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Synergistic Effects of Atrazine and Mesotrione on Susceptible and Resistant Wild Radish (*Raphanus raphanistrum*) Populations and the Potential for Overcoming Resistance to Triazine Herbicides

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The synergistic interaction between mesotrione, a hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicide, and atrazine, a photosystem II (PS II)-inhibiting herbicide, has been identified in the control of several weed species. A series of dose–response studies examined the synergistic effect of these herbicides on a susceptible (S) wild radish population. The potential for this interaction to overcome target-site *psb*A gene-based atrazine resistance in a resistant (R) wild radish population was also investigated. Control of S wild radish with atrazine was enhanced by up to 40% when low rates (1.0 to 1.5 g ha^{-1}) of mesotrione were applied in combination. This synergistic response was demonstrated across a range of atrazine–mesotrione rate combinations on this S wild radish population. Further, the efficacy of 1.5 g ha^{-1} mesotrione increased control of the R population by a further 60% when applied in combination with 400 g ha^{-1} of atrazine. This result clearly demonstrated the synergistic interaction of these herbicides in overcoming the target-site resistance mechanism. The mechanism responsible for the observed synergistic interaction between mesotrione and atrazine remains unknown. However, it is speculated that an alternate atrazine binding site may be responsible. Regardless of the biochemical nature of this interaction, evidence from whole-plant bioassays clearly demonstrated that synergistic herbicide combinations improve herbicide efficiency, with lower application rates required to control weed populations. This, combined with the potential to overcome *psb*A gene-based triazine resistance, and, thereby, regain the use of these herbicides, will result in more sustainable herbicide use.
Nomenclature: Atrazine; mesotrione; wild radish, *Raphanus raphanistrum* L. RAPRA.
Key words: Herbicide resistance, herbicide synergy.

La interacción sinérgica entre mesotrione, un herbicida inhibidor de hydroxyphenylpyruvate dioxygenase (HPPD), y atrazine, un herbicida inhibidor del fotosistema II (PS II), ha sido identificada en el control de varias especies de malezas. Una serie de estudios de dosis-respuesta examinó el efecto sinérgico de estos herbicidas en una población susceptible (S) de *Raphanus raphanistrum.* También se investigó el potencial de esta interacción para solventar la resistencia de sitio activo a atrazine basada en el gene *psb*A en una población resistente (R) de dicha maleza. El control con atrazine de *R. raphanistrum* (S) mejoró hasta 40% cuando se aplicó en combinación con dosis bajas de mesotrione (1.0–1.5 g ha^{-1}). Esta respuesta sinérgica se demostró a través de una gama de combinaciones de dosis atrazine-mesotrione en esta población de *R. raphanistrum* (S). Además, la eficacia de mesotrione a 1.5 g ha^{-1} incrementó el control de la población (R) en otro 60% cuando se aplicó en combinación con 400 g ha^{-1} de atrazine. Este resultado demostró claramente la interacción sinérgica de estos herbicidas para solventar problemas asociados a este mecanismo de resistencia en el sitio activo. Aún se desconoce el mecanismo responsable de la interacción sinérgica observada entre mesotrione y atrazine. Sin embargo, se especula que puede haber una asociación reducida por competencia entre atrazine y plastoquinone o un sitio alterno de asociación de atrazine puede ser el responsable. Sin importar la naturaleza bioquímica de esta interacción, la evidencia de los bioensayos con plantas enteras demostró claramente que las combinaciones sinérgicas de herbicidas mejoran la eficiencia de los herbicidas, con dosis de aplicación más bajas requeridas para controlar las poblaciones de malezas. Esta situación, combinada con el potencial para solventar la resistencia a atrazine basada en el gene *psb*A y por lo tanto poder recuperar el uso este herbicida, resultará en un uso de herbicidas más sostenible.

Although in use internationally for more than a decade, the relatively new mode-of-action 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides have only very recently been introduced into Australia for selective dicot weed control, particularly the troublesome wild radish. During the past 3 decades, with the intensification of Australian conservation cropping systems, wild radish control has been almost exclusively herbicide-reliant (Walsh et al. 2004). Subsequently, evolved herbicide resistance in wild radish populations is now common, especially in Western Australia (WA), where wild radish is particularly problematic. A large random survey of more than 500 crop fields, conducted in 2003, found herbicide-resistant (R) wild radish in more than 80% of collected populations (Walsh et al. 2007). Most of these populations displayed multiple resistance, with very high-frequency resistance to acetolactate synthase–inhibiting (54%), auxin analog (60%), phytoene desaturase–inhibiting (40%), and photosystem II (PS II)-inhibiting (15%) herbicides. Therefore, as well as a need for more diverse, integrated control strategies, there is also a requirement for highly effective herbicides with new modes of action.

HPPD- and PS II–inhibiting herbicides are known to be synergistic in their control of several weed species. There are

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also indications that this synergy can overcome *psb*A-gene based resistance to PS-II herbicides occurring in some of these species (Sutton et al. 2002). Combinations of the HPPD herbicide mesotrione and the PS-II herbicide atrazine are synergistic in common cocklebur (*Xanthium strumarium* L.), Canada thistle [*Cirsium arvense* (L.) Scop.], velvetleaf (*Abutilon theophrasti* Medik.), and Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Abendroth et al. 2006; Armel et al. 2005; Bollman et al. 2006; Hugie et al. 2008; Sutton et al. 2002). Surprisingly, mixtures of HPPD and PS-II inhibitors are effective on triazine-resistant (R) populations of amaranth, goosefoot (*Chenopodium* L.), and nightshade (*Solanum* L.) species (Sutton et al. 2002). Hugie et al. (2008) confirmed synergism between mesotrione and atrazine to control a redroot pigweed (*Amaranthus retroflexus* L.) and red morningglory (*Ipomoea coccinea* L.) population resistant to atrazine because of the commonly occurring *psb*A Ser264 to Gly264 mutation. HPPD and PS II–inhibitor herbicide synergy is believed to be due to complimentary modes of action of these herbicides (Armel et al. 2005; Hugie et al. 2008). PS II–inhibiting herbicides preferentially bind at the plastoquinone (PQ) binding site (QB) on the D1 protein of PS II (Trebst and Draber 1986). This prevents electron transport, halting photosynthesis and resulting in the generation of excess reactive oxygen species. Oxidative stress subsequently leads to chlorophyll destruction, lipid peroxidation, and membrane breakdown (Hess 2000; Lee et al. 1997). HPPD-inhibiting herbicides inhibit the synthesis of α-tocopherol and PQ by preventing the conversion of hydroxyphenylpyruvate to homogentisate. This causes reduced cellular PQ concentrations and light-dependent carotenoid destruction (Norris et al. 1995; Pallett et al. 1998). Here, we have examined synergy between mesotrione and atrazine in both triazine-R and triazine-susceptible (S) wild radish populations. An additional aim was to investigate whether this herbicide combination could overcome target-site *psb*A Ser264 gene-based resistance to triazine herbicides.

Materials and Methods

Wild Radish Populations. The R wild radish population was collected in 1998 from a cropping field near Mingenew, WA, in the northern wheat belt (29°7′12″S, 115°15′36″E). This population is target-site triazine-R because of the *psb*A gene Ser264 to Gly264 mutation (Friesen and Powles 2007). The S population was collected in 1999 from a reserve at Yuna, WA (28°20′23.994″S, 115°0′36″E), where there had been no known herbicide application (Walsh et al. 2004). Subsequent generations of seed from these populations have been produced on plants grown in isolation in pollen-proof enclosures at the University of Western Australia (UWA).

General Experimental Procedures. The following experimental procedures were used in all pot experiments. Wild radish seeds (12 pot^{-1}) were planted approximately 1 cm deep into plastic pots (15-cm diam) containing potting mix (25% peat moss, 25% sand, 50% mulched pine bark). Plants were grown (May to June) in an UWA glasshouse with natural sunlight and temperatures ranging from 20 to 25 C (maximum) and 5 to 10 C (minimum). Pots were arranged in a randomized block design and were watered to field capacity as required and fertilized weekly with a complete liquid fertilizer (N 19% [NH_2 15%, NH_4 1.9%, NO_3 2.1%], P 8%, K 16%, Mg 1.2%, S 3.8%, and Fe 400, Mn 200, Zn 200, Cu 100, B 10, and Mo 10 mg kg^{-1}).

Atrazine (Nu-trazine 900 g ai kg^{-1} DF, Nufarm Australia Limited, Lot 51, Mason Road, Kwinana, WA 6167, Australia) and mesotrione (Callisto 480 g ai L^{-1} SC, Syngenta Crop Protection Münchwilen AG, P.O. Box, CH-4333, Münchwilen, Switzerland) herbicide treatments included an adjuvant (1% v/v Hasten, 704 g L^{-1} esterified [ethyl-based] canola oil and nonionic surfactants, Victorian Chemicals, 83 Maffra St., Coolaroo, Victoria, Australia) whenever applied as a mixture or individually. Herbicides were applied to plants at the second true-leaf stage using a dual nozzle (XR11001 TeeJet flat fan spray nozzles, Spraying Systems Co., North Avenue, Wheaton, IL 60189) cabinet sprayer with a delivery rate of 112 L ha^{-1} (200 kPa, 4 km h^{-1}) and then immediately returned to the glasshouse. Surviving plants in each pot were counted 21 d after application of herbicide treatments, harvested by cutting at ground level, and then oven-dried for 72 h at 70 C before weighing.

Dose–Response Combinations of Mesotrione and Atrazine. To identify any synergistic interaction between mesotrione and atrazine for the S wild radish population, a dose–response experiment using rate combinations of these herbicides was conducted using the general procedures described above. Five different rates each of atrazine (0, 25, 50, 100, and 200 g ha^{-1}) and mesotrione (0, 0.5, 1.0, 1.5, and 3.0 g ha^{-1}) in combination, for a total of 25 POST herbicide treatments were applied to S wild radish plants at the two true-leaf stage. Atrazine and mesotrione rates were chosen following a series of preliminary dose–response studies (data not shown) aimed at identifying the doses that resulted in a 50% reduction in plant survival (ED_{50}) and biomass (GR_{50}) for each herbicide.

To explore the possible synergistic relationship between mesotrione and atrazine on R wild radish, combinations of these herbicides were applied in a dose–response format. Five rates of atrazine (0, 50, 100, 200, and 400 g ha^{-1}) and mesotrione (0, 1.5, 3.0, 6.0, and 12 g ha^{-1}) were applied in 25 combinations to two true-leaf R wild radish plants (May to June 2008). Mesotrione rates produced a greater-than-predicted effect on R wild radish survival and biomass, whereas atrazine treatments unexpectedly reduced R wild radish biomass. Subsequently, a second dose–response experiment was conducted exploring the effects of higher atrazine (0, 250, 500, 750, and 1,000 g ha^{-1}) and lower mesotrione (0, 0.5, 1.0, 1.5, and 3.0 g ha^{-1}) rates (May to June 2008).

Data Analysis. The S wild radish plant survival and biomass data were analyzed with nonlinear regression analyses using the open-source statistical software R 2.3.03 (R 2005) and drc package (Knezevic et al. 2007). The mesotrione ED_{50} and GR_{50} doses were determined for each atrazine rate by fitting a three-parameter log-logistic model to the data (Equation 1):

$$Y = \left\{ \left[A/(X-1) \times ED_{50}^{B} \right] \right\}^{1/B}, \quad [1]$$

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where Y represents reduction in plant survival or shoot biomass, X is the herbicide dose, A represents the maximum value of Y, ED_{50} is the application rate required to produce a 50% reduction in survival (or shoot biomass, GR_{50}), and B is the slope at ED_{50}. The ED_{50} values derived from S wild radish responses to dose–response herbicide treatments were used to create an isobole plot (Tammes 1964) to demonstrate the existence of synergistic, antagonistic, or additive effects of herbicide combinations. Because of the greater-than-expected effect of the chosen rate combinations on S wild radish plant biomass, valid GR_{50} values could not be derived from the biomass data ($P > 0.05$).

To further explore the possibility of synergism in S wild radish, the joint activity of each of the mesotrione plus atrazine combinations was examined using the multiplicative survival model (MSM)(Colby 1967) method, where estimated responses (Equation 2) are compared with actual results:

$$Y = (M_i \times A_j)/100, \quad [2]$$

where Y is the expected plant survival or biomass as a percentage of the control, M_i is the population response following the application of mesotrione at rate i, and A_j is the population response following the application of atrazine at rate j. The deviations between estimated and actual effects were analyzed in a two-tailed t test ($P = 0.05$) using SAS software (SAS Institute 2009; SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414). Survival and biomass data from the first dose–response experiment on the R wild radish population were analyzed using the nonlinear regression analysis procedures described above (Equation 1). The lower mesotrione rates used in the second dose–response experiment did not allow accurate curve fitting and ED_{50} estimation. Therefore, standard error values were used to indicate any differences between mesotrione rate responses plotted for each atrazine rate. Joint activity of mesotrione and atrazine on R wild radish for both dose–response studies was examined using the MSM (Colby 1967) described earlier (Equation 2).

Results and Discussion

Synergy between Atrazine and Mesotrione in S Wild Radish. A synergistic interaction between atrazine and mesotrione was observed when these herbicides were applied in combination (POST) to the S wild radish population. Dose–response curves were constructed for each atrazine rate showing the effect of increasing mesotrione rates on the survival and biomass of the S wild radish population (Figure 1). Synergistic effects were observed in both survival and biomass responses where combinations of atrazine plus mesotrione produced responses greater than those predicted from additive effects alone. These synergistic responses are most evident at lower rate combinations of mesotrione plus atrazine. For example, the low mesotrione rate (0.5 g ha^{-1}) and the lowest rate of atrazine (25 g ha^{-1}) resulted in 47% plant mortality (Figure 1A). When atrazine and mesotrione were applied individually at these same low rates, there was little mortality (< 10%). Therefore, the synergistic effect

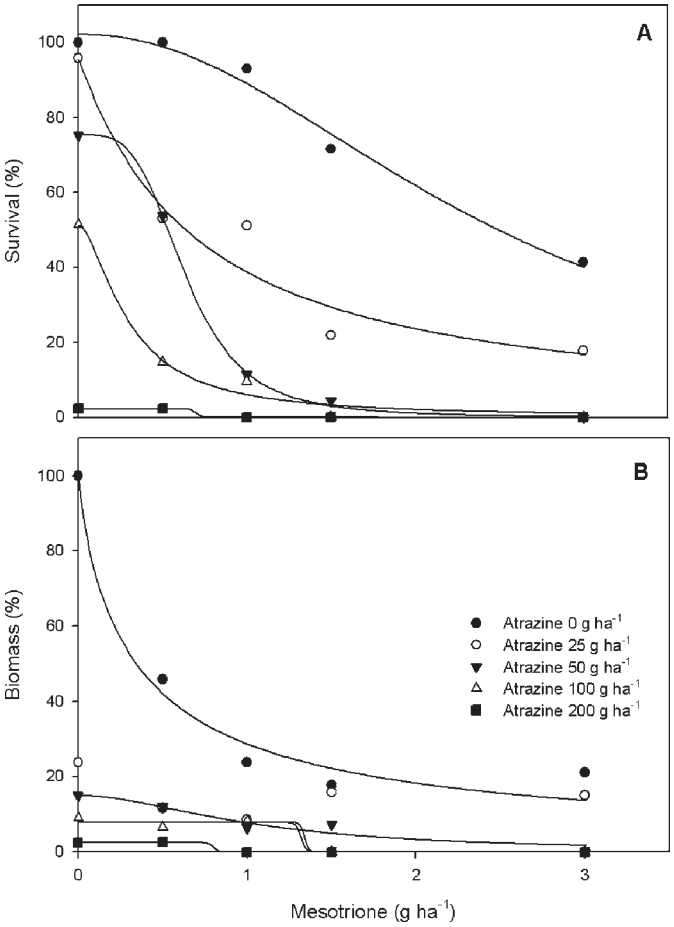

Figure 1. (A) Survival and (B) plant biomass responses of susceptible wild radish population treated with combinations of atrazine and mesotrione. Regression parameters are presented in Table 1.

resulting from the combination of these herbicide treatments was a 40% increase in plant mortality.

Plant survival responses, particularly for the low atrazine rate curves, allowed reasonably accurate estimation of ED_{50} mesotrione rates (Table 1). The isobole line, indicating additive treatment effects, is drawn between the ED_{50} rates for atrazine and mesotrione rates derived from the S survival responses to increasing application rates of these herbicides (Tammes 1964) (Figure 2). The points below the additive line are the estimated ED_{50} mesotrione rates derived from the atrazine 50 and 25 g ha^{-1} curves. These responses clearly show a greater-than-additive effect from the combinations of 25 g ha^{-1} atrazine + 0.7 g ha^{-1} mesotrione, and 50 g ha^{-1} atrazine + 0.6 g ha^{-1} mesotrione. The comparison of actual with estimated survival of the S population using the method of Colby (1967) across all rate combinations revealed that in most instances, a synergistic interaction was observed (Table 2). Unlike the isobole method, which examines synergistic responses around a selected response level (in this case, ED_{50}), the Colby method allows examination of response across a range of response levels. This method, as well as identifying a much broader range of synergistic reactions, also determined that it was only at the highest rates

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Table 1. Regression parameters (Equation 1) and estimated mesotrione (g ha^{-1}) rate that provided 50% reductions in susceptible wild radish population survival (ED_{50}) and plant biomass (GR_{50}). Values in parentheses are standard errors showing variation around the mean of four replicates.

	Plant survival			
Atrazine g ha^{-1}	A	B	ED_{50}	P value for ED_{50}
	% (SE)			
0	102.1 (5.2)	2.1 (0.5)	2.4 (0.3)	< 0.0001
25	95.3 (6.9)	1.1 (0.3)	0.7 (0.2)	0.0006
50	75.3 (5.8)	3.7 (1.0)	0.6 (0.1)	< 0.0001
100	51.3 (4.6)	1.7 (0.9)	0.3 (0.1)	0.04
200	2.3 (1.2)	9.2 (26.6)	0.7 (0.7)	0.3
	Plant biomass			
	% (SE)			
	A	B	GR_{50}	P value for GR_{50}
0	100.2 (4.8)	0.89 (0.2)	0.3 (0.1)	0.006
25	24.5 (1.6)	< 0.01 (< 0.01)	> 3 (35.4)	0.98
50	15.0 (2.7)	1.9 (0.9)	1.0 (0.4)	0.01
100	7.8 (1.2)	36.3 (345)	1.3 (2.3)	0.59
200	2.5 (1.1)	17.5 (180)	0.8 (2.1)	0.7

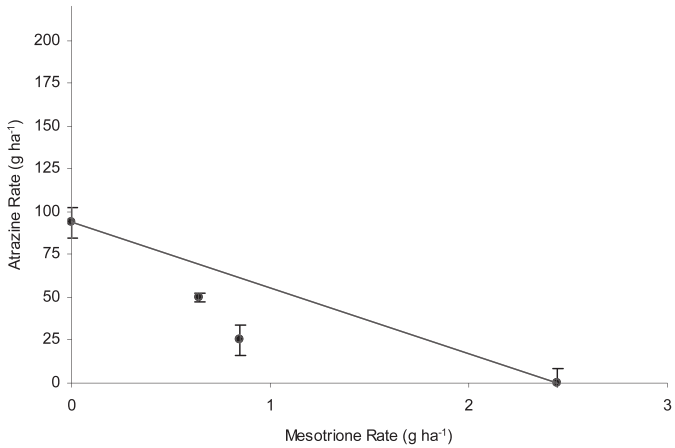

Figure 2. Isobole analysis of atrazine and mesotrione mixtures applied to susceptible wild radish plants. The isobole line indicating additive herbicide action was generated by linking ED_{50} survival values for atrazine and mesotrione applied alone. Plotted ED_{50} values for mesotrione plus atrazine combinations were determined from the regression analysis of S wild radish survival data (Table 1) with points below the isobole line indicating herbicide synergy. Capped bars represent the standard error values showing variation around the mean of four replicates.

of either atrazine or mesotrione that the combination responses became additive. As expected, at high rates, atrazine and mesotrione applied alone provided good control of the S population.

Despite plant survival responses providing evidence of synergy between mesotrione and atrazine, S wild radish plant biomass responses to the same combinations frequently displayed only additive or even antagonistic effects. The high efficacy of all atrazine plus mesotrione mixtures on S plant biomass prevented the accurate determination of GR_{50} mesotrione rates from the atrazine rate curves (Table 1). Therefore, the isobole method could not be used to identify synergistic effects on S wild radish biomass. Instead, the Colby (1967) method was used, which subsequently identified three rate combinations that produced a synergistic biomass response (Table 2). However, only one of these combinations (1.5 g ha^{-1} mesotrione + 100 g ha^{-1} atrazine) coincided with synergy in plant survival. In general, atrazine plus mesotrione combinations resulted in additive responses in S biomass levels. Therefore, as seen here, differences are likely to occur in joint-activity responses in plant survival and biomass following the application of herbicide combinations. However, in relating synergistic herbicide combinations to weed population control, plant mortality effects are of primary importance. Therefore, studies aimed at identifying synergistic interactions for the purposes of more-efficient weed control (maximum plant mortality at reduced herbicide inputs) should focus on plant-survival responses, not biomass.

Table 2. Comparison of actual atrazine and mesotrione combination effects on susceptible wild radish population survival and biomass compared with multiplicative survival model (Colby 1967)–derived estimates to identify herbicide joint-activity effects for selected rate combinations.

		Survival				Biomass			
Atrazine	Mesotrione	Actual	Estimate	P value	Joint activity	Actual	Estimate	P-value	Joint activity
g ha^{-1}		%				%			
25	0.5	53.0	95.6	0.018	Synergistic	11.6	10.9	0.658	Additive
50	0.5	53.9	75.2	0.015	Synergistic	11.9	6.8	0.038	Antagonistic
100	0.5	14.6	51.3	0.026	Synergistic	6.4	4.0	0.567	Additive
200	0.5	2.3	2.3	1.0	Additive	2.6	1.5	0.391	Additive
25	1.0	51.1	89.0	0.009	Synergistic	8.5	5.6	0.058	Additive
50	1.0	11.4	69.2	0.001	Synergistic	6.3	3.6	0.534	Additive
100	1.0	9.5	47.2	0.001	Synergistic	8.0	2.2	0.166	Additive
200	1.0	0.0	1.8	0.391	Additive	0.0	0.3	0.391	Additive
25	1.5	21.9	68.4	0.036	Synergistic	15.8	4.2	0.036	Antagonistic
50	1.5	4.4	52.8	0.005	Synergistic	7.3	2.7	0.343	Additive
100	1.5	0.0	36.3	0.003	Synergistic	0.0	1.6	0.001	Synergistic
200	1.5	0.0	1.3	0.391	Additive	0.0	0.5	0.391	Additive
25	3.0	17.7	39.3	0.145	Additive	15.0	4.8	0.007	Antagonistic
50	3.0	0.0	31.5	0.06	Additive	0.0	3.2	0.024	Synergistic
100	3.0	0.0	22.2	0.083	Additive	0.0	1.9	0.036	Synergistic
200	3.0	0.0	1.7	0.391	Additive	0.0	0.5	0.391	Additive

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Table 3. Regression parameters (Equation 1) and estimated mesotrione rate (g ha^{-1}) that provided 50% reductions in plant survival (ED_{50}) and biomass (GR_{50}) for resistant wild radish. Values in parentheses are standard errors showing variation around the mean of four replicates.

	Plant survival			
Atrazine g ha^{-1}	A	B	ED_{50}	P value for ED_{50}
	% (SE)			
0	100 (7.5)	1.8 (0.4)	2.9 (0.5)	< 0.001
50	100 (11.7)	1.2 (0.5)	1.6 (0.7)	0.01
100	98 (9.4)	1.2 (0.4)	1.6 (0.5)	0.005
200	100 (6.1)	1.7 (0.4)	1.7 (0.2)	< 0.001
400	98 (6.3)	0.4 (0.3)	0.05 (0.2)	0.76
	Plant biomass			
	A	B	GR_{50}	P value for GR_{50}
	% (SE)			
0	100 (8.3)	0.6 (0.4)	0.2 (0.4)	0.2
50	77 (5.3)	0.4 (0.3)	0.05 (0.2)	0.76
100	57 (3.9)	1.2 (0.4)	1.1 (0.3)	0.005
200	50 (5.3)	0.8 (0.5)	0.5 (0.5)	0.36
400	56 (5.1)	0.5 (0.4)	0.2 (0.4)	0.68

Synergy between Atrazine and Mesotrione in R Wild Radish. As expected, high-level resistance to atrazine in R wild radish was confirmed in this population known to have *psb*A gene-based triazine resistance (Friesen and Powles 2007). Unexpectedly, dose–response studies identified differences between atrazine curves plotted for R wild radish population survival responses to increasing rates of mesotrione (Figures 3 and 4). Previous studies confirmed that the high-level (> 16-fold) atrazine resistance in this R wild radish population is conferred by the *psb*A gene-based mutation (Friesen and Powles 2007). This *psb*A mutation has no effect on the activity of mesotrione; thus, this herbicide alone is very active on this population with complete control by 12 g ha^{-1} mesotrione (Figure 3). Therefore, given this response and the robust nature of the atrazine-resistance mechanism, it was expected that the dose–response curves constructed for each atrazine rate would be the same. However, there were clear differences between the curves (Figures 3A and 3B). Increasing rates of atrazine in combination with mesotrione resulted in higher R plant mortality, indicating some atrazine effect despite the presence of the robust atrazine-resistance mechanism. In the first dose–response experiment, the effect of atrazine on this population is clearly evident at 1.5 g ha^{-1} mesotrione, where the inclusion of 50 g ha^{-1} of atrazine reduced population survival by approximately 25% (Figure 3A). An atrazine rate of 250 g ha^{-1} at this same rate of mesotrione further reduced survival to around 50%. The combination of 400 g ha^{-1} atrazine and the same low 1.5 g ha^{-1} mesotrione rate reduced plant survival to just 13%. Joint-activity analyses identified synergistic plant mortality effects for several low mesotrione rate (≤ 3.0 g ha^{-1}) combinations (400 g ha^{-1} atrazine + 1.5 g ha^{-1} mesotrione [P = 0.003], 400 g ha^{-1} atrazine + 3.0 g ha^{-1} mesotrione [P = 0.04], and 200 g ha^{-1} atrazine + 1.5 g ha^{-1} mesotrione [P = 0.04]). At higher rates, the increased effect of mesotrione

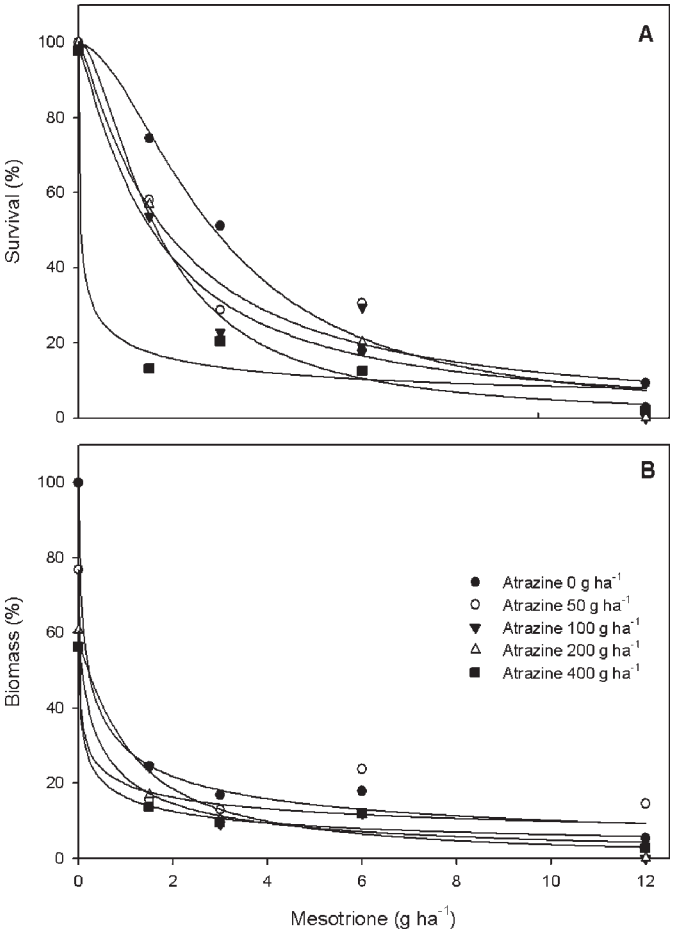

Figure 3. (A) Survival and (B) biomass responses of resistant wild radish population treated with combinations of atrazine and mesotrione in experiment 1. Regression parameters are presented in Table 3.

on R plant mortality masked the specific atrazine effects. The second dose–response experiment also identified clear synergistic responses in R plant survival to treatment combinations that included 1.5 g ha^{-1} mesotrione (Figure 4A). At that rate, there were three synergistic responses observed in the joint activity of these herbicides (500 g ha^{-1} atrazine + 1.5 g ha^{-1} mesotrione [P = 0.03], 750 g ha^{-1} atrazine + 1.5 g ha^{-1} mesotrione [P = 0.04], and 1,000 g ha^{-1} atrazine + 1.5 g ha^{-1} mesotrione [P = 0.048]). Curiously though, there were no synergistic responses in R wild radish survival to treatments based on mesotrione rates lower than 1.5 g ha^{-1}.

Although, as expected, atrazine alone did not cause any mortality in the R wild radish population, there was a reduction in plant biomass (Figures 3B and 4B). However, in the presence of mesotrione, the effect of atrazine was less clear, making it difficult to identify any combination effects on plant biomass. Additionally, the R wild radish plant biomass reductions from atrazine-alone treatments were larger in the first dose–response experiment than they were in the second, despite the higher rates used in the latter experiment. This difference may have been a temperature-related effect, with the second experiment exposed to a generally cooler growing season conditions. Joint-activity analyses failed to identify

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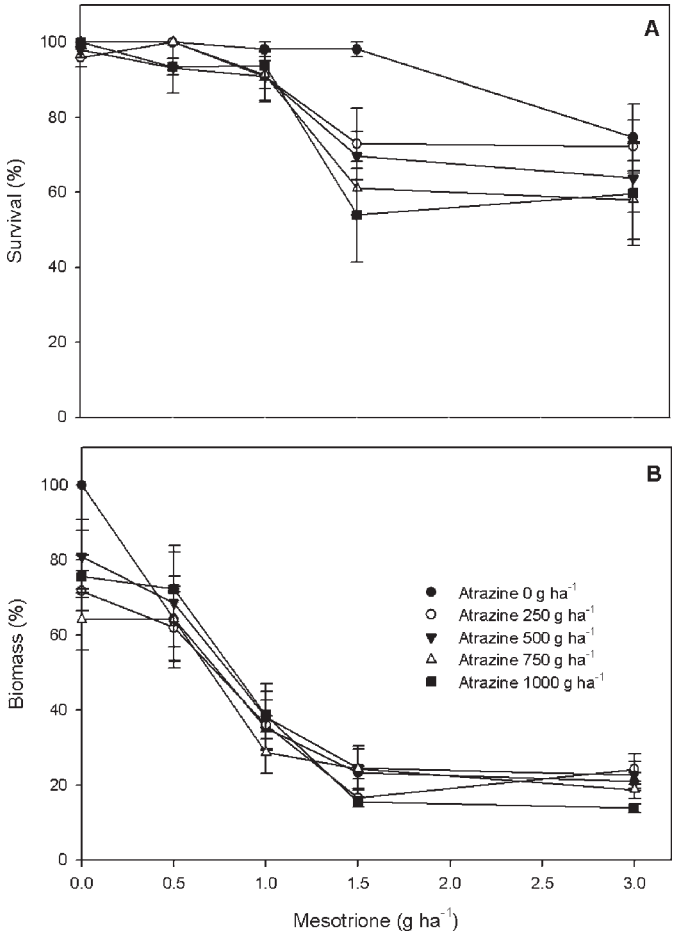

Figure 4. (A) Survival and (B) biomass responses of a resistant wild radish population following treatment with combinations of atrazine and mesotrione in experiment 2. Capped bars represent standard error values showing variation around the mean of four replicates.

synergistic effects on R wild radish biomass for the rate combinations used in both dose–response studies.

The observed higher mortality indicates the potential for these mixtures to overcome the *psb*A gene-based resistance mechanism. A similar synergistic response to these herbicide combinations has been recorded in a R redroot pigweed population with the same *psb*A gene-based mutation (Ser to Gly at residue 264) as the R wild radish used in this study (Hugie et al. 2008). Notably, it has been demonstrated that there is no synergism between atrazine and mesotrione in a velvetleaf population with a metabolism-based atrazine-resistance mechanism (Woodyard et al. 2009). This result suggests that binding of atrazine to its target site or sites is required for the synergistic interaction with mesotrione to occur. These studies cannot explain the biochemical basis of the synergy between mesotrione and atrazine or how that synergy overcomes atrazine target-site *psb*A gene-based resistance. However, it is widely understood that there is an interrelationship between the modes of action of the PS II- and HPPD-inhibiting herbicides (Fuerst and Norman 1991). These herbicides both act on biochemical processes directly related to the efficient activity and structural integrity of PS II.

We speculate that the mechanism for synergy in both R and S plants is via enhanced oxidative stress because of atrazine application, and the concurrent breakdown in synthesis of protective carotenoids resulting from mesotrione application. Although atrazine is unable to bind at the QB binding site in resistant plants, previous studies have demonstrated the presence of a second, low-affinity atrazine binding site (referred to as QC) on the D2 protein (Fuerst and Norman 1991). Atrazine is able to bind there, interrupt the flow of electrons, and thus cause accumulation of free radicals, similar to the effect caused by binding at QB. Because of the low affinity of that site, only small amounts of atrazine will bind, resulting in limited production of free radicals and, therefore, producing very little effect on a plant that has an R QB binding site. However, with the introduction of an HPPD inhibitor, there is no protection by PS II, and the originally low level of oxidative stress becomes compounded (Hess 2000; Lee et al. 1997). This hypothesis also explains the need for the much higher rates of atrazine required for a synergistic effect in R plants; a higher rate is required to achieve levels of binding at the low-affinity QC site, similar to those levels at the high-affinity QB site in S plants. The potential effects of increased free radical generation because of atrazine binding at a second site within the photosynthetic apparatus and reduced photosynthetic protection resulting from mesotrione application also warrant investigation.

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