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Confirmation of 4-Hydroxyphenylpyruvate Dioxygenase Inhibitor-Resistant and 5-Way Multiple-Herbicide-Resistant Waterhemp in Ontario, Canada

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Abstract

This is the first confirmation of 4-hydroxyphenylpyruvate dioxygenase-(HPPD)-inhibitor resistant waterhemp and the first report of 5-way multiple-herbicide-resistant (MHR) waterhemp in Ontario to Group 2, 5, 9, 14, and 27 herbicides. Seed was collected across southern Ontario in 2021 and tested for resistance to imazethapyr, atrazine, metribuzin, glyphosate, lactofen, mesotrione, and mesotrione + atrazine representing Group 2, 5, 5, 9, 14, 27, and 27 + 5 herbicides, respectively. All of the waterhemp populations collected in 2021 had 4-way resistance to imazethapyr, atrazine, glyphosate, and mesotrione. In seven Ontario counties, 5-way resistant waterhemp was confirmed which includes resistance to the HPPD-inhibiting herbicides.

Keywords: atrazine, glyphosate, imazethapyr, lactofen, mesotrione, metribuzin, waterhemp, *Amaranthus tuberculatus* (Moq.) J. D. Sauer

1. Introduction

Waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] is a prolific, dioecious, summer annual broadleaf weed found throughout much of the US corn belt and in the provinces of Quebec, Ontario, and Manitoba in Canada (Costea et al., 2005). Waterhemp begins emerging in the spring and continues emerging over the summer months and into the fall, allowing late emerging cohorts to escape control by chemical, cultural, or mechanical means. Vyn et al. (2007), and Schryver et al. (2017) in studies completed in Ontario, Canada, reported that waterhemp emergence was highest in June and declined over the remainder of the growing season with seedlings still emerging in September and October. In the US, waterhemp emergence begins in May and continues into the fall (Costea et al., 2005). Waterhemp interference in corn and soybean caused up to 74 and 73% yield loss, respectively, when no weed control measures were implemented (Steckel & Sprague, 2004; Vyn et al., 2007).

Female waterhemp plants produce seeds after fertilization by pollen from nearby male plants (Bell & Tranel, 2010). Seed is produced in copious amounts; Hartzler et al. (2004) reported that a single waterhemp plant produced 4.8 million seeds. Since waterhemp is a dioecious species, it results in greater genetic diversity compared to other weed species, allowing for more rapid evolution of herbicide resistance. The first report of herbicide-resistant waterhemp was in 1993, biotypes from Illinois and Iowa were resistant to the Group 2, acetolactate synthase (ALS) inhibiting herbicides (Heap, 2022). Multiple mechanisms of resistance confer resistance to the Group 2-herbicides; the most common, as identified by Patzoldt and Tranel (2007), is target-site resistance due to a tryptophan to leucine amino acid substitution at position 574 of the ALS enzyme. Other less common amino acid substitutions confer Group 2 resistance in waterhemp plus non-target site resistance by enhanced metabolism (Guo et al., 2015). Three years later in 1996, waterhemp from a field in Illinois field was confirmed to be resistant to the Group 2 and 5 (photosystem II (PSII) inhibiting) herbicides. Group 5 resistance in waterhemp is due to target-site resistance from a glycine to serine amino acid substitution at position 264 in the *psbA* gene which confers resistance to the Group 5 herbicides (symmetrical triazine and asymmetrical triazinone chemical families). Additionally, non-target site resistance via enhanced metabolism by glutathione-S-transferases confers resistance to symmetrical triazine herbicides such as atrazine but not to the asymmetrical triazinones such as metribuzin (Westerveld et al., 2021).

In Ontario, the first report of glyphosate-resistant waterhemp was from seed collected in 2014 (Schryver et al., 2017). In Ontario waterhemp populations, target-site resistance due to increased gene copy number and an amino acid substitution both confer resistance to glyphosate. Waterhemp biotypes from a Lambton county population have up to 29 copies of the EPSPS gene; this gene amplification was found in 70% of resistant individuals (Kreiner et al., 2019). In addition, a proline-106-serine amino acid substitution conferred resistance in some biotypes (Kreiner et al., 2019). Group 14-resistant waterhemp is due to target-site resistance, a glycine 210 codon deletion which resulted in a 53-fold resistance factor (Benoit et al., 2019). This mechanism of resistance confers resistance to the Group 14 herbicides applied postemergence whereas the soil-applied Group 14 herbicides are still effective.

Schryver et al. (2017) reported 3-way multiple-herbicide-resistant (MHR) waterhemp in Ontario; populations were resistant to the Group 2, 5, and 9 herbicides. Benoit et al. (2019) published the first incidence of Group 14-resistant waterhemp and 4-way resistance to Group 2, 5, 9, and 14 herbicides in Ontario. In the US, waterhemp has evolved resistance to the Group 2, synthetic auxins (Group 4), Group 5, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors (Group 9), protoporphyrinogen oxidase (PPO) inhibitors (Group 14), very long-chain fatty acid elongases (VLCFAE) inhibitors (Group 15), and the 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (Group 27) (Heap 2022). Shergill et al. (2018) reported that one Missouri waterhemp population has multiple resistance to the Groups 2, 4, 5, 9, 14, and 27 herbicides.

Waterhemp resistance to the Group 27 herbicides has been reported in multiple US states; however, Group 27 resistance had not been confirmed in Ontario (Heap, 2022). The mechanism of Group 27 resistance in waterhemp has not been elucidated; however, it is speculated to be enhanced herbicide metabolism by cytochrome P450 monooxygenases (Hausman et al., 2011). The objective of this research was to continue to document the spread of MHR waterhemp across southern Ontario and screen Ontario populations for resistance to Group 2, 5, 9, 14, and 27 herbicides.

2. Materials and Methods

2.1 Seed Collection

In 2021, waterhemp seed was collected from twelve fields across Ontario. Seed was collected in the fall prior to crop harvest, primarily from soybean fields although some seed was collected from corn and dry bean fields. Field locations were found via communication with growers, agronomists, extension personnel, agricultural retailers, and personnel from the herbicide manufacturers. Generally, a composite seed sample was collected from 10-20 female plants from different areas within the field.

Figure 1. Distribution of 5-way multiple-herbicide-resistant waterhemp to imazethapyr, atrazine, glyphosate, lactofen, and mesotrione in Ontario, Canada from seed samples taken in the fall of 2021

Source: https://commons.wikimedia.org/wiki/File:Canada_Southern_Ontario_location_map_2.png

2.2 Resistance Screening

Upon collection, seed was kiln-dried for 36 hours, threshed and cleaned using 16- and 28-mesh sieves and a seed cleaner. Seed was then transferred into nylon bags, labeled, and placed into trays with sand. The trays containing the bags of waterhemp seed were refrigerated and chilled at 4 °C for a minimum of 6 weeks; the trays were watered as required. After 6 weeks, seed from each population was tested for germination by placing 5 seeds into Berger growing media (potting soil). Once germination was deemed sufficient, 10 cm by 10 cm germination trays were half-filled with potting soil, waterhemp seed was spread on the soil surface, and then covered with 0.5 cm of potting soil. When waterhemp reached the 2-leaf stage they were transplanted into 8 cm diameter pots, with one waterhemp plant per pot. Plants were watered regularly to ensure sufficient soil moisture. When waterhemp reached an average of 10 cm in height, the most uniform plants were divided into groups of 12. Seven groups of 12 plants for a total of 84 plants per population were used. Two plants of each group of 12 were used as nontreated controls; the remaining 10 plants were treated with: imazethapyr (75 g ai ha^{-1}) + nonionic surfactant (Agral 90®, 0.2% v/v), atrazine (1000 g ai ha^{-1}) + paraffin-based mineral oil (83%)/surfactant (17%) (Assist, 1% v/v), metribuzin (560 g ai ha^{-1}), glyphosate (900 g ai ha^{-1}), lactofen (110 g ai ha^{-1}) + paraffin based mineral oil (83%)/surfactant (17%) (Assist, 1% v/v), mesotrione (100 g ai ha^{-1}) + nonionic surfactant (Agral 90®, 0.2% v/v), and mesotrione (100 g ai ha^{-1}) + atrazine (280 g ai ha^{-1}) + nonionic surfactant (Agral 90®, 0.2% v/v). All herbicide rates were determined based on the manufacturer's recommended rates. All herbicide treatments were applied in an enclosed sprayer (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN, USA) that was calibrated to deliver 200 L ha^{-1} at 240 kPa. The sprayer was equipped with a single even spray 8002 nozzle (TeeJet® Technologies, Springfield, IL, USA). Twenty-four hours after spraying, the pots were moved from the spray area and placed in the greenhouse.

At 1, 3, and 5 weeks after application (WAA) waterhemp injury was assessed. Plants were assigned a value between 0 and 100 (0—no injury; 100—complete plant death) as a visible estimation of the reduction in waterhemp biomass compared to the nontreated controls. At 5 WAA, each plant was rated as dead or alive to give a proportion of resistant plants. Plants were scored alive if there was any green, living tissue present. If one or more plants were alive at 5 WAA, the plants were considered resistant, thus implying that individuals within the population were resistant.

3. Results and Discussion

3.1 HPPD Resistance

All of the waterhemp populations that were collected in the fall of 2021 had at least one plant that was resistant to mesotrione, demonstrating that Group 27 resistance is widespread across southern Ontario. With the exception of one population, the proportion that was Group 27-resistant ranged from 50 to 100%, indicating that there is a high proportion of waterhemp in Ontario that is Group 27-resistant (Table 1). Mesotrione caused 2 to 15% waterhemp injury at 1 WAA which increased to 25 to 50% at 3 WAA (Table 2). Similarly, Hausman et al. (2011) reported that mesotrione (105 g ai ha^{-1}) caused 13 to 24% waterhemp injury at 3 WAA in HPPD-resistant waterhemp from Illinois, US. At 5 WAA, mesotrione caused as little as 15% waterhemp injury in HPPD-resistant waterhemp in contrast to 90 to 100% waterhemp injury in susceptible biotypes. Resistance to mesotrione was found in seven Ontario counties: Essex, Chatham-Kent, Lambton, Elgin, Middlesex, Northumberland, and Stormont/Dundas/Glengarry. This is the first record of Group 27-resistant waterhemp in Ontario.

Table 1. Locations and percent of waterhemp alive 5 WAA for populations within Ontario treated with imazethapyr (75 g ai ha^{-1}), atrazine (1000 g ai ha^{-1}), metribuzin (560 g ai ha^{-1}), glyphosate (900 g ae ha^{-1}), lactofen (110 g ai ha^{-1}), mesotrione (100 g ai ha^{-1}), and mesotrione (100 g ai ha^{-1}) + atrazine (280 g ai ha^{-1})

Location	Percent plants surviving 5 WAA						
	Imazethapyr[a]	Atrazine[b]	Metribuzin	Glyphosate	Lactofen[b]	Mesotrione[a]	Mesotrione + atrazine[a]
	%						
Glengarry	100	40	0	80	80	50	10
Elgin	100	100	40	100	90	100	60
Glengarry	100	20	20	10	20	10	20
Chatham-Kent	80	10	0	70	10	30	0
Lambton	100	70	0	90	10	80	30
Essex	100	30	0	90	0	20	0
Stormont	100	100	50	100	30	90	60
Northumberland	100	50	0	60	20	90	10
Northumberland	100	100	70	100	90	90	80
Elgin	100	70	30	100	0	80	20
Middlesex	100	90	0	50	70	80	10
Essex	100	100	0	100	80	80	10
# of populations resistant	100	100	50	100	80	100	80

Note. WAA: weeks after application; [a]: Non-ionic surfactant, Agral 90®, was added at 0.2% v/v; [b]: Paraffin based mineral oil (83%)/surfactant (17%), Assist was added at 1% v/v.

Table 2. Percent waterhemp injury at 1, 3, and 5 WAA for populations treated with mesotrione (100 g ai ha^{-1}) postemergence

Location	% Waterhemp injury		
	1 WAA	3 WAA	5 WAA
Glengarry	5	51	87
Elgin	4	20	20
Glengarry	2	57	90
Chatham-Kent	3	25	50
Lambton	3	55	94
Essex	6	25	32
Stormont	2	30	43
Northumberland	5	27	15
Northumberland	12	48	64
Elgin	7	24	22
Middlesex	5	65	74
Essex	7	36	55

Waterhemp biotypes from ten of the twelve populations had at least one plant that survived the co-application of mesotrione and atrazine. Mesotrione + atrazine-resistant waterhemp is present in four Ontario counties: Lambton, Elgin, Northumberland, and Stormont/Dundas/Glengarry counties. Hausman et al. (2011) reported that mesotrione + atrazine (105 + 560 g ai ha^{-1}) caused 51 to 78% waterhemp injury at 3 WAA. Similarly, in the present study, there was greater waterhemp injury when mesotrione was co-applied with atrazine; however, some biotypes were able to survive similar to Hausman et al. (2011).

There were individual plants that were resistant to imazethapyr, atrazine, glyphosate, and lactofen representing Groups 2, 5, 9, and 14, respectively in all 10 populations evaluated. One population from Northumberland county had 100, 100, 70, 100, 90, and 90% of plants survive the applications of imazethapyr, atrazine, metribuzin, glyphosate, lactofen, and mesotrione, respectively; this is the first record of 5-way resistant waterhemp in Ontario, Canada.

Waterhemp seed has been collected from 137 populations across Ontario since 2014. Not all populations have been screened for resistance to Group 2, 5, 9, 14, and 27 herbicides. Based on research completed to date, of the 137 populations, the percentage of populations with resistance to Group 2, 5, 9, 14, and 27 herbicides is 97%, 81%, 91%, 40%, and 8%, respectively. Research is needed to screen all of the earlier seed collections for resistance to the Group 27 herbicides.

This is the first record of HPPD-resistant waterhemp and the first record of 5-way MHR waterhemp in Ontario. HPPD-resistant waterhemp is present in seven Ontario counties. Weed management practitioners must combine cultural, mechanical, biological, and chemical waterhemp control programs to reduce the over-reliance on any one weed management tactic. Due to the season-long emergence pattern of waterhemp, cultivation will not provide complete control. Crop rotation with winter cereals and the use of cover crops have been shown to be useful in reducing waterhemp density and biomass (Davis, 2010). Of utmost importance is ensuring that all equipment entering the field is clean to prevent the spread of seed from field-to-field and county-to-county.

The repeated use of the HPPD-inhibitors in corn and cereals is not recommended. The inclusion of multiple herbicide modes of action over time is recommended including Group 4, PSII-inhibitors (Group 6), soil-applied Group 14, and Group 15; however, resistance to some of these herbicides has already been reported (Heap, 2022). The judicious use of these chemicals cannot be stressed enough to maintain their effectiveness in current production systems. The co-application of multiple effective modes of action can also be useful in delaying resistance and should be used when possible. The ultimate goal of waterhemp management programs should be zero weed seed return to the soil seed bank thereby reducing future weed problems.

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