



You cannot fight fire with fire: a demographic model suggests alternative approaches to manage multiple herbicide-resistant *Avena fatua*

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Summary

Multiple herbicide-resistant (MHR) weed populations pose significant agronomic and economic threats and demand the development and implementation of ecologically based tactics for sustainable management. We investigated the influence of nitrogen fertiliser rate (56, 112, 168, or 224 kg N ha⁻¹) and spring wheat seeding density (67.3 kg ha⁻¹ or 101 kg ha⁻¹) on the demography of one herbicide susceptible and two MHR *Avena fatua* populations under two cropping systems (continuous cropping and crop-fallow rotation). To represent a wide range of environmental conditions, data were obtained in field conditions over 3 years (2013–2015). A stochastic density-dependent population dynamics model was constructed using the demographic data to project *A. fatua* populations. Elasticity analysis was used to identify demographic processes with negative impacts on population growth. In both cropping systems, MHR seedbank densities were negatively impacted by increasing nitrogen

fertilisation rate and wheat density. Overall, MHR seedbank densities were larger in the wheat-fallow compared with the continuous wheat cropping system and seedbank densities stabilised near zero in the high nitrogen and high spring wheat seeding rate treatment. In both cropping systems, density-dependent seed production was the most influential parameter impacting population growth rate. This study demonstrated that while the short-term impact of weed management tactics can be investigated by field experiments, evaluation of long-term consequences requires the use of population dynamics models. Demographic models, such as the one constructed here, will aid in selecting ecologically based weed management tactics, such as appropriate resource availability and modification to crop competitive ability to reduce the impact of MHR.

Keywords: agroecology, ecologically based weed management, elasticity analysis, nitrogen, population modelling, resistance, *Triticum aestivum*, wild oat.

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Introduction

Weedy plants, as the main biotic cause of crop yield loss, have played a central role in agriculture since its beginning about ten thousand years ago (Delye *et al.*, 2013). In the early 1950s, synthetic herbicides revolutionised agriculture and have been at the foundation of both weed science research and the intensification and expansion of industrialised agriculture (Liebman *et al.*, 2001). However, their extensive usage worldwide imposes strong selection for resistant weed populations, threatening their continued efficacy (Menalled *et al.*, 2016). To date, approximately 250 species have evolved resistance to 23 of the 26 known herbicide modes of action, representing over 160 different herbicides (Heap, 2017). Furthermore, 37% of these species are resistant to two or more modes of action, a phenomenon known as multiple herbicide resistance (MHR) and considered a ‘wicked problem’ because of the biological, technological and social complexities involved in its management (Shaw, 2016). As a result of MHR, there are increasingly fewer herbicides to kill important weeds.

Avena fatua L. (wild oat), an annual, autogamous, allohexaploid monocotyledon, is an important weed worldwide. Across the Northern Great Plains and Pacific Northwest of North America, *A. fatua* infests over 11 million ha, causing annual crop losses of over \$1 billion (Beckie *et al.*, 2012). Due to extensive use of herbicides that inhibit acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS), and shoot growth, several populations of resistant *A. fatua* have evolved worldwide (Heap, 2017). We recently characterised two MHR *A. fatua* populations in Montana, USA, that are resistant to members of all selective herbicide families available for *A. fatua* control in cereal crops, representing five modes of action. Glasshouse experiments have demonstrated that fitness costs are limited in these MHR populations (Lehnhoff *et al.*, 2013b). We further showed that MHR plants did not possess known target site mutations, suggesting the involvement of non-target site mechanisms (Keith *et al.*, 2015).

Multiple herbicide-resistant populations pose significant agronomic and economic threats to agriculture and demand the development and implementation of alternative, ecologically based tactics for sustainable management. One such widely successful tactic involves maximising the competitive ability of the crop through increasing planting densities, planting competitive crop cultivars, fertiliser management and decreasing crop row spacing (Liebman *et al.*, 2001). For example, planting competitive barley (*Hordeum vulgare* L.) varieties at high seeding rates can reduce *A. fatua*

seed production by 60% (O’Donovan *et al.*, 2007). Nitrogen (N) is the main form of fertiliser added to increase crop yield, but the effects of added N on crop-weed interactions are often species-specific, site-specific, year-specific and field history-specific (Blackshaw & Brandt, 2008).

The short-term impact of ecologically based weed management tactics can be investigated by field experiments, but evaluations of their long-term consequences require the use of population dynamics models that allow for the range of outcomes based on variability in demographic data (Holst *et al.*, 2007). Previous studies have used population dynamics models to understand the impact of various management strategies on weed population growth (Davis *et al.*, 2004) and the evolution and spread of herbicide resistance (Maxwell *et al.*, 1990). Furthermore, elasticity analysis of the models can be used to identify demographic processes with the largest negative impact on population growth and thus can be the focus of management efforts (Caswell, 2001). The objective of this study was to construct a population dynamics model incorporating inter- and intra-population variability to provide a realistic basis for simulating response to agroecosystem management factors that could differentially influence population dynamics. Ultimately, knowledge gained from these models can lead to the design of ecologically based weed management programmes that reduce MHR weed population growth.

Materials and Methods

Experimental site

The experiment consisted of three trials conducted during the 2013–2015 growing seasons at the Montana State University Arthur H. Post Research Farm near Bozeman, Montana, USA. The soil at the site is an Amsterdam-Quagle silt loam, pH 7.8 (NRCS-USDA, 2008). Long-term average annual precipitation (1966–2015) at the site was 410 mm and total precipitation in 2013, 2014 and 2015 was 341, 408 and 361 mm respectively (Western Regional Climate Center, 2016). Temperature and precipitation data are presented in Fig. 1.

Population selection

The MHR populations used in this study, MHR3 and MHR4, were derived from seeds collected in 2006 from two *A. fatua* populations not controlled by 60 g a.i. ha^{-1} pinoxaden (Axial XL, 50.3 g a.i. L^{-1} , EC, Syngenta) in two barley production fields separated by 8 km in Teton County, Montana, USA. Field-collected

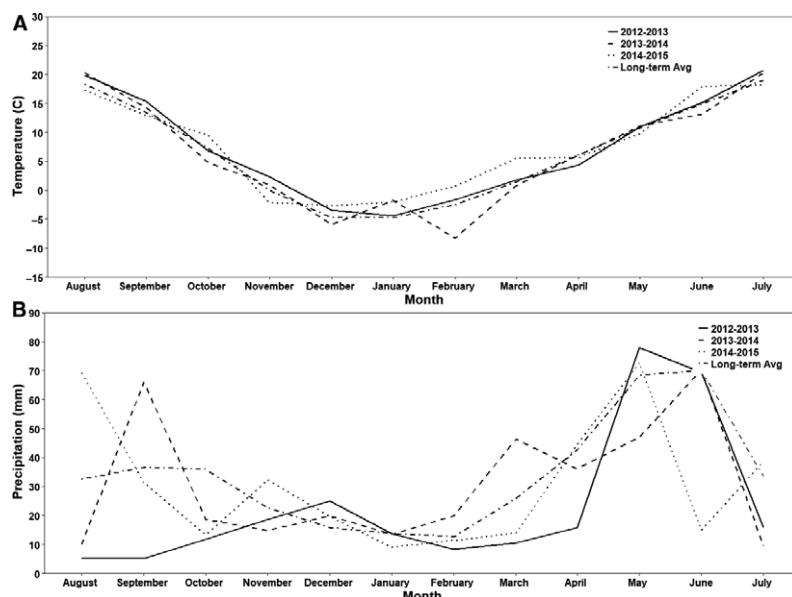


Fig. 1 Mean monthly air temperature (A) and total monthly precipitation (B) in 2012–2015 and long-term averages (1966–2016) at the research site near Bozeman, Montana, USA.

MHR3–4 seeds (90% resistant to 60 g a.i. ha⁻¹ pinoxaden, data not shown) were subjected to two generations of recurrent group selection (50 plants each generation) by spraying with the same dose of pinoxaden, after which 100% of plants were confirmed to be homozygous resistant to pinoxaden via dose-response experiments (Lehnhoff *et al.*, 2013b; Keith *et al.*, 2015). From each generation of 50 plants, all seeds were harvested and a random selection of 50 seeds was used to initiate five additional generations without herbicide selection to homogenise the genetic background. These two populations were used to successfully characterise the biochemical mechanisms responsible for MHR (Burns *et al.*, 2017; Keith *et al.*, 2017), as well as the physiological and ecological consequences of MHR (Lehnhoff *et al.*, 2013a,b). The susceptible HS1 population was derived from seeds of untreated plants bordering an adjacent barley production field, grown for seven generations as described for MHR3–4 except without herbicide selection, and was confirmed to be 100% susceptible to field rates of the herbicide used in this study (Lehnhoff *et al.*, 2013b).

To increase seed stocks, populations were grown at the Arthur H. Post Research Farm in 2012. Seeds were planted into bare ground covered with black landscape fabric in the spring. *Avena fatua* is mostly self-pollinating with outcrossing estimated at 0.05% when grown in crop (Murray *et al.*, 2002). To minimise cross-pollination, populations were separated by 5 m buffers planted with maize (*Zea mays* L.). *Avena fatua* plants were watered regularly and seeds harvested in the fall and stored at 10°C until use. This approach was taken

not only to increase seed stocks, but also removed maternal collection site differences from seed physiological status, thus isolating the effect of genotype in this study (Galloway, 2001).

Experimental design and data collection

Each year, the study followed a split-plot randomised block design with four replications. Whole plots (23 × 23 m) were assigned hard red spring wheat (*Triticum aestivum* L.) var. Duclair seeded at a density of 67.3 kg ha⁻¹ or 101 kg ha⁻¹. Sub-plots (3 × 3 m) were combinations of one of four targeted nitrogen (N) levels (56, 112, 168 or 224 kg N ha⁻¹) and one of three *A. fatua* treatments (HS1, MHR3 or MHR4). Additionally, fallow plots planted with *A. fatua* only were assigned to two sub-plots per replication.

Prior to the initiation of each year's study, the *A. fatua* germinable seedbank abundance was assessed by sampling soil at three random locations per plot using handheld bulb planters (10 cm deep, 6.5 cm in diameter). Exhumed soil was placed in trays (30.5 × 21.5 cm), watered regularly, and *A. fatua* emergence monitored for 30 days in the glasshouse. Each year, *A. fatua* germinable seedbank populations were found to be negligible (average <1 seed m⁻², data not shown). To estimate N availability prior to planting, soil cores (three per plot) were taken each fall to a depth of 61 cm, and N was analysed by AgVise laboratories (Northwood, North Dakota). Soil nitrate N ranged between 2 and 19.5 mg L⁻¹ (data not shown), and these values were taken into account when

calculating target N levels. In October of each year, *A. fatua* seeds were planted 3 cm deep in the centre (1.5×1.5 m) of each sub-plot at a targeted density of $1000 \text{ seeds m}^{-2}$ to represent a realistic *A. fatua* seedbank density (Beckie *et al.*, 2012). The following May of each year, wheat was planted 2.5 cm deep utilising a no-till drill seeder with 25.4 cm row spacing. Starter fertiliser (N-P-K at 6-19-28 kg ha $^{-1}$) was applied in the furrow at seeding. Nitrogen treatments in the form of urea were broadcasted by hand at the sub-plot level following wheat planting. After wheat and *A. fatua* emergence in the spring, the entire study, excluding the fallow plots, was sprayed with flucarbazone (30 g a.i. ha $^{-1}$; [Everest, 70%WDG, WDG, Arysta LifeScience North America LLC, Cary, North Carolina, USA]) at the *A. fatua* three-leaf stage to represent common producer practices. Bromoxynil plus MCPA (689 g a.i. ha $^{-1}$; Bronate Advanced, 299.6 g a.i. L $^{-1}$, L, Bayer CropScience, Monheim am Rhein, Germany) was applied at the same time to control broad-leaved weeds.

To assess overwinter changes in seed germinability (hereafter termed overwinter survival), three nylon mesh bags per block containing either 100 HS1, MHR3 or MHR4 seeds with percentage viability known and 98 cm 3 of field soil were buried each fall 3 cm deep in random locations within blocks. Each spring, bags were exhumed and *A. fatua* seeds were placed on moistened filter paper lined Petri dishes (100 × 15 mm) and held at 21°C for 30 days. Germinated seeds were removed and counted daily.

Every year and prior to herbicide application, *A. fatua* emergence was estimated in each sub-plot via nine randomly placed 100 cm 2 metal rings. After herbicide application, *A. fatua* seedling survivors were counted within rings. In the fall of each study year, *A. fatua* reproductive tiller and seed counts were recorded. In the spring of each

year, wheat emergence estimates were made by counting the number of plants per 40 cm in five random locations within each split-plot. In the fall of each year, wheat yield was measured by harvesting all individuals within the centre (1.5×1.5 m) of each sub-plot. Plants were dried for 1 week at 40°C, threshed using a Vogel thresher, and resulting seeds weighed (Table S4).

Parameterisation of the population dynamics model

A stochastic density-dependent population dynamics model was constructed using a series of equations to project *A. fatua* populations forward 20 years under the previously described treatment combinations. The model parameters included seedling emergence, seedling establishment, seedling survival, asymptotic density-dependent reproductive tiller number, asymptotic density-dependent seeds produced per plant, seed predation, year-long seedbank survival, overwinter seedbank survival and over summer seedbank survival (Table 1; equations in Table 1 build sequentially to form the annual projection equation presented in the last line of the table. Detailed R code is provided in the Code S1 and S2). Year-long seedling survival was estimated using data from an 11 year (1993–2004) *A. fatua* seedbank dynamics experiment conducted at the same location (Maxwell *et al.*, 2007b). Seed predation was quantified using data from an experiment conducted at the same location from 2004 to 2006 (Harbuck, 2007). Estimates of year-long seedbank survival and seed predation were drawn from distributions constructed with mean and standard error values reported in Maxwell *et al.* (2007b) and Harbuck (2007) for each simulation year. All other parameters were fitted using data obtained from this experiment. Non-linear least-squares estimates of the asymptotic density-dependent parameters for

Table 1 Abbreviations and estimators for *Avena fatua* parameters used in population dynamics model*

Parameter	Abbreviation	Equation
Emergence	estab	Number of seedlings/1000
Seedling density	sdl	seedbank density*estab
Reproductive tiller density	rprt	(z*sdl)/(1 + z*sdl/q) †
Seeds produced per plant	spp	sppmax*(1 - (i*rprt)/(1 + i*rprt/asym)) ‡
Seeds produced m 2	sp	rprt*spp
Seeds in seedbank in previous fall	seeds_fall1	(seedbank/owss) $^{\$}$
Seeds remaining in seedbank current fall	seeds_fall2	(seeds_fall1*yss)-sdl $^{\ \}$
Over summer seed survival rate	osss	seeds_fall2/seeds_fall1
Seeds in next spring's seedbank	seed	((seedbank-sdl)*osss) + (sp*(1 - pred)))**

*Equations in the table build sequentially to form the annual projection equation presented in the last line of the table. Detailed R code is provided in the Code S1 and S2.

\dagger z, q asymptotic density-dependent shape parameters.

\ddagger sppmax maximum carrying capacity; i, asym asymptotic density-dependent shape parameters.

$\$$ owss overwinter seedbank survival rate.

$\|$ yss year-long seedbank survival rate (Maxwell *et al.*, 2007b).

**pred seed predation rate (Harbuck, 2007).

reproductive tillers (z , q) and seeds produced per plant (i , $asym$) were fitted using the `nls` function in R (R, 2016).

The population dynamics model was constructed under two scenarios: continuous wheat cropping and a wheat-chemical fallow rotation. Within each scenario, populations were projected forward for each one of the eight seeding density and N rate combinations with a starting *A. fatua* seedbank population of 1000 seeds m⁻². From this initial population, the model randomly chose vital rates from a sub-plot and year within that seeding density and N rate combination and then projected the population forward 1 year. The second year started with year one projected seedbank population and the model once again randomly chose vital rates from a sub-plot and year and then projected the population forward 1 year. The utilisation of independent plots for each year of the simulation served to maximise potential variation and relieved the time series analysis constraint associated with sequential population dynamics. All parameters (except year-long seedling survival and seed predation outlined above) for a given simulation year were from the randomly chosen sub-plot to maintain the covariances among vital rates (Van Tienderen, 1995). This process was repeated over 20 years which constitutes one simulation. The population projection was modelled for 500 simulations for each population and treatment combination. Under the continuous wheat cropping scenario, randomly selected sub-plots planted with *A. fatua* and wheat were used in each year of the model. Under the wheat-chemical fallow cropping rotation, the model randomly selected a sub-plot planted with wheat and *A. fatua* for the first year of the projection, followed by a randomly selected *A. fatua* only sub-plot (i.e. fallow plot). This pattern was repeated over 20 years. In the fallow years, a simulated glyphosate application was performed and assumed to have an efficacy rate of $90 \pm 5\%$ (Adkins *et al.*, 1998).

Elasticity of population growth rate to model parameters

Elasticity analysis was used to quantify the proportional change in the *A. fatua* seedbank resulting from a proportional change in vital rates (Caswell, 2001). Elasticities were calculated as:

$$E_{ij} = \frac{a_{ij}}{\text{SB}} \frac{\delta \text{SB}}{\delta a_{ij}} \quad (1)$$

where E_{ij} is the elasticity of the seedbank (SB) to a proportional change in the parameter a_{ij} (Caswell, 2001) where i and j represent rows (years) and columns (treatments). Elasticity analysis was performed using the population dynamics model described above except

mean demographic vital rates from each year of data collect were used and modelled for 500 simulations.

dN/dt analysis

To assess the overall impacts of wheat seeding density and N fertilisation on the dN/dt (the change in the number of individuals [dN] per change in time [dt]) for each *A. fatua* populations, a linear mixed effect model was constructed with fixed effects for the three-way interaction between *A. fatua* population, N rate, wheat seeding density and random intercept effect for cropping system. The model was fitted using the `lmer` function from the `lme4` package in R (R, 2016). Differences in means were further investigated using Tukey's HSD post hoc tests in the `lsmeans` package in R (R, 2016).

Results

Demographic parameters and results are presented in Appendix S1.

Continuous wheat cropping system

Overall, HS1 seedbanks declined within 3–5 years, regardless of N fertilisation rate and wheat seeding density treatments (Fig. 2). The MHR3 population indicated a consistent advantage over MHR4 and HS1 based on simulated seedbank densities and the advantage was amplified with increased N fertilisation rate and crop seeding density.

After 20 years of low wheat seeding density and 56 kg ha⁻¹ N rate scenario, MHR seedbank densities were larger than the initial 1000 seeds m⁻² (Fig. 2A). Under the same N fertilisation treatment, but high wheat seeding density, MHR3 populations increased slightly over time; however, MHR4 populations declined steadily to 77 ± 3 (mean \pm one standard error) seeds m⁻² by year 20 (Fig. 2B).

Under the second lowest N fertilisation treatment, 112 kg ha⁻¹ N and low wheat seeding density, MHR3 and MHR4 simulated seedbank densities were lower than at the 56 kg ha⁻¹ N rate with 1048 ± 36 and 47 ± 1 seeds m⁻² respectively (Fig. 2C). Increasing wheat seeding density combined with the 112 kg ha⁻¹ N resulted in a decrease in the MHR3 seedbank density that stabilised at 868 ± 14 seeds m⁻² at the end of the simulation (Fig. 2D). Increasing the wheat seeding density at 112 kg ha⁻¹ N had little additional impact on MHR4.

Increasing the N fertilisation rate to 168 kg ha⁻¹ N under the low wheat seeding density resulted in a steady decline of the MHR3 population, stabilising at 92 ± 5 seeds m⁻² (Fig. 2E). The MHR4 seedbank

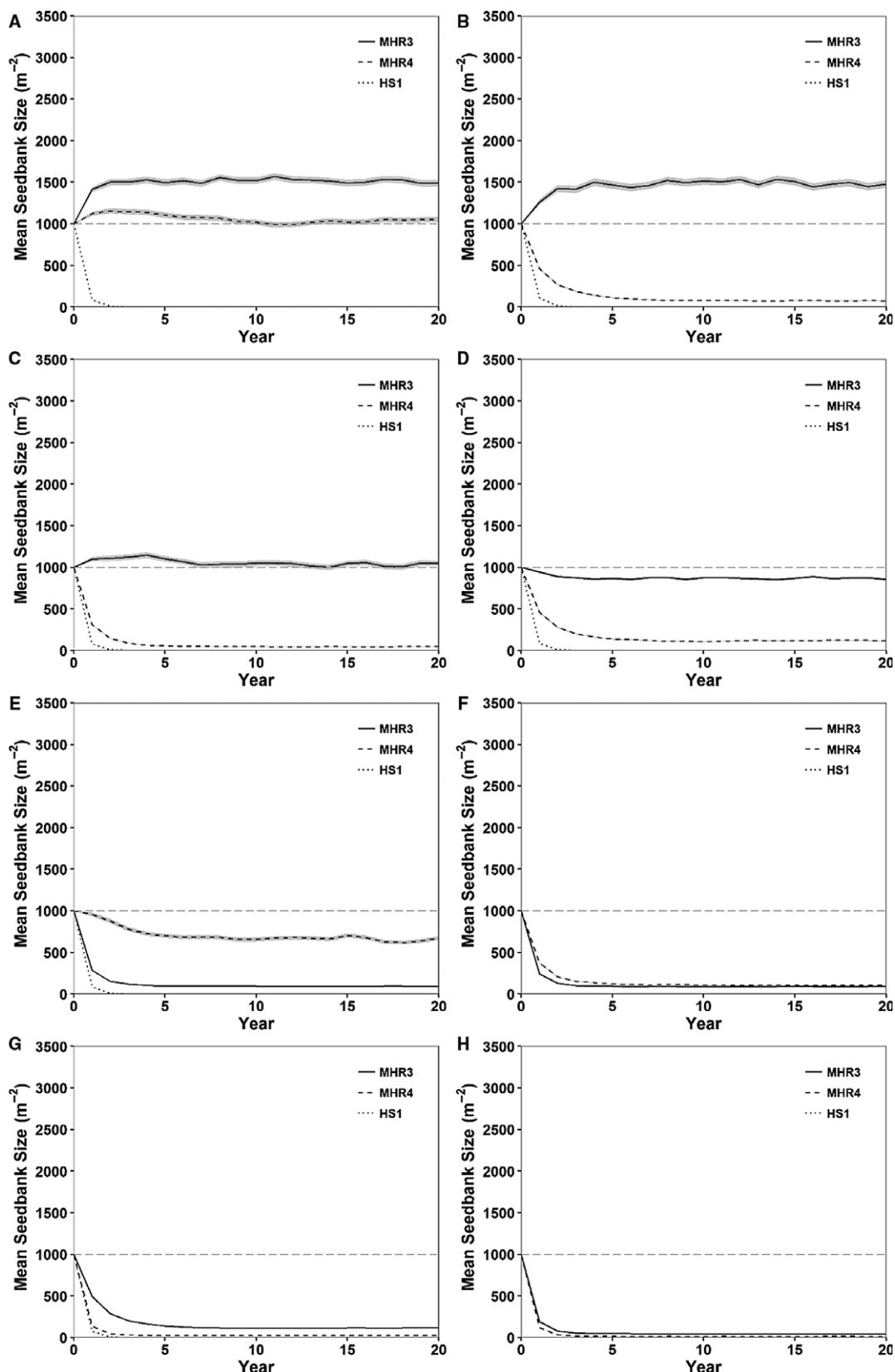


Fig. 2 Mean density-dependent population projections for multiple herbicide-resistant (MHR3–MHR4) and herbicide susceptible (HS1) *Avena fatua* populations under two spring wheat seeding densities (kg ha^{-1}) and four nitrogen fertiliser rates (kg ha^{-1}) in a continuous wheat cropping system. Lines represent the mean and shading $\pm 1 \text{ SEM}$. A–B 56 N. C–D 112 N. E–F 168 N. G–H 224 N. A, C, E, G 67 seeding density. B, D, F, H 101 seeding density.

declined slightly (671 ± 26 seeds m $^{-2}$) in contrast with the rapid decline observed in the low wheat seeding density – 112 kg ha $^{-1}$ N treatment. Under the same fertilisation rate, 168 kg ha $^{-1}$ N, but high wheat seeding density, MHR3 and MHR4 seedbanks declined within 5 years (Fig. 2F) to 88 ± 7 and 103 ± 11 seeds m $^{-2}$, respectively, at the end of the simulation. Under the highest N fertiliser rate, MHR3 and MHR4 seedbanks declined rapidly in both wheat seed density simulations, stabilising below 120 seeds m $^{-2}$ (Fig. 2G and H).

Elasticity analysis in the continuous wheat cropping system

Relative rankings of the seedbank density elasticity to a 10% change in model parameters were similar in all HS1 population models, regardless of treatment (Table S2). Overwinter seed survival had the greatest impact on *A. fatua* seedbank density of this herbicide susceptible population, followed by over summer seed survival and maximum seed produced per plant. Regardless of treatment, the most elastic parameter in both MHR populations was asym, the shape parameter in the asymptotic density-dependent seed production function, which dictates the maximum proportional seed production reduction at high densities (Table S2). Overwinter seed survival was the second most elastic parameter across treatments, followed closely by the maximum seed produced per plant.

Wheat-fallow cropping system

Overall, HS1 populations declined to near zero within 3–5 years, regardless of N fertilisation rate and wheat seeding density (Fig. 3). MHR seedbank populations differed from each other in response to treatments. The fallow years of the rotation were easily distinguishable due to the large increase in MHR seedbanks by individuals that survived simulated glyphosate treatment and lack of wheat competition (Fig. 3). Although the HS1 population survived the simulated glyphosate treatment at the same rate as the MHR populations, the increase is not evident because HS1 seedbanks were already low after the herbicide treatment applied in the wheat year.

As in the continuous wheat cropping system, MHR3 and MHR4 populations had the largest seedbank densities in the lowest N and low wheat seeding density treatment with 1580 ± 77 and 2158 ± 118 seeds m $^{-2}$ in year 20 respectively (Fig. 3A). Increased wheat seeding density at the lowest N treatment did not negatively impact the MHR3 seedbank (Fig. 3B). Unlike MHR3, the increase in wheat seeding density at the lowest N rate resulted in a decline of the MHR4 seedbank to 347 ± 31 seeds m $^{-2}$.

Increasing the N fertilisation rate to 112 kg ha $^{-1}$ N reduced the seedbank density of both MHR populations (Fig. 3C and D). MHR3 mean seedbank density at the end of the simulation was similar under low and high wheat seeding densities with 964 ± 54 and 1045 ± 35 seed m $^{-2}$ respectively. MHR4 mean seedbank density was lower than MHR3 in both wheat seeding densities, although there were slight differences in the rate of decline. In the low wheat seeding density, MHR4 declined more rapidly (101 ± 10 seeds m $^{-2}$; Fig. 3C) than in the high wheat seeding density (436 ± 32 seeds m $^{-2}$; Fig. 3D).

Similar to the continuous wheat cropping system, MHR4 had a larger seedbank density than MHR3 in the 168 kg ha $^{-1}$ N-low wheat seeding density treatment (Fig. 3E). MHR3 population declined gradually for the first 5 years to 234 ± 13 seeds m $^{-2}$, while MHR4 populations slightly increased to 1023 ± 62 seeds m $^{-2}$ (Fig. 3E). Increasing the wheat seeding density had a slight negative impact on MHR3 populations, resulting in a seedbank density of 136 ± 8 seeds m $^{-2}$ (Fig. 3F). Increasing wheat seeding density led to a large decline in MHR4 seedbank density to 248 ± 17 seeds m $^{-2}$. Under the highest N fertiliser rate, MHR3 and MHR4 seedbanks declined rapidly in both wheat seed density simulations, stabilising below 270 seeds m $^{-2}$ (Fig. 3G and H).

Elasticity analysis in the wheat-fallow cropping system

Relative rankings of the seedbank elasticity to a 10% change in model parameters were similar in HS1 populations, irrespective of treatment (Table S3). The most elastic parameter in the HS1 population was the glyphosate kill rate. All other elasticities were generally very low. Similar to the continuous wheat cropping system and regardless of treatment, for MHR populations, the most elastic parameter was asym (Table S3). Glyphosate kill rate was the second most elastic parameter across treatments for all MHR populations, except the MHR4 population in the 56 kg ha $^{-1}$ N-low wheat seeding density treatment in which it was second. Overwinter seed survival was the third most elastic parameter for both MHR populations in all N rate and seeding density combinations.

Nitrogen and wheat seeding density impacts on dN/dt across rotations

There was a significant three-way interaction between *A. fatua* population, N rate and wheat seeding density on the simulated *A. fatua* dN/dt ($P \leq 0.0001$). This interaction was further supported by the visual model

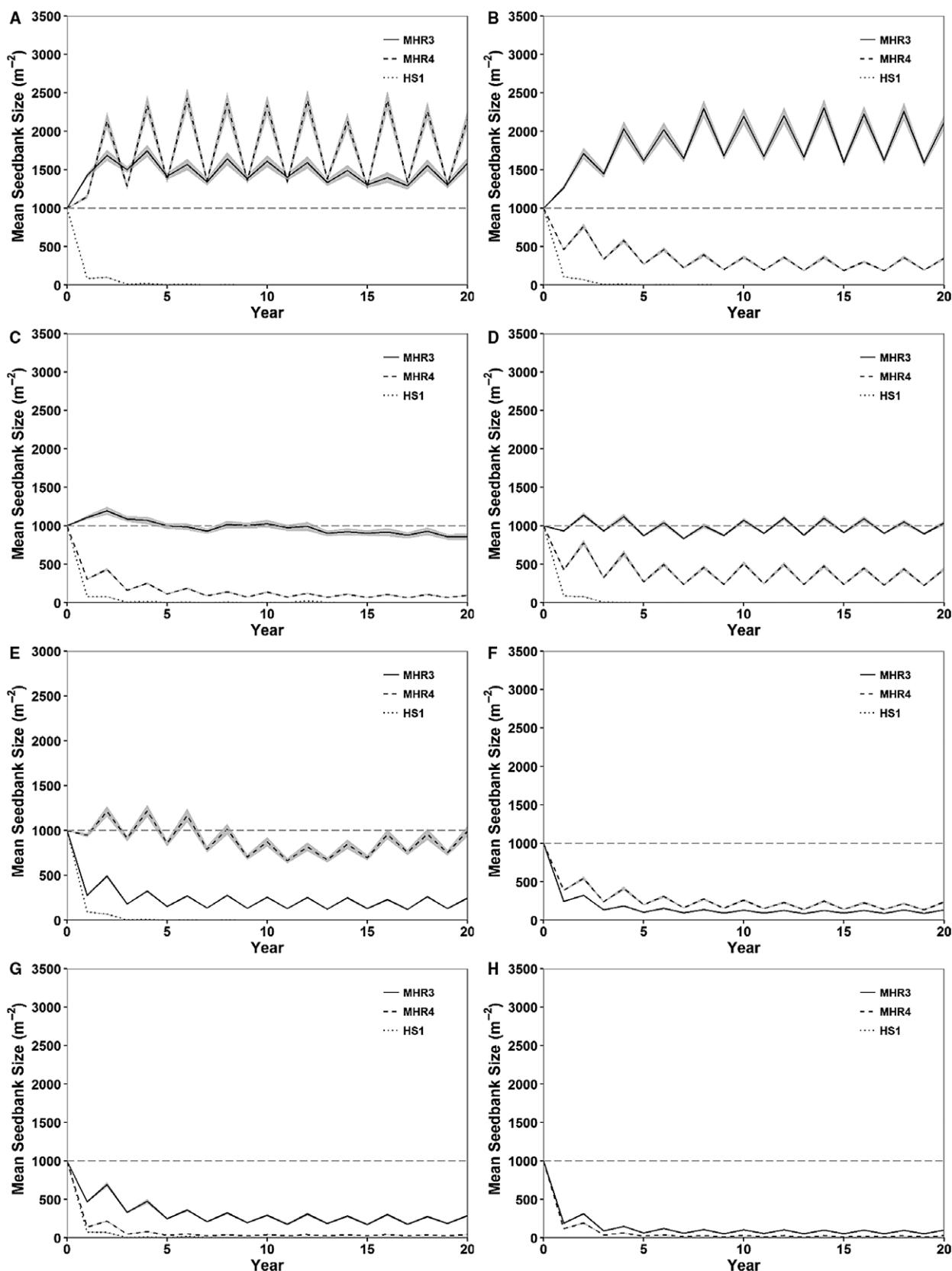


Fig. 3 Mean density-dependent population projections for multiple herbicide-resistant (MHR3–MHR4) and herbicide susceptible (HS1) *Avena fatua* populations under two spring wheat seeding densities (kg ha^{-1}) and four nitrogen fertiliser rates (kg ha^{-1}) in a wheat-fallow cropping system. Lines represent the mean and shading $\pm 1 \text{ SEM}$. A–B 56 N. C–D 112 N. E–F 168 N. G–H 224 N. A, C, E, G 67 seeding density. B, D, F, H 101 seeding density.

projections in Figs 2 and 3. The simulated MHR3 dN/dt was larger when the wheat seeding density increased under 56 kg ha⁻¹ N and smaller under 112 and 224 kg ha⁻¹ N (Table 2). There was no difference in dN/dt when the wheat seeding density increased under 168 kg ha⁻¹ N. Within an N rate, the simulated MHR4 dN/dt was smaller when the wheat seeding density increased in 56 and 168 kg ha⁻¹ N and larger in 112 kg ha⁻¹ N (Table 2). There was no difference in dN/dt when the wheat seeding density increased in 224 kg ha⁻¹ N. Within any wheat seeding density and N rate, the simulated dN/dt was larger for MHR3 compared with MHR4 for all treatment combinations except the low wheat seeding density – 168 kg ha⁻¹ N, in which MHR4's dN/dt was larger and the high wheat seeding density – 168 or 224 kg ha⁻¹ N treatments in which there was no difference (Table 2).

Discussion

Population dynamics models are useful tools in weed management (Davis *et al.*, 2004; Tidemann *et al.*, 2016). Specifically, our model sought to incorporate inter- and intra-population variability by constructing *A. fatua* population models for one susceptible and two MHR populations with vital rates randomly chosen from a spatially replicated 3-year field study. This approach allowed us to provide a realistic basis for simulating response to agroecosystem management

factors that could differentially influence population dynamics (Neve *et al.*, 2009).

In the continuous wheat and wheat-fallow cropping systems, HS1 seedbank densities rapidly declined regardless of N fertiliser rate and wheat seeding density, due to their high susceptibility to the herbicide applied in this study (Lehnhoff *et al.*, 2013b). In the continuous wheat and wheat-fallow cropping systems, MHR mean seedbank densities of both populations were negatively impacted by increasing N fertilisation rate. While previous glasshouse (Blackshaw & Brandt, 2008; Lehnhoff *et al.*, 2013a) and field (Carlson & Hill, 1986; Ross & Van Acker, 2005) research determined that N fertilisation has a neutral or positive effect on *A. fatua* growth and competitive ability with wheat, our study demonstrated the opposite.

Although this study is in contrast with the above findings, our results consistently demonstrated a decrease in *A. fatua* seed production with increasing N fertilisation rate over the 3-year field study. It is possible the field conditions in which previous studies were conducted resulted in contradictory findings. For example, Carlson and Hill (1986) in California, USA, had plots that were flood irrigated and lower N rate treatments were used and Ross and Van Acker (2005) used lower N rates and the method of N application differed from this study. Furthermore, it is possible that the lower N rates used in previous studies, together with the moisture, light intensity and temperature controlled glasshouse conditions where they were conducted masked the mechanisms driving crop-weed competition occurring in field settings (Freckleton & Watkinson, 2000). Overall, wheat *A. fatua* competition appears to be very sensitive to resource availability modified by environmental conditions, thus future research determining why differences in response to N fertilisation occurs is needed.

In both the continuous wheat and wheat-fallow cropping systems, MHR mean seedbank density was generally negatively impacted by increasing wheat seeding rate. Improving crop competitiveness via increasing crop seeding densities is a well-known strategy to reduce weed interference (Maxwell *et al.*, 2007a).

The relative impact of N fertiliser rate and wheat seeding density on MHR *A. fatua* seed production differed among populations. This differential impact implied that while the MHR3 seedbank density was not reduced by the increase in wheat seeding density at the two lowest N fertiliser rates, MHR4 populations experienced a large decline in mean seedbank size within the first 5 years of the simulation at high wheat seeding density. Previous population dynamics models used data collected from a single population and thus inter-population variability was not incorporated

Table 2 Multiple herbicide-resistant (MHR3–MHR4) *Avena fatua* mean (standard error) dN/dt under two spring wheat (*Triticum aestivum*) seeding densities and four nitrogen fertiliser rates

Population	Seeding density kg ha ⁻¹	Nitrogen rate	dN/dt
MHR3	67	56	87 (5.9)a*
MHR4	67	56	-38 (5.2)b
MHR3	101	56	114 (6.8)c
MHR4	101	56	-161 (1.4)de
MHR3	67	112	10 (5.3)f
MHR4	67	112	-184 (0.5)ghi
MHR3	101	112	-23 (2.3)j
MHR4	101	112	-159 (1.3)de
MHR3	67	168	-176 (0.5)egh
MHR4	67	168	-43 (4.4)k
MHR3	101	168	-180 (0.4)ghi
MHR4	101	168	-170 (0.8)deg
MHR3	67	224	-163 (1.1)de
MHR4	67	224	-194 (0.1)i
MHR3	101	224	-186 (0.7)hi
MHR4	101	224	-194 (0.6)i

*Means followed by the same letter are not significantly different ($P \leq 0.05$).

(Neve *et al.*, 2009). Our findings highlight the importance of using multiple locally adapted MHR populations, thus enhancing the capability of simulation models to realistically predict long-term dynamics under field conditions.

In general, MHR seedbank densities at year 20 of the projection were larger in the wheat-fallow compared with the continuous wheat cropping system. We utilised a simulated glyphosate efficacy $90 \pm 5\%$ (Adkins *et al.*, 1998) and the large numbers of seeds produced by MHR plants escaping glyphosate treatment in the fallow year resulted in the simulated peaks in seedbank density. These simulated peaks were not as defined in HS1, due to the extremely low survival rate resulting from the flucarbazone application during the wheat phase. Although MHR populations were smaller in the continuous cropping system, there are many negative effects of continuous cropping, including insect pest and disease outbreaks, depleted soil quality, reduced soil organic carbon, soil moisture depletions and general lack of resilience in a changing climate (Miller *et al.*, 2013) that were not incorporated into the model.

In both cropping systems, the maximum proportional impact of crop and weed density on seed production was the most influential parameter impacting *A. fatua* population growth rate. As these MHR populations were not affected by the flucarbazone application, the numbers of seeds MHR populations could produce became an important life stage parameter. Thus, our results suggest that management practices should target *A. fatua* seed production, a goal that can be reached by maximising the competitive effect of the crop (Liebman *et al.*, 2001).

Overwinter seed survival was the second and third most impactful parameter on MHR population growth rates across agronomic treatment combinations in the continuous and crop-fallow systems respectively. This is not surprising given *A. fatua*'s lifecycle of a summer annual in which all individuals must survive overwinter in the seedbank to be part of the following year's population. Previous studies have also identified this life stage as highly sensitive to changes in management of annual weeds (Davis *et al.*, 2004; Tidemann *et al.*, 2016), representing a potential 'choke-point' in the MHR populations in which management would be most effective. Studies evaluating burial depth and *A. fatua* seed survival indicated that 80% of seeds placed on the soil surface were characterised as non-viable after 6 months (Zorner *et al.*, 1984). No-till and conservation tillage practices would greatly increase the number of seeds remaining on the soil surface, improve soil quality, enhance post-dispersal predation (Menalled *et al.*, 2007) and improve the soil microbial

community, therefore exposing more seeds to microbial decay (Gallandt *et al.*, 1999).

Fallow year weed management efficacy was the second most elastic parameter for all MHR treatment combinations in the wheat-fallow cropping system, even though MHR populations were not resistant to the imposed glyphosate management. *Avena fatua* seedlings escaping management in the fallow year of the rotation produced large amounts of seeds; chemically based or other weed management in the fallow year must be strategically applied to minimise seed production without increasing selection for multiple herbicide resistance.

Conclusion

Multiple herbicide resistance is a growing problem that threatens the utility of conventional agricultural practices (Powles & Yu, 2010). Particularly, troublesome is the characterisation of two MHR *A. fatua* populations, utilised in this study, that are resistant to members of all selective herbicide families available for *A. fatua* control in cereal crops (Lehnhoff *et al.*, 2013b; Keith *et al.*, 2015). Therefore, alternative ecologically based approaches for sustainable weed management are required. Our results suggest that increasing N fertilisation rate and wheat seeding density are efficient tools to manage MHR *A. fatua* populations in the tested environmental conditions. In addition, decreasing seed production and increasing mortality of *A. fatua* in the fallow years using cover or pulse crops may represent alternatives to using a herbicide, reducing the risk of selecting for additional resistance associated with the tested MHR populations.

To our knowledge, this study was the first to utilise HS and MHR *A. fatua* field data obtained in a range of environmental conditions to construct a stochastic population dynamics model aimed at providing management recommendations. While other studies have evaluated the impacts of resistance on population dynamics, they did not directly use seed of resistant plants, but rather they simulated herbicide resistance using reduced herbicide rates (Tidemann *et al.*, 2016). The use of field evolved MHR population dynamics modelling represents a novel practice that can inform proactive ecological weed management strategies. Duke (2012) stated that herbicides appear to be non-renewable resources and alternative weed control methods must be investigated before the utility of herbicides is lost. Our results suggest that while herbicide resistance may be a 'wicked problem' (Shaw, 2016) from the social perspective, there are solutions available, as long as they are expanded beyond herbicide based approaches to weed management (Menalled

et al., 2016). Demographic models such as the one constructed in this study will aid in selecting ecologically based weed management tactics to both control MHR weed populations and extend the utility of weed sciences' non-renewable resources.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Appendix S1 *Avena fatua* demographic data.

Table S1 Multiple herbicide resistant (MHR3 and MHR4) and herbicide susceptible (HS1) *Avena fatua* mean emergence, reproductive tillers, and seed production under two wheat seeding densities and four nitrogen fertilisation rates in a 3 year study (2012–2015).

Table S2 Seedbank elasticity under continuous spring wheat cropping system.

Table S3 Seedbank elasticity under spring wheat-fallow cropping system.

Table S4 Spring wheat mean (standard error) yield under two wheat seeding densities and four nitrogen fertilisation rates in a 3 year study (2012–2015).

Code S1 R code for continuous wheat cropping system.

Code S2 R code for wheat-fallow cropping system.