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Enquête sur la petite herbe à poux (*Ambrosia artemisiifolia* L.) résistante à l’imazéthapyr au Québec

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Article abstract

Common ragweed (*Ambrosia artemisiifolia* L.) is widespread in soybean (*Glycine max* L.) fields in southern Québec. Biotypes resistant to ALS (group 2) herbicides are commonly reported in conventional crops where these herbicides are used. Reported cases are voluntary and potentially underestimate the occurrence of resistance. A survey was therefore undertaken in 2014 and 2015 in soybean fields treated with a Group 2 herbicide. Common ragweed seeds were collected from 123 fields. Seedlings were grown and tested for resistance using the recommended rate of imazethapyr (100.8 g a.e. ha^{-1}). Weed populations were classified as susceptible, developing resistance (less than one third of plants classified as resistant) or resistant (at least one third of plants were resistant). Twenty populations were then selected based on these resistance levels and treated with four doses of the herbicide (0, 100.8, 201.6, and 403.2 g a.e. ha^{-1}). Resistance to imazethapyr was detected in 81% of samples (21.1% were classified as developing resistance and 59.4% were classified as resistant). Populations classified as developing resistance had a resistance factor of 1.04, while populations classified as resistant had a resistance factor greater than 5. These results confirm the presence of multiple populations of imazethapyr-resistant common ragweed in Quebec.

Survey of imazethapyr-resistant common ragweed (*Ambrosia artemisiifolia* L.) in Quebec

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Keywords: acetolactate synthase (ALS) inhibitor, herbicide, resistance, weed.

[Enquête sur la petite herbe à poux (*Ambrosia artemisiifolia* L.) résistante à l'imazéthapyr au Québec]

La petite herbe à poux (*Ambrosia artemisiifolia* L.) est très fréquente dans les champs de soya (*Glycine max* L.) du Québec méridional. Des biotypes résistants aux herbicides qui inhibent l'acétolactate synthase (ALS) (Groupe 2) sont fréquemment signalés dans les champs où ces herbicides sont utilisés. Les cas rapportés se font sur une base volontaire et sous-estiment potentiellement la fréquence réelle de la résistance. Une enquête a donc été réalisée en 2014 et en 2015 dans des champs de soya traités avec un herbicide du groupe 2. Des graines ont été récoltées dans 123 champs. Des plantules ont été testées pour leur résistance avec une dose recommandée d'imazéthapyr (100,8 g e.a. ha^{-1}). Les populations ont été classifiées sensibles, résistantes (au moins un tiers des plants étaient résistants) ou présentant une résistance en développement (moins du tiers des plants étaient résistants). Vingt populations ont ensuite été sélectionnées selon leur degré de résistance et traitées avec quatre doses d'herbicide (0; 100,8; 201,6 et 403,2 g e.a. ha^{-1}). De la résistance à l'imazéthapyr a été détectée dans 81 % des échantillons (21,1 % classifiés avec une résistance en développement et 59,4 % classifiés résistants). Le facteur de résistance des populations avec de la résistance en développement était de 1,04 et celui des populations résistantes était supérieur à 5. Ces résultats confirment la présence de multiples populations de petite herbe à poux résistantes à l'imazéthapyr au Québec.

Mots clés : inhibiteurs de l'acétolactate synthase (ALS), herbicide, résistance, mauvaise herbe.

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INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia* L.) is an annual plant that is native to central North America and is now found in South America, Australia, Europe, and Asia, where it is considered an invasive species (Basset and Crompton 1975; Gaudeul *et al.* 2011). Common ragweed grows along roadsides, on waste ground, in borders, and on cropland. This weed is widespread in southern Quebec and southern Ontario, particularly in soybean crops (Basset and Crompton 1975).

Group 2 herbicides (Group B, international classification system) are frequently used to control common ragweed in conventional (non-genetically modified) soybean. These herbicides inhibit acetolactate synthase (ALS), a key enzyme for the formation of branched-chain amino acids, namely, leucine, isoleucine, and valine (Shaner and Singh 1997). Since the mutations that confer resistance to ALS inhibitors are found in the nucleus DNA, resistance can spread via pollen and seeds (Tranel and Wright 2002). Resistance is also dominant, meaning that a plant is resistant if it has just one allele with a mutation that confers resistance. If a population is resistant to Group 2 herbicides, it may be resistant to one, several, or all of the herbicides in the group (Beckie and Tardif 2012). To date, only the Trp574Leu mutation has been identified as conferring resistance to Group 2 herbicides in common ragweed, and this mutation is broad-spectrum, meaning that it confers resistance to a number of chemical families in the group (Beckie and Tardif 2012; Patzoldt *et al.* 2001).

Since the detection of the first case of Group 2 resistance in Quebec in 2006–2007, the number of cases has increased to more than 33 for all species and herbicide groups combined, with more than half of those cases being common ragweed (Bernier 2012, 2015; Cuerrier 2017; Simard and Bernier 2014). Given that the number of reported cases relies on voluntary reporting and that the majority of cases of resistance have been found in conventional soybean in Montérégie, a more comprehensive inventory was warranted.

The main objective of this project was to conduct a survey of imazethapyr-resistant common ragweed in fields of conventional soybean varieties in the Montérégie region of Quebec. The secondary objective was to characterize the level of resistance of the populations by means of response curves.

MATERIALS AND METHODS

Detection and identification

The survey project was widely publicized from the spring of 2014 until the fall of 2015. Common ragweed populations were selected in conventional (non-genetically modified) soybean fields that had been treated with various Group 2 herbicides and that were located (1) near fields where cases of resistance had already been confirmed in the past; (2) in fields suspected by stakeholders to contain a resistant population; (3) in fields where clumps of common ragweed were visible from the road; and (4) in fields with a history of equipment sharing (including contract work) with other fields containing populations that had already been confirmed to be resistant. In all cases, the common ragweed populations had to have survived herbicide treatments (plant height and stage are used as indicators of survival). Initial field visits were done in July and August in both 2014 and 2015.

Harvesting and seeding

Common ragweed populations identified during the initial summer visit were sampled during September and October before soybean harvest. A total of 123 populations were sampled (78 in 2014 and 45 in 2015). A minimum of 40 plants were collected per field. All plants were shaken above a plastic tub in order to collect mature seeds. Seeds were stored at 4 °C until use. Ten weeks before seeding, 15 g of seeds per population (inserted in organza bags [Uline, Milton, Ontario]) was placed between two 5-cm layers of moist sand and kept at 4 °C (Rousonelos *et al.* 2012; Willemsen 1975). Seeds from control herbicide susceptible populations were harvested from isolated organically managed fields located in the Montérégie area (pooled as one population). Seeds from control herbicide resistant populations were obtained from populations located in Ontario (François Tardif, University of Guelph, Guelph, ON, Canada). The seeds from each population were sown in multi-cell plant trays containing a mixture of one-part organic black earth to three parts Agro-Mix G5 transplanting mix (Fafard, Agawam, Massachusetts) (Saint-Louis *et al.* 2005). The trays were randomly placed (and relocated every week) in a greenhouse with a 16-h photoperiod (150 to 180 µmol $m^{-2} s^{-1}$) and a day/night temperature regime of 23 °C/21 °C. Four replicates of 15 seedlings per population and per treatment were tested for herbicide resistance, for a total of 14,760 seedlings (4 replicates × 15 seedlings × 123 populations × 2 treatments [0X–1X]). Plants from the two control populations, one susceptible and one resistant, were also treated (4 replicates × 15 seedlings × 2 controls × 2 treatments).

Treatments

The treatments were applied at the cotyledon-two leaf stage of common ragweed. Imazethapyr (Pursuit®, 240 g L^{-1}; BASF Canada, Mississauga, ON, Canada) was applied at a rate of 100.8 g a.e. ha^{-1}. Application was performed in the greenhouse by means of a backpack sprayer at a volume of 200 L ha^{-1}, a pressure of 165 kPa, and a travel speed of 3.2 km h^{-1}. The spray mixture with herbicide (1X) and without herbicide (0X) contained a nonionic surfactant at 0.25% v/v and a nitrogen solution (28-0-0; 2 L ha^{-1}).

For the response curves, 20 populations sampled in the first year were treated again in the second year with four imazethapyr treatments: no herbicide (0X), the standard rate (100.8 g a.e. ha^{-1}) (1X), twice the standard rate (201.6 g a.e. ha^{-1}) (2X), and four times the standard rate (403.2 g a.e. ha^{-1}) (4X). The 20 populations were selected based on seed availability and on the previously obtained diagnostic results, so that populations with different levels of resistance could be tested. Therefore, the selected populations consisted of two that were diagnosed as susceptible, six with developing resistance (defined below), and ten classified as resistant (defined below), in addition to one susceptible control and one resistant control, for a total of 4,800 treated seedlings (4 replicates × 15 seedlings × 20 populations × 4 treatments). For all the treatments, the plants were fertilized twice, one and three weeks after treatment (WAT), with a soluble mineral fertilizer (20-20-20) at a rate of 1 g L^{-1} of water.

Assessments

Two and four WAT, the plants were assessed visually on a graded injury scale from 0 to 100 (Brown and Farmer 1991; Ellis *et al.* 2010; Grey *et al.* 2006). At 4 WAT, the aboveground portion of each plant was harvested and dried at 55 °C for 24 h or until constant weight, in order to obtain aboveground dry biomass.

Diagnosis

A plant was declared resistant if, at 4 WAT, its visual assessment score was less than or equal to 20% injury. That threshold was established based on the visual assessment of the susceptible and resistant controls in the first trial. An entire population was diagnosed as susceptible if it contained no more than one resistant plant across all four replicates (Llewellyn and Powles 2001). A population was classified as developing resistance when more than one but less than one third of all the plants in a population were classified as resistant. Finally, a population was declared resistant if one third or more of its plants were classified as resistant (Llewellyn and Powles 2001).

Statistics

For all populations tested with two treatments (0X and 1X of imazethapyr standard rate), the mean visual injury score, the mean aboveground dry biomass, and the number of plants declared resistant were calculated. Data were analyzed using the MIXED procedure in the SAS software package, version 9.4, with populations treated as a fixed effect and replicates treated as a random effect. The analyses were carried out for each year separately. For the response curves for injury and biomass as a function of herbicide rate, the means were compared using a protected Fisher's LSD (least significant difference) test with a minimum threshold of $p = 0.05$. The curves for biomass reduction as a function of herbicide application rate were created in SAS using a logistic regression procedure (Knezevic *et al.* 2007; Seefeldt *et al.* 1995; Streibig 1980). The reduction in biomass as a function of herbicide application rate was fitted using the following equation for each classification (susceptible, developing resistance and resistant):

$$\frac{a}{(1 + exp^{-k(r-b)})}$$

where a is the asymptote, k is the growth rate, r is the herbicide application rate (g a.e. ha^{-1}), and b is the inflection point. The resistance factor was calculated by dividing the herbicide rate that reduces aboveground biomass by 50% (GR_{50}) for a resistant population by the GR_{50} for the susceptible control.

RESULTS

2014 and 2015 survey

During the survey, 78 and 45 populations of common ragweed were harvested in 2014 and 2015, respectively. In total, 19.5% of all the populations were diagnosed as susceptible, 21.1% were diagnosed as developing resistance, and 59.4% were diagnosed as resistant to imazethapyr (Table 1). Thus, 80.5% of the tested populations contained resistant common ragweed plants. This high rate of resistance is explained by the high frequency in natural populations of the allele that confers resistance and by the repeated use of Group 2 herbicides (Thill *et al.* 1994; Van Wely *et al.* 2015). Nevertheless, these results are lower than those obtained in the study by Van Wely *et al.* (2015), in which 100% of the 24 populations tested in Ontario were declared resistant.

Scouting and monitoring in the field are very important for ensuring the rapid identification and detection of populations that are developing resistance, because although crop yield will not be significantly affected by the first generations of such populations, their growth is exponential. A single resistant plant can produce offspring with the potential to cover 95% to 100% of the field in only three years (Norsworthy *et al.* 2014).

Response to herbicide rate

The response to increasing rates of imazethapyr was verified across 20 populations of common ragweed. The mean visual injury percentage for the susceptible populations was 70% after the application of the standard rate and it increased to 89% when the rate was four times the standard rate (Fig. 1).

In the populations developing resistance, the injury percentage was 63% when the standard rate was applied, reaching 74% and then 78% when the double and quadruple rates were applied, respectively (Fig. 1). There was a significant difference at the standard rate between the injury to the populations diagnosed as susceptible ($p = 0.009$) and control susceptible population ($p < 0.001$) and the injury to the populations developing resistance

Table 1. Number of fields containing susceptible, developing resistance, and resistant populations to imazethapyr in Montérégie, QC in 2014 and 2015

	2014		2015		2014 and 2015	
Common ragweed populations	Number of fields	Total (%)	Number of fields	Total (%)	Number of fields	Total (%)
Susceptible[a]	19	24.4	5	11.1	24	19.5
Developing resistance[b]	17	21.8	9	20.0	26	21.1
Resistant[c]	42	53.8	31	68.9	73	59.4
Total	78	100.0	45	100.0	123	100.0

[a] For a population to be classified as susceptible, no more than one plant could be resistant to imazethapyr.
[b] For a population to be classified as developing resistance, more than one but less than one third of its plants had to be resistant to imazethapyr.
[c] For a population to be classified as resistant, one third or more of its plants had to be resistant to imazethapyr.

(Fig. 1), and that difference was maintained as the rates increased, because of a few resistant plants that lower the mean population injury for the populations developing resistance (Fig. 1). The injury levels remained low in the populations classified as resistant, at 22%, 30%, and 34%, as herbicide rates increased. These levels did not differ from those observed in the resistant control population ($p > 0.227$).

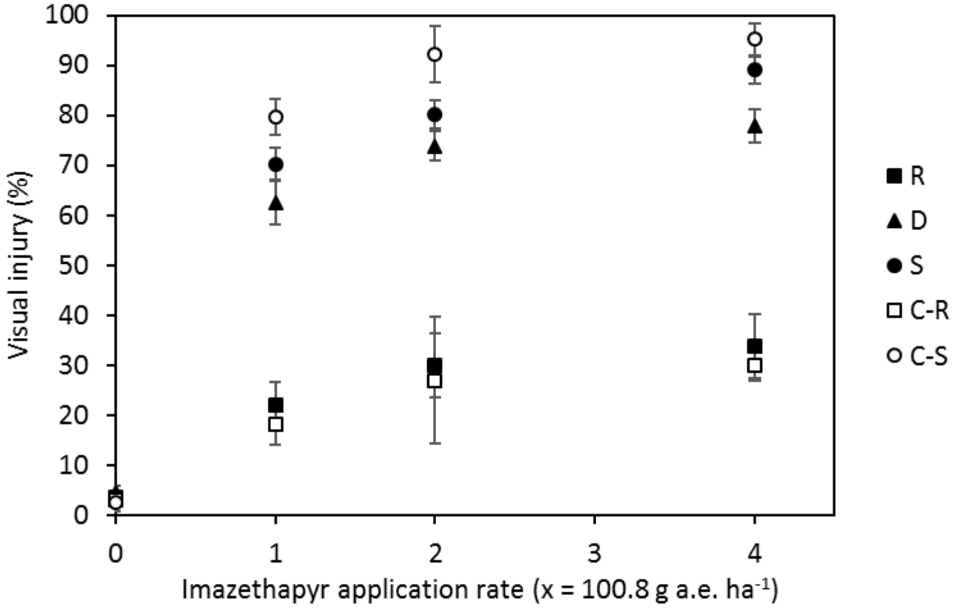

Figure 1. Percentage of visual injury four weeks after treatment on common ragweed as a function of imazethapyr application rate. The treated populations were classified as susceptible (S) (*n* = 2), developing resistance (D) (*n* = 9), or resistant (R) (*n* = 7). The trial also included two control populations, one that was susceptible to imazethapyr (C-S) and one that was resistant to it (C-R). Bars indicate 95% confidence intervals, *n* is the number of populations.

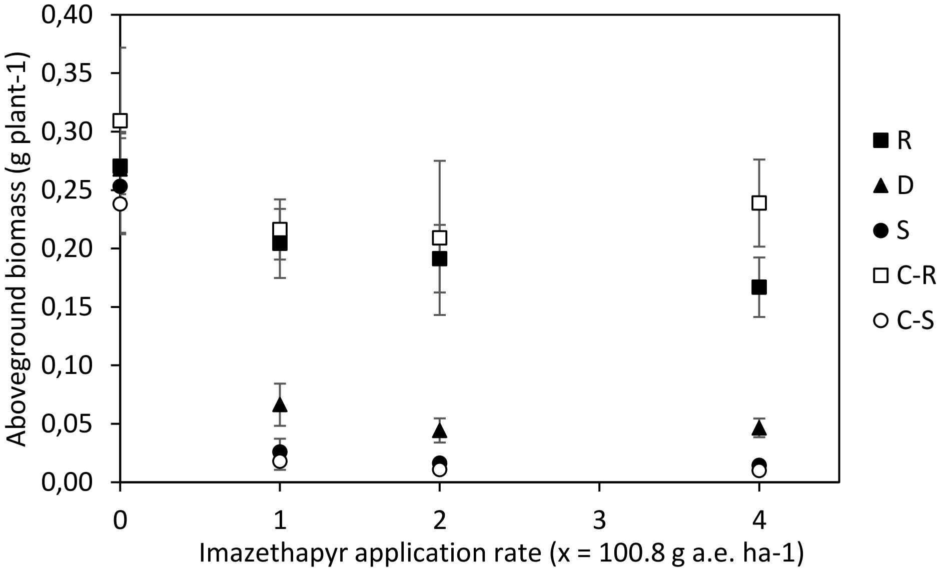

Figure 2. Common ragweed aboveground dry biomasses four weeks after treatment as a function of imazethapyr application rate. The treated populations were classified as susceptible (S) (*n* = 2), developing resistance (D) (*n* = 9), or resistant (R) (*n* = 7). The trial also included two control populations, one that was susceptible to imazethapyr (C-S) and one that was resistant to it (C-R). Bars indicate 95% confidence intervals, *n* is the number of populations.

Dry weight as a function of imazethapyr rate for each population type is presented in Fig. 2. At the standard and double rates, there was again no significant difference between the resistant control and the populations classified as resistant ($p = 0.560$). However, a significant difference was observed at four times the standard rate. With respect to the populations developing resistance, their dry biomass after application of the herbicide differed from the dry biomasses of the resistant populations and the susceptible populations ($p < 0.001$).

Although aboveground biomass can reveal a trend, biomass reduction is a better variable to consider for comparing different populations. The mean biomass reduction for the susceptible populations was 89.7% at 4 weeks after application of the standard rate and 93.7% when the quadruple rate was used (Fig. 3a). For the plants developing resistance, the biomass reduction was 76.6% with the standard rate and it increased to 83.5% with the quadruple rate (Fig. 3b). For these populations, a reduction of 75% or more in dry biomass, supported by a high injury percentage, as shown in Fig. 1, was reflected visually by plants that were dead or unable to recover, given that the biomass reduction cannot be 100% with a postemergence herbicide, since some dead or dying plant material always remains. However, despite a mean biomass reduction of 76.6% for the populations developing resistance, it is necessary to consider that the remaining 23.4% came at 75.6% from resistant plants which survived the herbicide treatment.

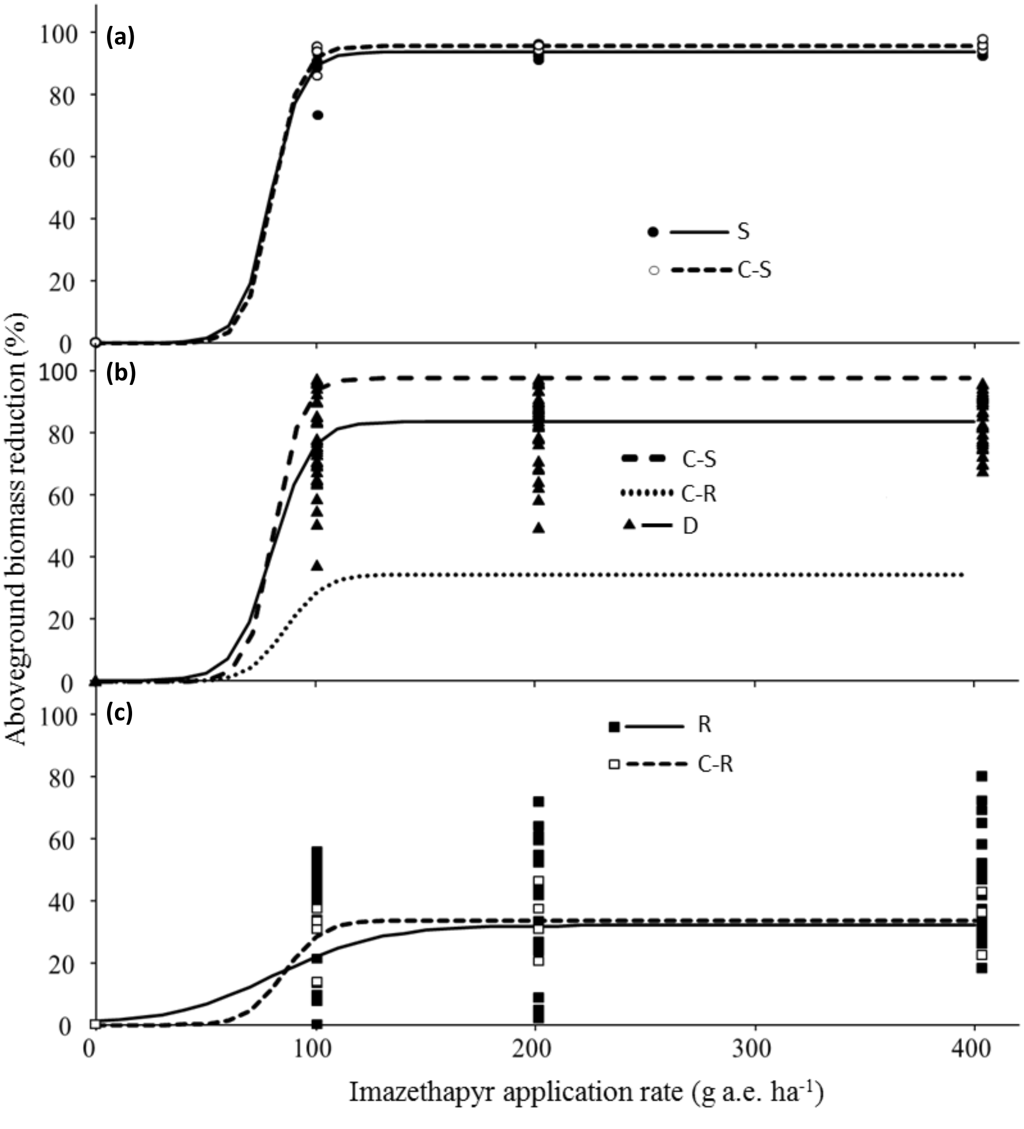

Figure 3. Aboveground dry biomass reduction curves in comparison with untreated controls for five biotypes of common ragweed following one of four treatments with the herbicide imazethapyr. The populations were classified as **(a)** susceptible (S), **(b)** developing resistance (D), or **(c)** resistant (R). The trial also included two control populations, one that was susceptible to imazethapyr (C-S) and one that was resistant to it (C-R). The points show the percentage of biomass reduction in the 20 tested populations for the four replicates of each of the four treatments.

For the resistant populations, the biomass reduction was 22.2% at the standard rate and it increased to 31.8% at 2X and 32% at 4X (Fig. 3c). In our study, these biomass reductions did not lead to additional plant death or increase plant injury between two and four WAT (data not shown). The resistant plants could therefore survive and reproduce even after application of the quadruple rate, which is in agreement with various studies that also showed a high level of resistance to Group 2 herbicides in common ragweed. In the state of Delaware, the aboveground dry biomass of a resistant common ragweed population was reduced by 33%, in comparison with untreated control, three weeks after the application of imazethapyr at 100 times the standard rate (Rousonelos *et al.* 2012). In field trials with resistant common ragweed populations in Ohio, the control percentage varied between 1% and 7% with the standard imazethapyr application rate and between 1% and 14% with the double rate (Taylor *et al.* 2002). These results are supported by results obtained in Ontario where the mortality percentage did not reach 20% in 20 of the 24 populations diagnosed as resistant tested for resistance using three Group 2 chemical families (cloransulam-methyl, chlorimuron, and imazamox) (Van Wely *et al.* 2015).

Determination of GR_{50}

The imazethapyr rates required for a 50% biomass reduction were slightly more than 80 and 84 g a.e. ha^{-1} for the susceptible populations and the populations developing resistance, respectively, which are still below the manufacturer's recommended rate (100.8 g a.e. ha^{-1}) (Fig. 3, Table 2). The Canadian federal standards for herbicide efficacy require "At least 80% consistent reduction in weed stand and/or growth, when compared to untreated control plots" (Health Canada 2003) in order for a weed species to be included on the label of a herbicide, as is the case for common ragweed on the Pursuit® label. In our study, if we consider the GR_{80} as an indicator of such a response, the GR_{80} for the susceptible populations would be 91.5 g a.e. ha^{-1}, which would still be below the standard rate and therefore would meet the federal standards. For the populations developing resistance, the GR_{80} would be 106.9 g a.e. ha^{-1}. The mean biomass for all the plants in the tested population is considered, regardless of whether some of the plants may have clearly resisted the herbicide treatment.

The resistance factor was greater than 5 in populations classified as resistant; the exact value cannot be specified since it was not possible to achieve a 50% biomass reduction even with the application of four times the standard rate (Table 2). Experiments with significantly higher rates of herbicides would be necessary in order for those results to be compared with results from other trials. In greenhouse trials using resistant populations in Ohio, it was not possible to determine the GR_{50} values for three Group 2 active ingredients, and resistance factors greater than 12,000 (cloransulam-methyl), 1,500 to 4,800 (chlorimuron), and 1,100 (imazamox) were calculated (Taylor *et al.* 2002). Patzoldt *et al.* (2001) also obtained high resistance factors to Group 2 herbicides in resistant common ragweed populations in Indiana, namely, 5,100, 4,100, and 110, respectively, for the same three chemical families tested in Ohio. The populations classified as resistant in our study could therefore have very high resistance factors.

Table 2. Logistic regression equations, acid equivalent rates per hectare required to reduce dry biomass by 50% (GR_{50}), and resistance factors for common ragweed as a function of imazethapyr application rate

Population[a]	Equation[b]	GR_{50} (g a.e. ha^{-1})	Resistance factor[c]
Susceptible $n^d = 2$	$\frac{93.6999}{(1 + exp^{-0.1449(r-79.2992)})}$	80.2	0.99
Developing resistance $n = 9$	$\frac{83.4735}{(1 + exp^{-0.1184(r-80.4169)})}$	83.8	1.04
Resistant $n = 7$	$\frac{32.0300}{(1 + exp^{-0.0423(r-81.4806)})}$	> 403.2	> 5.00
Susceptible control $n = 1$	$\frac{95.5598}{(1 + exp^{-0.1652(r-80.1277)})}$	80.7	1.00
Resistant control $n = 1$	$\frac{33.6469}{(1 + exp^{-0.1182(r-85.1667)})}$	> 403.2	> 5.00

[a] The treated populations were classified as susceptible, developing resistance, or resistant. The trial also included two control populations, one that was susceptible to imazethapyr and one that was resistant to it.

[b] In $\frac{a}{(1 + exp^{-k(r-b)})}$, a is the asymptote, k is the growth rate, r is the herbicide rate (g a.e. ha^{-1}), and b is the inflection point.

[c] Resistance factor = $\frac{GR_{50} population\ X}{GR_{50} susceptible\ control}$.

[d] Number of populations.

Distribution of cases of resistance

The presence of imazethapyr-resistant plants in more than 80% of the samples collected from across Montérégie (Fig. 4) demonstrates that resistance is common in that region. The random distribution of the cases of resistance suggests that there were multiple independent selection events or stochastic dispersal in time. A European study aimed at determining the origin and dispersal model of common ragweed revealed diversity within a given region and a given population (Gaudeul *et al.* 2011). The authors also indicated that long-distance dispersal occurred. A number of authors agree that common ragweed pollen can be transported over long distances by wind (Cecchi *et al.* 2006; Garneau *et al.* 2006; Grewling *et al.* 2016).

Management of resistance

Models predict that resistance to ALS inhibitors will appear after two to six seasons of herbicide application (Claude *et al.* 2004; Gressel *et al.* 1996). Once it has appeared, if only two resistant plants (the minimum number of resistant plants required to classify a population as developing resistance in this study) produce an average of 3,500 seeds in a soybean field (Simard and Benoit 2012), the frequency of resistant plants can significantly increase after only a few applications of the same herbicide.

Once resistant plants are present, increasing the imazethapyr application rate will have very little effect on them and will therefore not eliminate or reduce crop competition from resistant plants. The use of active ingredients belonging to chemical families in the same group (Group 2) is unlikely to provide better control because the mutation at the Trp574 codon, identified as conferring resistance to ALS herbicides in common ragweed, confers cross-resistance and a high to very high level of resistance to all the chemical families in this group (Beckie and Tardif 2012). Rotating herbicide groups is therefore an essential practice to adopt in order to reduce selection pressure. In order to prevent an increase in the number of resistant seeds in the seed bank, the herbicide to which a plant is resistant must not be used alone (Beckie and Reboud 2009). A mixture of several groups of herbicides in a single application can control resistant plants but is not a long-term solution as biotypes resistant to multiple herbicide groups can eventually be selected (Beckie and Reboud 2009; Gressel and Segel 1990; Henskens *et al.* 1996). Since herbicides that control broadleaf weeds in conventional soybean crops are limited, crop rotation is a practice that, in addition to its other already known benefits, could aid in the rotation of herbicide groups. Other methods, such as mechanical weeding when plants are small, could prove useful to reduce selection pressure and eliminate resistant plants without herbicide application. Using a combination of mechanical weeding tools (such as a tine harrow, rotary hoe, and cultivator) is most effective (Weill *et al.* 2007).

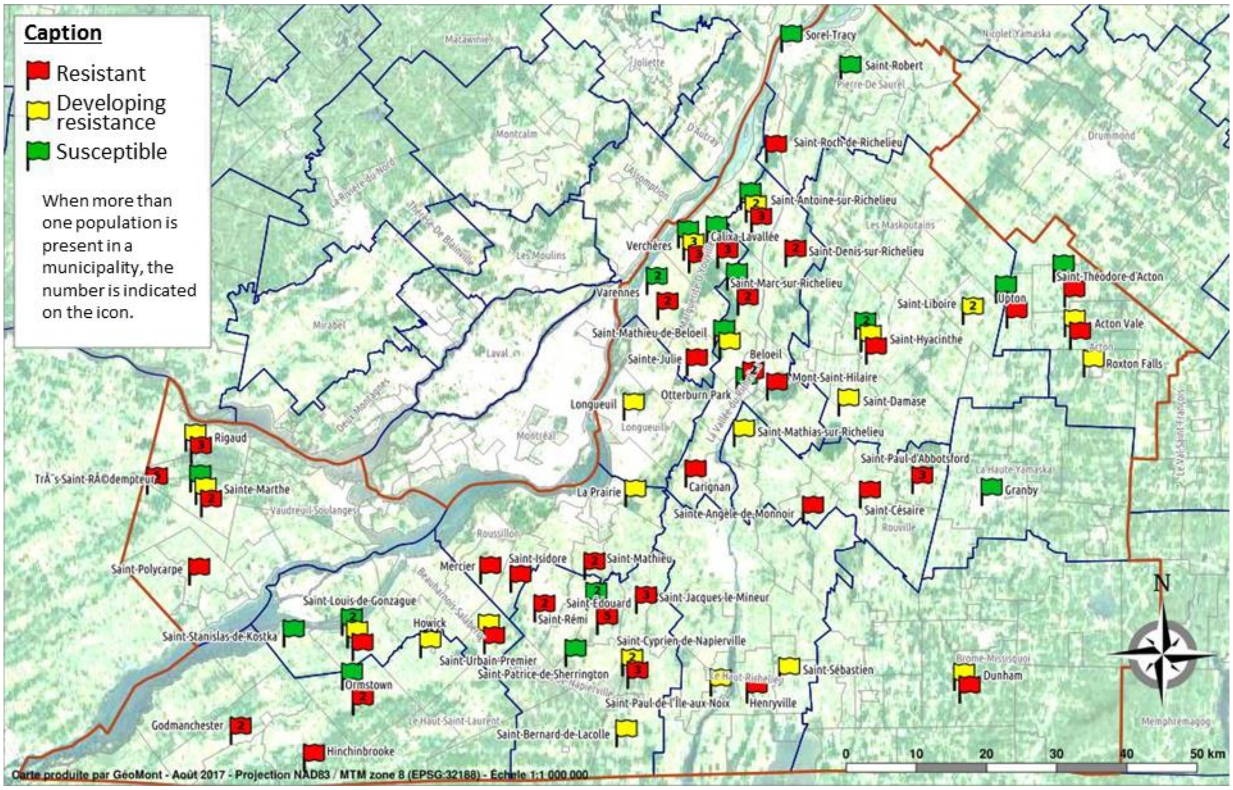

Figure 4. Location of the common ragweed populations tested for resistance to imazethapyr during the survey conducted in Montérégie, QC in 2014 and 2015. The treated populations were classified as susceptible (S), developing resistance (D), or resistant (R).

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