Multiple Herbicide Resistance in Wild Oat and Impacts on Physiology, Germinability, and Seed Production

Erik A. Lehnhoff,* Barbara K. Keith, William E. Dyer, Robert K. Peterson, and Fabian Menalled

ABSTRACT

The evolution of weed biotypes resistant to multiple herbicide modes of action, here termed *multiple herbicide resistance*, is a growing problem around the world. We investigated two multiple herbicide resistant (MHR) wild oat (*Avena fatua* L.) populations from Montana and hypothesized that they would exhibit fitness costs compared with two herbicide-susceptible (HS) populations. Dose-response tests showed that the MHR populations were resistant to difenzoquat (a membrane disruptor), imazamethabenz (an acetolactate synthase [ALS] inhibitor), flucarbazone (an ALS inhibitor), and tralkoxydim (an acetyl-CoA carboxylase inhibitor). In greenhouse studies, we assessed differences between MHR and HS populations in seed germination, photosynthetic parameters, plant growth, and reproduction. Seeds of one HS population germinated more at cold temperature (4.9°C) and less at high temperature (29.6°C) compared with the other populations. Plants of this HS population also had lower stomatal conductance (23%), intercellular CO_2 concentration (7.5%), and transpiration (15.3%) than the other populations, but there were no differences in photosynthetic rates between any populations. Also, there were no differences in relative growth rate among all HS and MHR populations. The MHR populations initiated seed production several days sooner than the HS populations; however, HS populations produced 67% more tillers, and one HS population ultimately produced 43% more seeds than the MHR populations, indicating a potential fitness cost of resistance. With the exception of seed production differences, our results do not indicate a consistent fitness cost. More research is needed in field settings and with resource competition to further evaluate fitness costs in MHR populations.

Herbicide resistance is a rapidly growing worldwide problem that causes significant crop yield losses, threatens our ability to successfully manage weed populations, and increases production costs (Owen, 2010; Powles and Yu, 2010; Tranel et al., 2011). Although the majority of the 388 herbicide-resistant weed biotypes documented worldwide to date are resistant to only one mode of action, approximately 30% are resistant to two or more modes of action, with 44% of these appearing since 2005 (Heap, 2012). Biotypes that are resistant to herbicides from two or more mode-of-action families as conferred by more than one physiological mechanism are termed multiple herbicide resistant (MHR) (Hall et al., 1994). This type of resistance makes weed management more challenging because changing to a different mode of action may not control MHR biotypes. We use the MHR acronym here because the wild oat populations characterized in this study are resistant to members of three different mode-of-action families, and we suspect the presence of different physiological mechanisms (Keith et al., unpublished data, 2012).

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In a review of fitness costs associated with herbicide resistance, Vila-Aiub et al. (2009) described fitness costs as arising when (i) novel, resistance-conferring mutations in herbicide target enzymes interfere with normal plant function or metabolism, (ii) altered ecological interactions occur such as herbicide-resistant plants becoming less attractive to pollinators or more susceptible to disease or herbivores, and (iii) the physiological mechanism(s) of herbicide resistance divert resources away from growth and reproduction. The last scenario, known as the *resource-based allocation theory* (Coley et al., 1985), results from individuals maximizing their ecological success under different environmental selective pressures by differentially allocating resources among growth, defense, and reproduction processes (Bazzaz et al., 1987; Herms and Mattson, 1992). The resource-based allocation theory provides a framework within which to study the evolutionary dynamics and impacts of herbicide resistance as an evolved defense mechanism that can alter the ecological fitness of resistant biotypes. The expression and magnitude of these fitness tradeoffs are mediated by the abiotic, biotic, and genetic environment where selection occurs (Vila-Aiub et al., 2009).

Many field and greenhouse studies support the phenomenon of fitness tradeoffs associated with herbicide resistance. In a greenhouse evaluation of the ecological cost of herbicide resistance due to enhanced metabolism, Vila-Aiub et al. (2005)

Abbreviations: ACCase, acetyl-CoA carboxylase; AIC, Akaike information criterion; ALS, acetolactate synthase; DAP, days after planting; HS, herbicide susceptible; MHR, multiple herbicide resistant; RGR, relative growth rate.

determined that herbicide-resistant rigid ryegrass (*Lolium rigidum* L.) individuals produced less aboveground biomass and had lower relative growth rates than susceptible biotypes. These differences, in turn, translated into weaker competitive responses to wheat (*Triticum aestivum* L.) by the resistant than the susceptible biotype (Vila-Aiub et al., 2009). Also, herbicide resistance to triazine, conferred by a point mutation in the chloroplast *psbA* gene, imposed a tradeoff of increased susceptibility to herbivorous insects in smooth pigweed (*Amaranthus hybridus* L.) (Gassmann, 2005; Gassmann and Futuyma, 2004). Park and Mallory-Smith (2005) demonstrated that a MHR downy brome (*Bromus tectorum* L.) biotype exhibiting both enhanced herbicide metabolism and the *psbA* gene mutation produced less shoot biomass, reduced leaf area, and smaller seeds than the susceptible biotype. In a review of glyphosate [*N*-(phosphonomethyl)glycine] resistance in ryegrass species conferred by both altered patterns of glyphosate translocation and target site mutations, Preston et al. (2009) reported reduced fitness in resistant biotypes, including lower seed production and a decline in population numbers with time. Sibony and Rubin (2003) showed that prostrate pigweed (*Amaranthus blitoides* S. Watson) resistant to both ALS inhibitors and triazine (photosynthetic inhibitor) herbicides produced less shoot biomass than susceptible biotypes when grown both in monocultures and in competition with the susceptible biotype; however, prostrate pigweed resistant only to triazine and redroot pigweed (*A. retroflexus* L.) resistant to ALS inhibitors did not exhibit fitness costs (Sibony and Rubin 2003).

The existence of fitness costs of herbicide resistance suggests that on the cessation of herbicide selection pressure, the proportion of resistant to susceptible individuals within a population should decrease (Holt and Thill, 1994; Maxwell and Mortimer, 1994; Park and Mallory-Smith, 2005; Tranel and Wright, 2002). Nevertheless, the relative impacts of herbicide resistance on fitness attributes can vary with the weed species, mechanism of resistance, and environmental context (Gassmann and Futuyma, 2004; Goss and Dyer, 2003; Menalled and Smith, 2007; Sibony and Rubin, 2003). Thus, understanding the fitness level consequences of evolved herbicide resistance under varying management practices has important implications for predicting the spread of resistant populations and developing effective weed management strategies.

The purpose of this research was to investigate a suspected case of MHR in wild oat (a problematic weed in cereal-growing regions throughout the world) from an irrigated barley (*Hordeum vulgare* L.) production area of Montana. Complaints by producers in 2006 led us to investigate wild oat populations that were not satisfactorily controlled by tralkoxydim (Achieve; 2-[1-(ethoxyimino)propyl]-3-hydroxy-5-(2,4,6-trimethylphenyl)-2-cyclohexen-1-one) or pinoxaden (Axial; 8-(2,6-diethyl-4-methylphenyl)-1,2,4,5-tetrahydro-7-oxo-7*H*-pyrazolo[1,2-*d*][1,4,5]oxadiazepin-9-yl 2,2-dimethylpropanoate). One specific field where complaints arose had been treated with imazamethabenz (Assert; 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-4(*or* 5)-methylbenzoic acid) from 1997 through 1999, tralkoxydim from 2000 through 2004 with 2 yr of tralkoxydim plus triallate (Fargo; *S*-(2,3,3-trichloro-2-propen-1-yl) *N,N*-bis(1-methylethyl)carbamothioate), and clodinafop-propargyl (Discover; 2-[4-[(5-chloro-3-fluoro-2-pyridinyl)oxy]-phenoxy]-2-propynyl ester) in 2005, all with reports of sporadic control. The use of pinoxaden, with its novel chemistry, in 2006 was expected to control wild oat, and the unsatisfactory control led us to suspect MHR. Our preliminary work showed that resistance to one or more, but not all, of the three modes of action described above is associated with higher constitutive and inducible expression of cytochrome P450 monooxygenase gene(s) (Keith et al., unpublished data, 2012). Therefore, we suspected that more than one resistance mechanism was involved. For the current work, our specific objectives were to determine (i) to which active ingredients these populations of wild oat were resistant, and (ii) if there were fitness tradeoffs associated with herbicide resistance.

MATERIALS AND METHODS

Population Selection and Plant Growth

The MHR populations MHR3 and MHR4 were derived from seeds collected in 2006 from two wild oat populations not controlled by 60 g a.i. ha^{-1} pinoxaden (Axial, Syngenta Crop Protection, Inc.; an acetyl-CoA carboxylase [ACCase] inhibitor) in two production fields separated by approximately 8 km in Teton County, Montana. Field-collected seeds (about 90% resistant to 60 g a.i. ha^{-1} pinoxaden, data not shown) were subjected to two generations of recurrent group selection (about 50 plants each generation) by spraying with the same dose of pinoxaden (for populations MHR3 and MHR4) or no herbicide treatment (HS1), followed by three additional generations with no herbicide selection in the greenhouse. From each generation of 50 plants, all seeds were harvested and a random selection of 50 seeds was used to initiate the next generation. Herbicide-susceptible population HS1 was derived from seeds produced by untreated plants in an adjacent field and was subsequently confirmed to be 100% susceptible to the herbicides used in these studies. A second susceptible population (biotype) HS2 used for these studies was the nondormant inbred SH430 line used in seed dormancy research (Naylor and Jana, 1976; Johnson et al., 1995). Plants were grown under a 16-h photoperiod of natural sunlight supplemented with mercury vapor lamps (165 $\mu E\ m^{-2}\ s^{-1}$) at 25 $\pm$ 4°C in a standard greenhouse soil mix (1:1:1 v/v/v Bozeman silt loam [a fine-silty, mixed, superactive Pachic Argicryoll]/Sunshine Mix no. 1 [Sun Gro Horticulture, Inc.]/perlite).

Herbicide Screening

To evaluate herbicide resistance, seeds of each population were sown 2 cm deep in the greenhouse soil mix (as described above) in 10- by 10- by 12-cm-deep pots and grown in a 35-m^2 bay of the Montana State University Plant Growth Center under light and temperature conditions as described above. Seedlings were treated with five doses of either imazamethabenz plus 0.125% nonionic surfactant (Assert, Nufarm, Inc.; an ALS inhibitor), flucarbazone (4,5-dihydro-3-methoxy-4-methyl-5-oxo-*N*-[[2-(trifluoromethoxy)phenyl]sulfonyl]-1*H*-1,2,4-triazole-1-carboxamide) plus 0.125% nonionic surfactant (Everest 2.0, Arysta LifeScience North America; an ALS inhibitor), tralkoxydim (Achieve, Dow AgroSciences Canada; an ACCase inhibitor), or difenzoquat (Avenge, AMVAC; 1,2-dimethyl-3,5-diphenyl-1*H*-pyrazolium,

a membrane disruptor) using a moving nozzle sprayer at a rate of 94 L water ha^{-1}. Imazamethabenz, flucarbazone, and tralkoxydim were applied at the wild oat two-and-a-half- to three-leaf stage, while difenzoquat was applied to four-leaf-stage plants. Herbicides were applied at 0, 0.1×, 0.3×, 1×, 3×, and 6× rates, with 1× being the manufacturer's recommended application doses for small grains, which are 348 g ha^{-1} for imazamethabenz, 200 g ha^{-1} for tralkoxydim, 340 g ha^{-1} for difenzoquat, and 30 g ha^{-1} for flucarbazone. When the spray solution had dried, the plants were returned to the greenhouse conditions described above for 6 wk, after which the aboveground biomass was harvested, dried for 1 wk at 40°C, and weighed. There were four replications of each herbicide dose, and the experiments were conducted twice.

The dose response was analyzed using the drc package in R (R version 2.12.1, The R Foundation for Statistical Computing), and three-parameter log-logistic curves were fit to the data for each herbicide and population:

$$y = \frac{d}{1+\exp\left\{b\left[\log(x)-\log(e)\right]\right\}} \quad [1]$$

where y is the biomass response (dry matter), x is the dose, d is the upper limit, b is the relative slope around e, and e is the dose causing 50% injury (ED_{50}) (Streibig, 1988). Relative population differences in ED_{50} values for each herbicide (based on a t statistic with $P \leq 0.05$) were evaluated by comparing selectivity indexes (Knezevic et al., 2007), which are the ratios of two effective doses from two different dose-response curves.

Primary Physiological Analysis

Primary photosynthetic parameters were measured with an LI-6400 portable photosynthesis system (Li-Cor Inc.) on eight plants grown from seed from different plants of each population. Responses measured included photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol CO_2 m^{-2} s^{-1}), intercellular CO_2 concentration (μmol mol^{-1}), and transpiration rate (mmol H_2O m^{-2} s^{-1}). Measurements were taken at approximately the three-leaf stage (17 d after planting [DAP]) and again when the plants were in the reproductive stage (49 DAP). Measurements were made on the uppermost fully expanded leaf of each plant. Following Peterson et al. (2005), leaves were illuminated with a light intensity of 1400 μmol photons m^{-1} s^{-1} from a light source inside the leaf chamber (10% blue light, 90% red light), an air flow rate of 500 μmol s^{-1}, and a reference CO_2 concentration of 400 μmol CO_2 mol^{-1} generated from an integral 12 g CO_2 cylinder. The light intensity was chosen to ensure saturating light conditions. Leaves were retained in the leaf chamber until conditions were stable, as indicated by the software and the display on the LI-6400.

Photosystem II photochemistry was estimated by chlorophyll *a* fluorescence measurements. Chlorophyll *a* fluorescence parameters were estimated from the same leaves used for gas exchange measurements using a modulated chlorophyll *a* fluorometer (Model OS1-FL, Opti-Sciences) and the light-adapted test (modulation intensity = 200 μmol electrons m^{-2} s^{-1}, saturation intensity = 230 μmol electrons m^{-2} s^{-1}, duration = 8 s, and detector gain = 80; default photosynthetic active radiance value = 1100 μmol electrons m^{-2} s^{-1}). Parameters measured were steady-state fluorescence, steady-state maximum fluorescence, and overall photochemical quantum yield.

The experiment was conducted twice simultaneously in different greenhouse bays. Differences in photosynthetic rates and gas exchange and chlorophyll fluorescence parameters were evaluated separately after repeated measures analysis for early and late growth using ANOVA with experimental run as a random error term and Tukey's honestly significant difference (HSD) test to compare populations. All analyses were conducted in R.

Impact of Multiple Herbicide Resistance on Wild Oat Fitness

A germination test was conducted using a thermal-gradient table consisting of six parallel aluminum plates (101.6 cm long by 15.2 cm wide by 1.0 cm thick). Temperatures were controlled by a 200-W fluid loop chiller and a 260-W fluid heater at opposite ends to create a temperature gradient. Temperatures at five locations along the length of the plates were maintained at 4.9, 12.3, 18.5, 24.0 and 29.6 (±0.1)°C. For each wild oat population, six seeds were placed in separate covered 5-cm-diameter plastic boxes lined with one sheet of germination paper (Anchor Paper Co.), wetted with distilled water, and kept moist by the addition of distilled water as needed. There were six replications of each population at each of the five temperatures. Germination was recorded at the same time daily for 14 d and germination rates were calculated as

$$\text{Germination rate} = \frac{\sum_{i=1}^{14}\left(N_i/i\right)}{\sum_{i=1}^{14}N_i} \quad [2]$$

where N is the number of seeds germinating on the ith day (Weaver and Thomas, 1986) for 14 d. The experiment was conducted twice and differences in seed germination rates were evaluated via a linear mixed-effects model with temperature as a block effect, trial as a random effect, and population as a fixed effect in R.

The effects of soil N level on the ecological fitness of MHR wild oat was determined in a greenhouse experiment using a randomized complete block design with five replications under the environmental conditions listed above. Plastic pots (17.8 cm diameter by 15.2 cm deep) were filled with a mixture of 1:1:1 (v/v/v) sphagnum moss, sand, and greenhouse soil (Sunshine Mix no. 1) and leached to reduce N levels by draining four pot volumes of water through the soil during 2 d. The pots were then fertilized with $(NH_4)_2SO_4$ to create N application rates of 5, 25, 75, or 150 kg ha^{-1}. In each pot, six seeds of one of the four populations were planted 2 cm deep, and seedlings were thinned to the single largest individual per pot at approximately 10 DAP.

Five weeks after planting, plants in one-half of the pots from each replication were randomly chosen and harvested by cutting the plant at the root crown, washing soil from the roots, drying the roots and shoots separately at 40°C for 10 d, and weighing. The remaining pots were refertilized at rates of 0, 12.5, 37.5, and 75 kg N ha^{-1}, or approximately one-half of their original rates, to

provide the plants with enough N to keep growing but maintain a gradient of N stress. At 9 wk after planting, the number of tillers and seeds per plant were counted, and plant roots and shoots were harvested, dried, and weighed as above. Relative growth rates (RGR) were calculated following Hunt (1982):

$$\mathrm{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad [3]$$

where W_2 and W_1 are the final and first harvest biomasses, respectively, and t_2 and t_1 are the final and first harvest times (in d).

Table 1. Effective dose for 50% plant injury (ED_{50}) of herbicide-resistant (MHR3 and MHR4) and herbicide-susceptible (HS1 and HS2) wild oat populations (n = 7 for each population, herbicide, and rate [0, 0.1×, 0.3×, 1×, 3×, and 6×] combination).

Wild oat population	Effective dose			
	Difenzoquat (Avenge) (1× = 340 g a.i. ha^{-1})	Imazamethabenz (Assert) (1× = 348 g a.i. ha^{-1})	Flucarbazone (Everest) (1× = 30 g a.i. ha^{-1})	Tralkoxydim (Achieve) (1× = 200 g a.i. ha^{-1})
	g a.i. ha^{-1}			
HS1	51.0 (23.8) a†	66.1 (10.4) a	9.9 (3.6) a	412 (136) a
HS2	54.4 (10.2) a	41.8 (13.9) a	6.6 (2.1) a	360 (62.0) a
MHR3	462 (136) c	1395 (174) c	179 (45.3) b	990 (144) b
MHR4	312 (74.8) b	828 (473) b	120 (28.2) c	696 (134) b

† Standard errors in parentheses; population differences in ED_{50} within herbicide groups are indicated by different letters at $P < 0.05$.

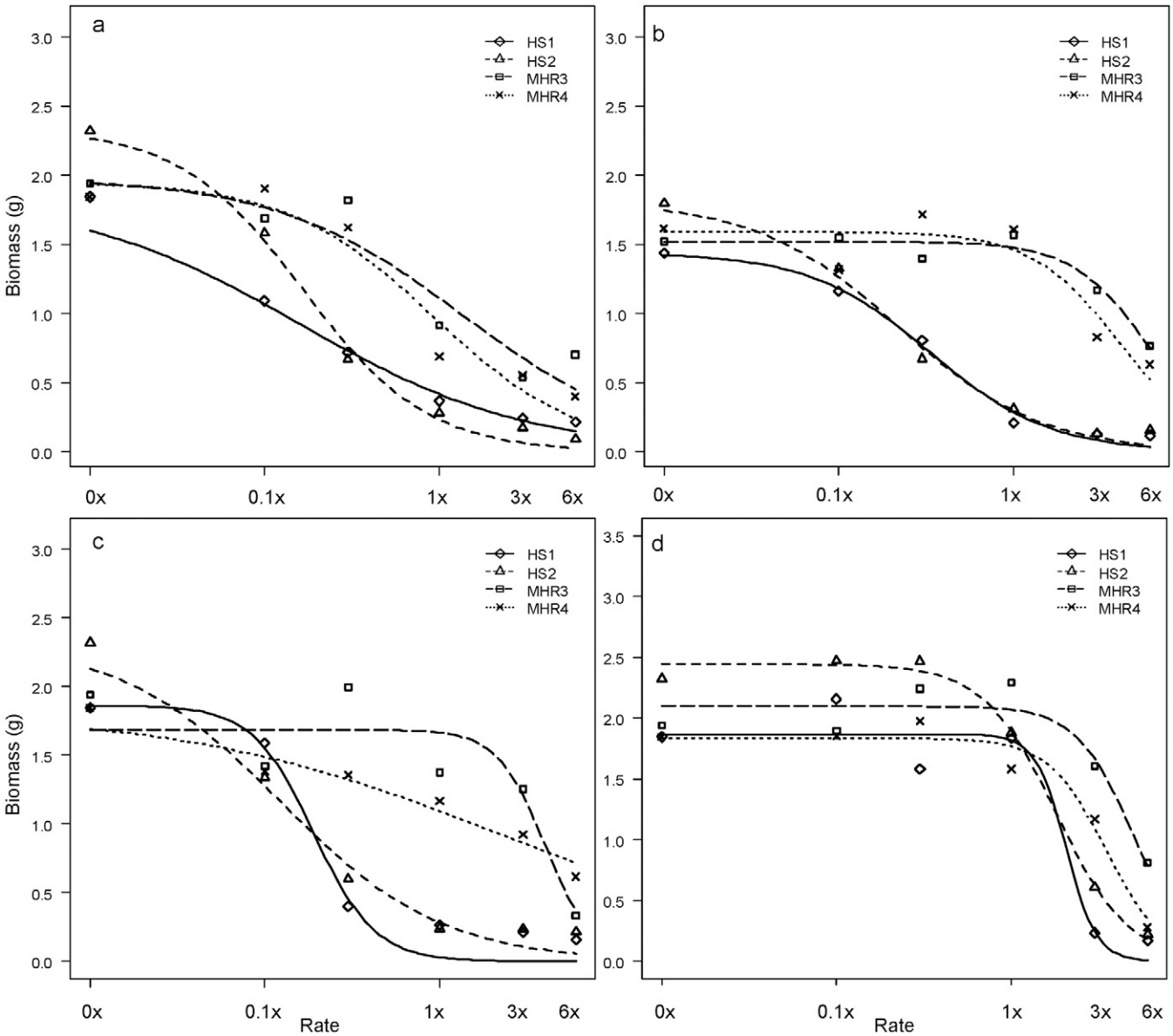

Fig. 1. Dose-response curves of herbicide-resistant (MHR3 and MHR4) and herbicide-susceptible (HS1 and HS2) wild oat populations to (a) difenzoquat (Avenge), 1× rate = 340 g a.i. ha^{-1}, (b) flucarbazone (Everest), 1× rate = 30 g a.i. ha^{-1}, (c) imazamethabenz (Assert), 1× rate = 348 g a.i. ha^{-1}, and (d) tralkoxydim (Achieve), 1× rate = 200 g a.i. ha^{-1}.

Five additional replicates of each population were grown under the same greenhouse conditions at a N rate of 75 kg ha^{-1} to evaluate total tiller and seed production. These plants were grown for 73 d and the numbers of tillers and seeds were counted when the plants senesced. This study was conducted using a completely randomized design and repeated twice; combined data were analyzed by ANOVA, with experimental replicate as a random effect.

Resource allocation (ratio of above- to belowground biomass or shoots to roots), RGR, and tiller and seed production at the time of harvest of MHR and HS populations were compared via analysis of covariance, with population as a factor and N level as a continuous variable. For RGR and resource allocation, both linear and polynomial models were fit, and the best models were selected based on the lowest Akaike information criterion (AIC) score using the package pgirmess in R.

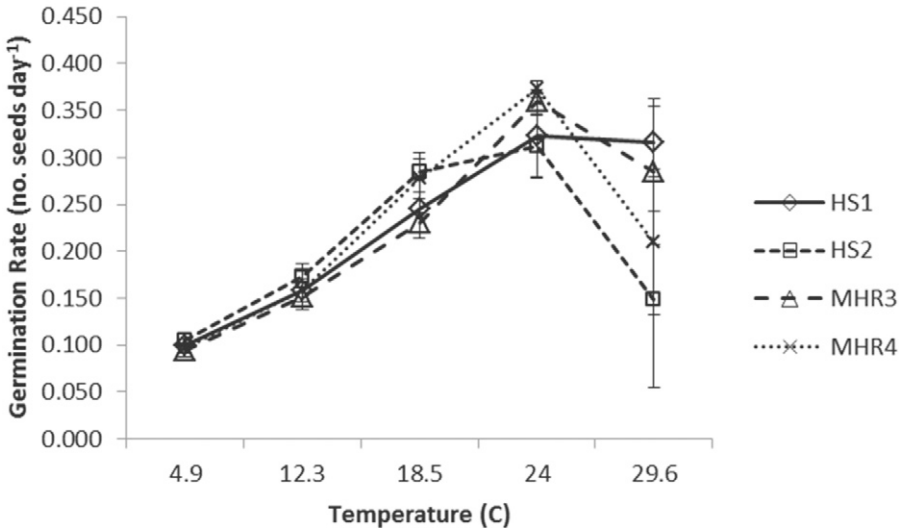

Fig. 2. Germination rates of herbicide-susceptible (HS1 and HS2) and herbicide-resistant (MHR3 and MHR4) wild oat populations across a temperature gradient. Error bars represent one standard error.

RESULTS AND DISCUSSION

Population Selection

Original MHR seed collections from producers' fields contained about 90% resistant individuals (data not shown) and so recurrent group selection (with pinoxaden treatment of MHR populations) was conducted for two generations followed by three additional generations without herbicide treatment in the greenhouse. About 50 plants in each generation were allowed to randomly self- and cross-pollinate in an attempt to maintain a reasonable level of population heterogeneity while approaching homozygosity of the R genotype. Field estimates of wild oat outcrossing by wind pollination range from 1 to 12% (Sharma and Vanden Born, 1978), and outcrossing in the greenhouse would probably be near the lower end of this range. Thus, populations HS1, MHR2, and MHR3 represent the regional genetic backgrounds of the populations selected in the field, while population HS2 represents an unrelated inbred biotype.

Herbicide Screening

Based on differences in ED_{50} values and analysis of selectivity indices, we confirmed that wild oat populations MHR3 and MHR4 were resistant to multiple herbicides (Table 1; Fig. 1a–1d). Both MHR3 and MHR4 were resistant to difenzoquat (a membrane disruptor), flucarbazone (an ALS inhibitor), imazamethabenz (an ALS inhibitor), and tralkoxydim (an ACCase inhibitor) compared with HS1 and HS2, and MHR3 and MHR4 were different from each other in their ED_{50} values (Table 1). The MHR/HS (mean of HS1 and HS2) ratios of ED_{50} values were 8.8, 25.9, 21.6, and 2.6 for MHR3 and 5.9, 15.4, 14.6, and 1.8 for MHR4 for difenzoquat, imazamethabenz, flucarbazone, and tralkoxydim, respectively.

Seed Germination

We did not detect differences in germination rates across the four wild oat populations ($P > 0.5$); however, there was an interaction between HS2 and temperature ($P < 0.01$) (Fig. 2). This interaction was seen at extreme temperatures: at 4.9°C, HS2 had the most seeds germinate, while its germination was the lowest of all populations at the highest temperature tested (29.6°C) (Fig. 3). Maximum germination rates for all populations occurred at 24°C.

Primary Physiology

In the early stages of plant growth (17 DAP), there were differences in photosynthesis, CO_2 conductance, intercellular CO_2 concentration, and transpiration. The photosynthetic rate of MHR4 was higher than that of HS1 ($F_{3,56} = 2.97$, $P = 0.04$), HS2 had lower CO_2 conductance than HS1 ($F_{3,56} = 3.19$, $P = 0.03$), the intercellular CO_2 concentration of MHR3 was higher than that of HS2 ($F_{3,56} = 3.90$, $P = 0.01$), and transpiration was higher in HS1 than in HS2 and MHR4 ($F_{3,56} = 4.78$, $P < 0.01$). By 45 DAP, some differences were still apparent, but they were not consistent with the differences observed at 17 DAP. The MHR3 population had a higher photosynthetic rate than HS2 ($F_{3,56} = 3.56$, $P = 0.02$), MHR3 had higher CO_2 conductance than HS1 and HS2 ($F_{3,56} = 6.65$, $P < 0.01$), both MHR3 and MHR4 had higher intercellular CO_2 concentrations than did HS2 ($F_{3,56} = 5.09$, $P = 0.003$), and MHR3 and MHR4 had higher transpiration than HS2, with MHR3 also being greater than HS1 ($F_{3,56} = 7.38$, $P < 0.01$). Overall, based on repeated measures analysis, there were only minor differences in primary physiology parameters among the four populations, with HS2 having lower stomatal conductance, intercellular CO_2 concentration, and transpiration rate than the other populations (Table 2). Most importantly, there were no significant differences in photosynthetic rates (Table 2) or chlorophyll fluorescence ($P > 0.05$; data not shown) among the populations. Overall, we did not detect a consistent primary physiological cost to resistance in the absence of herbicide exposure.

Relative Growth Rate, Seed Production, and Resource Allocation

The RGR was better modeled with a polynomial model than a linear model (ΔAIC = 51.2). The highest RGR for all populations occurred at an N rate of 75 kg ha^{-1}, and there were no differences among populations ($P = 0.33$) or interactions between N rate and population ($P = 0.89$) (Fig. 4a). At the time of harvest (63 DAP), the MHR populations had produced more seeds than the HS populations ($P < 0.01$) at all N levels (Fig. 4b) even though they had produced fewer tillers ($P < 0.01$) at all N levels (Fig. 4c). Biomass allocation was better modeled with a linear model than a polynomial model (ΔAIC = 0.1). Resource allocation differed among populations ($P = 0.01$), and there were interactions with N level ($P = 0.02$) (Fig. 4d). The HS1 population had a lower ratio of shoots to roots than the other populations, while HS2 had a lower ratio of shoots to roots at low N levels but a higher ratio at high N.

While MHR populations produced more seeds than HS populations at 63 DAP, decreased tillering led to a potential fitness cost, as demonstrated when plants were grown to senescence (73 DAP). Ultimately, the HS populations produced more tillers than the MHR populations (HS1 = 9.3, SE = 0.8; HS2 = 8.2, SE = 1.0; MHR3 = 4.9, SE = 1.2; MHR4 = 5.6, SE = 1.1) ($P < 0.01$). The additional tillering resulted in HS1 producing more seeds than the MHR populations (HS1 = 533, SE = 135; MHR3 = 373, SE = 78; MHR4 = 370, SE = 79) ($P < 0.01$), but seed production from MHR3 and MHR4 were not different from HS2 (461, SE = 82) ($P > 0.12$). It should be noted that the rapid seed production and senescence patterns that we observed in the greenhouse were different from what is experienced under typical field conditions, where plant maturation is much slower, and it is unknown if these patterns would be consistent in the field.

With the exception of HS1 producing more seeds than the MHR populations, our results do not indicate a consistent fitness cost and thus generally do not strongly support expectations emerging from the resource-based allocation theory. Moreover, although reduced tiller and seed production by the MHR populations represents a fitness cost, this may not be an important factor in wild oat population dynamics, as Maxwell et al. (2007) showed that wild oat populations are not limited by seedbank abundance. Furthermore, the fitness cost of lower seed production in MHR wild oat may be offset by the fact that both MHR populations initiated anthesis and completed seed production earlier than the HS populations, similar to findings reported for hare barley (*Hordeum leporinum* L.) by Purba et al. (1996). This could have implications for seed dispersal if wild oat seeds are ripe and remain on the plants to be taken into the combine during harvest (Shirtliffe et al., 2000). The earlier seed shatter could, however, serve to limit the spread of the MHR wild oat in wheat crops. Wheat is usually harvested when the spike moisture content is <20% (Shirtliffe et al., 2000), and the earlier seed maturity of MHR wild oat seed may mean that the seeds would be shed from the panicle before wheat harvest, significantly reducing spread.

There were no consistent differences in the RGR or the ratio of root to shoot resource allocation among wild oat

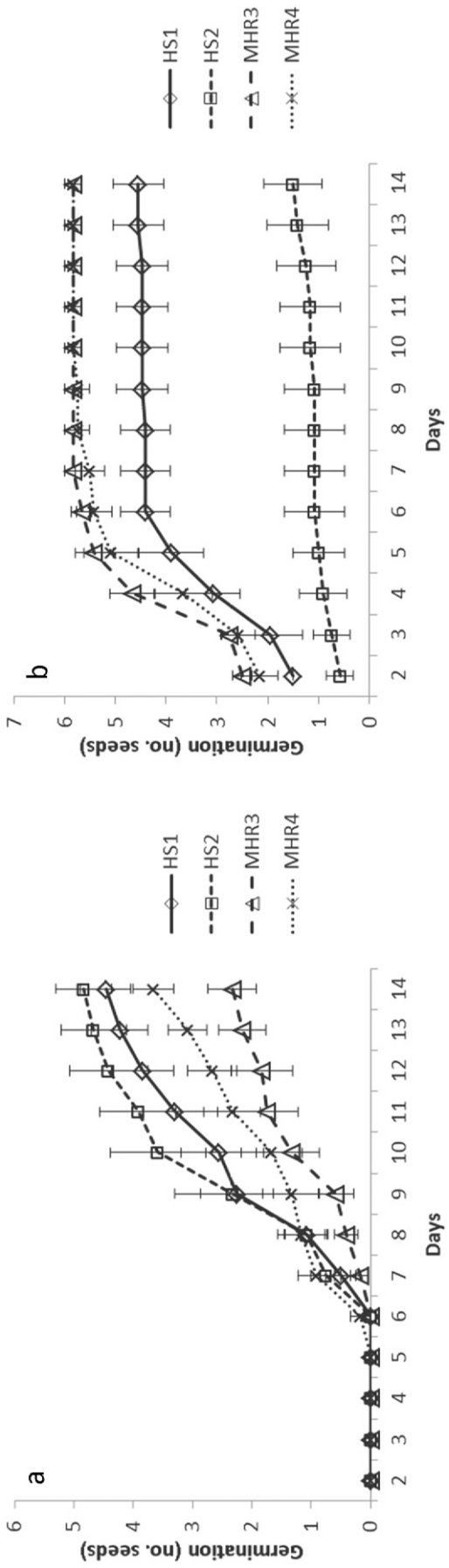

Fig. 3. Cumulative wild oat seed germination of herbicide-susceptible (HS1 and HS2) and herbicide-resistant (MHR3 and MHR4) and wild oat populations at (a) 4.9°C and (b) 29.6°C. Error bars represent one standard error.

Table 2. Primary physiology of herbicide-resistant (MHR3 and MHR4) and herbicide-susceptible (HS1 and HS2) wild oat populations.

Wild oat population	Photosynthetic rate	Stomatal conductance	Intercellular CO_2 concentration	Transpiration
	μmol CO_2 m^{-2} s^{-1}	mol CO_2 m^{-2} s^{-1}	μmol mol^{-1}	mmol H_2O m^{-2} s^{-1}
HS1	19.11 (0.99) a†	0.45 (0.06) ab	281.77 (10.28) ab	8.67 (0.76) ab
HS2	18.07 (0.87) a	0.37 (0.04) a	268.81 (12.46) a	7.67 (0.69) a
MHR3	19.84 (0.96) a	0.50 (0.03) b	299.07 (5.50) b	9.70 (0.41) b
MHR4	19.09 (0.81) a	0.46 (0.04) ab	291.28 (6.40) ab	8.79 (0.49) ab

† Standard errors in parentheses; population differences in photosynthetic rate, stomatal conductance, and transpiration are indicated by different letters at $P \leq 0.05$ based on repeated measures ANOVA with plants measured at 17 and 45 d after planting.

MHR and HS populations. Our results thus differ from those of Vila-Aiub et al. (2005), who documented a reduction in aboveground biomass in a *Lolium rigidum* biotype with a MHR phenotype. Also, our results are different from those of Stowe and Holt (1988), Reboud and Till-Bottraud (1991), and Purba et al. (1996), who showed that resistant plants exhibited slow early vegetative growth but their reproductive output was not different from susceptible plants.

Gassmann and Futuyma (2004) found that triazine-resistant and -susceptible smooth pigweed plants exhibited no significant differences in reproductive or vegetative biomass in the absence of herbivores, but the resistant biotype had reduced

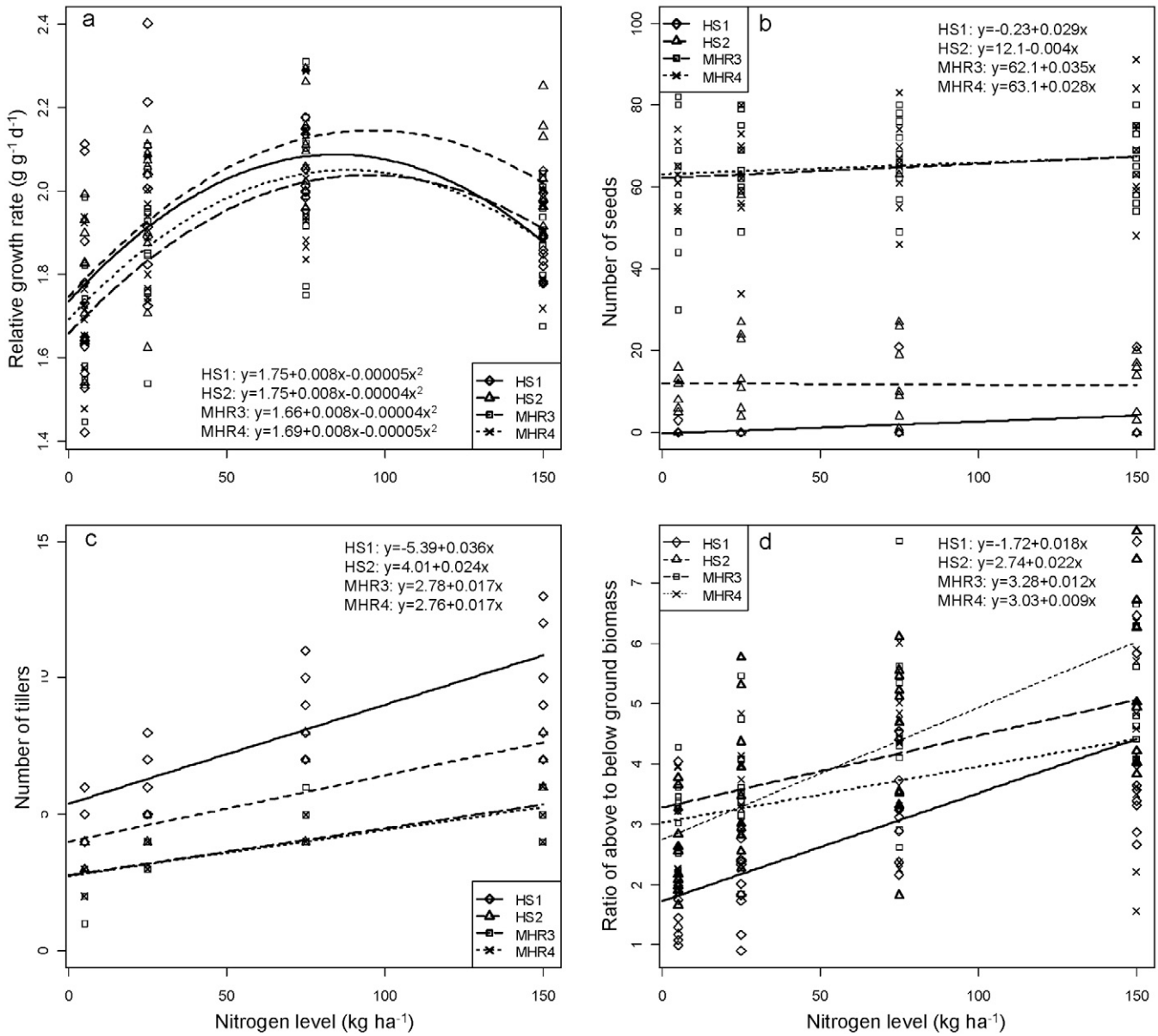

Fig. 4. Differences in (a) relative growth rate, (b) seed production, (c) number of tillers, and (d) ratio of above- to belowground biomass of herbicide-resistant (MHR3 and MHR4) and herbicide-susceptible (HS1 and HS2) wild oat populations as affected by soil N levels.

biomass when herbivores were present. While we did not have a biological stressor in our study, we evaluated the effects of N stress and found no interaction between population fitness and N level. Plants of all four populations exhibited reduced fitness under N stress, and the trends of lower fitness with reduced N were similar in both HS and MHR plants.

This study did not consider the effects of resource competition on wild oat fitness, which could be shown from experiments with HS and MHR populations grown in the presence of crop plants such as wheat or barley. The fitness cost of herbicide resistance may increase under intense resource competition (Vila-Aiub et al., 2009), and this relationship deserves further study to better understand how HS and MHR wild oat populations may persist after the cessation of herbicide use. Maxwell et al. (1990) suggested that, in the absence of herbicide use, fitness costs of resistance will cause resistant weed populations to decline while susceptible populations would increase with time, and we speculate that resource competition may further exacerbate the decline of MHR populations.

Many studies on herbicide resistance fitness costs have been difficult to interpret because they failed to control for genetic background, particularly when susceptible and resistant populations from different geographic locations were compared (Vila-Aiub et al., 2011). An alternative approach to overcome the unknown contributions of different genetic backgrounds is to create and compare isogenic lines developed through recurrent backcrossing (Bergelson and Purrington, 1996). Such comparisons can provide important insights into fitness costs associated with single-gene resistance mechanisms. Comparison of fitness parameters between isogenic lines, however, does not allow inference regarding the adaptation of resistant phenotypes in genetically diverse populations after the cessation of herbicide use.

In the current studies, our objectives were to determine the magnitude of resistance to several families of herbicides and determine if there were fitness tradeoffs in the resistant populations associated with herbicide resistance. This will allow us to estimate potential post-herbicide use changes in the population dynamics of wild oat containing a mixture of genetic backgrounds. Therefore, we purposefully maintained genetic diversity in two recently evolved resistant populations and one susceptible population that probably shared similar genetic backgrounds because they grew in nearby fields with at least 30 yr of similar cropping practices and herbicide selection pressures. Recurrent selection was conducted using the same herbicide and rate used by producers in the field to maintain a constant selection for the physiological mechanism(s) selected, as suggested by Vila-Aiub et al. (2011).

We acknowledge that this approach cannot distinguish among other genetic differences not related to herbicide resistance that may contribute to fitness. Additionally, we cannot discriminate between heterozygous and homozygous resistant individuals, thereby possibly underestimating resistance costs (Vila-Aiub et al., 2011). Dominance is a property of phenotypic characteristics and not alleles, however, and the dominance of resistance alleles for other characteristics, such as fitness in the absence of herbicide exposure, is not necessarily the same as dominance for the resistance phenotype (Roush and McKenzie, 1987). Therefore, maintaining genetic diversity within the HS and MHR populations more realistically represents populations as they exist under field conditions and allows us to better evaluate the population-level impacts of fitness costs and the persistence of such genetically diverse populations after the cessation of herbicide use. Further study on phenotypic variance within and among the populations could provide additional insight into population-level fitness costs. To specifically address fitness costs, generation of wild oat MHR and HS isogenic lines is underway in our laboratory, and these lines will be used in future work to address potential fitness costs associated with single-gene resistance mechanisms.

Fitness costs associated with one physiological mechanism of resistance may be less than those resulting from two or more mechanisms in the same plant. While we suspect that the MHR populations may contain more than one resistance mechanism, further research will be required before we can investigate this scenario.

In summary, this research did not detect consistent differences in fitness costs among MHR and HS wild oat populations in the greenhouse, although it is important to note that very similar patterns in growth and reproductive characters emerged when comparing HS and MHR populations. The field-collected HS1 mirrored the responses of genetically different HS2 (the inbred SH430 biotype used for dormancy research) in most experiments, and likewise the field-collected MHR3 and MHR4 exhibited responses similar to one another. Future work will make these same comparisons among isogenic lines, but the current studies provide an initial evaluation of populations that probably reflect field populations and, when retested in field studies, should provide relevant data needed to inform practical management strategies for producers.

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