighboring fields by thoroughly cleaning equipment before moving from one field to another, but also when moving from areas with high weed densities (Duary 2014). Growers can also rotate crops to clovers (*Trifolium* spp.) or other nongrass crops as means of increasing herbicide diversity and adding other mechanical control practices. Reicher et al. (2011) reported a reduction of roughstalk bluegrass as a seed contaminant in lots following a survey that raised awareness among seed producers and resulted in changed behavior.

Improvements in technology for seed cleaning may also help to reduce the spread of herbicide resistance by removing more weed seed before commercial distribution. A great deal of past effort has been made to develop efficient technology for removing weed seed from the harvested yield. Weed seed and other impurities are separated from harvested grass seed and by size, mass, length, surface texture, and shape. Using those methodologies, several machines have been constructed that work effectively in most cases (Wheeler and Hill 1957). However, grass seed cleaning technology has changed very little since the 1970s. One common method of removing more weed seed from crops is to "clean" the crop seed twice; however, this significantly increases the grower's cost of production. There may be potential to introduce newer, computer-aided optical technology to increase the future effectiveness of seed cleaning to remove weed seed (Heo et al. 2018), although distinguishing between seeds in the same genus or family is difficult (Luo et al. 2021). In the meantime, seed producers must rely on other IWM practices until seed cleaning technology allows for future improvements in weed seed detection and removal.

Practical Implications

Annual bluegrass populations from grass seed production systems with a low to medium magnitude of resistance (R/S 2 to 6) (Beckie and Tardif 2012) were discovered in all tested sources: production fields, weed seed removed during seed cleaning, and seed testing lots (seed being prepared for sale). Populations that can survive labeled application rates of ethofumesate exist in fields and produce seed that is harvested with the crop seed and can persist as a contaminant in commercial seed lots despite seed cleaning to remove weed seed. Therefore site-specific IWM strategies to prevent and manage herbicide-resistant annual bluegrass should be implemented by growers (McCurdy et al. 2023), and new nonchemical practices may need to be developed to aid in weed management. Furthermore, new technology that would allow for improved seed cleaning would help growers to remove any

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herbicide-resistant weed seed that escapes IWM in the field to prevent the retail distribution of weed seed, albeit at a higher cost to the grower and the consumer.

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