Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Glyphosate- and multiple-resistant waterhemp (*Amaranthus tuberculatus* var. *rudis*) in Ontario, Canada

Mike G. Schryver, Nader Soltani, David C. Hooker, Darren E. Robinson, Patrick J. Tranel and Peter H. Sikkema

Abstract: Waterhemp is one of the most troublesome weeds in the US and is spreading into Ontario. In 2014, a waterhemp population was not controlled with glyphosate in a field in Lambton County, Ontario. This population was the first confirmed glyphosate-resistant (GR) waterhemp in Canada. In 2015, waterhemp seeds were collected from 48 fields in Lambton (32), Chatham-Kent (2), and Essex (14) counties to determine the occurrence and distribution of GR waterhemp in Ontario. Waterhemp plants were grown in a greenhouse and sprayed at 10 cm in height. In addition to glyphosate (Group 9), collected populations were screened for resistance to imazethapyr and atrazine, representing herbicide Groups 2 and 5 respectively. Visual control estimates for biomass reduction were completed at 1, 3 and 5 weeks after application. Glyphosate-resistant waterhemp was confirmed in 40 fields, representing 82% of all sampled fields from three Ontario counties (Lambton, Chatham-Kent and Essex). Of the 49 populations collected, all were resistant to imazethapyr (Group 2), and 76% were resistant to atrazine (Group

---

Received 15 November 2016, accepted XX XX 2016.
M. G. Schryver, N. Soltani, D.C. Hooker, D.E. Robinson, and P.H. Sikkema. University of Guelph Ridgetown Campus, Ridgetown, ON N0P 2C0, Canada.
P.J. Tranel, Department of Crop Science. University of Illinois at Urbana-Champaign, 1201 W. Gregory Dr. Urbana, IL 61801, USA.
Corresponding author: Nader Soltani (e-mail: soltanin@uoguelph.ca).
Abbreviations: GR, glyphosate resistant; **WAA**, weeks after application; **WH**, waterhemp

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

5). Of all populations tested, 61% of all samples were found to resistant to all three herbicide groups. This study is the first to confirm glyphosate-resistant waterhemp in Ontario.

Key words: Glyphosate resistance, distribution, multiple-resistant, survey, waterhemp

Introduction

Two subspecies of waterhemp exist in Ontario. Waterhemp was first thought to be one diverse species by Unline and Bray in 1895 (Costea et al. 2005). Although some disagreement remains among botanists, the current botanical taxonomic understanding of waterhemp recognizes two distinct subspecies species in Ontario. The first is tall waterhemp (*Amaranthus tuberculatus* [Moq.] Sauer var. *tuberculatus*) which is native to Ontario and Quebec found in undisturbed habitats such as along waterways and beaches since the late 1800's (Costea et al. 2005). The second is common waterhemp (*Amaranthus tuberculatus* [Moq] Sauer var. *rudis* [Sauer] which is found in disturbed habitats such as agricultural land and is a relatively new weed in Ontario agricultural cropping systems (Costea et al. 2005). It has been suggested that it was introduced via a demonstration combine from Illinois between the late 1990s and early 2000s (Costea et al. 2005). In a greenhouse experiment conducted by Vyn et al. in 2006, differences were reported between the two subspecies in plant height and 100 seed weight with reductions of 32 and 39% in var. *tuberculatus* in comparison to var. *rudis*, supporting the notion var. *rudis* is more competitive (Costea et al. 2005). Although some disagreement remains among botanists, there are two subspecies of waterhemp found in Ontario with all further mention of waterhemp relating to that of the non-native, competitive sub species, common waterhemp.

Waterhemp is a small seeded, summer annual, broadleaf weed with many traits that make it particularly troublesome in agriculture. First, this weed can emerge in Ontario throughout the

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

entire growing season (Vyn et al. 2007), which makes control difficult since most herbicides are applied early in the growing season. Second, one female plant in a noncompetitive environment has been documented to produce up to 4.8 million seeds (Hartzler et al. 2004). In the soil, seeds has been found for up to 17 years with 3% viability (Burnside et al. 1996). Third, waterhemp is dioecious, which results in vast genetic diversity and rapid evolution with an increased the likelihood of evolution of herbicide resistance due to obligate outcrossing, unlike most other *Amaranthus* species (Costea et al. 2005). Past research in Ontario documented that waterhemp interference can reduce soybean yield by up to 73% (Vyn et al. 2007). Waterhemp has the potential to become one of the most problematic weeds in Ontario, as it is in the midwestern United States. Through waterhemp's ability to thrive in many agricultural production areas due to prolific seed production, extended emergence pattern, and high genetic diversity, it is also make it likely to evolve herbicide resistance.

In the U.S., GR waterhemp was first reported in Missouri (Legleiter and Bradley 2008) and has now been documented in 18 states (Heap 2016). Waterhemp is estimated to occur on 1.2 million hectares (Light et. al 2011) and is considered one of the most problematic weeds in the U.S. corn belt (Hager and Sprague 2002; Sarangi et al. 2015). Waterhemp has been found to be resistant to six unique herbicide groups, each with a different chemical mode of action – Group 2, 4, 5, 9, 14 and 27 (Heap 2016). A waterhemp population has been discovered in Illinios with multiple herbicide resistance to five modes of action, including Group 2 (ALS), Group 4 (growth regulator), Group 5 (triazine), Group 14 (PPO), and Group 27 (Heap 2016). Resistance has also been found in a population to four modes of action including Groups 2, 5, 9 and 14 (Tranel et al. 2011; Bell et al. 2013). Group 27 (HPPD) resistance has also been discovered, co-occurring with resistance from two additional sites of action, Group 2 (ALS) and Group 5 (triazine) herbicides

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

(McMullan and Green 2011). Past research in the U.S. has demonstrated the scale of herbicide resistant waterhemp and the concomitant decrease in the number of effective herbicide options for waterhemp control.

Past research demonstrates the shift in effective options of control in waterhemp over time through herbicide resistance. Research Vyn et al. (2007) identified the most efficacious herbicides for the control of Group 2 and 5 resistant waterhemp from studies conducted in Essex and Lambton counties. Control PRE with metolachlor, dimethenamid, and linuron was found to exceed 80% with metolachlor plus metribuzin providing 94% 10 WAA (Vyn et al. 2007). Control POST was achieved with acifluorfen, fomesafen, imazamox plus fomesafen, and glyphosate (Vyn et al. 2007). In 2014, a grower in Lambton County reported poor control of waterhemp with glyphosate in a soybean field. Glyphosate-resistant soybean were grown continuously on this field for nine years with glyphosate applied two times per year, with the exception of one application of imazethapyr in those nine years. Samples of waterhemp seed and leaf tissue were taken from the site in Lambton County for testing (see results below). Further tests revealed this was the first known case of GR waterhemp in Canada.

The objective of this survey was to document the distribution of waterhemp resistant to herbicide Groups 2, 5 and 9 across three counties in southwestern Ontario.

Materials and Methods

Seed collection

A total of 49 waterhemp seed samples were collected from fields in southwestern Ontario in 2014 and 2015. In 2014, only one seed sample was collected from the field with the first confirmed case of GR waterhemp on Walpole Island, ON. Of the 48 samples collected in 2015,

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

14 were from Essex, 2 from Chatham-Kent, and 32 from Lambton County. Of the 32 from Lambton County, 27 were collected in new, individual fields on Walpole Island.

Once waterhemp escapes were identified in a field the following information was recorded: date observed, GPS coordinates, field size, percent of field infested with waterhemp, other weed species present, and a photo was taken to ensure later screening information could be linked to that site. These methods are similar to the waterhemp survey conducted by Rosenbaum and Bradley (2013) in Missouri, USA. Waterhemp seed was collected and combined from at least 20 (where available) plants from mature soybean fields near harvest (September or October). All seed was collected by hand from mature female plants and placed in a paper bag; each bag was given a unique number to identify the field location. The survey first focused on Walpole Island near the initial confirmed site. In addition, seeds were collected from fields identified by agricultural retailers and growers in southwestern Ontario with poor control of waterhemp. Finally, seed was collected in areas of previously-known waterhemp populations in southwestern Ontario (Costea et al. 2005; Vyn et al. 2007). The survey methodology used was similar to previous published research for other herbicide resistant weeds in Ontario (Falk et al. 2005; Vink et al. 2012; Byker et al. 2013; Follings et al. 2013; VanWely et al. 2015).

Resistance screening

Waterhemp seeds were stratified by refrigeration at 4°C for 8 weeks. To prepare the seeds for refrigeration, seeds were placed in labeled nylon bags and buried in moist sand in plastic trays. After waterhemp seed was stratified, seeds were spread in germination trays that were half filled with soilless mixture (Pro-Mix PXG), and then covered with a thin layer of the soilless mixture. The trays were placed in a greenhouse with a 16-hour photoperiod with a day/night temperature of 25/18°C. When the seedlings reached the cotyledon stage, 60 plants from each population

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

were transplanted into 10-cm diameter individual pots. This provided adequate plants to select 42 uniform plants for herbicide (glyphosate, atrazine, imazethapyr) screening. Fourteen plants were used for each herbicide application, of which 12 were sprayed and two plants were unsprayed and served as the control. In addition to the seed samples collected for the survey, three populations were used as references, which included a GS waterhemp population from Petrolia, ON, a GR population, and a population with confirmed resistance to Group 2, 5 and 9 herbicides.

The primary objective of this survey was to ascertain the distribution of GR waterhemp in southwestern Ontario, but as previously mentioned, there is known multiple herbicide resistance in waterhemp. To gain a more complete understanding of the spectrum of herbicide resistance in waterhemp in Ontario, each population was screened for resistance to imazethapyr (75 g ha^{-1}) plus Agral 90 (0.2% v/v), atrazine (1000 g ha^{-1}) plus Assist (1.0% v/v) and glyphosate (900 g a.e. ha^{-1}) representing a Group 2, 5 and 9 herbicide, respectively. Herbicides were applied when waterhemp was approximately 10-cm in height in a spray chamber with a flat fan nozzle calibrated to apply 200 L ha^{-1} at 2.15 km hr^{-1} and 280 kPa. When the herbicide application was complete, sprayed plants were left to dry in the spray area before placing the plants in the greenhouse.

The assessments for resistance included a visual control estimate of biomass reduction at 1, 3, and 5 wks after application (WAA). Waterhemp biomass reduction was rated 0 to 100% scale relative to the untreated control, with 100% representing complete plant necrosis and death. At 5 WAA, each of the twelve plants from each screen were classified as either susceptible or resistant to obtain the frequency of resistance to each herbicide from each population (Beckie et al. 2000).

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Results and Discussion:

Preliminary tests

Studies included a greenhouse glyphosate biologically effective rate study at the University of Guelph, Ridgetown Campus (Schryver et al. 2017), and gene amplification testing conducted at the University of Illinois using a bio-leaf assay. The biologically effect rate study found a resistance factor ranging from 4.7 (Schryver et al. 2017). The leaf assay revealed that this waterhemp population had 6-13 extra copies of the EPSPS gene (Tranel, unpublished data). In addition, 60% of the waterhemp had Group 2 resistance due to an altered target site (Trp574Leu) (Tranel, unpublished data).

Resistant screening

The GS populations, following the application of glyphosate, behaved as expected with no surviving plants at the end of the assessment period. The symptoms included chlorosis beginning in the growing point, followed by necrosis, and plant death. For the GR populations, following the application of glyphosate, there was slight chlorosis and necrosis in the apical meristem.. Following the application of atrazine, the atrazine resistant plants developed marginal chlorosis and necrosis of the older leaves while there were no symptoms on the newly formed leaves. Following the application of imazethapyr, the resistant biotypes had slight, but transient, chlorosis in the growing point. Symptoms in all resistant biotypes were transient and decreased with time, with little to no injury observed 5 WAA.

Group 9 resistant waterhemp

Waterhemp from 40 of the 49 seed samples (82%) collected in 2014 and 2015 had at least some individuals that were resistant to glyphosate. Resistant populations were found in Essex,

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Chatham-Kent, and Lambton counties (Figure 1). This survey indicates that GR waterhemp is present in the three counties on at least 40 field sites. Resistance ranged in frequency from 8 to 100% in the collected fields with an average of 44% (Figure 2).

GR waterhemp can spread by both natural means as well as human activities. Dispersal of resistance genes through natural means is through pollen as well as seed movement by birds and water. Seed dispersal due to human activities include movement on equipment (combines, tillage equipment, trucks) and by removal of crops contaminated with waterhemp seed (see discussion below). Interestingly, some populations on Walpole Island within one kilometer of each other ranged from 0 to 100% resistant to glyphosate. This may be a reflection of field specific weed management practices over the past decade. Waterhemp pollen has been documented to travel up to 800 m but typically remains within 50 m of the male plant, which may explain the wide range in resistance in a very small area (Liu et al. 2012). Glyphosate-resistant waterhemp has not been found in a wide geographic area across all of southwestern Ontario. Instead, there are three primary areas where it has been observed: Walpole Island, Lambton County; near Petrolia, Lambton County; and near Cottam, Essex County. The distance between the two furthest locations is approximately 150 km. The rather wide geographical distribution suggests that there was independent selection in a number of fields in the province due to historical weed management practices.

Group 2 resistant waterhemp

All seed samples had individual plants that were resistant to the Group 2 herbicide imazethapyr. Group 2 resistant waterhemp has been documented in Essex, Chatham-Kent, and Lambton counties (Figure 3). The frequency of Group 2 resistance ranged from 42 to 100%

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

(Figure 4) among these populations with an average of 88%. The waterhemp populations collected near Petrolia in Lambton County had the highest level of Group 2 resistance with a range of 75-100% (data not shown).

Group 5 resistant waterhemp

Seventy-five percent (37 of 49 fields) of the seed samples had individuals that were resistant to the Group 5 herbicide atrazine. The Group 5 resistant populations were found in Lambton and Essex counties (Figure 5) and the proportion of waterhemp plants resistant to atrazine within each population varied between 0 and 100% with an average of 31% (Figure 6). This average is lower than Group 2 or 9 resistance frequencies and could be attributed to a potential increase in fitness cost of atrazine when comparing to that of imazethapyr or glyphosate. Interestingly, the highest proportion of Group 5 resistant individuals was from seed samples collected near Petrolia, Lambton County (83-100%); however, these seed samples had the lowest proportion (8-17%) of Group 9 (glyphosate) resistance (data not shown). It is hypothesized that this is a reflection of historical herbicide use patterns in this area with lower reliance on Group 9 herbicides and more frequent use of Group 5 herbicides.

Multiple resistant waterhemp

Seventy-six percent of the seed samples had individual plants that were resistant to both a Group 2 and 5 herbicide (Figure 7), 82% of seed samples contained resistance to both a Group 2 and 9 herbicide (Figure 8), 61% of the total fields contained resistance to both a Group 5 and 9 herbicide (Figure 9), and 61% of the seed samples contained 3-way resistance to a herbicide in each of Groups 2, 5 and 9 (Figure 10).

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Waterhemp seed was collected non-randomly when escaped waterhemp plants were visible from the road, which biased the current study towards sampling herbicide resistant populations rather than sensitive populations. The high proportion of GR waterhemp documented in this study is driven by the high usage of glyphosate in the survey area for weed control; this inference is supported by the pesticide use survey published by OMAFRA in 2010. In the provincial survey, glyphosate use had increased by 76% from 2003 to 2008, which was largely associated with the adoption of glyphosate resistant crops (OMAFRA 2010). Consequently, there is a high probability that the waterhemp plants from which seeds were collected were sprayed with glyphosate. However, it is important to note that waterhemp emerges over an extended period of time and some late emerging plants may not have been exposed to glyphosate. Waterhemp populations with the higher proportion of glyphosate resistant individuals were frequently from small patches or in strips in the field. A small patch of waterhemp in a field treated with glyphosate suggests incipient evolution of glyphosate resistance. Strips in the field may be attributed to dispersal with equipment such as a combine. It has been documented that the majority of waterhemp seed that passes through a combine is viable and is returned to the soil seedbank with very little seed destruction (Schwartz et al. 2016).

Dispersal and resistance evolution in waterhemp

Glyphosate resistant waterhemp may evolve within a field, be transported into a field by tillage and harvest equipment, or be transported by natural means such as birds. Research conducted by Davis et al. (2008) categorized four origins of weed seed in crop fields, including: 1) undispersed seeds that remained on the mother plant; 2) seed dispersed on the soil surface; 3) seed dispersed in the current year by harvest equipment; and 4) seed dispersed in prior years that

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

remained in the soil seedbank. Of the many species researched, waterhemp was in all four origin groups demonstrating the high possibility of field-to-field movement (Davis et al. 2008). Waterhemp seed can also be dispersed by waterfowl (Green and Martin 2015; Farmer et al. 2016). Research conducted on waterfowl in the midwestern U.S. found 8 *Amaranthus* seeds per duck, found in their digestive tract. With an estimated 49 million migrating ducks, an estimated 882 million *Amaranthus* seeds could be transported distances as far as 2800 km in one year (Farmer et al. 2016). The management of waterhemp seed dispersal is very complex due to the number of seeds one plant can produce and the number of methods by which viable seeds can be moved from field-to-field.

Due to prolonged viability in the soil, there is the potential of movement from one field to another with tillage equipment. In addition to tillage equipment, harvest equipment has the potential of moving waterhemp seed from field-to-field. Waterhemp seed may be lodged in a combine and become trapped before shaking free in another field. This is of increasing importance with the growth in farm size and the need to move equipment large distances. As growers increasingly adopt cover crops, many use a mixture of low-cost seed for these crops, which may contain weed seeds (Green and Martin 2015). Through genetics, future research could explore and track the origins of waterhemp seed and herbicide resistance gene flow aiding in the mitigation of further herbicide selection and waterhemp infestation. In summary, natural means and human actions contribute to the movement of GR waterhemp seed.

Implications

A reduction in the selection pressure for GR waterhemp and the reduction in spread of resistant populations are imperative to minimize economic losses. The overreliance on a single management strategy or a simplified crop rotation may have short-term advantages such as

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

simplicity and possible short-term profit maximization, but may have long-term detrimental effects due to the evolution of herbicide resistant weed populations. A more sustainable approach is to use a diverse crop rotation with multiple weed management tactics. A diverse crop rotation should include crops with different seeding and harvesting times, crops with different row widths and seeding densities, the inclusion of cover and companion crops, and the use of multiple herbicide modes of action. An important step in depleting waterhemp seed in the soil seed banks is to strive for near-perfect weed control and to remove any waterhemp escapes prior to seed maturation. Proper cleaning of tillage implements, equipment tires, and combines are recommended to reduce seed movement. The above strategies will limit the selection of herbicide resistant waterhemp and reduce its movement from field to field.

References

Beckie, H.J., Heap, I.M., Smeda, R.J., and Hall L.M. 2000. Screening for herbicide resistance in weeds. Weed Technol. **14:** 428-445.

Bell, S.M., Hager, G.A., and Tranel P.J. 2013. Multiple resistance to herbicides from four sites-of action groups in waterhemp (*Amaranthus tuberculatus*). Weed Sci. **61**: 460-468.Burnside, O.C., Wilson, R.G., Weisberg, S., and Hubbard, K.G. 1996. Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Sci. **44:** 74-86.

Byker, H.P., Soltani, N., Robinson, D.E., Tardif, F.J., Lawton, M.B., and Sikkema, P.H. 2013. Occurrence of glyphosate and cloransulam resistant Canada fleabane (Conyza canadensis (L.) Cronq.) in Ontario. Can. J. Plant Sci. **93**: 851-855.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Costea, M., Weaver, S.E., and Tardif, F.J. 2005. The biology of invasive alien plants in Canada. 3. *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif. Can. J. Plant Sci. **85**: 507-522.

Davis, A.S. 2008. Weed Seed Pools Concurrent with Corn and Soybean Harvest in Illinois. Weed Sci. **56**: 503-508.

Falk, J.S., Shoup, D.E., Al-Khatib, K., and Peterson, D.E. 2005. Survey of common waterhemp (*Amaranthus rudis*) response to protox- and ALS-inhibiting herbicides in northeast Kansas. Weed Technol. **19**: 838–846.

Farmer, J.A., Bish, M.D., Long, A.R., Biggs, M.E., and Bradley K.W. 2016. Next Day Air: Waterfowl and Weed Seed Distribution. University of Missouri. Poster session presented at: WSSA Annual Conference, Puerto Rico.

Follings, J., Soltani, N., Robinson, D.E., Tardif, F., Lawton, M., and Sikkema, P.H. 2013. Distribution of glyphosate and cloransulam-methyl resistant giant ragweed (*Ambrosia trifida* L.*)* populations in southern Ontario. Ag. Sci. **4**: 570-576.

Green, J.D., and Martin R.J. 2015. Introduction and spread of glyphosate-resistant palmer amaranth and waterhemp across Kentucky- The past five years. University of Kentucky. Presentation at NCWSS Dec. 2015.

Hager, A. and Sprague C. 2002. Weeds on the horizon. in Steffey K. The Pest Management and Crop Development Bulletin, 6 Champaign, IL University of Illinois.

Hartzler, R.G., Battles, B.A., and Nordby, D. 2004. Effect of common waterhemp (*Amaranthus rudis*) emergence date on growth and fecundity in soybean. Weed Sci. **52**: 242-245.

Heap, I. 2016. The International Survey of Herbicide Resistant Weeds. Available at www.weedscience.com (accessed July, 2016)

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Legleiter, T.R., and K.W. Bradley. 2008. Glyphosate and multiple herbicide resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. Weed Sci. **56**:582–587.

Light, G.G., Mohammed, M.Y., Dotray, P.A., Chandler, J.M., and Wright, R.J. 2011. Glyphosate-resistant common waterhemp (*Amaranthus rudis*) confirmed in Texas. Weed Technol. **25**: 480-485.

Liu, J., Davis, A.S., and Tranel, P.J. 2012. Pollen biology and dispersal dynamics in waterhemp (*Amaranthus tuberculatus*). Weed Sci. **60**(3): 416-422.

McMullan, P.M., and Green J.M. 2011. Identification of a tall waterhemp (*Amaranthus tuberculatus*) biotype resistant to HPPD-inhibiting herbicides, atrazine, and thifensulfuron in Iowa. Weed Technol. **25**: 514–518

OMAFRA. 2010. Economics Information - Survey of pesticide use in Ontario. 2008 Estimates of pesticides used on field crops, fruit and vegetable crops, and other agricultural crops. Available online: http://www.omafra.gov.on.ca/english/crops/facts/pesticide-use.htm#3

OMAFRA. 2016. Herbicide Resistant Weed Maps Field Crop News. Mike Cowbrough. Available online: http://fieldcropnews.com/2016/03/herbicide-resistant-weed-maps/

Rosenbaum, K.K., and Bradley, W.K. 2013. A Survey of Glyphosate-Resistant Waterhemp (*Amaranthus rudis*) in Missouri soybean fields and prediction of glyphosate resistance in future waterhemp populations based on in-field observations and management practices. Weed Technol. **27**: 656-663.

Sarangi, D., Sandell, L.D., Knezevic, S.Z., Aulakh, J.S., Lindquist, J.L., Irmak, S., and Jhala, A.J. 2015. Confirmation and control of glyphosate-resistant common waterhemp (*Amaranthus rudis*) in Nebraska. Weed Technol. **29**: 82–92.

Schryver, M.G., Soltani, N., Hooker, D.C., Tranel, P.J., Robinson, D. E., Sikkema P.H. 2017. Glyphosate resistant waterhemp (*Amaranthus tuberculatus* var. *rudis*) control in soybean (*Glycine max*) using one and two-pass weed control strategies in Ontario. Unpublished.

Schwartz, L.M., Norsworthy, J.K., Young, B.G., Bradley K.W., Kruger G.R., Davis V.M., Steckel L.E., and Walsh M.J. 2016. Waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*) seed production and retention at soybean harvest. Weed Technol. **30**: 284–290.

Tranel, P.J., Riggins, C.W., Bell, M.S., and Hager, A.G. 2011. Herbicide resistances in *Amaranthus tuberculatus*: a call for new options. J Agric Food Chem. **59**:5808–5812.

VanWely, A.C., Soltani, N., Robinson, D.E., Hooker, D.C., Lawton, M.B., and Sikkema, P.H. 2015. Glyphosate and acetolactate synthase inhibitor resistant common ragweed (*Ambrosia artemisiifolia* L.) in southwestern Ontario. Can. J. Plant Sci. **95:** 335-338.

Vink, J., Soltani, N., Robinson, D., Tardif, F., Lawton, M.L., and Sikkema, P.H. 2012. Occurrence and distribution of glyphosate-resistant giant ragweed (*Ambrosia trifida* L.) in southwestern Ontario. Can. J. Plant Sci. **92(3)**: 533-539.

Vyn, J.D., Sikkema, P.H., and Swanton, C.J. 2006. MSc.Thesis- Biology and control of common waterhemp (*Amaranthus Tuberculaus* Var. Rudis). Appendix 1: Growth comparison of common waterhemp (*Amartanthus tuberculatus* var rudis) and tall waterhemp (*Amaranthus tuberculatus* var. Tuberculatus). p 85-90.

Vyn, J.D., Swanton, C.J., Weaver, S.E., and Sikkema, P.H. 2007. Control of herbicide-resistant common waterhemp (*Amaranthus tuberculatus* var. *rudis*) with pre- and post-emergence herbicides in soybean. Can. J. Plant Sci. **87**(1): 175-182.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

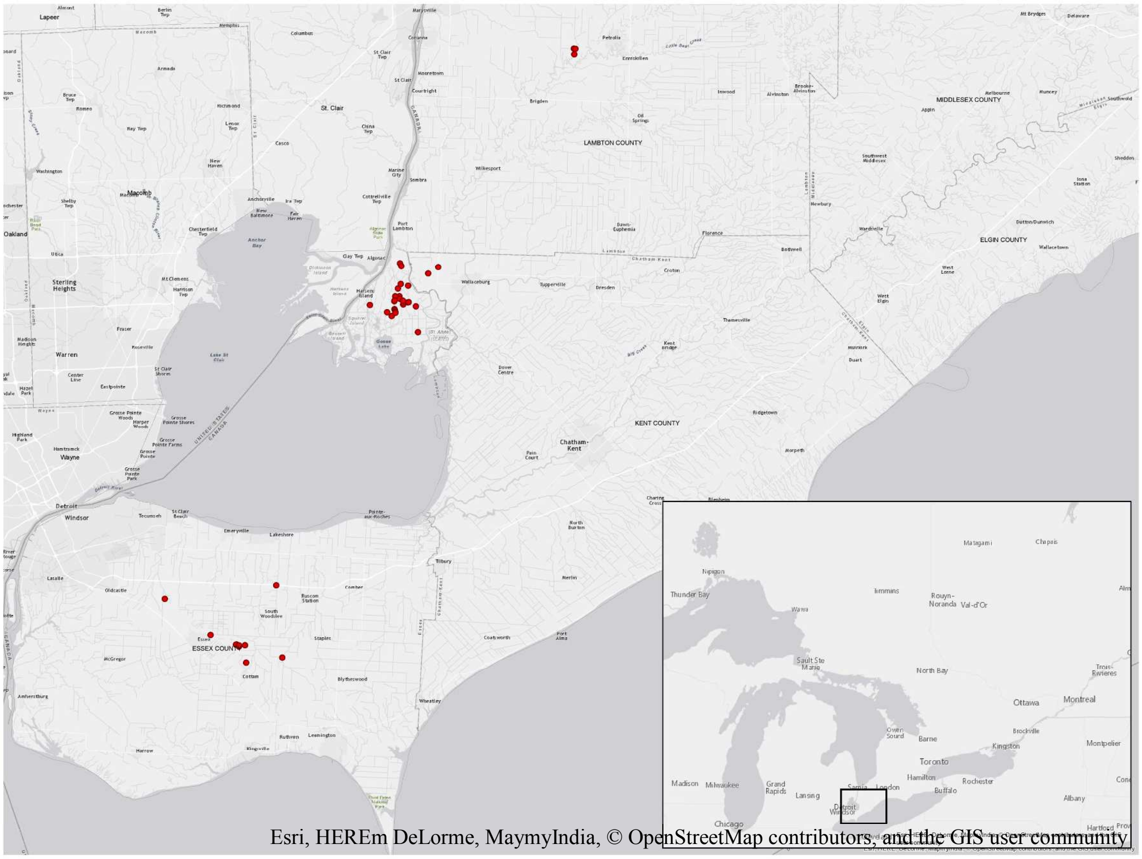

Figure 1. Location of 40 fields with Group 9 resistant common waterhemp in Essex, Chatham-Kent and Lambton counties in Ontario, Canada during 2014 and 2015.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

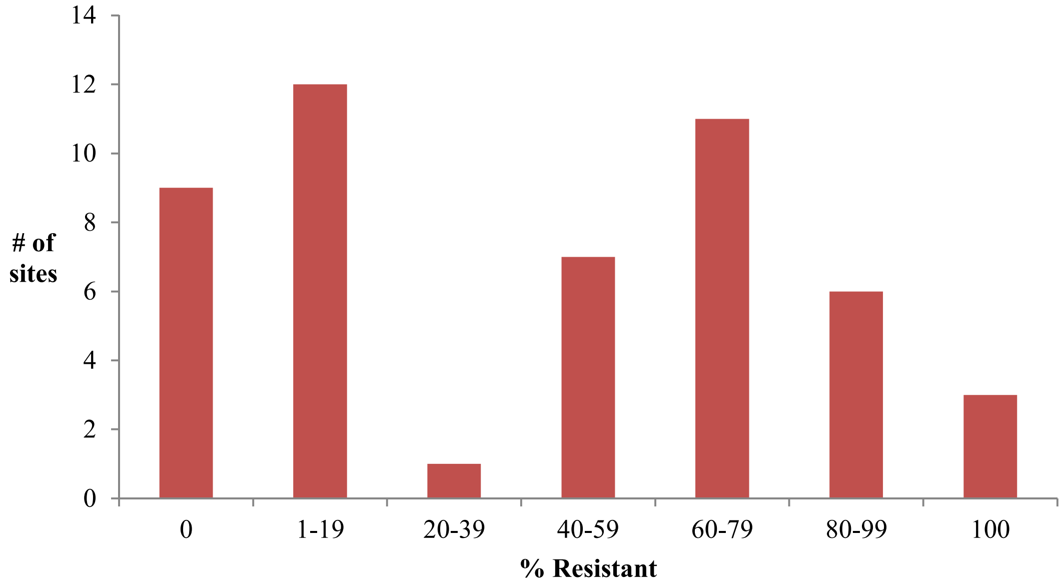

Figure 2. Incidence of resistance to glyphosate and frequency of GR individuals per population across 49 common waterhemp populations collected in Ontario, Canada during 2014 and 2015.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

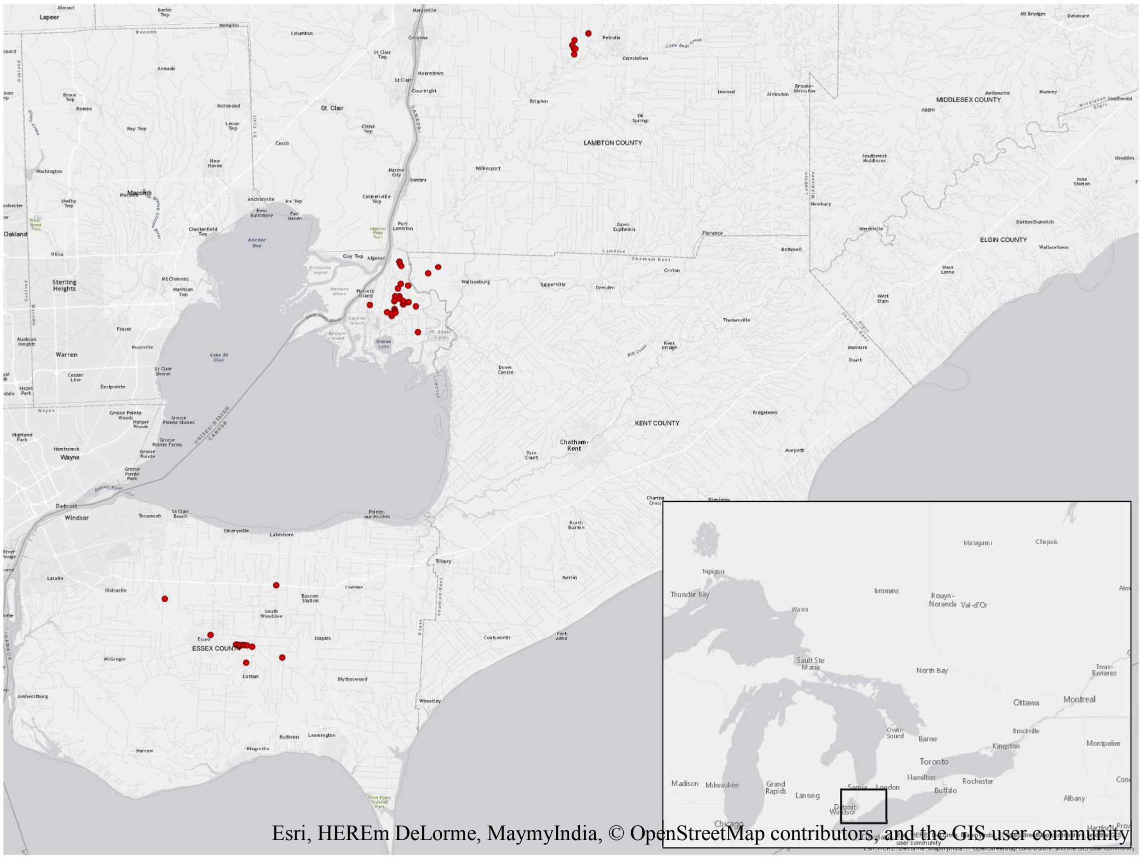

Figure 3. Location of 49 fields with Group 2 resistant common waterhemp in Essex, Chatham-Kent and Lambton, counties in Ontario, Canada during 2014 and 2015.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

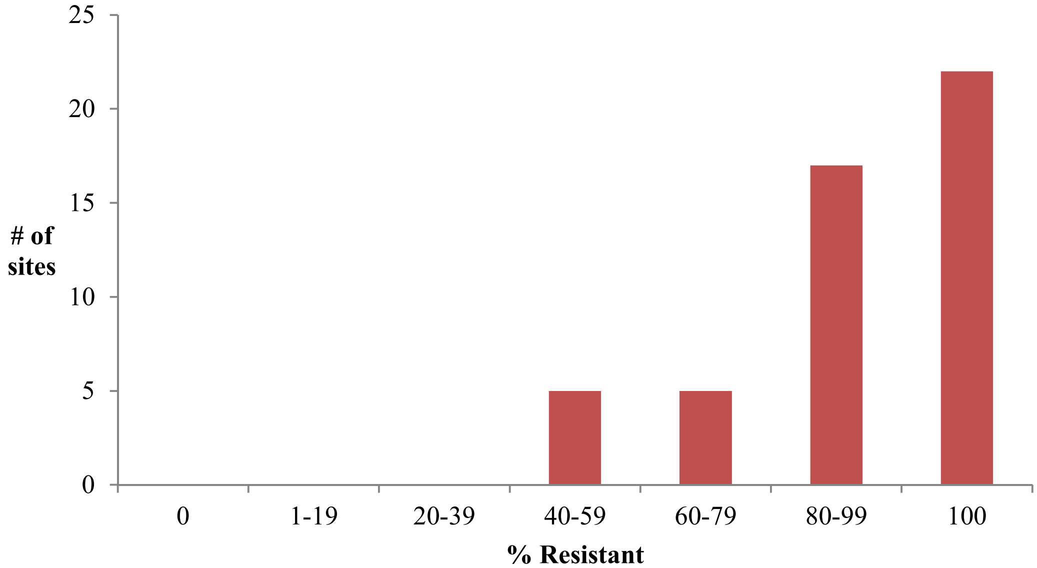

Figure 4. Incidence of resistance to imazethapyr and frequency of resistant individuals per population across 49 common waterhemp populations collected in Ontario, Canada during 2014 and 2015.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

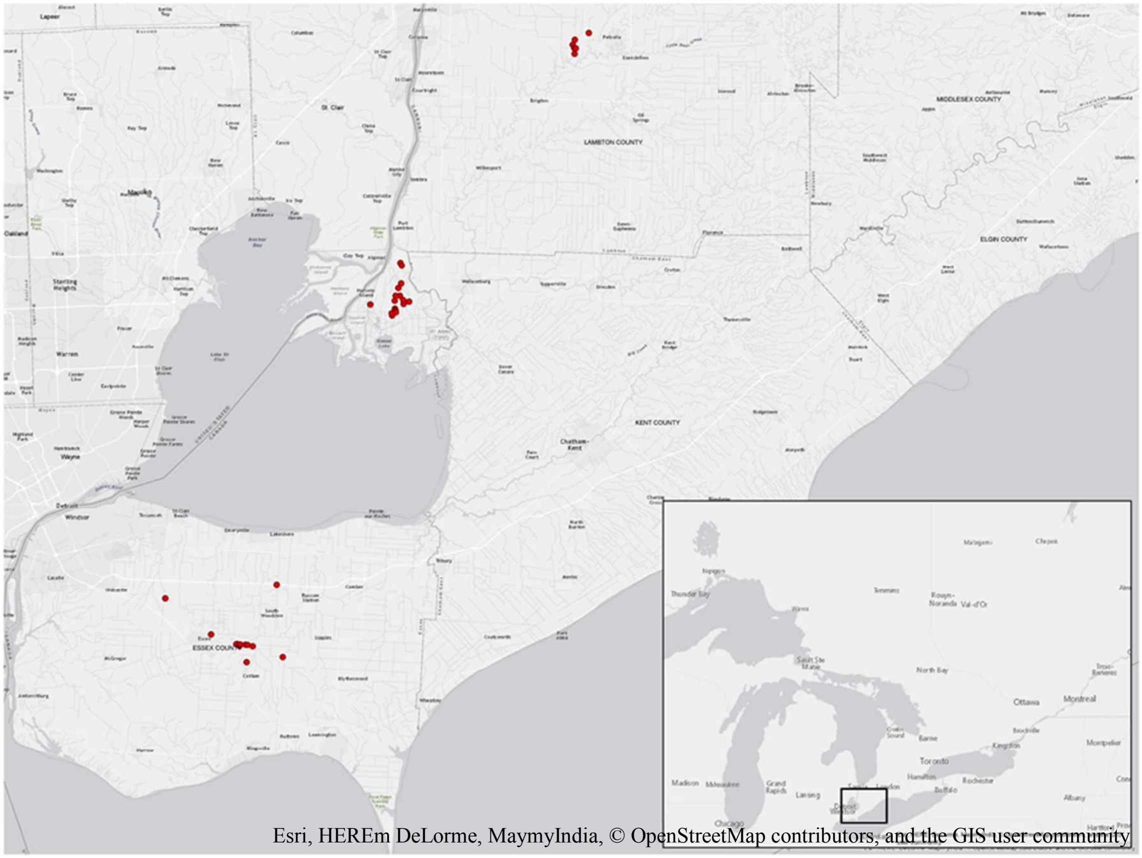

Figure 5. Location of 37 fields with Group 5 resistant waterhemp in Essex and Lambton counties in Ontario, Canada during 2014 and 2015.

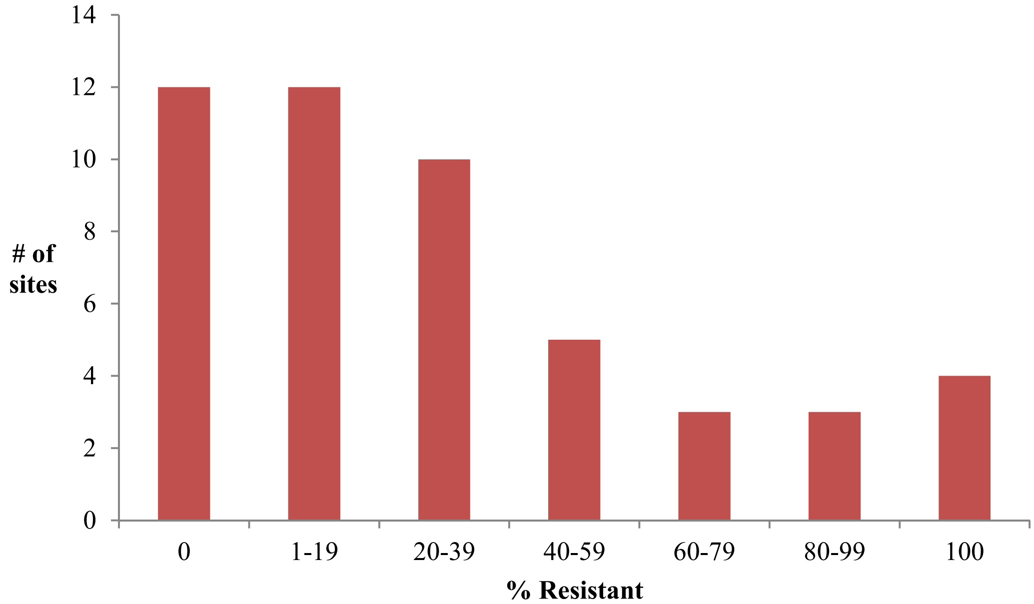

Figure 6. Incidence of resistance to atrazine and frequency of resistant individuals per population across 37 waterhemp populations collected in Ontario, Canada during 2014 and 2015.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

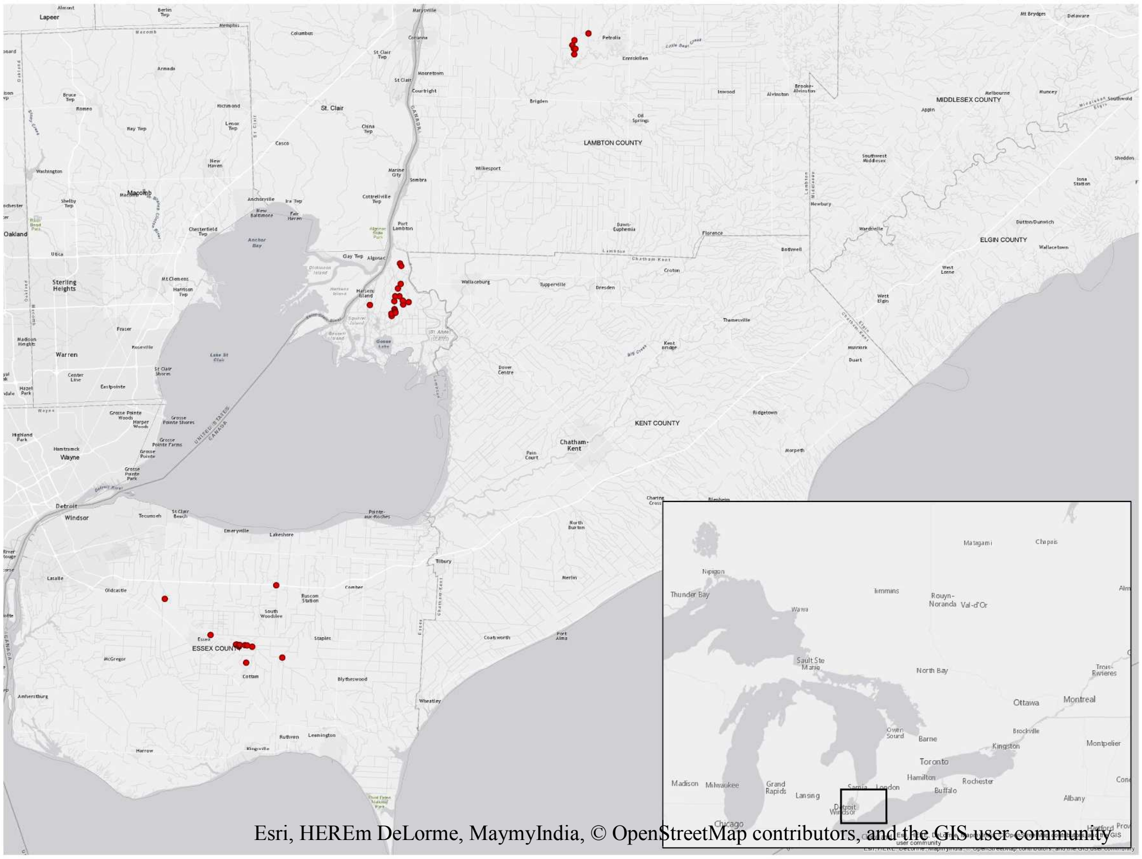

Figure 7. Location of 37 fields with Group 2 and 5 resistant waterhemp in Essex and Lambton counties in Ontario, Canada during 2014 and 2015.

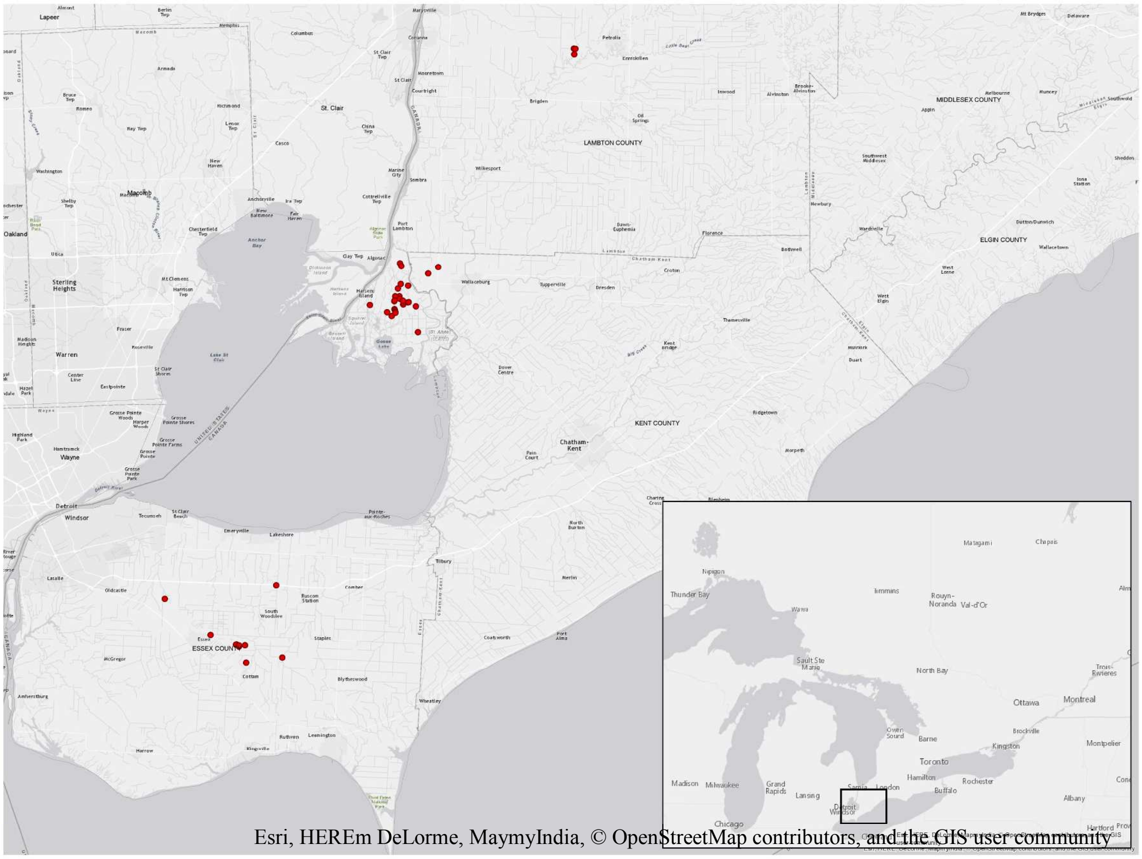

Figure 8. Location of 40 fields with Group 2 and 9 resistant waterhemp in Essex, Chatham-Kent and Lambton counties in Ontario, Canada during 2014 and 2015.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

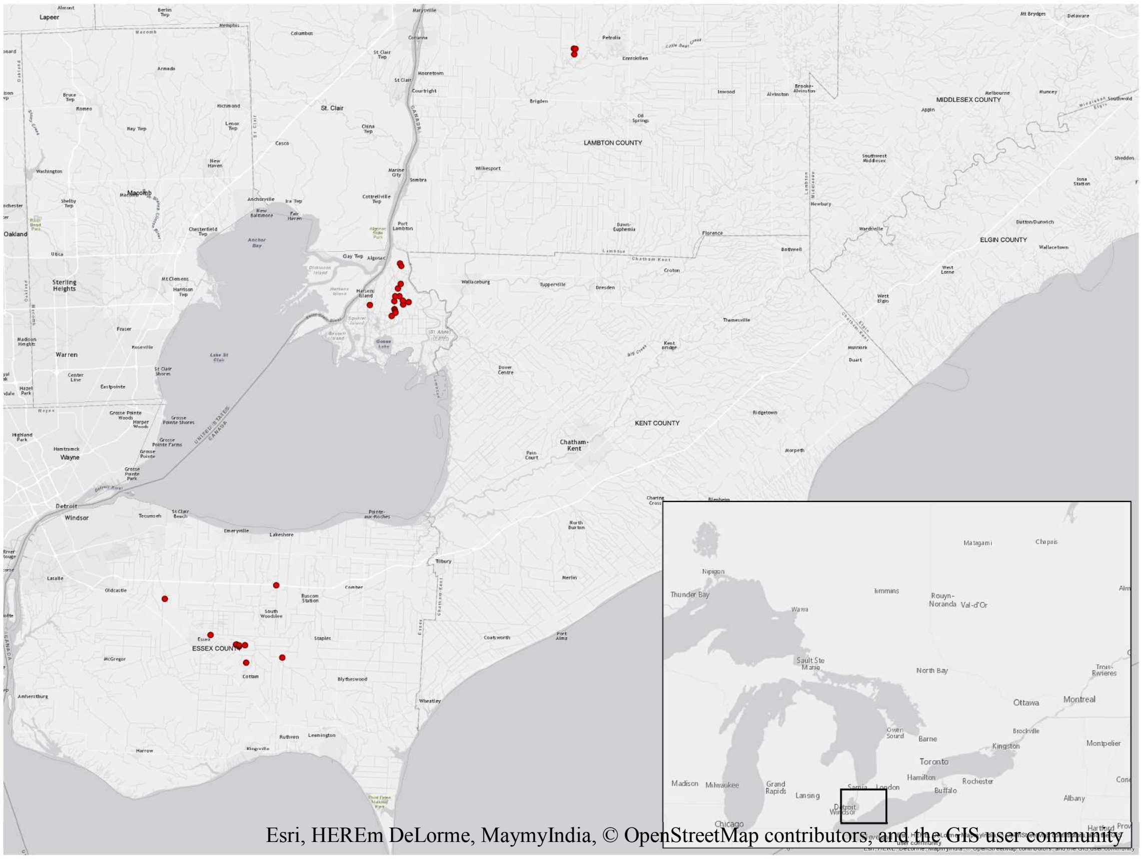

Figure 9. Location of 30 fields with Group 5 and 9 resistant waterhemp in Essex and Lambton counties in Ontario, Canada during 2014 and 2015.

Can. J. Plant Sci. Downloaded from www.nrcresearchpress.com by NANJING UNIV LIB on 05/12/17
For personal use only. This Just-IN manuscript is the accepted manuscript prior to copy editing and page composition. It may differ from the final official version of record.

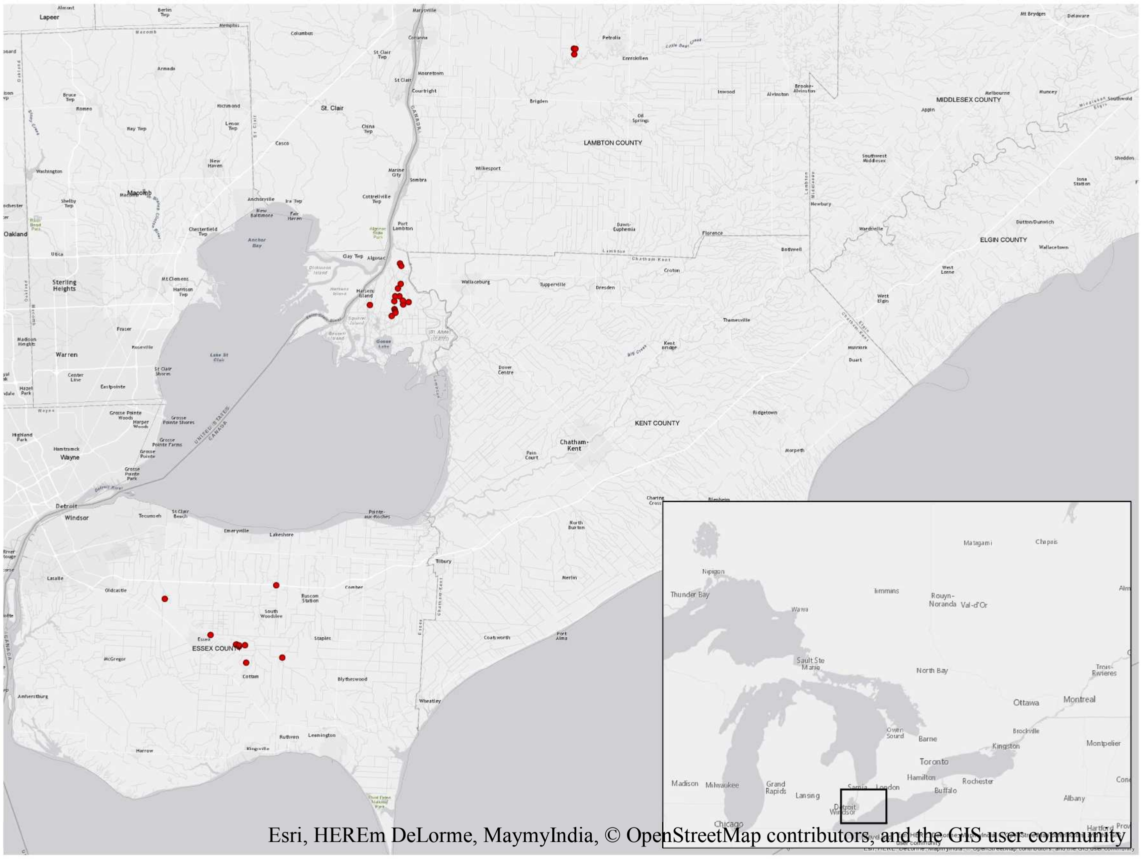

Figure 10. Location of 30 fields with Group 2, 5 and 9 resistant waterhemp in Essex and Lambton counties in in Ontario, Canada during 2014 and 2015.