# Canadian Weed Science Society
# Société canadienne de malherbologie

## Proceedings of the 2005 National Meeting

**59th Annual Meeting**
**November 28 – 30, 2005**
**Sheraton Fallsview Hotel**
**Niagara Falls, ON**

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**Niagara Falls, ON**

*Compiled, assembled and produced by*
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**Canadian Weed Science Society**
**Société canadienne de malherbologie**
**(CWSS-SCM)**

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# Introduction

## Canadian Weed Science Society
## Société canadienne de malherbologie
## 2005 National Meeting
## Réunion nationale 2005
## Niagara Falls, ON

There were 215 registered participants at the meeting and one symposium took place.

The 2005 Awards and Scholarships recipients were:

**Monsanto Scholarship:**
PhD: Delgermaa Chuluunbaatar, New Farming Technology for Sustainable Development in Mongolia. University of Saskatchewan
MSc: Paula Halabicki, Soil Properties and Environment Affect Odyssey and Everest Phytotoxicity and persistence in Soil. University of Manitoba

**Dow Agrosciences Travel Awards:**
PhD Mohammed Abudieyeh, Population Dynamics of Dandelion and Other Broadleaf Weeds in Turfgrass Systems as Influenced by *Sclerotinia minor* Jagger, Macdonald Campus, McGill University
MSc: Josh Vyn, Biology and Control of Common Waterhemp (*Amaranthus tuberculatus* var. *rudis*) in Corn and Soybeans, University of Guelph

**Syngenta Crop Protection Travel Awards:**
PhD: Jamshid Ashigh, Impact of Stress on Fitness of ALS-Inhibitor Resistant Eastern-Black Nightshade. University of Guelph
MSc: Scott White, Spatial and Temporal Variability of Vegetation in Wild Blueberry Production. Nova Scotia Agricultural College

**Dow AgroSciences Excellence in Weed Science Award**
The 2005 winner is Hugh Beckie, Weed Research Scientist, Agriculture and Agri-Food Canada, Saskatoon Research Centre.

**Bayer CropScience Best Student Presentation Award**
The Bayer Inc. Best Student Presentation Award was awarded to Jamshid Ashigh, University of Guelph, for his presentation titled "Characterization and Genetic Variation of ALS Inhibitor Resistance in Eastern Black Nightshade from Ontario".

**Outstanding Industry Member Award**
The 2005 winner of the Outstanding Industry Member award is Dr. Luc Bourgeois. Luc is regarded highly throughout Canada as a leader in the crop protection industry. He has also been a major contributor to the success of the Canadian Weed Science Society.

**BASF Canada Poster Award Winners**
1st Place:
Oviposition Preferences of *Trichoplusia* ni on Broccoli and Selected Agricultural Weeds.
J.H. Cameron, M.B. Isman and M.K. Upadhyaya

2nd Place:
W/O/W Emulsions – Formulation Development for Foliar Application of Bioherbicides.
R. K. Hynes, P. Chumala, D. Hupka, and G. Peng

3rd Place:
Crop Residues: an Obstacle to Emerging Weed Seedlings?
A. Légère, B. Gradin, A.G. Thomas, F.A. Holm, and F.C. Stevenson

**E.I. DuPont Canada Photo Contest Winners**
The judges were:
Dean Palmer – The Scenario
Virginia Govier - AdFarm, Production Manager
Saghir Alam - Dupont
Luc Bourgeois - Bayer CropScience - Photo contest chair for 2005

Winners in Niagara Falls were as follows:

**General agriculture**:
1) Ian Morrison - Spring wheat at three hills
2) Rick Holm - Sunflowers
3) Daniel Cloutier - Green onion harvest

**Weeds**:
1) Stephen Crozier - Rudbeckia
2) Venkata Vakulabharanam - Goatsbeard Head
3) Ian Morrison - Wooly Burdock

**Weeds in action:**
1) Peter Smith - Derelict combine infested with wild grape
2) Ian Morrison - Diffuse knapweed
3) Peter Smith - Harvesting grapes!

# 2005 Local Arrangements Committee Members

The committee members and their responsibilities were:

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**Sunday Pre-Conference Tour**

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# CWSS-SCM 2005 Annual Meeting Agenda

| Date | Time | Topic |
|---|---|---|
| Sunday November 27th | 9:00 am – 5:00 pm | Board of Directors Meeting. Lunch served at noon |
| | 1:00pm – 5:00 pm | Butterfly Conservatory and Winery Tour |
| | 5:00 pm – 10:00 pm | Registration – Oakes Foyer |
| | 5:00 pm – 10:00 pm | Poster and Commercial Display Setup – Oakes Foyer |
| | 5:00 pm – 10:00 pm | Grey Cup Party – in Oakes North |
| Monday November 28th | 8:00 am – 6:00 pm | Poster and Commercial Display Session – Oakes Foyer |
| | 9:00 am – 12:00 pm | Symposium Session |
| | 12:00 pm – 1:00 pm | Lunch – in Oakes North |
| | 1:00 pm – 5:00 pm | Symposium Session |
| Tuesday November 29th | 6:30 am – 8:00 am | Continental Breakfast for 2006 Program Committee - Huron |
| | 8:00 am – 6:00 pm | Poster and Commercial Display Viewing |
| | 8:00 am – 12:00 pm | Graduate Student Presentations |
| | 12:00 pm – 2:00 pm | Awards Banquet – in Oakes North |
| | 2:00 pm – 3:45 pm | Working Group Sessions – Weed Control in Corn, Soybeans and Edible Beans / Extension and Noxious Weeds / Integrated Weed Management |
| | 3:45 pm – 4:00 pm | Health Break |
| | 4:00 pm – 5:45 pm | Working Group Sessions – Weed control in Horticultural Crops / Herbicide Residues / Herbicide Resistance |
| | 6:30 pm – 12:00 am | CropLife Canada Reception |
| Wednesday November 30th | 7:30 am – 9:30 am | CWSS Annual Business Meeting Breakfast |
| | 9:30 am – 10:00 am | Health Break |
| | 10:00 am – 12:00 pm | Working Group Sessions – Crop Life / Physical Weed Control/ Application Technology |
| | 12:00 pm – 2:00 pm | Board Member Meeting/ Lunch - Huron |

**Transgenic HT Crops: Agronomy, Environment and Beyond**
**Symposium Session**

## AGENDA

| Time | Topic | Speaker | Affiliation |
|---|---|---|---|
| 9:00 am – 9:05 am | Welcome and Announcements | Denise Maurice | AgricoreUnited – Calgary, Alberta |
| 9:05 am – 9:10 am | Local Arrangements | Al Hamill | Agriculture and Agri-Food Canada- Harrow, Ontario |
| 9:10 am – 9:20 am | Introduction to Symposium | Clarence Swanton | University of Guelph |
| 9:20 am – 9:40 am | Sowing the Seeds of Acceptance | Ray Mowling | Executive Director of the Council of Biotechnology Information |
| 9:40 am – 10:00 am | Ten Years of Biotechnology – a Historical Perspective of Science, Politics and Trade | Connor Dobson | Bayer Canada |
| 10:00 am – 10:20 am | Health Break | | |
| 10:20 am – 10:40 am | Weed Management with Herbicide Tolerant Crops – Eastern Canada | Peter Sikkema | Ridgetown College – University of Guelph |
| 10:40 am – 11:00 am | Weed Management with Herbicide Tolerant Crops – Western Canada | Neil Harker | Agriculture and Agri-Food Canada – Lacombe, Alberta |
| 11:00 am – 11:20 am | Selection of Herbicide Resistance and Tolerance in Weeds; the Influence of Herbicide Resistant Crops | François Tardif | University of Guelph |
| 11:20 am – 11:40 am | Herbicide Tolerant Canola – the View from the Farm Gate | Joanne Buth | Canola Council of Canada |
| 11:40 am – 12:00 pm | Panel discussion with all morning speakers | Clarence Swanton | University of Guelph |
| 12:00 pm – 1:00 pm | Lunch – in Oakes North | | |

**Transgenic HT Crops: Agronomy, Environment and Beyond**
**Symposium Session**

## AGENDA
**(Continued)**

| Time | Topic | Speaker | Affiliation |
|---|---|---|---|
| 1:00 pm – 1:20 pm | Intraspecific Gene Flow: Influencing Factors and Consequences | Linda Hall | University of Alberta |
| 1:20 pm – 1:40 pm | Gene Flow Between GM Crops and Related Species in Canada | Suzanne Warwick | Agriculture and Agri-Food Canada – Ottawa, Ontario |
| 1:40 pm – 2:00 pm | Monitoring and Persistence of rDNA in Soil and Water | Rob Gulden | University of Guelph |
| 2:00 pm – 2:20 pm | Non-Target Impacts on Soil Fungi of Roundup Ready Cropping Systems | Jeff Powell | University of Guelph |
| 2:20 pm – 2:40 pm | Non-Target Impact of Herbicide Tolerant Crops on Soil Bacterial Communities | Kari Dunfield | University of Guelph |
| 2:40 pm – 3:00 pm | Genetically Modified Feed and the Fate of Recombinant DNA Through the Digestive Tract of Livestock | Tim McAllister | Agriculture and Agri-Food Canada – Lethbridge, Alberta |
| 3:00 pm – 3:20 pm | Health Break | | |
| 3:20 pm – 3:40 pm | GM Crops are Not Containable. | Ann Clark | University of Guelph |
| 3:40 pm – 4:00 pm | The Potential for Co-Existence of GM and non-GM Crops in Canada. | Rene Van Acker | University of Manitoba |
| 4:00 pm – 4:20 pm | Implications of Genetically Modified Crops for the Canadian Seed Industry – Challenge or Opportunity? | Henry Olechowski | Chair of BioTech Committee of Canadian Seed Trade |
| 4:20 pm – 4:40 pm | Incorporating Rapidly Evolving Scientific Knowledge into Risk Assessment for Plants with Novel Traits | Phil MacDonald | Canadian Food Inspection Agency – Saskatoon, Saskatchewan |
| 4:40 pm – 5:00 pm | Summary and panel discussion with all afternoon speakers | Clarence Swanton | University of Guelph |

**Working Groups**

## AGENDA

| Date | Time | Working Group |
|---|---|---|
| Tuesday November 29th | **Session I/II/III – Concurrent Working Groups** | |
| | 2:00 pm – 3:45 pm | A) Weed Control in Corn, Soybeans, and Edible Beans – Mike Cowbrough |
| | | B) Extension and Noxious Weeds – Clark Brenzil |
| | | C) Integrated Weed Management – Paul Watson |
| | **Session IV/V/VI – Concurrent Working Groups** | |
| | 4:00 pm – 5:45 pm | A) Weed Control in Horticultural Crops – Darren Robinson |
| | | B) Herbicide Residues – Eric Johnson |
| | | C) Herbicide Resistance – François Tardif |
| Wednesday November 30th | **Session VII/VIII/IX – Concurrent Working Groups** | |
| | 10:00 am – 12:00 pm | A) CropLife – Joe McNulty and Bill Summers |
| | | B) Physical Weed Control – Maryse Leblanc |
| | | C) Application Technology – Helmut Speiser |

**Transgenic HT Crops: Agronomy, Environment and Beyond**

The symposium has been published separately. The full reference is:

Gulden, R. H. and C. J. Swanton, eds.  2007.  The first decade of herbicide-resistant crops in Canada. Topics in Canadian Weed Science, Volume 4.  Sainte Anne de Bellevue, Québec: Canadian Weed Science Society – Société canadienne de malherbologie. 176 pp. ISBN 978-0-9688970-4-1.

## Graduate student presentations

Because of a hard disk crash, some graduate students presentations might be missing.

# Effect of host plant age and biotypes on efficacy of *Sclerotinia minor* for dandelion control

Mohammed H. Abu-Dieyeh and Alan K. Watson

Department of Plant Science, McGill University, 21,111 Lakeshore Road, Ste.-Anne-de-Bellevue, Quebec, Canada, H9X 3V9

**Abstract**

Fourteen dandelion (*Taraxacum officinale*) biotypes were assessed for their susceptibility to a granular formulation of *Sclerotinia minor*. Although the biotypes of dandelion were found to be morphologically variable, *S. minor* reduced the above ground and below ground biomass by 94% and 96%, respectively, without significant differences among biotypes. Foliar damage and dandelion mortality caused by *S. minor* was significantly affected by plant age, grass competition and the interaction of both factors. All plant ages were more severely affected by *S. minor* treatment in the presence of grass competition. Without grass competition, *S. minor* treatment caused 100% mortality of 4-wk-old plants, but 6-wk-old and older plants (up to 13 wk) showed different degrees of recovery after considerable initial foliar damage two weeks after application. With grass competition, the fungal treatment caused 100% mortality of 4- and 6-wk-old, and 90% aboveground damage up to 10-wk-old. Six weeks after application, foliar and root biomass was severely reduced and survival was significantly less for all plant ages in the presence of grass competition than in the absence of grass competition.

**Introduction**

Understanding the components of a plant pathogen system is required to maximize the success of biocontrol (Cousens & Croft, 2000). The extensive intraspecific variations in *Taraxacum officinale* (common dandelion) are well documented (Stewart-Wade et al., 2002) and a mixture of dandelion genotypes could colonize a small area (Solbrig, 1970). Within population genetic diversity and host plant growth stage (age) can alter the efficacy of a biological control agent (Cousens & Croft, 2000). However, crop interspecific competition favours biocontrol success (Kennedy & Kremer, 1996).

The fungus *Sclerotinia minor* is being studied as a possible biological control for dandelion and other broadleaf weeds in turfgrass environments (Ciotola *et al.*, 1991; Riddle *et al.*, 1991; Brière *et al.*, 1992). The objectives of this research were to assess the susceptibility of different dandelion biotypes and ages to *S. minor* (IMI 344141) and to quantify the relative importance of turfgrass competition and the biological stress of *S. minor* on dandelion survival and biomass reduction.

**Methods**

The *S. minor* (IMI 344141) granular formulation was freshly prepared and assayed for virulence on detached dandelion leaves prior to application (Abu-Dieyeh & Watson, 2005). Seeds of dandelion biotypes were collected from 14 regions in Europe, Canada and USA. These biotypes were grown in greenhouse conditions and morphological variations of 8-week-old plants were recorded. The susceptibility of seven biotypes to spot application of 0.2 g/plant of the *S. minor* formulation was also assessed under greenhouse conditions. In a separate greenhouse experiments, dandelion seeds, previously collected from Macdonald campus lawns, were sown in plastic containers (40x32x20 cm) at different planned times to get to different plant ages (4, 6, 8, 10 and 13 wks age) at a specific time period. Four replications of a split plot design experiment with the weed control treatments (untreated or spot application of 0.2 g/plant *S. minor* formulation) as the main plots and the grass factor (present or absent) as the subplots. Each experiment was conducted twice and treatment efficacy, biomass reduction, and dandelion survival were reported and analyzed using ANOVA of SAS and Tukey's test at $P = 0.05$ (SAS Institute Inc, Cary, NC, USA 2001).

## Results

The dandelion biotypes were readily distinguished by one or more morphological character including: rosettes growth form, number of leaves, leaf length, leaf length: breadth ratio, leaf trichome density, blade margin incisions, petiole length, redness of the midrib, tap root length, and leaf and root biomass. These variations were better explained by genotypic variation instead of phenotypic plasticity as all plants were grown under the same growth conditions. Despite this variation *S. minor* as a necrotrophic fungus exerted similar damage and biomass reduction on all biotypes (Figure 1) indicating the high susceptibility to *S. minor* of the diversified genotypic population of dandelion.

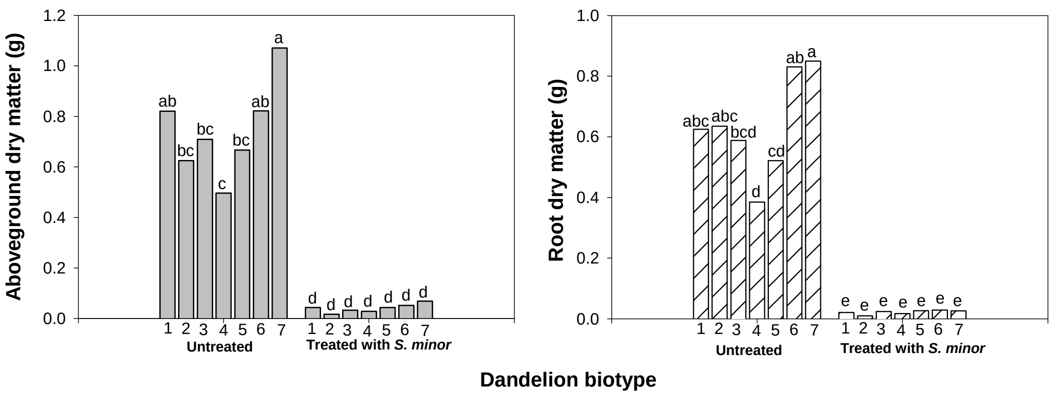


Figure 1. Effect of *Sclerotinia minor* (IMI 1344141) on foliar and root biomass of different dandelion biotypes six weeks after spot application with 0.2 g/plant of a *S. minor* granular formulation. Within each graph, bars with similar letters are not significantly ($P = 0.05$) different according to Tukey test.

In the presence of grass competition, 4- and 6-wk-old plants completely collapsed without any recovery, while older plants, after almost 100% damage recorded in the second week after application, showed some degree of recovery proportionally correlated to plant age (Figure 2). For all ages up to 10-wk-old plants, the fungus caused severe cumulative damage of ~ 90% and only the 13-wk-old plants were able to recover partially with 50% damage six weeks after application. In the absence of grass competition, 4-wk-old plants were highly susceptible with 100% collapse of all tested plants (Figure 2). Other plant ages showed 80-95% aboveground damage two weeks after application. Subsequently, the level of damage decreased with corresponding less damage with higher aged plants. Incomplete damage of plant leaves and/or vegetative regrowth was the cause of decreasing damage values. There was significantly less damage to the 13-wk-old plants than other ages one week after application. The 6-wk-old plants responded similarly to the 8- and 10-wk-old plants from the first to the fifth week after application. Within the same plant age, no significant differences were obtained for efficacy on 4-wk-old plants due to grass competition. However, differences were significant ($P \leq 0.01$) on 6- and 13-wk-old. Eight- and 10-wk-old showed no significant difference between the two grass treatments up to two weeks after application, subsequently the differences were significant (Figure 2). The biomass of leaves and roots were severely diminished by combining grass competition and *S. minor* treatments (Figure 3). The grass competition alone exerted similar biomass reduction as the fungus, without grass treatment (Figure 3). Our findings indicate the synergetic interaction of *S. minor* treatment with grass competition even on the resilient root system of 13-wk-old dandelion and highlight the importance of proper grass management to enhance the efficacy of *S. minor* on such a tenacious, perennial weed.

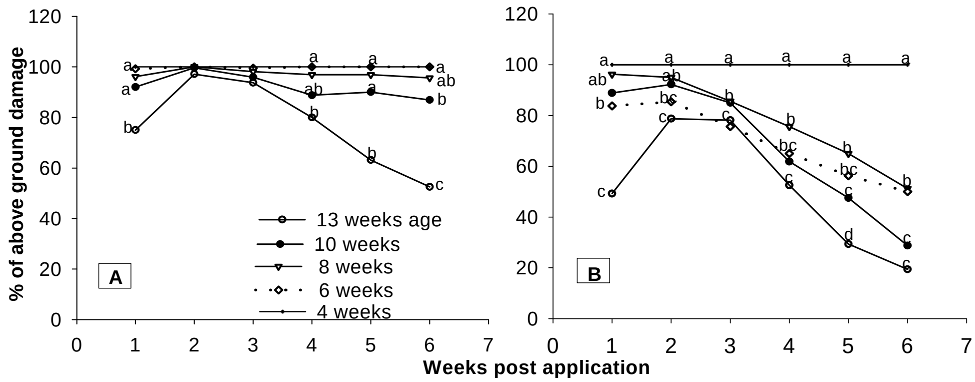


Figure 2. Effect of plant age and grass competition on the control of dandelion using *Sclerotinia minor* (IMI 1344141). The means were separated using Tukey's test at *P* = 0.05, within each graph, and at any time post application, values with similar letters are not significantly different.

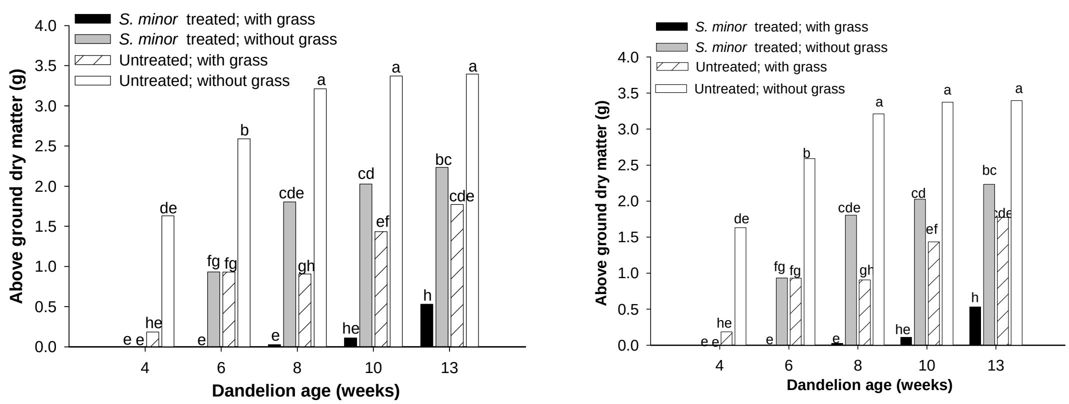


Figure 3. The effect of plant age and grass factor on aboveground and root biomass of dandelions six weeks after *Sclerotinia minor* (IMI 1344141) application. Values with similar letters are not significantly different according to Tukey's test at *P* = 0.05.

## References

Abu-Dieyeh MH & Watson AK (2006) *Biocontrol Sci. & Technol*. (*Accepted November 2005).*
Brière SC, Watson AK & Paulitz TC (1992) *Phytopathology* **82**, 1081.
Ciotola M, Wymore L & Watson AK (1991) *WSSA Abstracts* **31**: 242.
Cousens R & Croft AM (2000) *Weed Res.* **40**, 63-82.
Kennedy AC & Kremer RJ (1996) *J. Prod. Agric.* **9**, 480-485.
Solbrig OT (1970) *Isozyme Bulletin* **3**, 43-44.
Stewart-Wade SM, Neumann S, Collins L & Boland GJ (2002) *Can. J. Plant Sci.* **82**, 825-853.

# Characterization and Genetic Variation of ALS Inhibitors Resistant and Susceptible Populations of Eastern black nightshade (*Solanum ptycanthum*) from Ontario

Jamshid Ashigh [1] and François J. Tardif [1]

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**Key Words:** *Solanum ptycanthum*, acetolactate synthase, herbicide resistance, genetic variation, feed back inhibition, point mutations

Abstract

Acetolactate synthase (ALS) inhibitor resistance has been rising rapidly in populations of eastern black nightshade from Ontario. To determine the molecular bases of resistance in thirteen confirmed resistant populations, the *ALS* gene from all resistant populations were sequenced and compared with a susceptible *ALS*. The results indicated that resistance in twelve populations was due to an $Ala_{205}$Val substitution, while in one population was caused by an $Ala_{122}$Thr substitution. In vitro enzyme assays of one resistant population with $Ala_{205}$Val substitution showed that the ALS enzyme in that population was 67-, 60-, and 60-fold less sensitive than that of susceptible population to imazethapyr, imazamox, and primisulfuron, respectively. Furthermore, the resistant enzyme was less sensitive to feedback inhibition from branched-chain amino acids compare to susceptible enzyme. Moreover, RAPD technique was employed to detect the genetic variability of twenty-five resistant and susceptible populations, and results showed that both local selection and gene flow explain the spread of resistance in Ontario.

## Introduction

Evolution of resistance to ALS inhibiting herbicides has been rapid and possibly faster than for several other modes of action herbicides (Saari et al., 1994). Major factors influencing the evolution of herbicide resistance, including the intensity of selection by herbicides, the initial frequency of herbicide resistant individuals in the population (Jasieniuk et al., 1996), gene flow, persistence in the soil seed bank, and relative fitness of resistant biotypes (Maxwell et al., 1990; Mortimer et al., 1992). In most cases the biochemical mechanism of ALS-inhibitor herbicide resistance is a herbicide-resistant ALS enzyme (Saari et al., 1994). Within the *ALS* gene there are several conserved regions or domains, which at the amino acid level, are nearly conserved 100% in susceptible species (Devine and Eberlein, 1997; Chong and Choi, 2000). Since ALS inhibitors have a single site of action, a single point mutations in one of the conserved domains in the *ALS* gene is typically responsible for conferring resistance to ALS inhibitors, (Guttieri et al., 1995; Bernasconi et al., 1995; Boutsalis et al., 1999). To date, target site resistance to ALS inhibitors in weed species has been caused naturally by a substitution at one of the six conserved locations in *ALS* (Tranel and Wright, 2002). The six conserved amino acids and their position based on the precursor ALS from *Arabidopsis thaliana*, from amino-terminal to carboxy-terminal include: $Ala_{122}$, $Pro_{197}$, $Ala_{205}$, $Asp_{376}$, $Trp_{574}$, and $Ser_{653}$ (Tranel and Wright, 2002; Whaley et al., 2004).

ALS catalyses the formation of both acetohydroxybutyrate and acetolactate and therefore is the first enzyme unique to leucine, isoleucine, and valine biosynthesis. The synthesis of branched chain amino acids is regulated, in part, by control of this enzyme through end product (amino acids) feedback inhibition (Duggleby and Pang, 2000). Furthermore, it has been indicated that the sensitivity of the ALS enzyme to feedback regulation could be reduced by mutations causing ALS-inhibitors resistance in higher plants (Duggleby and Pang, 2000). However, the insensitivity to feedback inhibition from some or all branched chain amino acids is profoundly affected by the specific site and type of substitution that confers resistance (Eberlein et al., 1997).

Since 2001, ALS inhibitor resistance has been confirmed in thirteen populations of eastern black nightshade from different locations in Ontario. Our whole plant dose response experiments showed that, compared to a susceptible (S) population, one of the resistant (R) populations had 726-, 31-, 6-, and 4-fold resistance to post applied imazethapyr, imazamox, primisulfuron, and flumetsulam, respectively. Furthermore, fitness experiments under various light, watering, and temperature regimes, as well as competition indicated that resistance to ALS inhibitors in eastern black nightshade populations comes at a fitness cost. This fitness cost does not involve a reduction in aboveground vegetative biomass accumulation but rather total berries production as well as a delay in the maturation of berries. This would mean that at any given time, under optimal conditions, the resistant plants would produce fewer seeds than the susceptible plants. Furthermore, the differences in reproductive ability between the susceptible and resistant populations tended to decrease and become not significant under stress conditions.

Therefore the objectives of this study were to determine the molecular bases of resistance in all confirmed resistant populations of eastern black nightshade. We also aimed at determining how resistant ALS behaves in response to different herbicide and end product concentration compared to susceptible ALS. Finally, we determined the genetic variability of twenty-five resistant and susceptible populations of eastern black nightshade from Ontario using RAPD technique.

## Materials and Methods

### Plant material.

Seeds of the resistant (R) and susceptible (S) populations of eastern black nightshades were collected from different locations in Ontario.

### DNA extraction and sequencing

DNA from two individuals of all S and all R populations of eastern black nightshade was isolated. Polymerase chain reaction (PCR) primers were designed to amplify the six highly conserved areas of *ALS* from one S and all thirteen R populations. PCR fragments were sequenced to determine the molecular basis of resistance in the R biotypes.

### ALS kinetics

ALS from one S and one R population of eastern black nightshade was extracted. The activity of ALS enzyme from both populations in presence of different herbicide and end product concentration was detected and compared as a colored complex (A530 nm). The experimental design in this experiment was a randomized complete block with three replications.

### RAPD Markers

Initial RAPD profiles were generated using 160 decamer primers, and one randomly chosen individual from four populations. 15 primers were selected for analysis of entire sample set of the populations. DNA from the individuals of all R and S populations was pooled within the populations. Based on reproducible banding patterns between reactions, the banding patterns of six primers were chosen for final analysis (Table 1). Furthermore, DNA from hairy nightshade (*Solanum sarrachoides)* was used as an out group to verify the reliability of RAPD results. Genetic similarity dendrogram was constructed by using the simple matching coefficient and the UPGMA cluster analysis in the NTSYS-PC computer program.

| Primer | Nucleotide sequences (5' to 3') |
|---|---|
| OPB-05 | TGCGCCCTTC |
| OPE-06 | AAGACCCCTC |
| OPE-11 | GAGTCTCAGG |
| OPE-15 | ACGCACAACC |
| OPG-18 | GGCTCATGTG |
| OPH-14 | ACCAGGTTGG |

Table 1. RAPD primers used in the final study

**Results and Discussion**

The results of *ALS* sequencing has indicated that thus far, resistance in twelve populations was due to an alanine to valine substitution at position 205, while in one population was caused by an alanine to threonine substitution at position 122. In vitro enzyme assays of one resistant population with $Ala_{205}$Val substitution showed that the ALS enzyme in that population was 67-, 60-, and 60-fold less sensitive than that of susceptible population to imazethapyr, imazamox, and primisulfuron, respectively (Table 2). Furthermore, it was shown that the resistant enzyme was less active and less sensitive to feedback inhibition from branch chain amino acids compare to susceptible enzyme (Figure 1).

Table 1. Response of resistant (R) and susceptible (S) ALS to ALS-inhibiting herbicides.

| Herbicide | **$I_{50}$ µM**[a] **R** | **S** | (a) **R/S**[b] |
|---|---|---|---|
| Imazethapyr | 645.7 ± 199 | 9.7 ± 4 | 67 |
| Imazamox | 923.4 ± 546 | 15.4 ± 6 | 60 |
| Primisulfuron | 3.7 ± 2 | 0.06 ± 0.02 | 60 |

[a] $I_{50}$ values are the herbicide concentrations required to reduce ALS activity by 50% compared to control treatment (±95% confidence interval).
[b] The resistance factor (R/S) is obtained by dividing the R $I_{50}$ by the S $I_{50}$.

The results of genetic similarity dendrogram indicated that all populations of eastern black nightshade were more related to each other than to hairy nightshade, confirming the reliability of RAPD results. The RAPD profile of the eastern black nightshade populations indicated four groups of populations, in which resistance seems to have arisen independently. However, resistance within the three of clusters, based on high levels of similarity, could have occurred by dispersal (Figure 2).

Of thirteen resistant populations, twelve had the same mutation ($Ala_{205}$Val). This could lead to believe that resistance arose from one founder event and further spread on multiple farms. The results of our genetic markers study showed that both local selection and gene flow explain the spread of resistance in Ontario. The lower activity of resistant ALS compared to susceptible ALS, could explain lower fitness of resistant populations. However, lower sensitivity to feed back inhibition in resistant ALS may compensate for the lower activity of the enzyme by increasing the production of branch chain amino acids, which may moderate the fitness differences among the plants.

Figure 1. Inhibition of ALS activity by different branch chain amino acids valine (V), leucine (L) and isoleucine (I) and their combinations, compare to untreated control, at concentration of 1 mM.

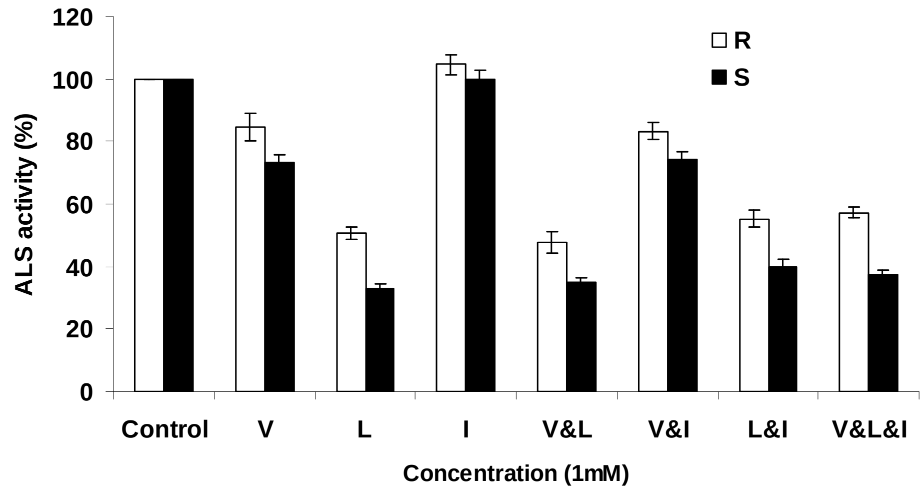
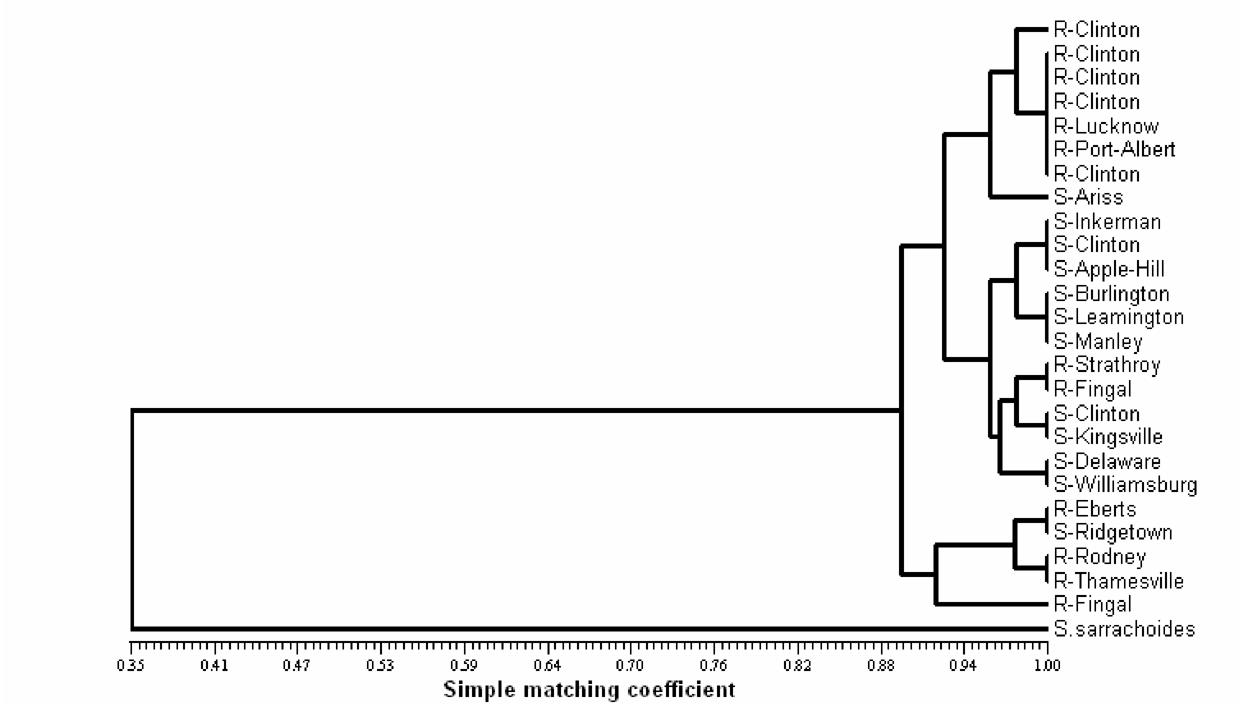



Figure 2. Genetic similarity dendrogram showing the relationships between the analyzed populations of eastern black nightshade and hairy nightshade.

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# Effets allélopathique et de compétition du seigle d'automne (*Secale cereale*) contre les mauvaises herbes annuelles dans la citrouille

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## 1. Introduction

Les mauvaises herbes causent depuis toujours des ennuis aux producteurs agricoles. De lourdes pertes de rendements et de qualité des récoltes résultent de la compétition des mauvaises herbes. Depuis le milieu du 20ième siècle, les herbicides sont couramment utilisés. Cependant, certaines cultures maraîchères comme la citrouille (*Curcubita pepo*) ne possèdent pas toujours des herbicides homologués et efficaces pour contrôler les diverses espèces de mauvaises herbes présentes. La citrouille à un jeune stade de croissance est très peu compétitive due en partie au grand espacement entre les rangs favorisant ainsi la prolifération des mauvaises herbes. Des moyens alternatifs de désherbage efficaces et économiques sont nécessaires dans cette culture.

Depuis les années 1960, l'allélopathie suscite l'attention des scientifiques pour son application en agriculture (4). L'allélopathie réfère à tout processus impliquant des métabolites secondaires produits par des plantes, microorganismes, virus et champignons qui influencent la germination, la croissance et le développement d'une plante avoisinante (2). Le seigle (*Secale cereale*) est une espèce reconnue comme ayant des propriétés allélopathiques (3). Le DIBOA et le BOA sont deux composés allélochimiques du seigle ayant un fort potentiel de répression des dicotylédones annuelles, modérément aux graminées annuelles et très peu aux espèces vivaces (1). Les toxines naturelles son principalement relâchées durant la décomposition des résidus dans le sol et par exudation racinaire (3). Le seigle d'automne peut être ensemencé soit à l'automne ou au printemps, mais doit être détruit avant le semis de la culture principale. Jusqu'à maintenant, il y a eu très peu de recherche scientifique au Québec sur l'activité allélopathique du seigle pour lutter contre les mauvaises herbes.

## 2. Méthodologie

Ce projet de recherche comprend trois volets. Le premier volet évalue l'activité allélopathique du seigle d'automne 'Gauthier' pour le contrôle des mauvaises herbes. Le deuxième volet sert à vérifier la réponse de la citrouille au seigle d'automne utilisé comme culture de couverture. Les travaux expérimentaux ont été mis en place à la Station Agronomique de l'Université Laval à St-Augustin à l'été 2004 et 2005. Les expériences utilisent quatre répétitions d'un dispositif en blocs complets aléatoires. Les données récoltées ont été soumises à l'analyse de la variance.

### *2.1. Volet 1 : Exploration de l'activité allélopathique du seigle d'automne 'Gauthier'*

Ce volet exploratoire compare un semis de seigle d'automne 'Gauthier' réalisé soit à l'automne, soit au printemps suivant et des parcelles témoins sans seigle. Le seigle 'Gauthier' a été ensemencé en rangs espacés de 18 cm au taux de 400 grains/m$^2$. La destruction du seigle semé à l'automne a été réalisée à deux moments soit à la fin du mois de mai et à la mi-juin alors que la destruction du seigle semé au printemps a été réalisée à la mi-juin. Diverses méthodes de destruction du seigle ont été évaluées; le seigle a été soit fauché, fauché et roulé, fauché et motoculté ou détruit avec un herbicide non sélectif (glyphosate). Le protocole expérimental comporte un total de dix-neuf traitements.

Diverses variables ont été mesurées afin d'évaluer le contrôle des mauvaises herbes. Des évaluations visuelles du recouvrement total des mauvaises herbes par classe (dicotylédones annuelles (DA) et vivaces (DV), graminées annuelles (GA) et vivaces (GV)) et de la reprise du seigle après sa destruction ont été réalisées à divers moments. La biomasse sèche des mauvaises herbes (DA, DV, GA et GV) dans un quadrat de 50 X 50 cm ainsi que la biomasse du seigle dans un quadrat de 30 X 30 cm ont été réalisées en juillet et en septembre. Les quadrats ont été placés de façon aléatoire dans le centre de la parcelle.

### *2.2. Volet 2 : Réponse de la citrouille au seigle d'automne utilisé en culture de couverture.*

Le seigle d'automne 'Gauthier' a été ensemencé à l'automne au taux de 400 grains/$m^2$ espacés de 18 cm sur 6 m de longueur. Quelques jours avant le semis de la citrouille, le seigle a été fauché en totalité à l'aide d'une fourragère à fléaux et a été motoculté selon quatre largeurs de travail : 1 cm (semis-direct), 40 cm, 80 cm et 120 cm. Un témoin sans seigle a servi de traitement comparatif. Un rang de citrouille 'Connecticut Field' a été semé vers la mi-juin au centre des bandes motocultées. Les graines de citrouille ont été semées manuellement.

Diverses variables ont été évaluées à plusieurs reprises durant la saison de végétation. Des évaluations visuelles de la phytotoxicité du seigle sur la citrouille, du recouvrement total des mauvaises herbes et de la reprise du seigle après sa destruction ont été réalisées. La biomasse sèche des mauvaises herbes et du seigle dans deux quadrats de 30 X 30 cm a été mesurée. Les quadrats ont été placés de façon aléatoire près des plants de citrouille. Le rendement total de citrouille en catégories vendables et non-vendables a été déterminé.

## 3. Résultats

### *3.1. Premier volet : Exploration de l'activité allélopathique du seigle d'automne 'Gauthier'*

De fortes différences sont observées selon les années et le site expérimental. En 2004, une très forte pression des graminées annuelles était présente alors qu'en 2005, il y avait une forte présence de dicotylédones vivaces. Les deux années ont dû être analysées séparément. Cependant, certaines similitudes sont retrouvées. En 2004 et 2005, le recouvrement des mauvaises herbes est significativement plus faible pour des semis de seigle 'Gauthier' à l'automne ou au printemps par rapport à un témoin sans seigle. De plus, lorsque le seigle est fauché et enfoui en juin, le recouvrement des mauvaises herbes est beaucoup plus faible que dans le témoin sans seigle et motoculté à la même date. La période de semis du seigle 'Gauthier' (automne ou printemps) n'a aucune influence sur la biomasse des mauvaises herbes si le seigle est fauché et roulé en juin ou s'il est enfoui en juin.

De plus, en 2004 et 2005, le moment de faucher et de rouler le seigle 'Gauthier' semé à l'automne, n'a aucune influence sur la biomasse des mauvaises herbes. Par ailleurs, aucune différence entre le traitement fauché et le traitement fauché et roulé n'est observé sur un semis de seigle 'Gauthier' semé soit à l'automne ou au printemps.

*3.2. Deuxième volet : Réponse de la citrouille au seigle d'automne utilisé en culture de couverture*

De fortes réductions de croissance sont observées sur les plants de citrouille en présence du seigle d'automne à chaque année. En 2005, le seigle d'automne a réduit la levée de la citrouille. Aucune citrouille n'a émergé lorsque le seigle est détruit selon une largeur de 1 cm. La réduction de croissance des plants de citrouille est inversement reliée à la largeur de destruction du couvert de seigle d'automne.

En 2005, le rendement vendable en citrouilles est beaucoup plus élevé dans les parcelles sans seigle comparativement aux parcelles avec seigle d'automne. Cependant, le poids moyen des citrouilles vendables ne diffère pas significativement lorsque le seigle est détruit sur une largeur de 80 cm et 120 cm par rapport à la parcelle témoin sans seigle. Ce qui indique que la grosseur des citrouilles ne diffère pas mais le nombre de citrouilles vendables par hectare est inférieur dans les traitements de seigle.

## 4. Conclusion

Le seigle d'automne 'Gauthier' possède des propriétés d'interférence procurant une répression des mauvaises herbes. Cependant, la méthode de destruction du seigle influence beaucoup la pression subséquente des mauvaises herbes. L'enfouissement du seigle par le motocultage en juin est la méthode de destruction ayant procuré une plus faible pression des mauvaises herbes ainsi qu'une biomasse faible. Le seigle peut être considéré comme mauvaise herbe si la destruction du seigle n'est pas efficace. Le seigle d'automne 'Gauthier' peut s'intégrer dans une régie de citrouille s'il est détruit avant le semis. Cependant, des réduction de rendements de 40% sont observés par rapport au traitement sans seigle.

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# Emergence periodicity of volunteer flax (*Linum usitatissimum* L.) in conventional and direct seeding

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## Introduction

Flax or linseed (*Linum usitatissimum* L.) is an established crop on the Canadian prairies, and Canada is a world leader in flax production and flaxseed exports (Lay and Dybing 1989). Bio-based products are a rapidly emerging opportunity in the agricultural sector and there is a strong need to create novel germplasm for the Canadian flax industry to provide high-value added bioproducts for nutraceutical and pharmaceutical markets. However, the ecological and food safety concerns associated with large-scale production of plant made industrial products has not been evaluated. These concerns focus on the segregation of flax varieties that contain an industrial trait from the food system. The Canadian Food Inspection Agency stipulates that before a plant with a novel trait can be released into the environment, the associated risk to the environment including human health is required (Canadian Food Inspection Agency, 2000). Contamination of the food system can occur via pollen movement to conventional flax fields, by volunteers in subsequent crops and by mixing of seed in handling. Currently little is known about the reproductive biology of flax and flax volunteers and how crop management practices influence flax seed germination, seedling mortality and fecundity in succeeding grain and oilseed crops. All of these factors influence gene flow via pollen and seed, which may result in contaminating conventional harvested flax seed.

Volunteer flax initially arises from seed losses incurred during harvest and although annual flax acreage has not changed to any extent over the past two decades, the relative abundance of volunteer flax has increased from 2.0 to 15.3 over the same time period (Thomas et al. 1997). In recent Manitoba field surveys, volunteer flax was present in twice as many fields under zero tillage, but were present at much lower average densities (17.43 plants $m^{-2}$) compared to conventional tillage systems (54.7 plants $m^{-2}$) (Thomas et al. 1997). In Saskatchewan, volunteer flax was present in 3.5% of fields surveyed and occurred at an average density of 9.4 plants $m^{-2}$ (Leeson et al. 2003). Registered herbicides for volunteer flax control are limited, but generally provide consistent control of this weed over a wide range of growing conditions (Manitoba Agriculture 2005).

Weed species exhibit species-specific emergence periodicity, the time when weed seedlings typically emerge during the year (Egley and Williams, 1991; Stoller and Wax 1973). Weed seedling emergence (seed germination plus early shoot elongation) varies according to environmental conditions (Forcella et al. 1992; Moore et al. 1994), including soil temperature, soil moisture and seed depth (Clements et al. 1996; King and Oliver 1994). Tillage affects microsites or conditions within the seedling recruitment zone (Cousens and Moss 1990; du Croix Sissons et al. 2000) which, in turn, affects the time of emergence of weed seedlings in the field (Anderson and Neilsen 1996; Mohler 1993; Oryokot et al. 1997). Tillage systems influence soil temperature, soil moisture (Addae et al. 1991; Johnson and Lowery 1985; Mahli and O'Sullivan 1990) and the vertical distribution of weed seed in soil (Buhler 1992; Clements et al. 1996).

The purpose of this study was to (1) characterize the emergence periodicity of volunteer flax both before and after crop seeding, relative to site specific meterological events (rainfall) and environmental conditions within the weed seed germination zone (soil temperature and moisture) in central Alberta and (2) to determine the influence of tillage system (conventional vs. direct seeding) on its emergence periodicity. This study is a component of a 3 year, extensive project to monitor the frequency and persistence of volunteer flax in 20 commercial fields in Alberta.

**Materials and Methods**

The volunteer flax emergence periodicity study was established in a direct-seeded and a conventionally-tilled spring wheat field north of Armena, AB and northwest of Holden, AB respectively. The experiments were completely randomized designs with 10 blocks at each location. Each block consisted of a 1 $m^2$ quadrat randomly selected on May 14-16 from the center meter of a grid of 2 x 34 m rows at least 20 m from the field perimeter. The experiments were established after hard red spring wheat had been sown by growers in commercial fields. In the direct-seeded commercial field near Armena, AC Splendor was seeded to a depth of 3-4 cm at a rate of 12.5 kg/ha using a minimal-disturbance air seeder equipped with double shoot single side band openers and individual row packers. Fertilizer was placed with the seed and consisted of 100 kg N $ha^{-1}$ and 25 kg P $ha^{-1}$ ($P_2O_5$). In the conventionally-tilled commercial field near Holden, Parkland wheat was seeded to a depth of 3-4 cm at a rate of 12 kg/ha using a double disc drill. Fertilizer was applied prior to seeding and consisted of 33.6 kg N $ha^{-1}$ and 30 kg P $ha^{-1}$ ($P_2O_5$) deep banded 4-5 cm beneath the seed rows in the spring. Precipitation, soil moisture and soil temperature at 2.5 cm and 10 cm depth were recorded hourly using on-site data loggers (HOBO Micro Station) equipped with programmable sensors and rain gauges. Metrological data collection at both locations began on May 26, 2005 and was terminated August 22, 2005.

Volunteer flax plants in each quadrat were counted and recorded weekly. Newly emerged volunteer flax plants were hand weeded within each established quadrat. The emergence assessments of volunteer flax plants began immediately following snow melt and were recorded from 1 week after quadrat establishment. Counts were initiated on May 18, 2005 and terminated August 22, 2005 at both locations.

**Results and Discussion**

Total accumulated precipitation at Armena and Holden for the 2005 growing season was below normal (174 mm and 181 mm respectively) compared to the 1961-1990 precipitation normal of 317 mm (Alberta Agriculture, Food and Rural Development 2001). Both upper and lower soil temperatures recorded at Armena, were slightly higher than those recorded at Holden, throughout the growing season with the exception of weeks 4-6 in which soil temperatures were considerably warmer in the conventionally tilled plots than in the direct seeded plots (Table 1).

Weekly volunteer flax emergence varied throughout the growing season from 0 to 189 plants $m^{-2}$ in the direct seeded plots at Armena to 1 to 1510 plants $m^{-2}$ in the conventionally-seeded plots Holden. Volunteer flax reached peak emergence 2 weeks later at Armena compared to Holden (Figure 1). However, volunteer flax emergence continued over a longer period of time at Holden, at a low frequency.

Preliminary results indicate that volunteer flax emergence was poorly linked with meteorological variables. Volunteer flax emerged more rapidly (weeks 1-3) at Armena, where warmer upper and lower soil temperatures were recorded (Table 1), however in the weeks following; there was a poor correspondence between soil temperature and germination. Weeks of higher emergence were not uniformly proceeded by rainfall event (Table 1). Volunteer flax emergence ceased in the first week of August at Armena, possibly limited by reduced soil moisture, but late season emergence (weeks 11-15) continued at Holden possibly because of cooler soil temperatures and moist environmental conditions (Table 1).

At the period of peak emergence, total volunteer flax was 7-fold less in direct seeded Armena plots (189 plants $m^{-2}$) than in conventionally tilled plots at Holden (1510 plants $m^{-2}$). These fields presumably had different number of flax seeds in the soil seed bank, but differences may also be associated with the larger number of safe sites created by tillage in the conventionally seed plots or differences in vertical distribution.

In this study we found similar results to those reported in the Manitoba weed survey in which direct seeded fields had lower densities of volunteer flax compared to conventionally tilled fields (Thomas et al. 1997). Differences in seedling emergence associated with tillage for many weed species has been reported previously. Mulugeta and Stoltenberg (1997) reported that common lambsquarters

(*Chenopodium album* L.) seedling emergence increases as a result of soil disturbance, but only if there is adequate soil moisture for germination. In contrast, Ried and Van Acker (2005) reported that false cleavers (*Galium spurium* L.) seedling recruitment was due to the effect of tillage on the vertical distribution of false cleavers in the soil and not due to the effect of tillage on soil conditions associated with recruitment microsites. In a recent field survey of southern Manitoba, du Croix-Sissons (2000) reported that seedling recruitment originated from deeper soil depths in fields that received a minimum of 2 tillage passes than those fields that did not receive a tillage pass due to favorable microsite conditions below the soil surface. A better understanding of the germination behavior of volunteer flax in relation to management practices and meteorological variables presents a number of opportunities to maximize mechanical and chemical weed control efficacy and to limit opportunities for gene flow via pollen and seed in cereal crops.

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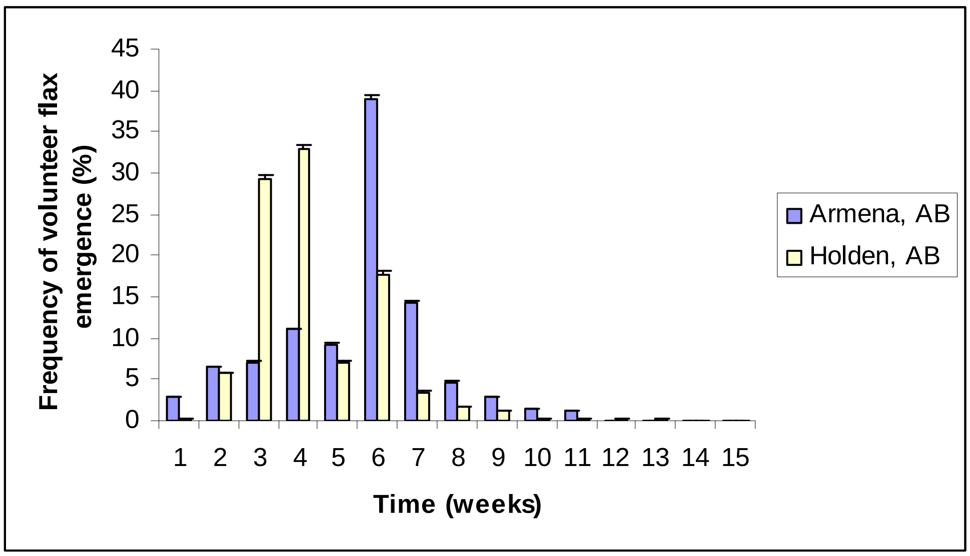


Figure 1. Frequency of volunteer flax emergence, expressed as a percentage of the total emergence, at Holden and Armena over the course of the 2005 growing season.

Table 1. Volunteer flax emergence, soil temperature, soil moisture and rainfall at Armena and Holden. Data are means (and standard errors) of 10 replicates.

| Week | Flax Emergence | Soil Temperature 2.5 cm depth | Soil Temperature 10 cm depth | Rainfall |
|---|---|---|---|---|
| | Plants m$^{-2}$ | ----------------°C | ------------------ | mm |
| Armena | | | | |
| 1 | 14 (2.67) | | | |
| 2 | 31 (7.46) | 16.8 (0.47) | 15.9 (0.42) | 0.2 |
| 3 | 34 (6.30) | 16.4 (0.28) | 17.8 (0.70) | 8.8 |
| 4 | 54 (6.50) | 14.3 (0.43) | 14.0 (0.28) | 24 |
| 5 | 45 (6.05) | 13.2 (0.43) | 13.7 (0.34) | 16 |
| 6 | 189 (28.16) | 14.2 (0.71) | 14.6 (0.49) | 3 |
| 7 | 69 (10.16) | 15.5 (0.11) | 15.1 (0.22) | 12 |
| 8 | 22 (7.88) | 17.6 (0.34) | 17.3 (0.25) | 20 |
| 9 | 14 (6.05) | 17.8 (0.68) | 16.6 (0.23) | 15 |
| 10 | 7 (2.04) | 16.5 (0.75) | 15.8 (0.36) | 4 |
| 11 | 6 (1.84) | 17.1 (0.67) | 15.6 (0.39) | 1 |
| 12 | 0 | 19.9 (0.69) | 17.1 (0.27) | 6 |
| 13 | 0 | 13.52 (0.45) | 13.3 (0.22) | 1 |
| 14 | 0 | 13.01 (1.38) | 11.8 (0.58) | 34 |
| 15 | 0 | 13.47 | 13.7 | 0.2 |
| Holden | | | | |
| 1 | 10 (3.99) | | | |
| 2 | 266 (52.99) | 15.0 (0.16) | 14.9 (0.20) | 0.8 |
| 3 | 1348 (147.37) | 16.7 (0.20) | 16.6 (0.20) | 2 |
| 4 | 1510 (275.17) | 15.8 (0.32) | 15.8 (0.28) | 11 |
| 5 | 326 (79.79) | 15.2 (0.52) | 14.9 (0.49) | 43 |
| 6 | 815 (139.13) | 15.5(0.55) | 15.5 (0.53) | 1 |
| 7 | 158 (36.35) | 14.9 (0.10) | 14.7 (0.08) | 4 |
| 8 | 81 (17.83) | 16.8 (0.30) | 16.5 (0.27) | 22 |
| 9 | 50 (15.65) | 16.7 (0.19) | 16.4 (0.19) | 1 |
| 10 | 6 (1.53) | 15.9 (0.42) | 15.7 (0.38) | 4 |
| 11 | 7 (4.05) | 16.0 (0.45) | 15.8 (0.42) | 48 |
| 12 | 12 (4.07) | 16.8 (0.33) | 16.6 (0.32) | 8 |
| 13 | 7 (1.88) | 12.9(0.22) | 12.9(0.23) | 8 |
| 14 | 1 (0.51) | 11.8 (0.57) | 11.2 (0.55) | 28 |
| 15 | 1 (0.27) | 13.9 | 14.2 | 0 |

# The Residual Effect of Sequential ALS Inhibiting Herbicide Applications

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The group of herbicides that inhibit acetolactate synthase (ALS) enzyme have become increasing popular in Western Canadian production agriculture. Imazamox/imazethapyr, a common herbicide used in Western Canada for peas, along with the cereal herbicides imazamethabenz, flucarbazone-sodium, sulfosulfuron, and florasulam all potentially have soil residual properties. These ALS inhibiting herbicides are predominantly degraded by soil microbes and hydrolysis (Vencill 2002). Certain soil factors including microbial composition and activity, moisture, organic matter, pH, temperature, and soil texture have shown to influence the persistence of herbicides (Ayeni et al. 1998). Especially under conditions of drought and/or cool temperatures these herbicides have the potential to persist past the season of application. The objective of this study was to determine the extent to which ALS inhibiting herbicides interact and influence phytotoxicity when applied sequentially.

## Materials and Methods

### Field Trial Study

Three locations were selected in Saskatchewan, Saskatoon, Melfort, and Scott, with the experiment starting in 2002. The experiment was set up as an RCBD with four replications of ten treatments. In the first year of the experiment all the treatments were seeded to peas (*Pisum sativum* L. ‘Swing’), with treatments one through five being sprayed with a non-residual herbicide and six through ten being sprayed with imazamox/imazethapyr. In year two all the treatments were seeded to wheat (*Triticum aestivum* L. ‘Eatonia’) with treatments one and six sprayed with a non-residual herbicide, two and seven with imazamethabenz, three and eight with flucarbazone-sodium, four and nine with sulfosulfuron, and five and ten with florasulam. Between the second and third year growing seasons soil samples were taken from each treatment. The third year had all treatments seeded to Roundup Ready$^{tm}$ canola (*Brassica napus* L. ‘DKL 3455’) and sprayed with a non-residual herbicide.

The soil samples were air dried and passed through a 2mm sieve. These soils were then used to perform a root inhibition bioassay to test for residual herbicides (Eliason et al. 2004). Oriental mustard (*Brassica juncea* L. ‘Cutlass’) was the selected plant for the residual herbicide root length inhibition bioassays. The seeds were pregerminated for 24 hours prior to seeding. For each field treatment 100 g of soil was measured and placed into 6 Styrofoam cups. The soil was then wetted to ¾ water holding capacity. Five pregerminated seeds of similar size and radicle protrusion were selected and placed into the Styrofoam cups, covered with a small amount of soil and were lightly packed. The soil was then covered with plastic beads to reduce evaporation losses. The cups were then wetted to full water holding capacity, randomized, placed under a fluorescent canopy, and covered with a plastic sheet for 24 hours.

The plants were allowed to grow for 5 days. On the fifth day after seeding the plants were manually removed from the soil and the root lengths were then measured.

### Controlled Interaction Study

One 70 L can of untreated soil was collected from each of the three sites. The soil was air dried and passed through a 2mm sieve. Stock solutions of the herbicides to be tested were created by placing a known quantity of herbicide in approximately 50 ml of methanol then diluting with water to the 1L mark in a volumetric flask (Eliason et al. 2004). Standard solutions were created from the stock solution resulting concentrations of 0.2, 0.4, 0.8, 1.2, 1.6, 2.4 a.i. mg $L^{-1}$ of imazamox/imazethapyr; 10, 20, 30, 40, 60, 80 a.i. mg $L^{-1}$ of imazamethabenz; 0.2, 0.5, 1, 1.5, 2, 3 a.i. mg $L^{-1}$ of flucarbazone-sodium; 0.38, 0.75, 1.13, 1.5, 2.25, 3 a.i. mg $L^{-1}$ of sulfosulfuron; and 0.025, 0.05, 0.1, 0.2, 0.3, 0.4 a.i. mg $L^{-1}$ of florasulam.

The bioassay was set up similar to with the field trial samples. One hundred grams of untreated soil was weighed into 6 Styrofoam cups. For part one of this study, 1 ml of the standard solution was added to the untreated soil for each of the concentrations of all five different herbicides. For part two the same 1 ml of each concentration of imazamethabenz, flucarbazone-sodium, sulfosulfuron, and florasulam were utilized, in combination with another 1 ml of standard solution of imazamox/imazethapyr. The imazamox/imazethapyr concentration which yielded about 30% root inhibition for that specific soil, was the concentration utilized. The remainder of the bioassay followed the procedure as stated previously.

### Colby's Equation

In order to determine if the interaction between two different herbicide residues in the soil is synergistic, additive, or antagonistic, the observed values need to be compared to expected values generated from Colby's equation (Colby 1967). Colby's equation states that $E = (XY)/100$, where E is the expected growth as a percent of the check caused by 2 combined herbicides, X is the growth as a percent of the check caused by herbicide A, and Y is the growth as a percent of the check caused by herbicide B. To be able to compare the expected results to percent root inhibition given by the bioassay, $100 – E$ must be utilized to calculate the expected inhibition. When the expected root inhibition is compared to observed root inhibition, the type of interaction can be interpreted. If observed is greater than expected there is a synergistic interaction, if observed is equal to expected there is an additive interaction, or if observed is less than expected there is an antagonistic interaction.

## Results and Discussion

### Field Trial Study

There were two ways of determining if the were any interactions between the herbicide residues in soil. The first involved comparing the yields of the Roundup Ready[tm] canola from in the ten treatments from each location. The canola yields from the Saskatoon and Melfort sites harvested in 2004 showed no significant difference between treatments that had only residual herbicides in year 1 compared to the treatments that had two residual herbicides in years 1 and 2. The Scott trial did show a significant difference in yield in the treatments with imazamethabenz and sulfosulfuron alone compared to combination with imazamox/imazethapyr (Fig. 1).

The second measurement of herbicide interactions for the field trials was application of the root inhibition bioassay. In the field trial soil samples, the bioassay could detect soil residues from all five of the tested herbicides (Fig. 2). In all cases the combined residues of imazamox/imazethapyr and either imazamethabenz, flucarbazone-sodium, sulfosulfuron, or florasulam resulted in greater root length inhibition than these herbicides alone, although the difference was not always statistically significant.

The yield data and the root inhibition bioassay results were then examined using Colby's equation. In all cases, the observed interactions between the imazamox/imazethapyr residues and the residues from the other four herbicides were not statistically different from the expected values generated.

Therefore the interactions of the herbicides in the field trials appear to be additive in terms of phytotoxic effects.

**Controlled Interaction Study**

The bioassays for each concentration of the five individual herbicides yielded response curves for each soil type. These values were utilized for Colby's equation to be compared against the bioassays that received two herbicides (Fig. 3). The amount of imazamox/imazethapyr that was required to cause significant reduction in root length varied with each soil collected for the experiment. The Saskatoon soil required 2 a.i. µg $kg^{-1}$ of imazamox/imazethapyr and 8 a.i. µg $kg^{-1}$ of imazamox/imazethapyr was required for the Melfort soil. In the comparisons of the observed root inhibition to the expected inhibition, all except one showed additive interactions in the Saskatoon and Melfort soils. In the case of imazamox/imazethapyr and flucarbazone in Melfort soil, there was a significant difference between the observed and expected inhibition at the flucarbazone concentrations of 10, 15, and 20 a.i. µg $kg^{-1}$, suggesting a synergistic response between these two herbicides at these concentrations.

The results of this experiment tend to predict an additive interaction between the residues of imazamox/imazethapyr and the residues of imazamethabenz, flucarbazone-sodium, sulfosulfuron, and florasulam. This still can lead to problems with sensitive crops because the two herbicides together may cause greater damage than if only one of the herbicides is present. Future work will include complete bioassay analysis for the rest of the field trial samples and the remaining controlled interaction studies to determine if these interactions are consistent for all soil types.

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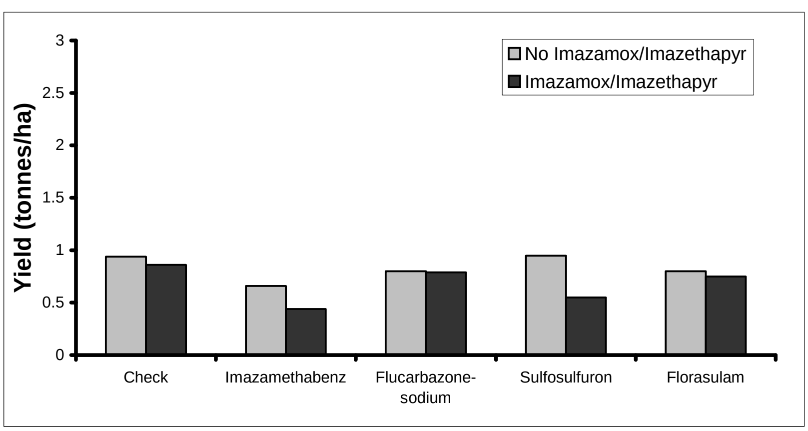


Figure 1: Yield of Roundup Ready canola from the Scott location field trial harvested 2004.

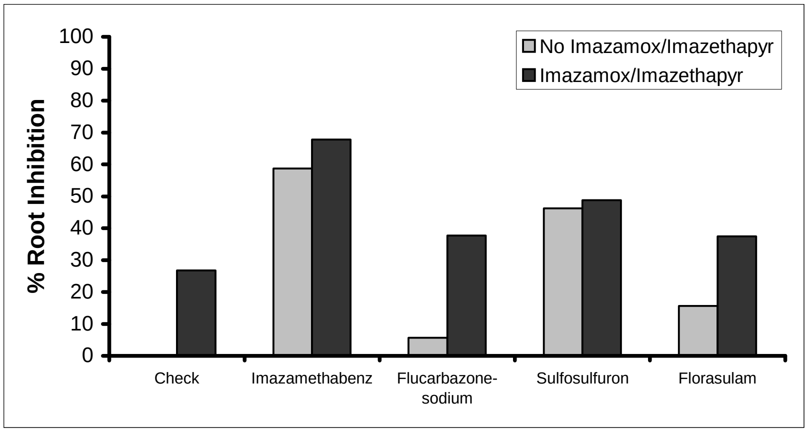


Figure 2: The percent root inhibition of the bioassay from soil samples taken from the Saskatoon field trial after the 2003 growing season.

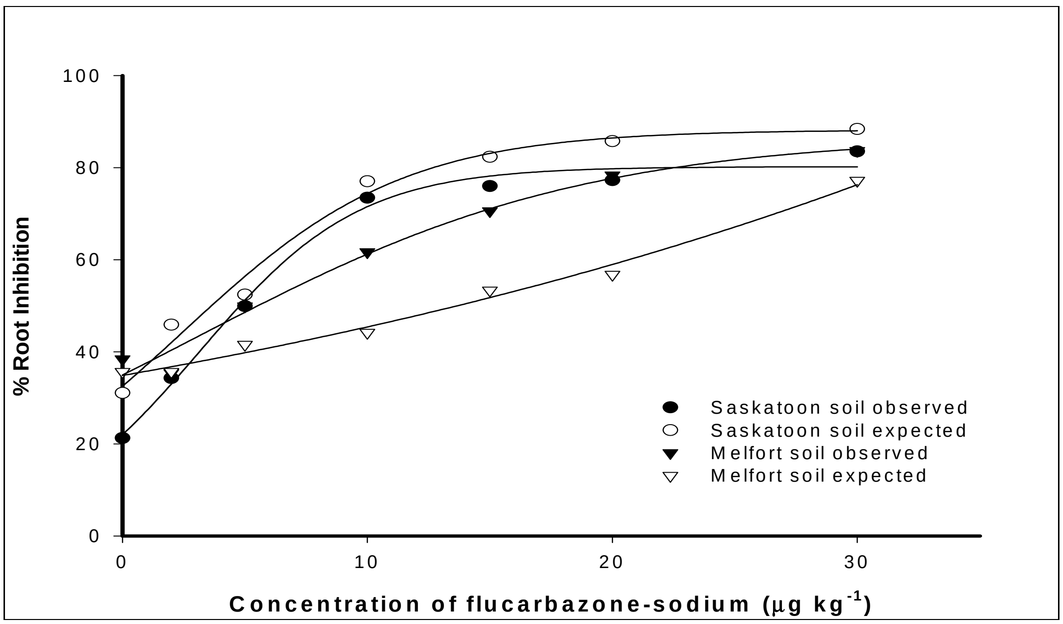


Figure 3: The observed percent root inhibition at various concentrations of flucarbazone-sodium with 2 µg kg$^{-1}$ of imazamox/imazethapyr in the Saskatoon soil and with 8 µg kg$^{-1}$ of imazamox/imazethapyr in the Melfort soil compared to the expected results derived from Colby's equation.

# Soil properties affect Odyssey and Everest phytotoxicity

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**Abstract**

Odyssey and Everest are ALS inhibitor (Group 2) herbicides containing active ingredients that have a high potential to persist in soil. These herbicide residues may damage subsequent sensitive crops when they are bioavailable to the plant by root uptake. Since there are limited studies on the phytotoxicity of Odyssey and Everest in Manitoba soils, this project conducted an oriental mustard root bioassay on four Manitoba soil series spiked with known concentrations of Odyssey and Everest. Root lengths of plants grown at seven application rates of each of Odyssey and Everest were measured and compared to root lengths of plants grown without herbicide. $GR_{50}$ (herbicide rates causing a 50% growth reduction in root length) were calculated. $GR_{50}$ values were significantly less for Odyssey (increased activity/phytotoxicity) than for Everest. Both Odyssey and Everest phytotoxicity were well correlated with soil organic carbon content, and negative correlations with pH were not significant. It is hypothesized that the differences in phytotoxicity observed between soil types are related to the sorption of Everest and Odyssey to soil. Specifically, it is likely that as herbicide sorption increases, the bioavailability of herbicide residues decreases, resulting in a lower phytotoxicity. Results of this and other studies will help identify which Manitoba soils have greater risk of crop injury following applications of Odyssey or Everest.

**Introduction**

Odyssey (imazamox:imazethapyr 1:1) and Everest (flucarbazone-sodium) are ALS inhibitor (Group 2) herbicides frequently used in Western Canada. Odyssey, belonging to the imidazolinone class of herbicides, contains 35% imazamox and 35% imazethapyr formulated as a dispersible granule. It is applied post emergence to field peas, Clearfield canola and alfalfa to control both grassy and broadleaf weeds (Vencill 2002; Anonymous 2003). Everest is a relatively new post emergence chemical used to control grassy and some broadleaf weeds in wheat (Vencill 2002; Anonymous 2003). Its active ingredient is flucarbazone-sodium (70%), formulated as a water dispersible granule, and it is chemically classified as a sulfonylamino carbonyltriazolinone.

Odyssey, Everest, and certain other Group 2 herbicides have a high potential to persist in soil past the season of application, potentially damaging subsequent sensitive crops (Loux et al. 1989; Moyer and Esau 1996; Jourdan et al. 1998; O'Sullivan et al. 1998). Herbicide residues in soil can be phytotoxic when they are bioavailable to the plant by root uptake, and this bioavailability is dependent on soil chemical and physical properties. Bioassays are sensitive, simple techniques that can measure bioavailable herbicide residues in soil and aid in understanding the relation between soil properties and herbicide phytotoxicity. Eliason et al. (2004) tested various crops to determine which could provide a sensitive bioassay for the detection of flucarbazone-sodium in soil. Of the five crops they tested, oriental mustard (*Brassica juncea*) root length was found to be the best indicator. Eliason et al. (2004) measured flucarbazone-sodium phytotoxicity in five Saskatchewan soils and one Manitoba soil, and found that phytotoxicity in the Manitoba soil was much lower than in the others tested. Thus, the objective of this study was to gain a better understanding of the effect of soil properties on the phytotoxicity of Odyssey and Everest in Manitoba soils using the oriental mustard root bioassay.

## Materials and Methods

*Soil Sampling and Characterization*

Four surface soils (0-10 cm) with varying properties and no history of Odyssey or Everest application were collected from Southern Manitoba (Figure 1, Table 1). Soils were identified by their soil series classification and soil texture: Lundar Clay Loam, Manitou Silty Loam, Red River Clay and Stockton Loamy Sand. Soils were air-dried and sieved (< 2 mm) prior to soil property (measured in duplicates) and bioassay analyses. Soil texture was measured using the hydrometer method (Gee and Bauder 1986). Soil organic carbon content was determined first by removing inorganic carbon by digestion with 6 N HCl (Tiessen et al. 1983) and then by dry combustion of 0.12 g oven-dried soil using a Leco model CHN 600 C and N determinator (Nelson and Sommers 1982). Soil pH was quantified using 20 mL of 0.01 M $CaCl_2$ and 10 g soil (Hendershot and Lalande 1993). Field capacity (as a percent) was measured by determining the weight of water required to completely wet a sample of air-dried soil to the bottom of a plastic vial without leaving standing water in the bottom of the vial after a 24-hour period (Eliason et al. 2004).

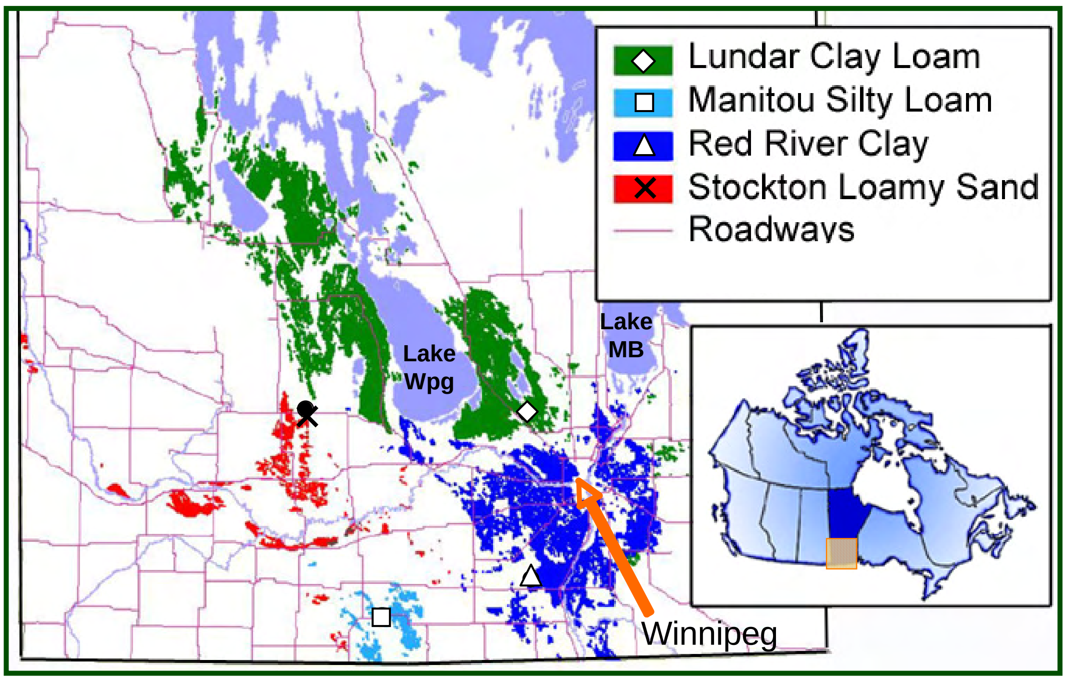



Figure 1. Map of Southern Manitoba identifying the geographical location of the four sampling points and the area of each soil series.

Table 1. Selected soil properties for the four soil series studied.

| Soil Series | Clay Content (%) | Organic Carbon Content (%) | pH (in $CaCl_2$) | Field Capacity (%) | Bulk Density (g $cm^{-3}$) |
|---|---|---|---|---|---|
| Lundar Clay Loam | 30.6 | 3.7 | 7.3 | 34 | 0.97 |
| Manitou Silty Loam | 25.7 | 4.5 | 5.8 | 42 | 0.84 |
| Red River Clay | 53.1 | 3.9 | 7.4 | 37 | 0.95 |
| Stockton Loamy Sand | 9.7 | 0.5 | 7.2 | 19 | 1.21 |

*Chemical Solutions and Root Bioassay*

The oriental mustard root bioassay described below was adapted from Eliason et al. (2004). All solutions were made from herbicide formulated products (f.p.). From a stock solution of 100 mg f.p. $L^{-1}$, standard solutions containing 0.15, 0.30, 0.60, 1.25, 2.50, 5.00, and 10.00 mg f.p. $L^{-1}$ were prepared in deionized water. In order to account for differences in soil bulk densities, weights of air-dried soil equivalent to 89 $cm^3$ were measured into 207 mL clear plastic Dixie cups (87 g Lundar Clay Loam, 75 g Manitou Silty Loam, 85 g Red River Clay, 108 g Stockton Loamy Sand). Aliquots (0.75 mL) of each standard solution were added to the calculated volumes of distilled water required to bring each cup of soil to 100% of its field capacity. These solutions were added to the cups of soil and mixed thoroughly by hand using a metal spatula. For the control (untreated) treatments, only distilled water was used to bring the soil to the desired moisture level. Each combination of soil series and herbicide was replicated six times, and the entire experiment was duplicated.

Application rates were 0, 1.3, 2.5, 5.0, 10.5, 21.0, 42.0 and 84.0 mg f.p. $m^{-3}$ where 42.0 mg f.p. $m^{-3}$ is approximately equivalent to the field application rate of 30 g a.i. $ha^{-1}$ for each herbicide, assuming the chemical is distributed through the top 10 cm layer of soil. For the purposes of this paper, these concentrations will be expressed as 0, 3, 6, 12, 25, 50, 100 and 200% of the field application rate.

Spiked, mixed soil cups were placed in plastic trays, covered, and left overnight in the dark to equilibrate. Meanwhile, oriental mustard seeds (variety AC Vulcan) were distributed into Petri dishes lined with wetted filter papers. Dishes were covered and seeds left in the dark to germinate. After 24 hours, seven pre-germinated seeds with radicles 2-3 mm long were planted into each cup of spiked soil to a depth of 5 to 10 mm. Soil surfaces were covered with 15 g polyethylene plastic pellets to minimize moisture loss.

Seedlings were grown for five days at room temperature under fluorescent lights and were watered daily to maintain 100% field capacity (by weight). After five days, whole seedlings were carefully removed from the soil and root lengths were measured. For each cup/replicate, root lengths were averaged over the seven plants, and percent of control was calculated for each:

$$L_t / L_0 \text{ x } 100\% \qquad [1]$$

where $L_t$ is the root length measured in the Odyssey- or Everest-treated soil, and $L_0$ is the average root length measured in the untreated soil.

*Statistical Analyses*

In order to compare dose responses for each soil and herbicide combination, data were subjected to nonlinear regression analysis using a 4 parameter log-logistic model (Seefeldt et al. 1995):

$$y = C + \frac{D - C}{1 + \exp[b(\log(x) - \log(I_{50}))]} \qquad [2]$$

where $y$ = oriental mustard root length (percent of untreated control), $x$ = herbicide dosage (percent of field application rate; a small positive value of 1.0 was assigned to 0 % dosage to calculate natural logarithms), $C$ = lower limit (asymptote) of the response curve, $D$ = upper limit, $I_{50}$ = x-axis value that corresponds to the inflection point at the centre of the curve (i.e. “drop line”) and $b$ = slope of the curve at the $I_{50}$ value. For each herbicide, individual curves for each soil type were statistically tested systematically for common $C$, common $D$, common $b$, and common $I_{50}$, using the lack-of-fit $F$ test at the 0.05 level of significance as outlined by Seefeldt et al. (1995).

The $I_{50}$ value corresponds to the inflection point of the curve, but because in most instances the curves’ upper and lower limits are not 100 and 0, respectively, fitted $I_{50}$ values do not necessarily represent the dosage of herbicide required to reduce root length by 50 % relative to the untreated control. Thus, $GR_{50}$ values were calculated for each herbicide/soil combination by solving equation 2 for $x$ at $y$ = 50 %:

$$x = I_{50} [((D - C) / (y - C) - 1)^{(1/b)}] \quad [3]$$

where $x = GR_{50}$, which is the herbicide dosage at $y$ = 50% of the untreated root length. These $GR_{50}$ values were then correlated to soil clay content, organic carbon content, and soil pH by determining Pearson correlation coefficients.

## Results and Discussion

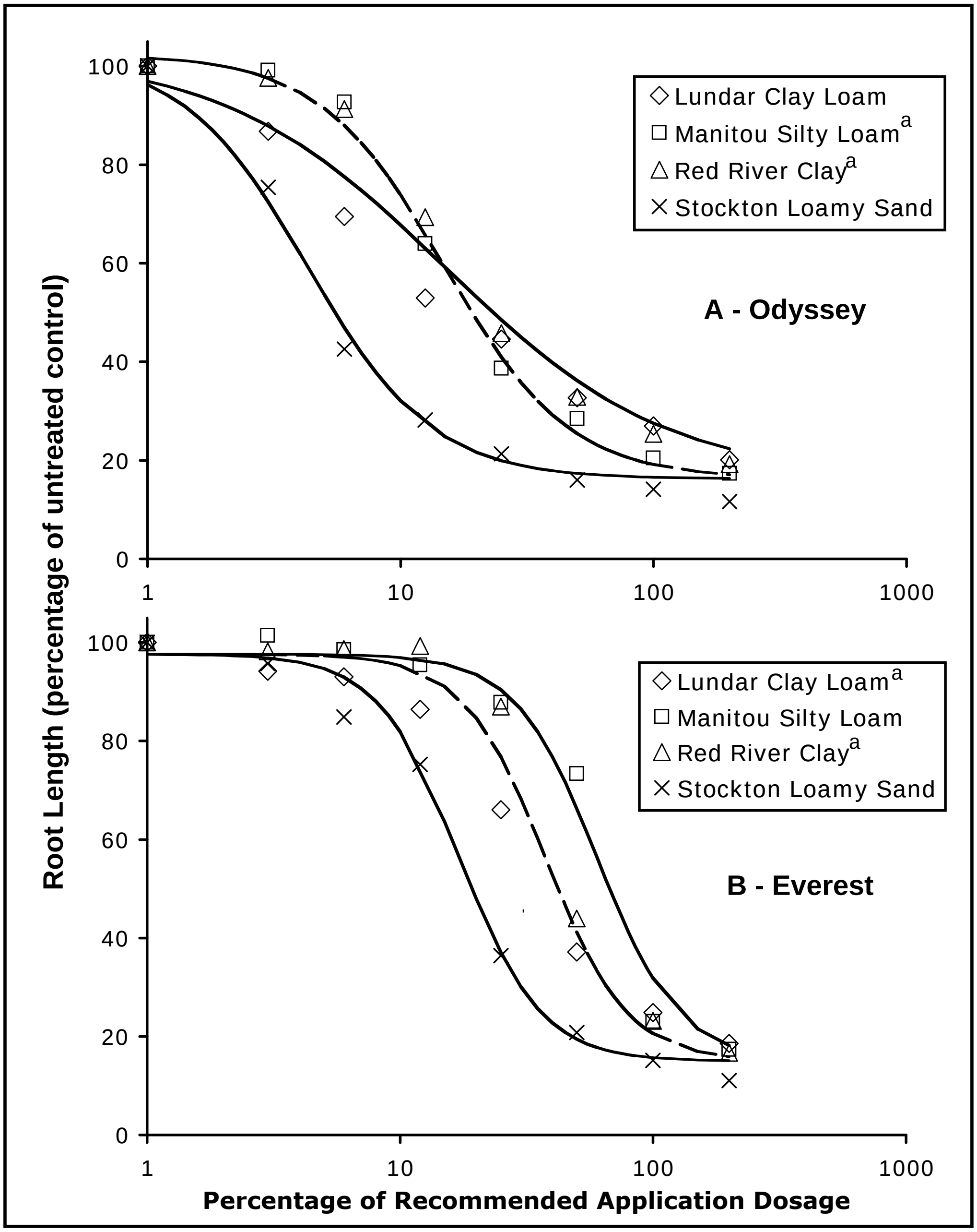
The response of oriental mustard root length to increasing dosages of Odyssey or Everest was described very well by the log-logistic model, as indicated graphically by the high $R^2$ values (Figure 2, Table 2). For response to Odyssey, all dose response curves had the same lower (*C*) and upper (*D*) limits. Three of the four curves (Manitou Silty Loam, Red River Clay, Stockton Loamy Sand) had the same slope (*b*), as depicted by the parallel curves (Figure 2A). The Stockton Loamy Sand $I_{50}$ value was significantly lower than the other three soils, indicating that Odyssey is more phytotoxic to oriental mustard in this soil as compared to the others. For response to Everest, all dose response curves had the same lower (*C*) and upper (*D*) limits and all curves were parallel, sharing the same slope (Figure 2B). However, three different $I_{50}$ values were fitted, with Lundar Clay Loam and Red River Clay having all parameter estimates common. $I_{50}$ values in increasing order are Stockton Loamy Sand < Lundar Clay Loam = Red River Clay < Manitou Silty Loam. Overall, Everest was less phytotoxic to oriental mustard than Odyssey by at least a factor of two (Table 2), and in all soils, Odyssey phytotoxicity was observed at even the lowest rate applied (Figure 2A).

Table 2. Parameter estimates for log-logistic dose response curves of oriental mustard grown in four Manitoba soils containing either Odyssey or Everest. Data fitted to the model were oriental mustard root lengths expressed as a percentage of untreated controls. Refer to Materials and Methods for a description of the log-logistic model fitted.

| Herbicide | Soil Series | $C^a \pm$ SE | $D \pm$ SE | $b \pm$ SE | $I_{50} \pm$ SE | $GR_{50}{}^b$ |
|---|---|---|---|---|---|---|
| Odyssey | Lundar Clay Loam | 16.2 ± 1.8 | 102.4 ± 2.5 | 1.0 ± 0.1 | 14.9 ± 1.1 | 23.2 |
| $R^2$ = 0.99 | Manitou Silty Loam | 16.2 ± 1.8 | 102.4 ± 2.5 | 1.8 ± 0.2 | 14.9 ± 1.1 | 19.1 |
| | Red River Clay | -------------------same as Manitou Silty Loam -------------------------- | | | | |
| | Stockton Loamy Sand | 16.2 ± 1.8 | 102.4 ± 2.5 | 1.8 ± 0.2 | 4.3 ± 0.4 | 5.5 |
| Everest | Lundar Clay Loam | 15.0 ± 2.4 | 97.6 ± 1.6 | 2.7 ± 0.3 | 37.6 ± 2.4 | 42.1 |
| $R^2$ = 0.98 | Manitou Silty Loam | 15.0 ± 2.4 | 97.6 ± 1.6 | 2.7 ± 0.3 | 60.1 ± 5.0 | 67.4 |
| | Red River Clay | ------------------ same as Lundar Clay Loam ---------------------------- | | | | |
| | Stockton Loamy Sand | 15.0 ± 2.4 | 97.6 ± 1.6 | 2.7 ± 0.3 | 17.2 ± 1.4 | 19.24 |

[a] Statistical differences between parameter estimates were determined using the lack-of-fit *F* test at the 0.05 level of significance (refer to Materials and Methods).
[b] $GR_{50}$ values were calculated by solving the log-logistic model for $x$ at $y$ = 50 % (refer to Materials and Methods).

Correlation analysis was conducted between $GR_{50}$ values and soil properties. $GR_{50}$ was used rather than $I_{50}$ for consistency, since $I_{50}$ values did not all occur at $y$ = 50%. No significant correlations were found at the 0.05 level, probably because only four soils were studied. Additional soils are needed to obtain more reliable correlations. However, at the 0.10 level, some significance was observed (Table 3). Both Odyssey and Everest showed strong correlations between $GR_{50}$ and organic carbon content, however no significant correlation was observed between $GR_{50}$ and clay content or pH (Figure 3). These findings are in agreement with Eliason et al. (2004) who observed a strong significant correlation ($p < 0.01$) between $I_{50}$ values for Everest and organic carbon content, but no significant correlation with clay content (p=0.90) or pH (p = 0.39). As Everest is a recently commercialized herbicide, no other studies examining the




correlation of Everest phytotoxicity and soil properties have been published to date.

Figure 2. Dose response curves of oriental mustard root lengths (% of untreated) grown in four Manitoba soils containing either A) Odyssey or B) Everest herbicide. Symbols are means of twelve replicates. The curves of soil series followed by the same letter are not significantly different according to the lack-of-fit *F* test (refer to Materials and Methods). Refer to Table 2 for parameter estimates of the log-logistic model fitted.

Table 3. Correlation analysis between Odyssey or Everest calculated (using Equation 3) $GR_{50}$ values and soil properties. Correlation coefficients are followed by probabilities in parentheses.

| Soil Property | Odyssey $GR_{50}$ | Everest $GR_{50}$ |
|---|---|---|
| Clay Content | r = 0.67 (0.33) | r = 0.34 (0.66) |
| Organic Carbon Content | r = 0.91 (0.09) | r = 0.90 (0.10) |
| Soil pH | r = -0.14 (0.86) | r = -0.79 (0.21) |

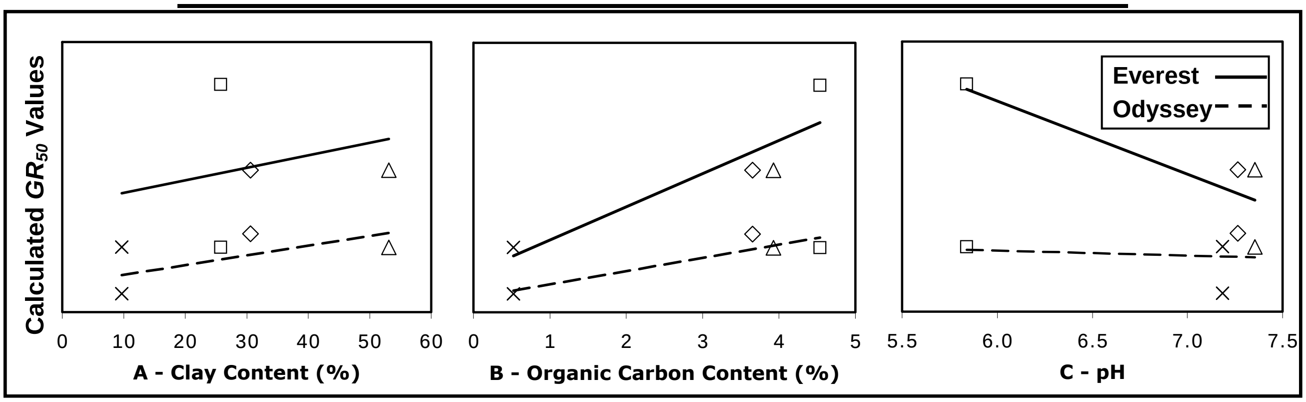



Figure 3. Graphical representation of the relation between Odyssey and Everest $GR_{50}$ values and A) percent clay content, B) percent organic carbon content, and C) soil pH.

It is likely that the observed phytotoxicity is related to Odyssey and Everest sorption to soil. As herbicide sorption to soil increases, the bioavailability of herbicide residues for plant uptake decreases, resulting in lower phytotoxicity and greater $I_{50}$ and $GR_{50}$ values (when modeled) (Eliason et al. 2004). Sorption of both imazamox and imazethapyr (active ingredients in Odyssey) has been found to increase with increasing soil organic matter and clay contents, and decreasing pH below 6.5 (Vencill 2002). Loux et al. (1989) and Goetz et al (1990) found that imazethapyr was more persistent in soils with higher clay and organic matter contents, which would have greater adsorptive potential compared to those soils with lower contents. Although clay content, organic carbon and pH did not significantly influence herbicide phytotoxicity in this study (at the 0.05 level), this result may have been different if addition soil types had been included in the experiments.

*Conclusion*

In this study, Odyssey and Everest phytotoxicity as assessed by the oriental mustard root bioassay procedure differed between herbicides and soils. Both Odyssey and Everest phytotoxicity decreased with increasing soil organic carbon content. This relation probably is a result of the increased sorption of the herbicides to soil, thus decreasing the bioavailability to plant roots. Since increased sorption also increases the persistence of Odyssey and Everest in soil, additional studies are needed to fully understand differences in carry-over risks among Manitoba soils. However, recropping decisions can be improved through knowledge of soils' properties and use of the oriental mustard root bioassay to detect bioavailable residues prior to planting sensitive crops.

## Acknowledgements

This study was supported by an NSERC Post-Graduate Scholarship. A special thanks is extended to Lyle Friesen for help with data analysis.

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# Resistance to Acetolactate Synthase Inhibitors in Green Foxtail

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**Abstract**

Five green foxtail populations were found to be resistant to imazethapyr in Ontario from 2001 and 2003. Acetolactate synthase (ALS) enzyme assays were conducted to determine resistance level to imazethapyr, nicosulfuron, pyrithiobac, and flucarbazone. ALS gene sequencing was performed with those populations. Ensyme assays indicated that the five resistant green foxtail populations were significantly resistant to imazethapyr compared to the susceptible population. All resistant populations had cross-resistance to nicosulfuron and flucarbazone. Only three populations had cross-resistance to pyrithiobac. Sequence analyses revealed single base-pair mutations were present in the resistant populations of green foxtail. These mutations coded for Thr, Asn or Ile substitution at $Ser_{653.}$ In addition, a new mutation was found in one of the population. It coded for an Asp substitution at $Gly_{654}$. There is agreement between the spectrum of resistance observed at the enzyme and the type of resistance known to be conferred by these substitutions.

## Introduction

The ALS enzyme is the target site of five chemical classes currently commercialized in agriculture: sulfonylureas (SU), imadazolinones (IMI), triazolopyrimidines (TP), pyrimidinyl-oxybenzoates (POB) and sulfonylamino-carbonyl-triazolinones (SCT) (Saari et al., 1994).
Because of their widespread usage, ALS inhibitors have imposed high selection pressure for resistance. The most important mechanism of resistance is an insensitive ALS enzyme. Six conserved amino acids have been identified in ALS in higher plants that are linked to resistance (Tranel and Wright, 2002; Tharayil-Santhakumar, 2004). Depending on the amino acid substitution, different cross-resistance patterns occur (Saari et al., 1994). For example, the $Trp_{574}$Leu substitution confers resistance to all classes of ALS inhibitors while substitutions at $Ala_{122}$ or $Ser_{653}$ confer resistance to IMIs with cross-resistance to POBs, but not to SUs and TPs (Duggleby et al., 2000).
In Ontario, the first resistance cases selected with ALS inhibitors were reported in Powell amaranth and redroot pigweed in 1997 (Ferguson et al., 2001). Since then, it has been confirmed in other broadleaf weeds: common ragweed, eastern-black nightshade, common waterhemp, common lambsquarters and common cocklebur (Heap, 2004). In 2001, the first grass weed resistant to ALS inhibitor was reported in Ontario (green foxtail population 01). Subsequently, four more populations (green foxtail populations 15, 16, 17, and 19) were reported from three other farms. All these populations survived field application of imazethapyr.
The objectives of this research are: (1) to characterize the level of resistance to imazethapyr and cross-resistance to nicosulfuron, pyrithiobac, and flucarbazone; and (2) to determine the genetic and biochemical basis of resistance to the ALS inhibitor herbicides.

## Materials and Methods

### Resistance level

ALS enzyme was extracted in all populations. The crude enzyme was assayed with imazethapyr, nicosulfuron, pyrithiobac, and flucarbazone. The ALS activity was converted to a percentage of the mean control and analyzed using a loglogistic statistical model. The resistance factors were calculated for each population by dividing the dose required to reduce activity by 50% ($I_{50}$) for the resistant population by the $I_{50}$ of the susceptible population.

Mechanism of resistance

DNA was extracted in all populations and ALS was polymerase chain reaction (PCR) amplified. PCR products were sequenced to determine molecular basis of resistance.

## Results and Discussion

Resistance level

ALS enzyme inhibition curves showed that the five green foxtail populations were significantly resistant to imazethapyr with higher $I_{50}$ values compared to the susceptible population (Figure 1). Resistance factors ranged from 15 to 260-fold. Resistant populations showed various pattern of cross-resistance to POBs, SUs, and SCTs. All resistant populations presented cross-resistance to nicosulfuron (11 to 140-fold) and flucarbazone (2 to 4-fold) (Figures 2 and 3). Cross-resistance to pyrithiobac was found in only three populations with resistance factors ranging from 5 to 190-fold (Figure 4).

Mechanism of resistance

Sequence analysis revealed mutations in the resistant populations compared to wild type susceptible. These mutations coded for substitution at $Ser_{653}$. The serine residue at position 653 is known to be conferring resistance when changed (Sibony et al., 2001). Three different substitutions at this position were found in four populations. A substitution of $Ser_{653}$Thr was observed in populations 01 and 19, while population 16 had $Ser_{653}$Asn. These substitutions have been seen previously in other species and confer the same spectrum of resistance we observed. In addition, a $Ser_{653}$Ile substitution was seen in population 15. This change has been identified in a spontaneous mutant of rice subsp. *japonica* (Ohshima et al., 2003) but has never been seen in a weed population. Interestingly, populations 15 and 16 were both from the same location (Arthur, ON). Finally, population 17 had a mutation one codon downstream from the other populations. It coded for a $Gly_{654}$Asp substitution which has not been reported before. Since no other mutation were found in the gene of population 17 and as it is located near an imidazolinone resistance site, this new mutation is very likely the cause of resistance in this population.

These results are significant in many aspects. First, four different mutations were observed in five different populations, all selected mostly with imazethapyr. This highlights the high variability in possible mutations in the ALS. This also reinforces the fact that it is very difficult to predict what mutation might be selected just by knowing the selective agent. Furthermore, two new mutations, one of which having never been documented before, were observed. This shows that there is still potential for weeds to develop resistance through means that we did not know. It is also interesting that two populations from the same farm had two distinct mutations: this emphasizes the inherent variability in response to ALS inhibitors selection pressure.

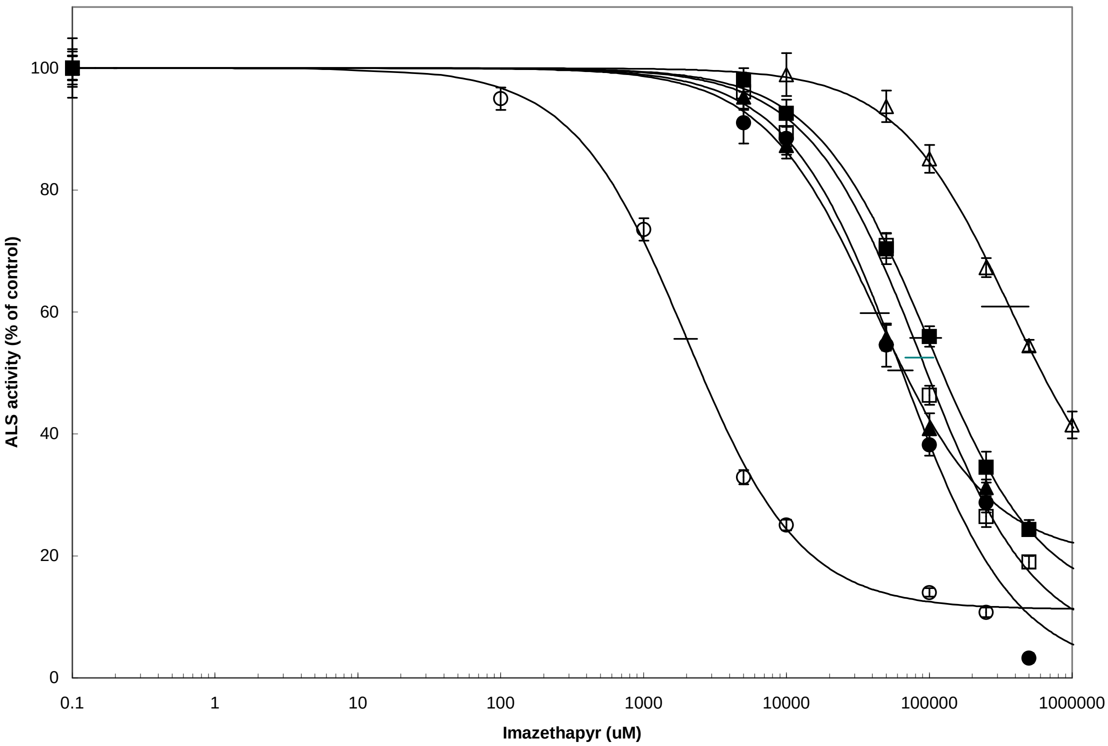


Figure 1 ALS activity of *S. viridis* population 04(○)(wildtype), population 15(●), population 16(△), population 17 (▲), population 19 (■), and population 01(●) after treatment with imazethapyr. ALS activity was measured after completion of the enzyme assay and results were expressed as the percentage of the untreated control for each biotype. Inhibition curves were generated by calculating values for the log-logistic formula $y=11.2 + (((99.9-11.2)/ (1+(x/2\ 003)^{1.09}))$, $y=0.83 + (((99.9-0.83)/ (1+(x/63\ 849)^{1.09}))$ $y=21.8 + (((99.9-21.8)/ (1+(x/364\ 078)^{1.09}))$ $y=19.7 + (((99.9-19.7)/ (1+(x/42\ 652)^{1.09}))$ $y=11.6 + (((99.9-11.6)/ (1+(x/97\ 253)^{1.09}))$ $y=5.03 + (((99.9-5.03)/ (1+(x/87\ 654)^{1.09}))$ where y is the ALS activity value and x is the herbicide dose, for populations 04, 15, 16, 17, 19, and 01, respectively. Horizontal error bars represent the 95% confidence interval of the $I_{50}$ values. Each point is the mean of twelve replicates, plotted with the standard errors.

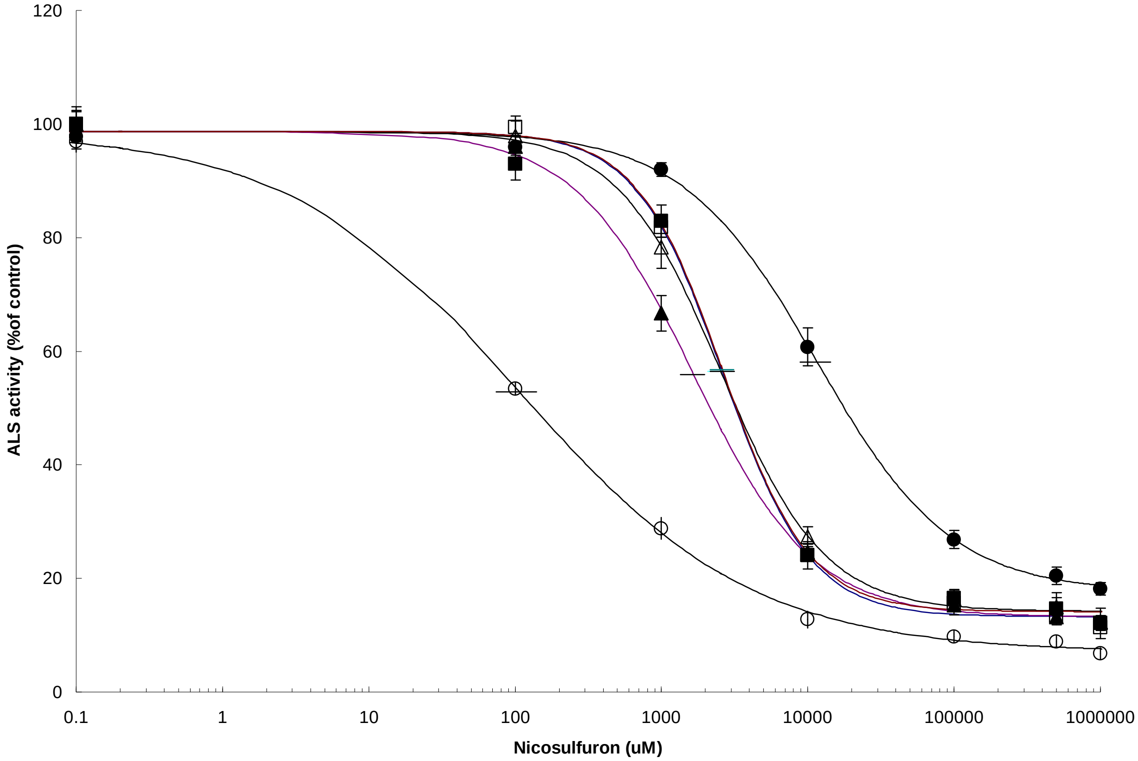


Figure 2 ALS activity of *S. viridis* population 04(○)(wildtype), population 15(●), population 16(△), population 17 (▲), population 19 (■), and population 01(●) after treatment with nicosulfuron. ALS activity was measured after completion of the enzyme assay and results were expressed as the percentage of the untreated control for each biotype. Inhibition curves were generated by calculating values for the log-logistic formula $y=6.97 + (((98.7-6.97)/ (1+(x/107)^{0.54}))$, $y=17.6 + (((98.7-17.6)/ (1+(x/11\ 557)^{0.95}))$ $y=14.2 + (((98.7-14.2)/ (1+(x/2\ 550)^{1.23}))$ $y=13.2 + (((98.7-13.2)/ (1+(x/1\ 660)^{1.07}))$ $y=14.1 + (((98.7-14.1)/ (1+(x/2\ 643)^{1.47}))$ $y=13.3 + (((98.7-13.3)/ (1+(x/2\ 644)^{1.45}))$ where y is the ALS activity value and x is the herbicide dose, for populations 04, 15, 16, 17, 19, and 01, respectively. Horizontal error bars represent the 95% confidence interval of the $I_{50}$ values. Each point is the mean of twelve replicates, plotted with the standard errors.

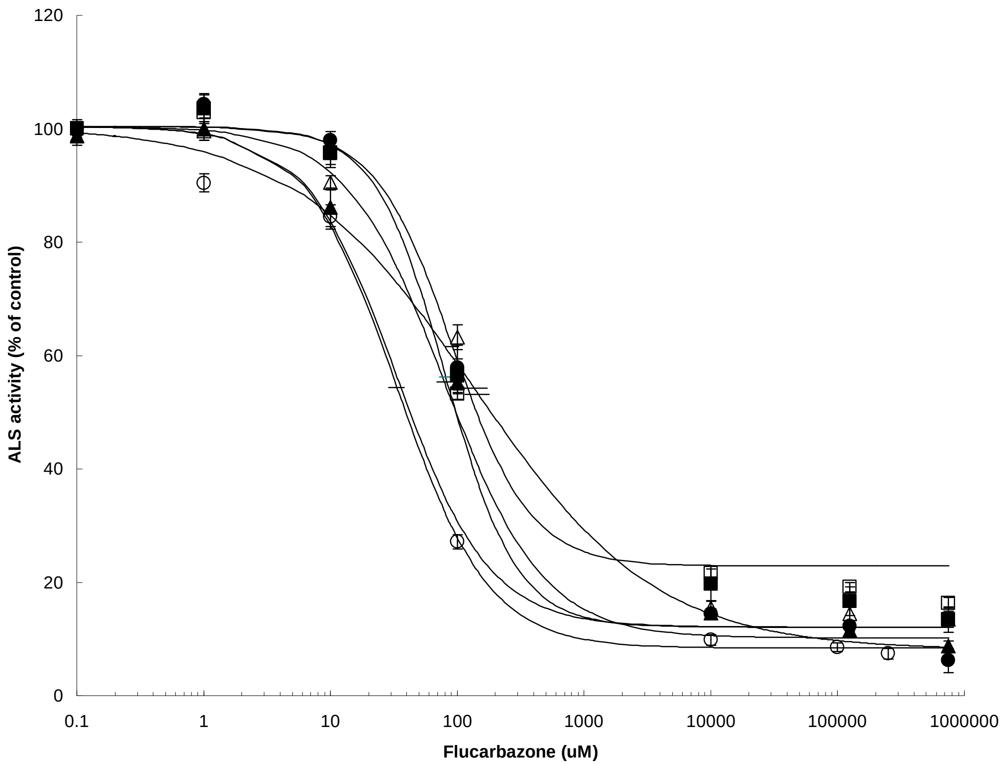


Figure 3 ALS activity of *S. viridis* population 04(○)(wildtype), population 15(●), population 16(△), population 17 (▲), population 19 (■), and population 01(●) after treatment with flucarbazone. ALS activity was measured after completion of the enzyme assay and results were expressed as the percentage of the untreated control for each biotype. Inhibition curves were generated by calculating values for the log-logistic formula $y=8.40 + (((100.4-8.40)/(1+(x/33.4)^{1.20}))$, $y=16.5 + (((100.4-16.5)/(1+(x/147)^{1.28}))$ $y=8.05 + (((100.4-8.05)/(1+(x/136)^{0.61}))$ $y=10.2 + (((100.4-10.2)/(1+(x/79.4)^{1.11}))$ $y= 22.9+ (((100.4-22.9)/(1+(x/91.8)^{1.42}))$ $y=12.1 + (((100.4-12.1)/(1+(x/81.4)^{1.55}))$ where y is the ALS activity value and x is the herbicide dose, for populations 04, 15, 16, 17, 19, and 01, respectively. Horizontal error bars represent the 95% confidence interval of the $I_{50}$ values. Each point is the mean of twelve replicates, plotted with the standard errors.

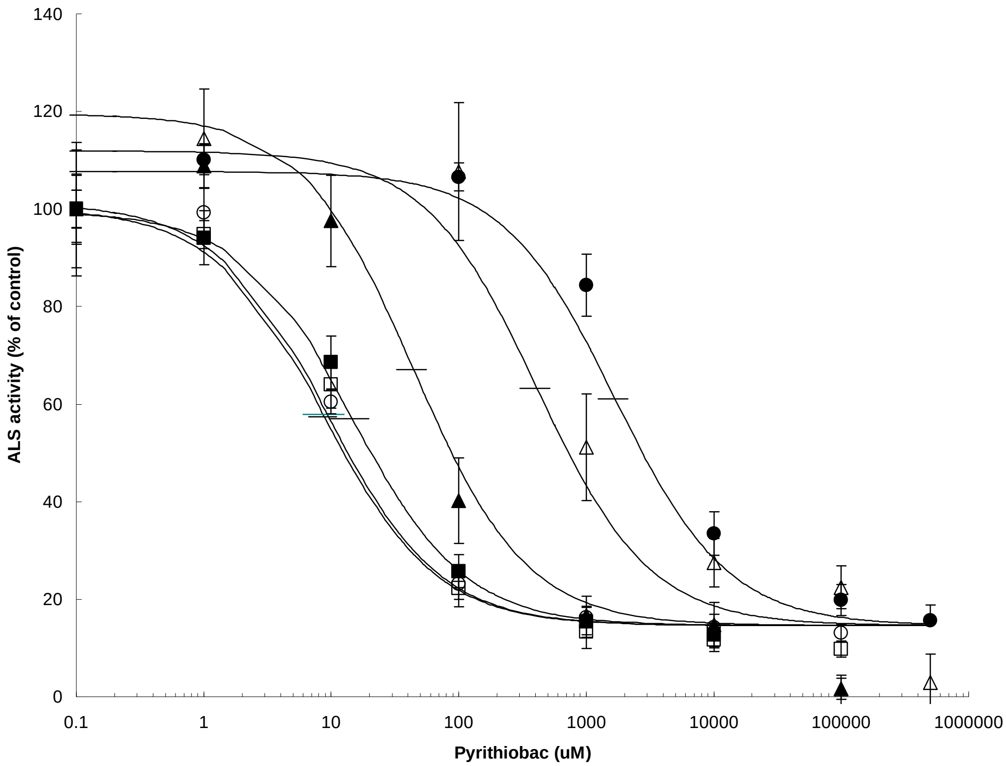


Figure 4 ALS activity of *S. viridis* population 04(○)(wildtype), population 15(●), population 16(△), population 17 (▲), population 19 (■), and population 01(●) after treatment with pyrithiobac. ALS activity was measured after completion of the enzyme assay and results were expressed as the percentage of the untreated control for each biotype. Inhibition curves were generated by calculating values for the log-logistic formula $y=14.6 + (((100.2-14.6)/ (1+(x/8.82)^{0.98}))$, $y=14.6 + (((107.7-14.6)/ (1+(x/1\ 684)^{0.98}))$ $y=14.6 + (((111.9-14.6)/ (1+(x/411)^{0.98}))$ $y=14.6 + (((119.5-14.6)/ (1+(x/44.3)^{0.98}))$ $y= 14.6 + (((99.5-14.6)/ (1+(x/14.6)^{0.98}))$ $y=14.6 + (((101.2-14.6)/ (1+(x/9.30)^{0.98}))$ where y is the ALS activity value and x is the herbicide dose, for populations 04, 15, 16, 17, 19, and 01, respectively. Horizontal error bars represent the 95% confidence interval of the $I_{50}$ values. Each point is the mean of twelve replicates, plotted with the standard errors.

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# Volunteer wheat seed fecundity: Contributions to a mechanistic agronomic model

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**Abstract**
A mechanistic model is being developed to assess the amount of admixture and volunteer fecundity of glyphosate resistant (GR) wheat volunteers in western Canadian cropping rotations. Field trials were conducted to investigate the effect of pre-seeding and post-seeding herbicide applications and crop competition on volunteer wheat fecundity and density in canola and pea crops. GR volunteer wheat fecundity (seed production plant$^{-1}$) was greater than wheat grown as a crop, in the absence of herbicides. GR volunteer wheat fecundity was reduced as herbicide rates increased. Pre-seeding herbicide application had a greater effect on volunteer densities, and in-crop herbicides had a greater effect on fecundity. The data derived from these field trials will be used to develop a wheat fecundity submodel to more accurately predict seedbank longevity and the degree of admixture in crops.

**Introduction**
Glyphosate resistant (GR) wheat was used to model the significance of crop volunteers to seed admixture within western Canadian crop rotations. Volunteer fecundity influences seed bank replenishment and thus the amount of admixture of seeds in subsequent crops. Volunteers may be less fecund than crops due to less favorable microsites. However, volunteer fecundity is influenced by both crop competition and herbicides applied prior to and after seeding. Data quantifying the fecundity of volunteer wheat under field conditions is lacking for modeling purposes. Field trials to assess the contribution of these factors on volunteer wheat fecundity were conducted to aid modeling parameterizations. A mechanistic population model similar to that described by Hansen *et al*. (2002) is being developed to predict the influence of agronomic parameters on GR wheat volunteer longevity and seed admixture.

**Methods and Materials**
Field trials were conducted in 2004 near Edmonton, Alberta, Canada to quantify the fecundity of volunteer wheat within pea and canola crops. GR volunteers were seeded at a depth of 2.5 cm at a rate of 75 seeds m$^{-2}$ prior to the crop. Herbicide treatments were applied pre-seeding and post-seeding each at four rates in a factorial, randomized complete block replicated design (Table 1). Permanent 2 m$^{-2}$ quadrats were randomly positioned within the plot for data collection. Glyphosate (444 g ai/ha) + quizalofop-p-ethyl (0, 12, 18, 24 g ai/ha) was applied at the 2-3 leaf stage of the volunteer wheat and prior to crop seeding. Glufosinate tolerant canola and conventional peas were seeded at 150 and 75 seeds m$^{-2}$, respectively, perpendicular to the volunteer seeding direction. Glufosinate (0, 300, and 500 g ai/ha and 300 + sethoxydim 211 g ai/ha) or imazamox/imazethapyr (0, 14.7, 22.5 and 29 g ai/ha) was applied to the canola or peas, respectively. Surviving GR volunteer wheat plants were hand harvested and the volunteer density, spikes plant $^{-1}$, seeds plant $^{-1}$, and kernel weights assessed. Plots were harvested using a plot combine and GR wheat admixture assessed.

**Results and Conclusions**

Pure stands of four spring wheat cultivars in commercial fields in Canada averaged 104 seeds plant$^{-1}$ with a seed kernel weight of 31 mg (Wang *et al*. 2002). Preliminary data from field trials indicate volunteer wheat plants produced 138 and 168 seeds plant$^{-1}$, with an average seed weight of 25 and 31 mg in the absence of herbicides in canola and peas, respectively. Volunteer wheat fecundity may be associated with the relative time of emergence of the wheat and the crop.

In the absence of a pre-seeding herbicide application, the highest in-crop herbicide rate reduced the volunteer fecundity by 48 % and individual seed weight by 20 % in canola. When combined with the highest rate of pre-seeding herbicide, the in-crop applications had the greater influence on individual volunteer fecundity, reducing the seeds plant$^{-1}$ from 101 to 0. (Table 1). Similar results were observed in peas (Table 2), illustrating the importance of the interaction on volunteer fecundity.

Average volunteer densities in quadrats prior to herbicide application were 69 and 63 plants m$^{-2}$ in canola and peas, respectively. Pre-seeding herbicide applications had a greater effect on plant densities in both crops. In canola, volunteer density was reduced to16 plants m$^{-2}$ by in-crop herbicides alone, but when combined with the full rate of pre-seeding herbicides, was reduced to 0 plants m$^{-2}$ (Table 1). In peas, volunteer densities were reduced to 6 plants m$^{-2}$ by in-crop herbicides alone and to 0.5 plant m$^{-2}$ with the combination of both pre-seed and in-crop herbicides (Table 2). Imazamox/imazethapyr used in peas provided more effective control in-crop alone of GR wheat than glufosinate used in Liberty Link canola.

When whole plots were harvested, all crop samples contained some level of GR wheat (Figure1, A and B). In-crop herbicides had the greatest effect on wheat admixture. In the absence of pre-seeding herbicides, in-crop applications reduced GR wheat admixture from 1700 to 250 seeds m$^{-2}$ in canola. By combining the highest rates of in-crop and pre-seeding herbicide treatments, GR wheat seeds recoved was reduced to 8 seeds m$^{-2}$ (Figure 1 A). In peas, the in-crop treatments alone reduced admixture from 3300 to less than 50 seeds m$^{-2}$. When both high rates were applied, GR admixture was less than 15 seeds m$^{-2}$ in peas (Figure 1 B).

This data provides support for model development. Volunteer seed fecundity is a key component to accurately model GR wheat persistence and the amount of admixture. Herbicides reduce volunteer wheat fecundity, and decrease seed bank replenishment, plant densities in subsequent years and admixture in harvested seed. The model approximates volunteer densities derived from agronomic field trials in which volunteer GR wheat populations were virtually eliminated two years following GR wheat production (Harker *et al*., 2004).

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Table 1. Volunteer wheat surviving treatments, seeds per plot, kernel seed weight, and total seed weight in Liberty Link canola. Values are averaged from data collected in two locations in 2004.

| | Herbicide Treatment | | | | |
|---|---|---|---|---|---|
| Treatment | Pre-Seed | In-Crop | Number of Survivors | Seeds Plant$^{-1}$ | Kernel Weight |
| | ---Herbicide Rate--- | | $m^{-2}$ | | mg |
| 1 | Zero | Zero | 69 | 136 | 25 |
| 2 | Zero | Low | 42 | 103 | 18 |
| 3 | Zero | Medium | 33 | 60 | 14 |
| 4 | Zero | High | 16 | 71 | 20 |
| 5 | Low | Zero | 5 | 94 | 23 |
| 6 | Low | Low | 0.5 | 40 | 22 |
| 7 | Low | Medium | 0.5 | 55 | 16 |
| 8 | Low | High | 0 | 21 | 21 |
| 9 | Medium | Zero | 4 | 49 | 16 |
| 10 | Medium | Low | 1.5 | 43 | 19 |
| 11 | Medium | Medium | 0 | 18 | 14 |
| 12 | Medium | High | 0 | 15 | 31 |
| 13 | High | Zero | 2.5 | 101 | 25 |
| 14 | High | Low | 0.5 | 33 | 14 |
| 15 | High | Medium | 0.5 | 12 | 18 |
| 16 | High | High | 0 | 0 | 0 |

Table 2. Volunteer wheat surviving treatments, seeds per plot, kernel seed weight, and total seed weight in peas. Values are averaged from data collected in two locations in 2004.

| | Herbicide Treatment | | | | |
|---|---|---|---|---|---|
| Treatment | Pre-Seed | In-Crop | Number of Survivors | Seeds Plant$^{-1}$ | Kernel Weight |
| | ---Herbicide Rate--- | | $m^{-2}$ | | mg |
| 1 | Zero | Zero | 63 | 168 | 31 |
| 2 | Zero | Low | 29 | 104 | 19 |
| 3 | Zero | Medium | 16.5 | 65 | 25 |
| 4 | Zero | High | 6 | 53 | 24 |
| 5 | Low | Zero | 6 | 114 | 23 |
| 6 | Low | Low | 4 | 126 | 26 |
| 7 | Low | Medium | 1.5 | 69 | 27 |
| 8 | Low | High | 1 | 46 | 25 |
| 9 | Medium | Zero | 4.5 | 103 | 28 |
| 10 | Medium | Low | 1.5 | 84 | 28 |
| 11 | Medium | Medium | 1 | 66 | 27 |
| 12 | Medium | High | 0.5 | 29 | 14 |
| 13 | High | Zero | 1 | 114 | 30 |
| 14 | High | Low | 1 | 93 | 25 |
| 15 | High | Medium | 2 | 0 | 0 |
| 16 | High | High | 0.5 | 14 | 21 |

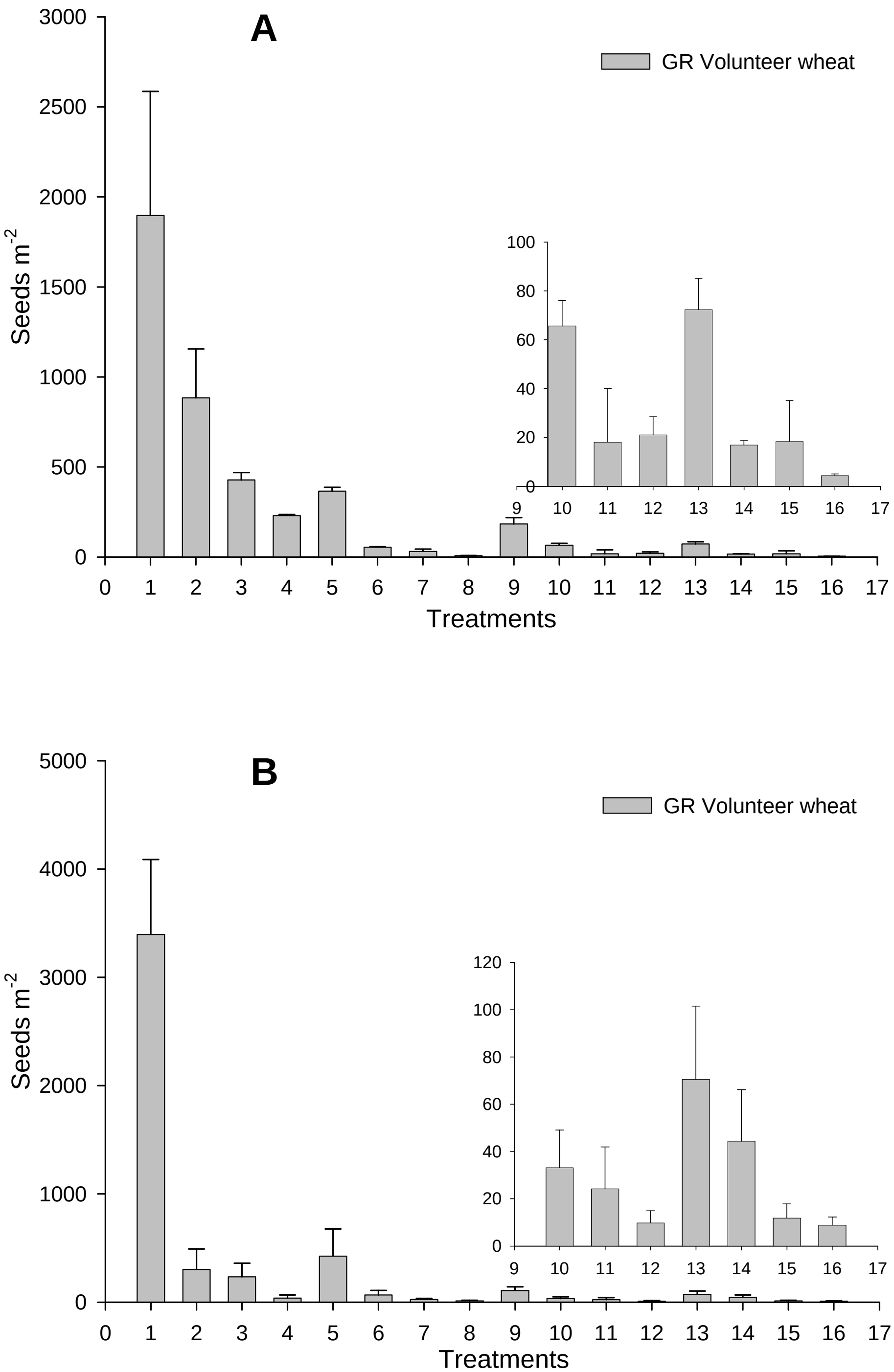


Figure 1. Admixture of volunteer GR wheat harvested from whole plots of Liberty Link canola (A) and peas (B). Values are averages from data collected at two locations in 2004. Vertical bars indicate ± one standard error.

# Control of Common Waterhemp (*Amaranthus tuberculatus* var. *rudis*)

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## Introduction

Common waterhemp (*Amaranthus tuberculatus* var. *rudis*) is an aggressive annual broadleaf weed in several American states. Waterhemp is an upright, branching plant, structurally similar to two other members of the amaranth family common to Ontario, redroot pigweed (*Amaranthus retroflexus*) and green pigweed (*Amaranthus powelli)*. It is difficult to differentiate waterhemp seedlings from those of *A. retroflexus* and *A. powelli*. However, the first leaves of waterhemp are more ovate than those of smooth and redroot pigweed, and waterhemp is distinguished by a complete lack of hair (Hager et al. 1997). Positive identification can be made at flowering as waterhemp is a dioecious species.

*A. tuberculatus* is one of the most troublesome weeds in agricultural production systems (Horak and Loughin 2000, Nordby 2003), due to the fact that it is difficult to control and it is extremely competitive with crops. The delayed and extended emergence pattern of waterhemp (Hartzler et al. 1999) compared to other common agricultural weeds makes herbicide application timing difficult. In addition, multiple herbicide resistances make herbicide selection difficult. In the US, biotypes of waterhemp exist that are resistant to the acetolactate synthase (ALS) inhibitors (Horak and Peterson 1995), the photosystem II (PSII)(site A) inhibitors (Anderson et al. 1996), and the Protox inhibitors. Biotypes with two or three way resistances also exist (Patzoldt et al. 2005). Waterhemp is more competitive than redroot pigweed (*Amaranthus retroflexus*) (Bensch et al. 2003), the more common amaranth weed species in Ontario. Yield losses in corn and soybeans due to waterhemp competition can be up to 23% (Sprague 2003) and 56% (Bensch et al. 2003), respectively. No research has yet been done on this weed in Ontario, therefore herbicide efficacy trials were established to determine the most efficacious herbicides to control waterhemp in Ontario.

## Methods

Herbicide efficacy trials were established in 2003 and 2004 in Essex and Lambton Counties to determine which herbicides are most efficacious for the control of this weed. Waterhemp at the Essex location was resistant to the ALS inhibitor herbicides, while waterhemp in Lambton County was resistant to both the ALS inhibitors and the PSII inhibitors. Four trials were established at each location, testing pre-emergent and post-emergent in both corn and soybeans. Imazethapyr was applied at 100 g ai/ha on each trial to control all other weeds.

Each trial was established with a RCBD design with four replications. Herbicide treatments were applied at the highest recommended label rate. Pre-emerge treatments were applied within 5 days of planting, and post-emerge treatments were applied at five to ten centimeter waterhemp. Herbicide treaments were applied with a $CO_2$ pressurized backpack sprayer, calibrated to apply 200 L/ha of water at 207 kPa. The plot size was two meters by eight meters.

Visual weed control ratings were conducted 28 and 70 days after crop emergence for the soil applied herbicide treatments, and 14, 28, and 70 days after treatment for the foliar applied treatments. In addition to the visual control ratings, waterhemp density, average height, and weed dry weight were determined at 70 days after herbicide application. The center crop row of all plots was harvested in the fall to determine the effect of the herbicide treatment on crop yield. Data was analyzed across years and means were compared using the mixed procedure in SAS v. 8.2 (SAS Institute, Cary, NC). The type I error rate for all statistical tests was 0.05.

**Results and Discussion**

Measurements of weed density, biomass, and height coincided with visual percent control data; therefore only 70 day visual control data will be discussed. Waterhemp control differed by location due to the presence of different resistance patterns, therefore results are separated by location. Pre and post-emergent treatments containing atrazine were very effective in controlling waterhemp at the Cottam location, but not at the Petrolia location. Table 1 shows visual percent waterhemp control with pre-emergent herbicides in 2003 and 2004. Isoxaflutole plus atrazine, s-metolachlor/atrazine, mesotrione, and s-metolachlor/atrazine plus mesotrione are the only treatments which provided an acceptable level of waterhemp control at both locations. Table 2 shows that, regardless of location, dicamba, dicamba/atrazine, and mesotrione plus atrazine all provided excellent control of waterhemp in corn when applied post-emergent.

In soybeans, s-metolachlor plus metribuzin was the only pre-emergent treatment which consistently provided good waterhemp control, as seen in Table 3. There were no post-emergent treatments in soybeans that provided season long control of waterhemp. Table 4 shows that acifluorfen, fomesafen, imazamox plus fomesafen, and glyphosate all provided some control of waterhemp at both locations. Multiple applications per season are likely necessary to achieve acceptable control with post-emergent treatments in soybeans (Hager and Sprague 2001).

**Table 1: Means for percent waterhemp control 70 days after application for pre-emerge treatments in corn at Petrolia and Cottam in 2003 and 2004**

| | Rate (g ai ha$^{-1}$) | Petrolia | Cottam |
|---|---|---|---|
| Non-treated | 0 | 0 d | 0 d |
| Weed Free | 0 | 100 a | 100 a |
| Atrazine | 1500 | 0 d | 100 a |
| Pendimethalin | 1680 | 71 b | 94 b |
| Dicamba | 600 | 60 c | 65 c |
| Dicamba/atrazine | 1800 | 68 bc | 80 c |
| Isoxaflutole<br>Atrazine | 2000<br>1063 | 97 a | 100 a |
| S-metolachlor/atrazine | 2880 | 97 a | 100 a |
| Mesotrione | 175 | 97 a | 100 a |
| S-metolachlor/atrazine<br>Mesotrione | 2520<br>175 | 99 a | 100 a |

**Table 2: Means for percent waterhemp control 70 days after application for post-emerge treatments in corn at Petrolia and Cottam in 2003 and 2004**

| | Rate (g ai ha$^{-1}$) | Petrolia | Cottam |
|---|---|---|---|
| Non Treated | 0 | 0 d | 0 d |
| Weed Free | 0 | 100 a | 100 a |
| Atrazine | 1500 | 0 d | 100 a |
| Dicamba | 600 | 91 ab | 98 ab |
| Dicamba/diflufenzopyr | 200 | 88 b | 98 ab |
| Dicamba/atrazine | 1800 | 91 ab | 100 a |
| 2,4-D/atrazine | 1404 | 86 b | 100 a |
| Bromoxynil<br>Atrazine | 280<br>1500 | 56 c | 100 a |
| Prosulfuron<br>Dicmaba | 10<br>140 | 45 c | 91 b |
| Primisulfuron/dicamba | 166 | 51 c | 82 c |
| Mesotrione | 100 | 87 b | 94 b |
| Mesotrione<br>Atrazine | 100<br>280 | 97 a | 99 a |

**Table 3: Means for percent waterhemp control 70 days after application for pre-emerge treatments in soybeans at Petrolia and Cottam in 2003 and 2004**

| | Rate (g ai ha$^{-1}$) | Petrolia | Cottam |
|---|---|---|---|
| Non-treated | 0 | 0 f | 0 c |
| Weed Free | 0 | 100 a | 100 a |
| S-metolachlor | 1600 | 81 c | 99 a |
| Dimethenamid | 1250 | 84 bc | 97 a |
| Flufenacet/metribuzin | 1000 | 77 c | 98 a |
| Metribuzin | 1120 | 24 d | 100 a |
| Linuron | 2250 | 86 bc | 100 a |
| Imazethapyr | 100 | 0 f | 34 b |
| Cloransulam-methyl | 35 | 2 ef | 48 b |
| Flumetsulam/metolachl | 1443 | 89 bc | 100 a |
| Imazethapyr<br>Metribuzin | 75<br>425 | 10 e | 96 a |
| S-metolachlor<br>Metribuzin | 1600<br>658 | 94 ab | 100 a |

**Table 4: Means for percent waterhemp control 70 days after application for post-emerge treatments in soybeans at Petrolia and Cottam in 2004 and 2005**

| | Rate (g ai ha$^{-1}$) | Petrolia | Cottam |
|---|---|---|---|
| Non Treated | 0 | 0 d | 0 d |
| Weed Free | 0 | 100 a | 100 a |
| Acifluorfen | 600 | 83 b | 83 a |
| Fomesafen | 240 | 80 b | 97 a |
| Bentazon | 1080 | 13 c | 31 c |
| Thifensulfuron-methyl | 6 | 10 cd | 54 b |
| Chlorimuron-ethyl | 9 | 0 d | 54 b |
| Cloransulam-methyl | 17.5 | 6 cd | 32 c |
| Imazethapyr | 100 | 0 d | 41 bc |
| Imazethapyr<br>Bentazon | 75<br>840 | 12 cd | 36 bc |
| Imazamox<br>Fomesafen | 25<br>200 | 79 b | 84 a |
| Imazamox<br>Bentazon | 25<br>600 | 8 cd | 33 bc |
| Glyphosate | 900 | 76 b | 97 a |
| Glyphosate | 1800 | 81 b | 98 a |

**Summary**

Waterhemp can be controlled in corn and soybeans by selecting appropriate herbicides. Treatment recommendations should be made specific to the biotype of waterhemp that is to be controlled, as PSII inhibitors are extremely effective options in biotypes which are not resistant. Future research should investigate the efficacy of sequential post-emergent herbicide applications.

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# Differences in translocation and metabolism pattern may account for MCPA-resistance in hemp-nettle (*Galeopsis tetrahit* L)

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Hemp-nettle (*Galeopsis tetrahit*) is a noxious weed of western Canada that infests cereals, canola, flax and forage crops. A hemp-nettle population from a field in Alberta was found to be resistant to MCPA after receiving repeated applications of various auxinic herbicides. A three-fold resistance factor was determined by comparing $GI_{50}$ values from a MCPA dose-response study. The resistant (R) and susceptible (S) biotypes were not different with regard to absorption rate of [$^{14}$C]MCPA, with 54% of the applied $^{14}$C being absorbed by the treated leaf (TL), 72 hours after treatment (HAT). However, the R biotype consistently exported less $^{14}$C from the TL in both acropetal and basipetal direction. Forty-five and 58% of absorbed $^{14}$C moved out of the TL, 6 and 13% moved up to the apical meristem of the shoot, and 32 and 38% moved to the root, in R and S respectively, 72 HAT. There were no differences in the total accumulation of [$^{14}$C]MCPA metabolites, with 20 and 22% of the recovered $^{14}$C detected as metabolites in R and S respectively, 72 HAT. However, metabolism rate in the roots was higher than the rest of the plant, and the proportions of metabolites were consistently higher in R, with 55 and 42% metabolites of total $^{14}$C recovered, in R and S roots, respectively, 72 HAT. It has been concluded that a combination of a lower rate of MCPA translocation and a higher rate MCPA metabolism in the roots may protect hemp-nettle from MCPA phytotoxicity.

**Nomenclature**: MCPA, hemp-nettle, *Galeopsis tetrahit* L.

**Keywords**: hemp-nettle, MCPA, metabolism, resistance, translocation,

The introduction of the auxinic herbicides during the 1940s has revolutionized modern agriculture and weed control. The ability of auxinic herbicides to selectively control dicotyledonous weeds in cereal crops and pastures has made these herbicides one of the most widely used group of herbicides in the world (Devine et al. 1993).

In susceptible species, auxinic herbicides cause continuous stimulation of the metabolic system resulting in the disruption of growth integrity. Cell division, growth and differentiation in meristematic and cambial tissues occurs at inappropriate times. This abnormal tissue acts as a strong sink that depletes carbohydrates and proteins from essentials tissues. Auxinic herbicides also cause lethal damage to the vascular system (Grossmann 2003). In addition, auxinic herbicides induce uncontrolled production of ethylene that is associated with other symptoms such as tissue swelling, leaf epinasty and accumulation of abscisic acid, which later cause inhibition of photosynthesis, formation of reactive oxygen species and ultimately destruction of cellular compartments (Grossmann 2003; Grossmann et al. 2001). Despite our extensive knowledge on the mode of action of the auxinic herbicides their primary biochemical site of action remains unknown.

Prolonged and repeated use of the same herbicide or herbicides sharing the same target site impose intense selection pressures that can result in the evolution of herbicide-resistance weed biotypes (Diggle and Neve 2001). To date, 24 species have developed resistance to auxinic herbicides (Heap 2005), e.g. resistance to picloram and dicamba in *Sinapis arvensis* or resistance to picloram and clopyralid in *Centaurea soltitialis* (Webb and Hall 1995; Fuerst et al. 1996). In 1998, a resistant biotype of hemp-nettle was found near Lacombe, Alberta, in a field subjected to repeated application of various auxinic herbicides.

## Material and Methods

**Growth conditions.** Resistant (R) and susceptible (S) hemp-nettle plants were grown in a growth room maintained at 21/16 ± 1 °C day/night temperature, 16 h photoperiod and relative humidity of 65% with constant light at 350 µEinstein $m^{-2}$ $s^{-1}$.

**MCPA dose-response experiments.** Plants were sprayed at the second opposite leaves stage. A commercial formulation of MCPA amine[1], at doses that ranged from 53 to 54400 g ai $ha^{-1}$, was applied [at 110 L $ha^{-1}$] with a track sprayer[2]. Plants were harvested 21 days after treatment (DAT), and dry weight (DW) of the shoot and the root were recorded. Data were subjected to Log-logistic analysis to calculate $GI_{50}$s of R and S (Seefeldt et al. 1995).

**Uptake, translocation and metabolism of [$^{14}$C]MCPA.** Plants at the second opposite leaves stage were treated with a mixture of formulated MCPA and [$^{14}$C]MCPA (2 KBq), by applying 10 µL per plant to the adaxial side of leaf #2. Plants were harvested from 6 to 72 hours after treatment (HAT), and treated leaves (TL) were rinsed with 30 ml of an aqueous solution of ethanol (20%) and Tween 20 (0.5%). Plants were dissected into TL, shoot above TL, shoot below TL and roots. Dry plant sections were combusted with a biological oxidizer[3]. Radioactivity was determined by liquid scintillation spectrometry[4] (LSS). For the study of [$^{14}$C]MCPA metabolism, hydroponically grown plants were treated at the first leaf stage with 10 KBq [$^{14}$C]MCPA solution, harvested 12 to 72 HAT using similar methods previously described, then immediately plant sections were extracted with acetone. Metabolites were separated using normal phase TLC, and radioactivity along the TLC lanes was estimated at 1-cm segments using LSS. Data was subjected to ANOVA and means were separated using Duncan's multiple range test.

## Results and Discussion

**MCPA dose-response experiments.** The phenotypes of untreated R and S were different; the S biotype had a bushier and denser growth pattern, compare to R. The accumulation of DW in R (5.0 ±0.6 g) and S (5.4 ±0.5 g) was not different. Shortly after MCPA application, both biotypes showed injury symptoms as their petioles were bent downward, plant growth was stunted, affected leaves were curled, and at 10 DAT, plants started to die. The R biotype was three-fold more resistant to MCPA than the S using $GI_{50}$ comparisons based on total DW, and seven-fold more resistant using the root DW parameter (Table 1). These results suggested that the activity of MCPA was reduced in R roots compare to S.

Table 1. $GI_{50}$ values and resistance ratios (R/S) based on total plant DW and root DW, for hemp-nettle biotypes treated with MCPA amine.

| | Total DW | | | Roots ratio of total DW | | |
|---|---|---|---|---|---|---|
| | R | S | R/S | R | S | R/S |
| $GI_{50}$ (g ai $ha^{-1}$) | 438 | 134 | 3.3 | 1035 | 142 | 7.3 |
| 95% confidence limits | (347-528) | (116-152) | | (385-1686) | (91-149) | |

**Uptake, translocation and metabolism of [$^{14}$C]MCPA in hemp-nettle.** The absorption rate of [$^{14}$C]MCPA was not different in R and S biotypes regardless of harvest time, with 54% of the applied [$^{14}$C]MCPA being absorbed 72 HAT (Table 2). The R biotype consistently exported less $^{14}$C from the TL, with 45 and 58% of the total recovered $^{14}$C moving out of TL in R and S, respectively, 72 HAT (Table 2). Both biotypes exported the most $^{14}$C toward the root; however, the R moved less $^{14}$C to both acropetal and basipetal portions of the plant (Table 2). Approximately 20% of the recovered $^{14}$C was detected as

[$^{14}$C]MCPA metabolites in both biotypes, 72 HAT (Table 2). Nevertheless, the proportion of metabolites recovered from the roots alone were 3- and 2-fold more than the proportion found for the whole plant, in R and S, respectively (Table 2).

Enhanced translocation of [$^{14}$C]MCPA to the root was correlated with increased MCPA toxicity (Achhireddy et al. 1984). It seems that R hemp-nettle sustained a lower rate of MCPA translocation from the TL to the apical meristem and the roots, and a higher rate of MCPA metabolism in the roots, thus protecting the root system and the whole plant from MCPA toxicity.

Table 2. Uptake, distribution and metabolism of [$^{14}$C]MCPA in R and S hemp-nettle, 72 HAT.

| | % of applied | Distribution of $^{14}$C, % of recovered $^{14}$C *in planta* | | | | Metabolites, % of recovered $^{14}$C | |
|---|---|---|---|---|---|---|---|
| | Uptake | TL | Above TL | Below TL | Roots | *In planta* | In root |
| R | 54.4% | 54.8% | 5.6% | 7.8% | 31.8% | 19.6% | 55.0% |
| S | 54.4% | 41.8% | 12.9% | 7.0% | 38.3% | 22.1% | 42.3% |

## Source of Materials

[1] MCPA-amine, United Agri product, 789 Donnybrook Dr., Dorchester, ON N0L 1G5, Canada.
[2] RC-5000-100EP, Mandel Scientific Crop., 2 Admiral Pl., Guelph ON N1G 4N4, Canada.
[3] OX-300, R. J. Harvey Instrument corporation, 123 Patterson St., Hillsdale, NJ 07642.
[4] LS 6000SC, Beckman Instruments, Inc., 4300 N Harbor Blvd., Fullerton, CA 92835.


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# Spatial and temporal variability of vegetation in wild blueberry (*Vaccinium angustifolium,* Ait.) production

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## Introduction

Lowbush wild blueberries, *Vaccinium angustifolium*, are a native berry species of the Maritime Provinces, Quebec, and Maine (MacIsaac, 1997). Wild blueberries are one of the most important horticultural crops in Nova Scotia (MacIsaac, 1997). The province is the largest producer of wild blueberries in Canada (WBPANS, 2004).

Current management practices in wild blueberry production include a continuous cycle of weed and pest control, pruning in alternate years, ensuring good pollination of the crop, and application of fertilizer (McIsaac, 1997). Harvesting of the crop takes place during the second year of the production cycle.

Weeds are one of the major limiting factors in wild blueberry production (Jensen, 2003), and will often respond with more vigour to fertilizer than the existing blueberry stand (Barker et al., 1964). The most commonly used product for pre-emergence weed control is hexazinone (Velpar®) which can be used to control a large variety of annual and perennial weeds (Jensen and Yarborough, 2004). Initially registered in 1982, it has become the predominant herbicide used in wild blueberry production (McCully et al., 1996).

In a comparison of weed surveys of Nova Scotia blueberry fields conducted in 1984 and 1985 (McCully and Sampson, 1991) and 2001 and 2002 (Jensen and Sampson, unpubl. data), Jensen and Yarborough (2004) report a doubling of biennial and perennial broadleaf weeds, a near doubling of annual broadleaf weeds, and the first ever recording of annual grasses. These shifts have been attributed to the extensive use of hexazinone and adoption of other management practices such as flail mowing for pruning (Jensen and Yarborough, 2004).

The objectives of this study are to quantify weed and crop growth during the 2-year production cycle, assess the current weed response to applications of hexazinone and fluazifop-p-butyl, develop accurate maps of weed and crop growth, and begin preliminary assessment of spectral technology in wild blueberry production.

## Materials and Methods

Three field sites were established in the spring of 2004, one in Mount Thom and two in Farmington, Nova Scotia. Treatments used in this study were a control (no herbicide), PRE application of hexazinone (Velpar®) at 2.56 kg ai/ha, POST application of fluazifop-p-butyl (Venture® L) at 2 L/ha, and a PRE application of hexazinone with a POST application of fluazifop-p-butyl, both at the rates indicated.

Treatments were replicated four times in a Latin Square design for a total of 16 plots at each study site. Plot size was 10 X 10 meters.

Each plot contained 25 sampling points spaced 2 meters apart. These were arranged in rows of five to form a grid across each plot. Weed species density, height, and percent cover were determined at each point within a 30 X 30 cm quadrat. Blueberry stem density, stem height, and percent cover were determined as well. The percent bare soil within each quadrat was recorded, and point measurements of weed, blueberry, or bare soil were made at each corner of the quadrat. Data were collected in June, August, September, and October of 2004 and June, July, and August of 2005. Data was compiled in spreadsheets and incorporated into the ArcView Geographic Information System (GIS) program for mapping.

Spectral data was collected in September of 2004 and July and August of 2005. Data collected in September 2004 was preliminary and used as a guide for data collection in 2005. Patches of weed species in Mount Thom were marked during June, 2005 so that the same weed patches could be sampled in July and August.

## Results and Discussion

Sheep sorrel (*Rumex acetosella* L.) and poverty oat grass (*Danthonia spicata* L. Beauv. ex Roem.& Schult) were the most abundant weed species present at the study sites. Hexazinone provided good initial control of sheep sorrel at Farmington, but small populations of this weed had developed in treated plots towards the end of the study. Control was not as good in Mount Thom where sheep sorrel was able to recover from the initial hexazinone application and reestablish.

Poverty oat grass was most abundant in Farmington where large populations of this weed developed in hexazinone treated plots. Some initial control was obtained in June and July, 2004, but populations appeared to quickly re-establish throughout the remainder of the study. Hexazinone plots that received an application of fluazifop-p-butyl had fewer populations of poverty oat grass than did plots sprayed with hexazinone only. Plots receiving applications of fluazifop-p-butyl had large populations of poverty oat grass. Fluazifop-p-butyl is registered for suppression of poverty oat grass with no residual control (Jensen et al., 2003). Thus, there was no control of plants emerging in fluazifop-p-butyl plots after application.

Spectral data obtained in early September indicate great potential for application of this technology in wild blueberry production. Spectral signatures for various weed species, blueberry plants, and bare soil, indicate a variety of wavelengths where individual species may be distinguished. Preliminary analysis of data collected in 2005 is indicating more variable results, but complete analysis is pending data correction by the Applied Geomatics Research Group (AGRG).

Certain weed species appear to have adapted to hexazinone use in wild blueberry production. It is important to begin identifying these problem weed species and to determine their mechanisms for hexazinone tolerance. This will help prolong the usefulness of hexazinone and help to better manage herbicides in the future. Spectral data has potential in the wild blueberry industry, however, conclusions on feasibility are pending final analysis of data.

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# Posters

As in the past, some abstracts only were submitted while exceptionally, some complete posters were also submitted.

Because of a hard disk crash, some posters abstracts might be missing.

**Phenotypic differences between a coastal and an interior population of purple loosestrife (*Lythrum salicaria* L.) in British Columbia.** Clements, D.R., Campbell, K., Becker, A, and Bainard, J.D. Department of Biology, Trinity Western University, Langley, BC

Purple loosestrife is one of the most notorious invasive plants in North America, and is now found throughout the continent. Numerous scientific studies have focussed on purple loosestrife, but few have examined genetic differences among populations. Although plant species are frequently assumed to be relatively monotypic over a geographic region, numerous studies have documented ecotypic variation. Moving eastward along the southern border with the U.S., the relatively cool and moist coastal climate rapidly gives way to a dry interior climate with more marked seasonal temperature changes. The result is that the Okanagan Valley, just 230 km from the coast, experiences a very different climate. The purpose of our study was to compare characteristics of a coastal population of purple loosestrife with those of an interior population. Many phenotypic differences were observed between the coastal (Langley) population and the interior (Oliver) population grown under identical conditions, with the interior populations producing more vigorous and highly branched root systems and also faster growing plants with more branching. After nearly 4 months of growth under the same conditions, interior plants averaged 61.8 cm in height, significantly higher ($P < 0.05$) than the 41.7 cm measured in coastal plants. Leaf area, root size and root branching were also significantly greater for interior plants ($P < 0.05$), with interior root systems averaging 3.5 branches over 1 mm vs. 2.6 for coastal plants. Although historical factors related to the introduction of particular strains of purple loosestrife to these two regions are important, many of the observed phenotypic differences may have resulted from evolution in response to the distinct soil and climatic conditions of these sites.

**Effect of epicuticular wax on the susceptibility of weeds to clove oil and its primary constituent eugenol.** Bainard, L.D., M.B. Isman, and M.K. Upadhyaya, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC.

Herbicidal activities of clove oil and its primary constituent eugenol and the role of leaf epicuticular wax (LEW) in susceptibility and retention of these essential oils in broccoli, lamb's-quarters, and redroot pigweed were studied. Clove oil (2.5%) and eugenol (1.5%) were applied to leaves of greenhouse-grown broccoli, lamb's-quarters and redroot pigweed seedlings and effects on seedling growth and membrane integrity were studied. Membrane integrity was studied by incubating leaf discs (10 mm diam) excised from the treated seedlings into a bathing medium and monitoring the electrolyte leakage using a conductivity meter. The role of LEW was investigated by comparing responses of leaves with or without LEW to essential oils; LEW was removed using the cellulose acetate stripping method, and the retention of foliar sprays was quantified by mixing methyl orange (0.01% w/v) to spray solutions and measuring the absorbance of the leaf-wash at 465 nm. Compared to plants with LEW, plants without LEW were more susceptible to both clove oil and eugenol. In seedlings with LEW, clove oil caused greater inhibition of growth than eugenol. Both clove oil and eugenol caused greater electrolyte leakage in the leaves without LEW than in the leaves with LEW. Removal of LEW increased electrolyte leakage by 280% in eugenol-treated and 180% in clove oil-treated broccoli leaves. While the presence of LEW greatly reduced the retention of the essential oil solutions, there was no significant difference between the retention of clove oil and eugenol solutions indicating that differences in susceptibility of broccoli leaves to these essential oils was not due to differential foliar retention.

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# Predicting early phenological stages of six major weeds

Gaétan Bourgeois and Diane Lyse Benoit

Agriculture and Agri-Food Canada, Horticultural Research and Development Centre, Saint-Jean-sur-Richelieu, Québec

## Introduction

Successful weed control often results from proper timing of chemical and mechanical weeding strategies. Predicting phenological weed stages is expected to provide useful information on the timing of these strategies. A generic plant phenology model, based on temperature and photoperiod, was developed by Bourgeois et al. (2005) for head lettuce, carrot, and wheat. The objective of this project was to adapt this generic model to early phenological stages of six weeds: *Amaranthus retroflexus* (AMARE), *Ambrosia artemisiifolia* (AMBEL), *Chenopodium album* (CHEAL), *Chenopodium glaucum* (CHEGL), *Echinochloa crus-galli* (ECHCG), and *Setaria viridis* (SETVI)..

## Materials & Methods

Experiments in growth chambers: Seeds of AMARE, CHEAL, CHEGL, ECHCG, and SETVI harvested during the previous growing season were planted in 10 cm diameter pots. These pots were placed in growth chambers at constant temperatures of 10, 15, 20, 25, 30, and 35ºC and at a photoperiod of 16 hours. Each treatment was repeated twice. Observations on weed phenological stages were made three times a week until 50% of the plants have reached the 6$^{th}$ leaf stage. A given phenological stage was declared when 50% of the plants have reached this stage. All observations were transformed in the decimal code of the BBCH universal scale for crops and weeds (Lancashire et al., 1991). Coefficients for the phenological models of these five weeds were derived from these growth chamber experiments.

Non-linear response to temperature: For each temperature treatment, the number of days from seeding (BBCH=0) to 50% cotyledon stage (BBCH=10) was transformed in emergence rate ($d^{-1}$). The leaf appearance rate ($d^{-1}$) was obtained from the slope of the linear regression of the number of leaves against the number of days to reach 50% of each leaf stage (BBCH=12 to 16). The following non-linear equation (Brière et al., 1998) was used to express these developmental rates ($D_R$) as a function of temperature ($T$):

$$D_R = A\,T\,(T - Tbase)\,(Tmax - T)^{0.5}$$

where $A$ is a curve amplitude parameter, and $Tbase$, $Tmax$ are the temperatures of the lower and the higher developmental thresholds, respectively. Data to derive the non-linear response to temperature of AMBEL were obtained from the literature (Deen et al., 1998; Shrestha et al., 1999).

Field experiments: For two seasons (2000-2001), sequential emergence of AMBEL has been provoked by mechanical cultivation in 3 cropping systems - carrot, onion and lettuce. A split-plot design with two repetitions was set up with main plots allocated to soil disturbance and sub-plots to crops. Soil disturbance was timed on specific crop stage and obtained by the passage of a mechanical weeder (Buddingh model C). AMBEL was seeded on the same day as the crop within two 20 x 50 cm quadrats in between the rows. Seedling emergence was monitored and for 10 individual plants, weed stage (BBCH), height and leaf number were noted bi-weekly until they reach the 6-8 leaves stage. Coefficients of the AMBEL phenological model were calibrated with observed data from these field experiments.

Generic phenological model: The generic phenological model predicts the BBCH phenological stages of a given species from hourly temperatures, photoperiod, and a chronology factor for early post-emergence stages (BBCH 10 to 12) (Streck et al., 2003). For these six weeds, the photoperiod effect was considered as non-limiting. Furthermore, all seeds were assumed with no dormancy and no germination limitation. Development rates of both emergence and leaf appearance phases are computed on an hourly basis and the daily averages to these rates are used to simulate the evolution of the BBCH phenological stages. Non-linear responses to temperature, obtained for growth chamber experiments and from the literature, were integrated for each species in the database interface of the generic phenological model, which was implemented in the CIPRA (Computer Centre for Agricultural Pest Forecasting) software for easy access to weather database and mathematical modelling tools (Plouffe et al., 2004).

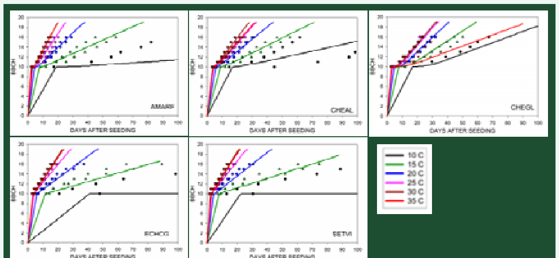


Figure 1. Comparison of BBCH predictions and observations of five weeds grown in growth chambers

## Results & Discussions

Table 1 shows the cardinal temperatures and the maximum development rate obtained from the non-linear regressions for the emergence and leaf development phases. In general, the emergence phase has lower Tbase, higher Topt, and higher Tmax than the leaf development phase for a given species. As observed in growth chambers, at higher temperatures, seedling of some weeds (e.g. CHEGL) did emerge from the soil but did not survive afterwards. For the leaf appearance rate, weeds studied in this project can be divided into three broad groups: 1) lower Tbase and lower Topt (CHEGL), 2) higher Tbase and higher Topt (AMARE, ECHCG, and SETVI), and 3) lower Tbase and intermediate Topt (AMBEL and CHEAL). This last group will respond to a wider range of temperatures than the other groups.

**Table 1. Results from non-linear regressions with the Brière et al. (1999) equation**

| Weed | Tbase | Topt | Tmax | $D_{Rmax}$ | Adj. $R^2$ |
|---|---|---|---|---|---|
| | | Sowing (0) to Cotyledon (10) | | | |
| AMARE | 3.4 | 36.1 | 44.7 | 0.48 | 0.89 |
| CHEAL | 0.0 | 33.4 | 42.1 | 0.28 | 0.80 |
| CHEGL | 1.6 | 32.2 | 40.1 | 0.36 | 0.80 |
| ECHCG | 6.9 | 32.8 | 40.0 | 0.33 | 0.83 |
| SETVI | 5.2 | 34.4 | 42.2 | 0.46 | 0.67 |
| | | Cotyledon (10) to 6th-leaf (16) | | | |
| AMARE | 8.8 | 32.6 | 39.4 | 0.60 | 0.97 |
| CHEAL | 2.6 | 30.8 | 38.2 | 0.40 | 0.96 |
| CHEGL | 0.0 | 27.7 | 35.3 | 0.37 | 0.90 |
| ECHCG | 10.8 | 32.2 | 38.7 | 0.49 | 0.98 |
| SETVI | 10.4 | 32.3 | 38.8 | 0.52 | 0.98 |
| AMBEL | 2.3 | 30.8 | 38.3 | 0.83 | 0.91 |

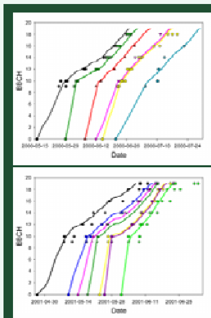


Figure 2. Comparison of BBCH predictions and observations of AMBEL observed in field conditions

Figure 1 illustrates the predictions of weed phenological stages obtained in growth chambers. In general, the generic model provided excellent predictions for all five weeds at T ≥ 15ºC. At T = 10ºC, the model underestimates leaf development of all weeds. For ECHCG and SETVI, the Brière et al. (1999) model estimated a Tbase > 10ºC (Table 1), which resulted in no leaf development at this temperature. The observed data at this temperature could be questionable. In the first repetition, only 12% of all weed seeds emerged and produced some leaves, compared to 95% in the second repetition. Furthermore, in this last repetition, major differences in days to reach a given phenological stage were observed.

Figure 2 illustrates the predictions of AMBEL phenological stages obtained in field experiments during summers 2000 and 2001. Days to soil emergence for this weed varied from 3 to 15 days and this variation could not be explained solely by soil temperature. Emergence rate at optimun soil temperature was then ajusted for each data set in order to evaluate the prediction of the leaf appearance rate. Excellent predictions of early leaf stages (BBCH from 11 to 19) were obtained by setting the maximun leaf appearance rate to 0.68 and 0.83 $d^{-1}$ in 2000 and 2001, respectively.

## Conclusion

In this project, we successfully implemented six major weeds in a generic plant phenology model. Leaf appearance rate was predicted adequately in growth cabinets for five weeds and under field conditions for AMBEL. At this stage, to predict BBCH stages, temperature and photoperiod are the main limiting factors introduced in the model. This is well suited for growth chamber studies, but additional factors, like soil temperature and moisture, will need to be integrated in the model in order to improve the prediction of seedling emergence. Furthermore, seed dormancy and germination potential will need to be investigated and implemented in the system. The processes are believed to be very specific for each species.

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Canada

**Stability and Shelf Life of a Pre-emergent Bacterial Bioherbicide in a Pesta Formulation**
Susan M. Boyetchko, Russell K. Hynes, Paulos Chumala, H. Jon Geissler, Karen C. Sawchyn, and Daniel J. Hupka. Agriculture and Agri-Food Canada, Saskatoon, SK  S7N 0X2

**Abstract**
A soil bacterium, *Pseudomonas fluorescens* strain BRG100, has demonstrated weed suppressive properties with green foxtail when applied as a pre-emergent bioherbicide. Application of BRG100 was by a granular formulation known as "pesta", which is a matrix consisting of cereal grain flour, polysaccharide and the bacterium. Mass production of BRG100 was carried out in a liquid minimal salts medium and the bacterium typically achieves a population density of 10 billion bacterial cells per mL after 48 h. Production of pesta with BRG100 can either be carried out by blending the bacterial culture directly with ingredients or concentrating the bacterial cells by centrifugation and then blending with pesta ingredients to make dough. The pesta dough is transferred to a single screw extruder modified to record temperature during extrusion of the pesta noodles. Pesta noodles are then transferred to a fluidized bed dryer, dried to 0.8 $a_w$ (water activity), and sieved to a granule size of 1 mm. The shelf life of BRG100 is correlated directly to the population size of BRG100 in pesta.
Stability of phospholipid and protein structure in bacterial membranes has been attributed to the addition of zinc to bacterial growth media and amending formulations with disaccharide sugars such trehalose, glucose or maltose. The objective of this study was to determine the effect of zinc (0, 0.2, 0.5, 0.9 m*M*) and maltose (10, 20% w/w) amendment to the fermentation medium and Pesta formulation, respectively, on the shelf life of BRG100. Addition of 0.2 m*M* zinc to the fermentation medium promoted the highest population of BRG100, exceeding 10 billion cells/mL, after 48 h of growth. The shelf life of BRG100 was greatest when 10% maltose (w/w) was included in the Pesta formulation and BRG100 was grown in medium amended with 0.2 or 0.9 m*M* zinc. These results suggest that modification of the fermentation and formulation processes are linked to advances in the shelf life of a formulated bioherbicide such as BRG100. Future studies intend to focus on extending shelf life, uniformity and dispersion of Pesta and validating product efficacy.

**Oviposition preferences of *Trichoplusia ni* on broccoli and selected agricultural weeds.** Cameron, J.H., M.B. Isman, and M.K. Upadhyaya, Faculty of Land and Food Systems, UBC

Egg-laying preferences of cabbage looper, *Trichoplusia ni* (a lepidopteran pest of crucifers) among broccoli and its selected weeds were studied to determine preference patterns and to identify weedy species as management tools for this insect in broccoli.  Common groundsel, lamb's-quarters, sheep sorrel, shepherd's-purse, and stinkweed were tested individually against broccoli in 48-h oviposition choice tests. Three- and four-species choice experiments were also conducted.  Since previous experience can influence oviposition preference of a pest, oviposition preferences of *T. ni* raised on broccoli or common groundsel were investigated. Broccoli was strongly preferred over common groundsel, lamb's-quarters, and shepherd's-purse, but stinkweed was preferred over broccoli. No preference was shown between broccoli and sheep sorrel. Broccoli was also preferred over lamb's-quarters and shepherd's-purse in four-species multi-choice tests. Exposure to common groundsel at the larval stage did not influence subsequent oviposition preference. The preference of some *T. ni* adults for stinkweed over broccoli suggests that it could have the potential for use as a dead-end trap crop, because larvae cannot survive on it.  If even some of the adult female *T. ni* choose to lay eggs on stinkweed rather than broccoli, those larvae will not survive and therefore not damage the crop.  This could be an ideal situation for a broccoli grower, because no chemical or biological inputs would be necessary to kill the larvae.  Field studies are needed to determine the level of weed presence required to attract insects away from the crop, and the impact of these weeds on crop yield.

# Weeding out the effects of crop residue:

## Crop residues effects on weed seedling emergence

**Christie L. Stewart and Paul B. Cavers**

University of Western Ontario, Biology Department, London, ON

### Introduction

➢Over-dependence on herbicides for weed management in no-till fields is partly a result of the lack of understanding of how weeds are affected by both type and amount of crop residues.
➢In previous studies in non-agricultural systems, leaf litter significantly affected seed production, seed-bank dynamics, and seedling emergence.
➢It is likely that crop residues will affect these aspects of weed growth, specifically in no-till systems where crop residue cover can be highly variable.

### Objective

➢To examine weed seedling emergence from a standard seed bank under different types of crop residue in a greenhouse environment
➢To provide much-needed information on the interaction between weed growth and crop residues for the purpose of improved weed management

### Methods

➢Seven common and problematic weed species were used as a standard seed bank: velvetleaf (*Abutilon theophrasti* Medik.), lamb's-quarters (*Chenopodium album* L.), common ragweed (*Ambrosia artemisiifolia* L.), redroot pigweed (*Amaranthus retroflexus* L.), lady's-thumb (*Polygonum persicaria* L.), barnyard grass (*Echinochloa crusgalli* (L.) P. Beauv.), yellow foxtail (*Setaria glauca* (L.) P. Beauv.) (common and Latin names according to Darbyshire *et al*, 2000).

➢Three major crops grown in Southwestern Ontario were used for crop residue applications: corn, soybean and wheat.

➢One hundred seeds of each species were sown on the surface of soil-filled pots, which were placed on greenhouse benches (Fig. 1).

➢Treatments involved four levels of percent cover of unweathered residue of each crop type (25, 50, 100 and 200%) placed on top of the seeds.

➢Each treatment was replicated five times. Three controls with no residue cover were also included in each replicate.

➢Percent cover was standardized by weight for each crop type across treatments.

➢Each seedling was numbered and tagged as it emerged, then identified at a minimum stage of 3 true leaves and removed.

Figure 1.

### Results

Pooled species effects

➢Percent cover of residue had a significant effect overall on the total number of weed seedlings emerging (p<0.001, Fig. 2)
➢Crop type did not have a significant effect overall on the number of weed seedlings emerging

Individual species effects

➢Percent cover of residue significantly affected the number of seedlings emerging per pot for common ragweed, redroot pigweed, velvetleaf, lamb's quarters, barnyard grass, and lady's thumb; (p<0.001 for all, Figs. 3-7).
➢Crop type had significant effects on redroot pigweed (p<0.001) and lamb's quarters (p<0.01, Figs. 8, 9).
➢Soybean and wheat residues had fewer redroot pigweed seedlings than corn residue, while soybean and wheat residues had more lamb's quarters seedlings than corn residue
➢A significant interaction between crop type and percent cover of residue affected the number of redroot pigweed seedlings emerging at the 100% (p<0.001) and the 200% (p<0.01) levels.
➢There were more redroot pigweed seedlings at 100% and 200% level under corn residue cover than at the same level of percent cover under soybean and wheat residues.
➢There was no effect of any treatment on yellow foxtail
➢Note: for each graph, different letters above the bars indicate significant differences

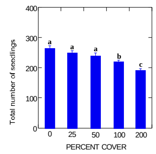


Figure 2. Effect of percent cover of crop residue on the total number of weed seedlings. Fifteen pots comprising 3 residue treatments, 7 weed species and 5 replicates.

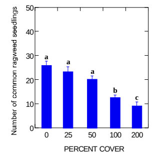


Figure 3. Effect of percent cover of crop residue on the number of common ragweed seedlings per pot.

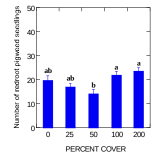


Figure 4. Effect of percent cover of crop residue on the number of redroot pigweed seedlings per pot.

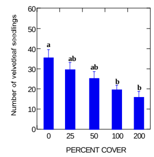


Figure 5. Effect of percent cover of crop residue on the number of velvetleaf seedlings per pot.

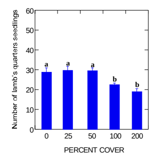


Figure 6. Effect of percent cover of crop residue on the number of lamb's quarters seedlings per pot.

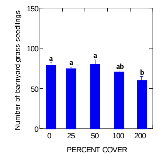


Figure 7. Effect of percent cover of crop residue on the number of barnyard grass seedlings per pot.

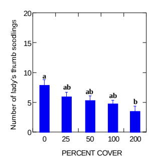


Figure 7. Effect of the percent cover of crop residue on the number of lady's thumb seedlings per pot.

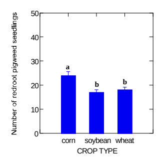


Figure 8. Effect of crop type on the number of redroot pigweed seedlings per pot.

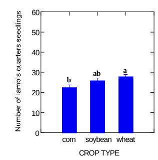


Figure 9. Effect of crop type on the number of lamb's quarters seedlings per pot.

### Conclusions and discussion

➢The amount of crop residue affected the total number of weed seedlings that emerged in six of the seven species studied.
➢The effect differed among weed species. Increasing the amount of crop residue decreased the number of common ragweed, velvetleaf, lamb's quarters, barnyard grass and lady's thumb seedlings per pot. Increasing the amount of crop residue to 100 and 200% increased the number of redroot pigweed seedlings per pot; even greater than the number of seedlings per pot that emerged in the control, thus indicating a stimulatory effect.
➢The amount of crop residue tested in this study had no effect on the number of yellow foxtail seedling that emerged per pot possibly as a result of insensitivity to light-limited conditions.
➢Other scientists have shown that different species show differential sensitivity to the amount of residue, related to each species' capacity to grow around obstructions under light-limited conditions caused by the presence of residue.
➢This effects have also been suggested to be a result of species' sensitivities to allelopathic chemicals released by the residues, changes in soil temperature, moisture and pH, residue structure and amount.
➢Corn residue had a stimulatory effect on redroot pigweed seedlings at the 100 and 200% levels of cover.
➢The interaction, stimulatory at the above levels of crop residue only, occurred with the corn residue, while the soybean and wheat residues at those levels of percent cover did not show stimulatory effects.
➢Crop type affected the number of seedlings of only two species, namely redroot pigweed and lamb's quarters.
➢Soybean and wheat residue treatments had fewer redroot pigweed seedlings than the corn residue ones, while soybean and wheat residues treatments had more lamb's quarters seedlings than the corn residue treatment.
➢Corn residue included both stalks and leaves, soybean residue contained stems and bean pods and wheat residue contained mostly stems.
➢Leaves are known to contain more nitrogen and break down faster, releasing this nitrogen more quickly than do other plants parts.
➢Redroot pigweed germination has been shown to be stimulated by the presence of nitrates.
➢The stimulatory effect of corn residue on redroot pigweed, particularly at higher amounts, is likely a result of the increase in nitrogen release from the corn leaves.
➢Other effects, e.g. fewer lamb's quarters seedlings under corn residues, may reflect sensitivity to pathogenic micro-organisms, allelopathic chemicals, etc.

**Acknowledgements**

We'd like to thank Darren Robinson, Peter Sikkema, Yvonne McLellan, Ridgetown College, Caroline Rasenberg, Peter Duenk, Greg Thorn, NSERC and many helpers.

# Tolerance of *Acinetobacter* sp. to Glyphosate

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## Background

- Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), preventing the biosynthesis of aromatic amino acids (Fig. 1). This enzyme is found in plants, fungi and bacteria (Padgette *et al.* 1995).

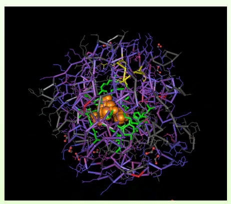

Fig. 1. Glyphosate occupying the active site (highlighted in green) of the EPSPS enzyme (Schönbrunn *et al.* 2001).

- The CP4 EPSPS gene construct is present in Roundup Ready® varieties, conferring tolerance to glyphosate.
- The recombinant DNA (rDNA) released by plants could be taken up by naturally competent bacteria such as *Acinetobacter*, which have been shown to take up DNA from other organisms (Nielsen *et al.* 1997).

## Objectives

- To determine the glyphosate concentration at which growth of the common soil bacteria *Acinetobacter* is inhibited.
- To use glyphosate selection to test for *in vitro* transformation of *Acinetobacter* with rDNA in soil.

## *Summary*

**To determine the glyphosate concentration that can be used to select *Acinetobacter* cells transformed with the CP4 EPSPS gene.**

- **20 mM glyphosate inhibited cell growth and replication completely.**
- **At increasing glyphosate concentrations reaching up to 15 mM, a delay in bacterial growth was found.**

***Conclusion*** **– 20 mM glyphosate is a useful concentration for monitoring *Acinetobacter* transformation events.**

## Results and Discussion

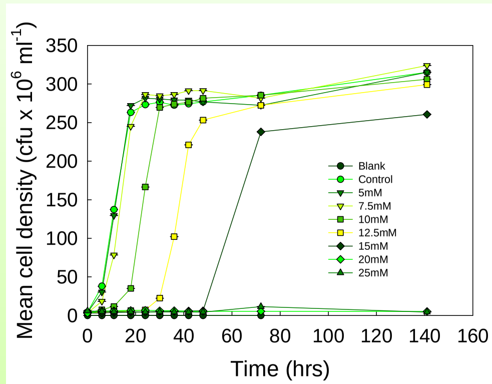


Fig. 2. Effect of glyphosate on replication rate of *Acinetobacter* over time.

- Up to 15 mM glyphosate, only a delay in bacterial replication was seen. The growth curves were similar but more delayed with increasing glyphosate concentration.
- Replication was strongly inhibited at 20 mM and 25 mM glyphosate.
- 20 mM glyphosate is likely a good level of selection to test for *Acinetobacter* transformants.
- It is crucial to monitor transformation events in soil to understand the role of uptake in the DNA cycle in soil. rDNA provides a marker to track such events.

## Methods

- Liquid cultures of *Acinetobacter* were exposed to glyphosate in 96-well plates.
- Kill curves were established by measuring optical density (OD) and converted to cfu ml$^{-1}$ using:

$$1.0 \text{ OD} \approx 2.5 * 10^8 \text{ cfu ml}^{-1}$$

## Future Research

To determine transformation efficiency

- The CP4 EPSPS was amplified from Roundup Ready® soybean genomic DNA with PCR primers:
  F – 5'GGAGGAGAATTCATGCTTCACGGTGCAAGCCGGCCC
  R – 5'GGTCCACAGCTGTCAGGCAGCCTTCGTATCGGAGAGTTCGAT,
  then ligated into the broad-host range vector pKT210 (Fig. 3).
- The ligation of CP4 EPSPS into pKT210 originally resulted in a non-functional protein due to a frameshift mutation.
- Research looking at *in vitro* transformation efficiency of *Acinetobacter* ongoing.

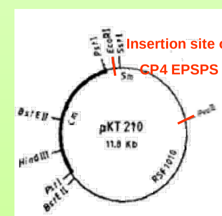


Fig. 3. pKT210 with insertion site of CP4 EPSPS between *Eco*RI and *Pvu*II (Bagdasarian *et al.* 1981)

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**Acknowledgements**
The authors wish to thank the following funding agencies: NSERC CRSNG

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

# Impact of dimethenamid use in onions on subsequent rotational crops in muck soil

Horticulture Research and Development Centre, Agriculture and Agri-Food Canada. Saint-Jean-sur-Richelieu (Québec) Canada J3B 3E6

Benoit Rancourt, Diane Lyse Benoit and Manon Bélanger

## Introduction

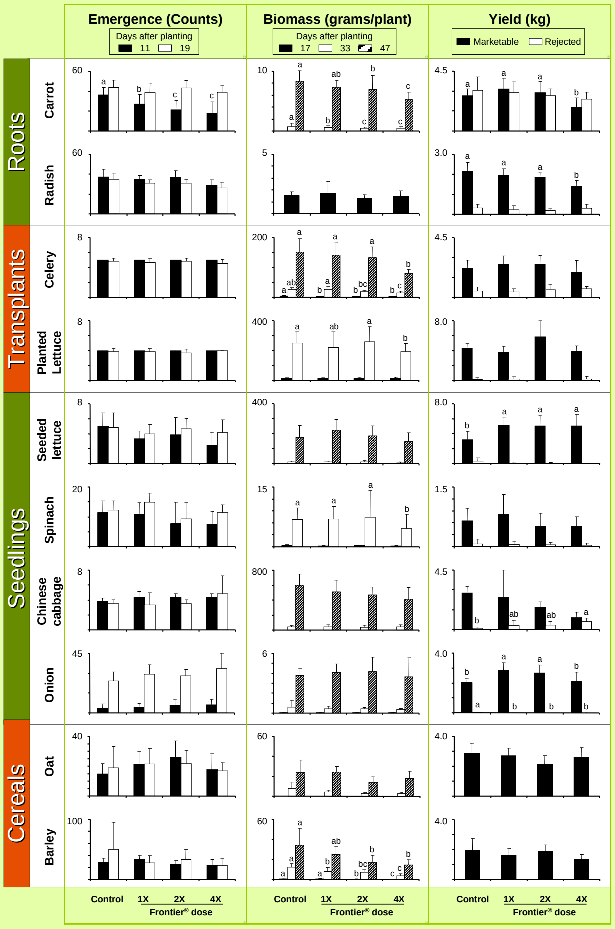
Yellow nutsedge (*Cyperus esculentus* L.) is a major weed in onion grown on muck soil in both Québec and Ontario. Herbicide screening trials in the 80's and 90's had identified dimethenamid (Frontier®) as a potential herbicide for yellow nutsedge suppression in onions. Its intensive degradation is generally over 60-80 days after application but may still pose a threat to rotational crops (Peneva 1999). The requirements of the Pest Management Regulatory Agency (PMRA) to proceed with the application for registration of Frontier® in onions (URMULE D.3.1-2002-0971) included a trial on recropping the year following application of dimethenamid in organic soil.

Consequently, an experimental site was set up in 2002 with the application of dimethenamid on muck soil and a trial was established in 2003 to observe whether the presence of residues of dimethenamid affected the development of rotational crops the year following its application in muck soils.

## Materials and methods

The trial was conducted at Agriculture and Agri-Food Canada experimental farm at Sainte-Clotilde-de-Châteauguay, Quebec. The statistical design was a split plot with three replications and four main plots 18 m long by 5.5 m wide, corresponding to the four herbicide treatments. These plots were divided into ten subplots 1.8 m wide by 5.5 m long for each of the ten crops studied. Frontier® treatments were applied on June 10, 2002 and included an untreated control, a 1x treatment (1.68 kg ai/ha), a 2x treatment (3.36 kg ai/ha) and a 4x treatment (6.72 kg ai/ha). The product was applied at 241 kPa and 275 L water/ha using a boom sprayer equipped with flat nozzles. In 2003, seeding and transplantation took place on May 16.

Crop emergence was measured 11 and 19 days following seeding, biomass after 17, 33 and 47 days and yield at crop maturity. Emerged seedlings were counted along a 1m row or within 25 x 50 cm quadrats for cereals; on each date, two counts were done in each plot. Crop biomass (number of harvested plants and weight) was quantified in two 25 cm x 50 cm quadrats. The above-ground fresh weight (leaves) was measured, except for the root vegetables (radishes and carrots), where the roots and leaves (fresh weight) were weighed. Crop yields were evaluated in duplicate over 2 m sections for row crops and in 25 cm x 50 cm quadrats for cereals. The harvests were classified by marketable and reject category, then counted and weighed. Cereal yields represent an overall assessment of the production of vegetative biomass and not commercial grain yield.




## Results and discussion

In the year following dimethenamid treatments, no difference in crop emergence was observed, except for carrots; in the latter case, the effect was temporary and completely disappeared a week later. Similarly, the residues still present in the soil 12 months after the applications had little effect on crop development compared to the control plots, except in the case of cereals such as oats, where perceptible symptoms of phytotoxicity (biomass) were observed with treatment 2X. Seeded lettuce, onions, carrots, celery and spinach had higher yields at recommended rate (1X) compared to the untreated control. For seeded lettuce and onions, this increase was significant, while for carrots, celery and spinach it was not. For all other crops (radishes, Chinese cabbage, transplanted lettuce and cereals), a non-significant decrease in yield was observed in plots which received the recommended rate of Frontier® (1X) the previous year.

**Radish:** The crop emergence, biomass and yield were lower at the maximum dose of the herbicide (4X rate) while the recommended rate (1X) showed no negative effect.

**Transplanted lettuce:** The 4X rate seems to have affected establishment of the plants but the differences between treatments had disappeared 1 month after transplantation.

**Celery:** Only 2X and 4X treatments seem to have affected biomass during the season. Yields at harvest were not significantly different from the control.

**Chinese cabbage:** All three herbicide rates affected crop emergence, but the values were not significantly different from the control.

**Onions:** Dimethenamid residue had little effect on seedling emergence or onion biomass, even at 4X treatment. At the end of the season, yields in 1X and 2X treatments were significantly higher than the control suggesting that dimethenamid residues present in the soil the year following its application have no effect on onion production.

**Cereals:** The effect of Frontier® on oats and barley was still perceptible the year following application of the herbicide, notably for biomass production. Only a high dose of dimethenamid (4X) significantly decreased barley biomass the year following its application.

## Conclusions

Rotational crops which can be grown safely the year following application of Frontier® recommended rates in onions are spinach, onions, carrots, transplanted celery and seeded lettuce.

## References

Peneva, A.A. 1999. Influence of environmental conditions and soybean agrotechniques on detoxification of herbicides in soil. Proceedings of 1999 Brighton Conference – Weeds 15-18 November 1999. Vol.2: 705-710.

## Acknowledgements

Authors acknowledge technical input from J. Chaput, Provincial Minor Use Coordinator (OMAFRA) and also extend their appreciation to the personnel of Ste-Clotilde experimental farm and summer students for their technical assistance.

 CWSS/SCM, Annual meeting, November 27 – December 1, 2005, Niagara Falls, Ontario

Canada

# Field Pea Response to Sequential Herbicides

K.Sapsford[1], F.A. Holm[1], E.Johnson[2]

[1] University of Saskatchewan, Saskatoon, SK; [2]Agriculture and Agri-Food Canada, Scott, SK

**Background:**

Field observations have led some producers to believe that field pea is more susceptible to injury (chlorosis, growth reduction, reduced yield) from post-emergence application of *imazamox/imazethapyr* (Odyssey®) when the crop is seeded in fields treated in the previous season with *flucarbazone sodium* (Everest®).

**Objective:**

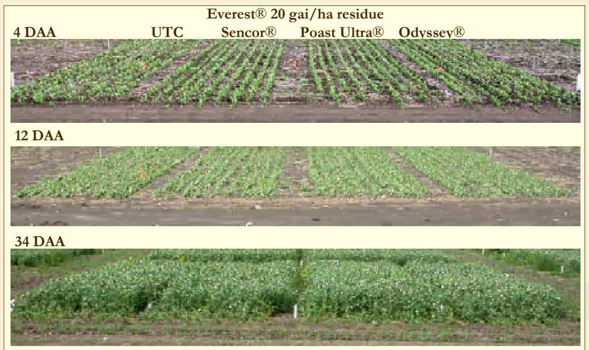
To determine if field pea planted into soil containing flucarbazone sodium residue is more susceptible to post-emergent herbicide injury.

**Materials and Methods:**

The trial was conducted in 2005 at the University of Saskatchewan Kernen Crop Research Farm and the Agriculture and Agri-Food Canada Scott Research Farm. In the fall of 2004 *flucarbazone sodium* was applied to the soil at 5 rates ranging from 33% to 200% of recommended rate. Peas were planted May 6 at Scott and May 16 at Saskatoon. Post-emergent applications of *metribuzin* (Sencor®), *sethoxydim* (Poast Ultra®) and *imazamox/imazethapyr* were applied to each of the *flucarbazone sodium* treated areas. The trial was set up as a 4 rep, 5 by 4 factorial with the *flucarbazone sodium* rates as the main plots and the post-emergent applications as the sub plots. Visual injury ratings were done at 4-5 and 22-34 days after post emergent application. Chlorosis was measured at 12 days after post emergent application with a SPAD meter. Weed pressure was not a factor in this trial. Seed yield was taken at Saskatoon but not at Scott due to hail in July.

**Treatments:**

| ***flucarbazone sodium*** | **Post-emergent Applications** |
|---|---|
| 1. Untreated Check | 1. Untreated Check |
| 2. 10 gai/ha ~ 0.33% of X rate | 2. *metribuzin* ………….…………….280 gai/ha |
| 3. 20 gai/ha ~ 0.66% of X rate | 3. *sethoxydim* ………………………..212 gai/ha |
| 4. 40 gai/ha ~ 1.33% of X rate | 4. *imazamox/imazethapyr* ……………..30 gai/ha |
| 5. 60 gai/ha ~ 2.0% of X rate | |




**Observations:**

Pea injury symptoms – stunting and chlorosis of the newest leaves

•Peas showed no chlorosis or growth reduction from the *flucarbazone sodium* residue prior to the post-emergent applications (2-3 above ground node) (data not shown)

• 4-5 DAA visual injury >10 was observed when all post-emergent herbicides were applied to peas grown on *flucarbazone sodium* residue of 60 gai/ha but not at lower *flucarbazone sodium* rates (Chart 1)

•12-22 DAA there was no visual difference in injury to peas with any treatment applied over 0, 10 or 20 gai/ha *flucarbazone sodium* residue. Visual injury >10 was observed when all post-emergent herbicides were applied to peas grown on *flucarbazone sodium* residue of 40 and 60 gai/ha. Injury from *imazamox/imazethapyr* was always slightly higher than the injury from other post-emergent herbicides. (Chart 2)

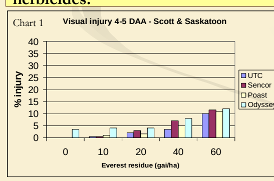
•5 DAA chlorophyll content in the newest pea leaves declined significantly as *flucarbazone sodium* residue increased. Chlorophyll content was significantly lower where *imazamox/imazethapyr* was applied compared to the other post-emergent herbicides, but there was no interaction between the two. (Chart 3)

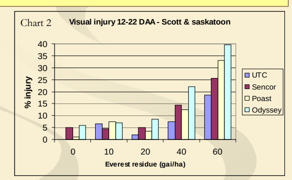
• Pea yields were reduced only when *metribuzin* , *sethoxydim* or *imazamox/imazethapyr* was applied to peas that were grown on 60 gai/ha *flucarbazone sodium* residue. (Chart4)

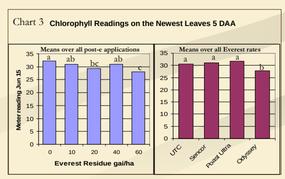
**Conclusions:**

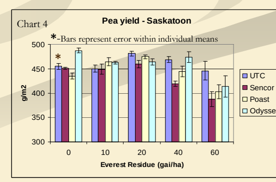
**•Increased injury to peas and reduced yield from sequential herbicide applications occurred only when extremely high rates of *flucarbazone sodium* (60 gai/ha) were present in the soil.**

**•Since *flucarbazone sodium* soil residue levels in a field would normally be much less than the 30 gai/ha (1X rate), normal levels of *flucarbazone sodium* residues in soil should not affect a pea crop's response to these post-emergent herbicides.**




**The authors acknowledge technical support provided by Gerry Stuber, Teri Ife, Herb Schell and Cindy Gampe and financial support from Saskatchewan Agriculture, Food and Rural Revitalization and the Saskatchewan Pulse Growers Association**

# Herbicide resistant weeds in Ontario

## Why won't herbicides work anymore?

Peter J. Smith and Dr. François Tardif – Department of Plant Agriculture, University of Guelph

**Herbicide resistance** in weed science is defined as the inherited ability of a localized plant sub-population to survive and reproduce after repeated exposure to herbicides normally lethal to the naturally occurring population. This happens after natural selection of mutants with repeated herbicide use.

### A brief history:

Following World War II, unprecedented advancements in mechanization and pesticide technology allowed Ontario's crop producers to grow better quality food much more efficiently. As well, improved plant breeding techniques facilitated the introduction of new crop traits (e.g. tolerance of colder climates, disease resistance) and ultimately new crops such as corn and soybeans. Subsequently, new classes of herbicides were developed to improve weed control and protect these new crops.

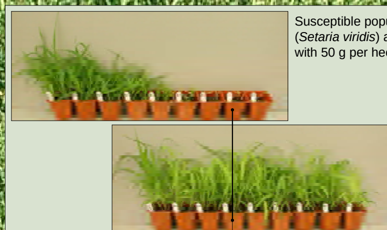
### How does resistance happen?

A single mutation in the DNA of many weeds enables them to survive normally lethal doses of commonly used herbicides. Through repeated use of similar chemistries, we are selectively breeding for herbicide resistant weeds by eliminating the susceptible portion of the population from the rural landscape.

Despite best management practices (e.g. yearly crop rotation, proper herbicide selection, timely application and rotation of herbicide modes of action) resistance occurs via the selection of these mutant plants.

Unfortunately, even our best farmers are at risk. Wind borne seeds and pollen will introduce new genetic material to even the most vigilant farmer's operation. Also, contaminated equipment and crop seeds have been identified as new point sources of resistant weed seed dispersal in Ontario.

There are currently 17 confirmed species of resistant weeds in Ontario and 174 species worldwide.



Susceptible populations of green foxtail (*Setaria viridis*) are completely killed with 50 g per hectare of Pursuit.

This resistant population shows minor injury following exposure to 3,200 g per hectare of Pursuit.

Full field rate of imazethapyr (Pursuit) which inhibits protein synthesis in susceptible plants

Susceptible populations of green pigweed (*Amaranthus powellii*) are completely killed with 250 g per hectare of Lorox.

This resistant population requires 8000 g per hectare of Lorox for effective control, a 32 fold increase in herbicide use.

Full field rate of linuron (Lorox) which inhibits photosynthesis in susceptible plants

### The human impact:

Cropping systems, and the infrastructure that supports the delivery of food to consumers, are incredibly complex. Approximately 1 of every 5 jobs in Ontario is connected to agriculture. We are all affected somehow. Any developments which destabilize the agrifood industry has the potential to affect the quality and quantity of food available to us.

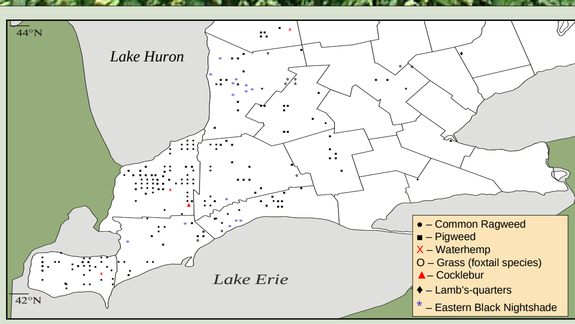
Our ability to develop practical tools for modern weed management will benefit other areas of the world as well. As effective stewards of the land, farmers around the world are entrusted with the safeguarding of the lands, both now and in the future.

### Current research:

Traditionally, whole plant dose responses to selected herbicides have determined if resistance exists in a test sample. The effective rate for control of these populations (compared to a susceptible population) establishes the degree of resistance.

New genetically based testing can rapidly and accurately identify genetic mutations. Researchers in the Ontario Agricultural College are working to sequence the DNA of many weeds. By cross referencing the DNA of test samples with normal populations, the pattern and level of resistance are quickly determined.

Farmers are then able to apply alternative herbicides, which effectively control these resistant weeds, in the same growing season in which they were discovered.




**Locations of resistant weeds discovered in Southwestern Ontario from 1997 to 2005**

### What's next?

Multi-year, large scale field studies will determine the effects of genetically modified crops and herbicide choices on the persistence of resistant populations.

Small plots trials will evaluate the additive effects of reduced rates of multiple herbicide combinations and their crop safety and weed control properties.

Researchers at the University of Guelph are working to develop effective methods to both delay the evolution of resistant weed populations and eradicate them from Ontario's rural landscape.

University of Guelph

Ontario

**Segmentation of remotely sensed imagery for discrimination of weed and crop species.** P.R. Eddy[1,2], A.M. Smith[1,2], C.A. Coburn[2], R.E. Blackshaw[1] and D.R. Peddle[2]. [1]Agriculture and Agri-food Canada, Lethbridge, AB. [2] Department of Geography, University of Lethbridge , AB.

Optimizing the placement of herbicides through site-specific application techniques can reduce both the cost of production and potentially harmful effects on the environment. Implementation of this technology requires information on the location and population density of weed species within a field. Through funding from the Improving Farming Systems and Practices Initiative of Agriculture and Agri-Food Canada, studies involving crop/weed discrimination using both spectral and spatial plant characteristics are under investigation. Hyperspectral imagery was acquired over greenhouse grown wheat, canola and redroot pigweed. In the first stage of analysis, thresholding and watershed transformation of hue images were compared with a “standard” three band (blue (460 nm), green (550 nm) and red (650 nm)) ISODATA classification for delineation of individual plant leaves. Variation in reflectance across the leaf surface caused by differences in leaf angle hindered the detection of plant matter using the ISODATA classification. Only vegetation of a certain brightness could be detected; while shaded vegetation could not be identified. The conversion of the non-normalized 460, 550 and 650 nm image data to hue colour space, moderated the effects of non-diffuse lighting. A high degree of leaf overlap in wheat and redroot pigweed confounded identification of single leaf segments using thresholding. In comparison, canola, characterized by widely spread leaves, was segmented relatively well although the entire plant rather than individual leaves was defined due to connecting petioles. In the watershed output, over-segmentation was reduced by quantizing the original 8-bit hue images to 4- and 3-bit grey-level depth, with the latter more effective in defining larger leaf regions. Detection of leaf edges was less distinct in the 3-bit images and a combination of watershed segmentation and thresholding proved more effective in delineating leaf tissue. Future research will focus on integrating spectral characteristics of the defined regions to further weed/crop discrimination.

**Influence of UV-B radiation on growth indices of broccoli and lambsquarters in mixtures.** Furness, N.H., P.A. Jolliffe and M.K. Upadhyaya, Faculty of Land and Food Systems, University of British Columbia.

Plant growth indices were used to assess the role of ultraviolet-B (UV-B) –induced changes in morphology and biomass partitioning to previously reported increased competitiveness of broccoli (*Brassica oleracea* L. var. *italica* cv. Purple Sprouting) relative to lambsquarters (*Chenopodium album* L.) at elevated UV-B radiation levels. Broccoli and lambsquarters monocultures (144, 256 and 400 plants $m^{-2}$) and binary mixtures were grown at 4 (ambient) and 7 (above-ambient) kJ $m^{-2}$ $d^{-1}$ biologically effective UV-B radiation ($UV\text{-}B_{BE}$) in a greenhouse. Plant growth indices, determined from per plant leaf area, and root, stem, leaf, and shoot biomass were subjected to analyses of variance. While morphology and biomass partitioning were sometimes influenced by UV-B, variation between species and years also occurred. UV-B did not influence the shoot:root ratio (SRR) of broccoli, but generally increased that of lambsquarters. UV-B effects on leaf area ratio (LAR), a measure of leafiness, of broccoli differed between years, while UV-B had no effect on LAR of lambsquarters. Therefore, shifts in competitive ability could not be attributed to differential sensitivity of LAR to UV-B radiation. UV-B did not affect biomass partitioning to leaves, as measured by leaf weight ratio (LWR), of either species. These results demonstrate that morphological responses observed at the per plant level do not necessarily directly translate into corresponding competitive responses. Leaf area index (LAI), representing the extent of leaf array available for photosynthesis, increased for broccoli, and declined for lambsquarters at elevated UV-B in both years. The influence of UV-B radiation on LAI may explain previously reported UV-B-induced shifts in competitive relationships.

**W/O/W emulsions- Formulation development for foliar application of bioherbicides.** Russell K. Hynes, Paulos Chumala, Daniel Hupka and Gary Peng. Agriculture and Agri-Food Canada, Saskatoon, SK. S7N 0X2

Biocontrol of weeds includes the use of naturally occurring microorganisms (ie. bioherbicides) to kill, suppress or reduce the vigour or reproductive capacity of the target. *Pyricularia setariae* and *Colletotrichum truncatum*, were selected from screening experiments as potential bioherbicide agents for post emergent control of green foxtail (*Setaria viridis*) and scentless chamomile (*Matricaria perforata*), respectively. Water/oil/water (W/O/W) emulsions[1] were explored using a variety of oils and surfactants to deliver spores of the bioherbicides to the target weeds in greenhouse experiments. Of the nineteen surfactant mixtures examined Spans 85/20/Tween 80 and Span80/Tween60 provided the best stability over 15 days. The droplet diameter, D[4,3], was 38-45 µm for the water/vegetable oil (canola and soybean)/water emulsions. Compatibility of crude, degumed and food grade canola and soybean oils with the bioherbicides as well as emulsion development and stability were examined. Degumed canola oil inhibited spore germination of *Pyricularia setariae* and *Colletotrichum truncatum* while the other oils had no adverse effect. A sub-lethal dose of the W/O/W formulated bioherbicides was applied using a cabinet track sprayer fitted with a XR8002 nozzle at 200 L/ha to green foxtail and scentless chamomile. Treated plants were provided with 24 h dew for infection. Green foxtail shoot fresh weight was significantly ($P \leq 0.05$) reduced after 7 d by *P. setariae* in the W/O/W emulsion when compared to the fungus in 0.1% Tween 80. Shoot fresh weight of scentless chamomile was reduced (not significantly $P=0.05$) by *C. truncatum* in the W/O/W emulsion when compared to the fungus in 0.1% Tween 80. No phytotoxicity was observed on green foxtail or scentless chamomile treated with the formulation alone. The W/O/W formulation appears compatible with the bioherbicide agents and its effectiveness should be further determined under sub-optimal dew conditions for infection by the bioherbicides.

**Decomposition Kinetics of Biomass and rDNA of Roundup Ready® Corn Roots.** David J. Levy-Booth. Department of Environmental Biology, University of Guelph, Guelph, ON, N1G 2W1.

The use of Roundup Ready® (RR) corn introduces recombinant DNA (rDNA) and organic material into soil environment during decomposition. Differences have been found in the decomposition kinetics of *Bt* and non-transgenic corn, possibly due to increased lignin concentration. We will investigate the decomposition of RR and isolinear, non-transgenic corn in a litterbag microcosm study for 60 d. The spatial and temporal persistence of the RR (*CP4 epsps*) gene in soil will be quantified using real-time PCR. This study will attempt to provide an assessment of RR corn root rDNA decomposition and persistence.

**Physiological Basis of Decreased Weed Sensitivity to Glyphosate Under Low Nitrogen Conditions.** J. Mithila, C.J. Swanton and J. Christopher Hall, University of Guelph, Guelph, ON, Canada

Herbicide efficacy is influenced by several environmental factors e.g. temperature, soil moisture, pH. Recently, we reported that herbicide efficacy is reduced when weeds are grown under low (1.5 mM N) versus high (15 mM N) nitrogen. To understand the physiological basis of nitrogen effect on glyphosate efficacy, growth room experiments were conducted using velvetleaf, lambsquarters and ragweed grown under high and low nitrogen concentrations. Higher doses (225 g ai/ha and above) of glyphosate was required for a significant reduction in plant biomass in plants grown under low nitrogen than in high nitrogen. Absorption and translocation pattern of $^{14}C$ glyphosate indicates that in velvetleaf plants grown under low nitrogen less herbicide was translocated to the actively growing meristem. Glyphosate is a phloem mobile herbicide and needs to be translocated along with photoassimilates from source to sink. It appears that low nitrogen conditions may decrease the net assimilated carbon in plants resulting in a decrease in the net export of glyphosate from mature leaves. Understanding the relationship between nitrogen levels and herbicide efficacy may help us understand weed-crop competition as well as some weed control failures.

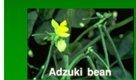
**The biology of invasive alien plants in Canada Series.** Warwick S.I., and Darbyshire S. Agriculture and Agri-Food Canada (AAFC)-ECORC, Ottawa, ON

A new series: *The Biology of Invasive Alien Plants in Canada* was initiated in 2003 in the Canadian Journal of Plant Science. To date, three species accounts have been published, two are in press and nine additional species have been assigned. The Series is designed to cover recently introduced plant species that pose a demonstrable economic or environmental risk. Invasive alien species are becoming a catastrophic problem to ecosystems throughout the world. Globalization and expansion of trade have greatly contributed to the increased rate at which species are being transported internationally. Presently an estimated 1-2 new alien plant species are becoming established in Canada each year and that rate of introduction and establishment will likely increase. Many of these new alien plants are likely to become widespread problematic weeds in the future. These new pests are generally poorly known and their weedy potential unrecognized by most Canadians. There is a need for information to assist with early detection and accurate identification of new infestations as well as diagnosis of their potential for detrimental effects. Contributions to the new series will serve as an alert of emerging problems, and will emphasize identification, occurrence, impact, effective control methods and future prognosis. The series will also engender research to fill important gaps in our knowledge of the biology and management of these species. For more information on the series, submission process and instructions to authors, see the CWSS web site (http://www.cwss-scm.ca/Biology_of_weeds/invasive.htm) or contact the associate editor at warwicks@agr.gc.ca for a pdf file.

**The IR-4 Project: Update of Weed Control Projects.** F.P. Salzman, M. Arsenovic,a nd D. L. Kunkel.

The IR-4 Project is a publicly funded effort to support the registration of pest control products on specialty crops. The Pesticide Registration Improvement Act (PRIA) is affecting IR-4 submissions and EPA review of packages. The IR-4 Project continues its role to meet grower’s needs for weed control options despite a climate in which fewer herbicides are available. IR-4 submitted herbicide petitions to the EPA from October 2004 to September 2005 for: clethodim on leafy greens subgroup, legume vegetables group, asparagus, hops, and sesame; ethofumesate on dry bulb onion; glyphosate on dry pea, safflower, and sunflower; lactofen on fruiting vegetables group; pendimethalin on green onion and perennial strawberry; and sethoxydim on root vegetables subgroup, pepper (to reduce PHI), okra, and buckwheat. From October 2204 through September 2005, EPA has published Notices of Filing in the Federal Register for ethalfluralin on rapeseed, canola, crambe, Mustard seed, and **potato,** flumioxazin on pome fruits group, stone fruits group, and strawberry; paraquat on *Brassica* leafy vegetables group, pome fruits group, stone fruits group, tree nuts group, berries group, edible-podded legume vegetables group, succulent shelled pea and bean subgroup, dried shelled pea and bean subgroup, cucurbit vegetables group, fruiting vegetables group, grape, cranberry, hops, ginger, okra, tanier, and dry bulb onion; and terbacil on watermelon. EPA established tolerances from October 2004 though September 2005 on 2,4-D on hop, wild rice, s-metolachlor on sweet corn, popcorn, garlic, dry bulb onion, green onion, safflower, shallots, head and stem *Brassica* subgroup, foliage of legume vegetables group, fruiting vegetables group, leaf petioles subgroup, edible-podded legume vegetables subgroup, dried shelled pea and bean subgroup, root vegetables (except sugar beet) subgroup, tuberous and corm vegetables subgroup, and tobasco pepper.

# CONTROL OF VOLUNTEER ADZUKI BEAN IN CORN AND SOYBEAN

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## INTRODUCTION

Adzuki bean originated in the Orient, where it has been cultivated and used for many centuries and is the sixth largest crop in Japan. It has since been introduced to North America in areas such as Florida, Minnesota, California, and Ontario (2). The adzuki bean plant grows approximately 1 to 2 feet high and produces pods, each containing two or three primarily dark red seeds with a white ridge on the side (4). In comparison to other beans, adzuki bean has a sweeter flavour with a nutty taste (1). In Japan, adzuki bean is used as a confectionary (3). However, the primary use of adzuki bean in North America is bean sprouts (2).

Adzuki bean provides an alternative high-value crop that growers can rotate with wheat, corn and soybean in Ontario. However, after an adzuki bean crop is grown, seeds that have remained in the soil after harvest are able to survive up to ten years, causing volunteer adzuki beans to appear in future crops (4). Effective adzuki bean control is essential especially in IP soybean production, which could stand to lose a substantial premium due to contamination of the crop with adzuki beans. There is currently little knowledge of herbicides that may provide effective control of volunteer adzuki bean in soybean or corn under Ontario environmental conditions.

**The objective of this study was to evaluate the performance of various pre- and post-emergence herbicides to control volunteer adzuki beans in soybean and corn.**

## MATERIALS AND METHODS

**Study establishment:** Field studies were conducted in 2005 at Exeter and Ridgetown, Ontario. The experiments were arranged in a randomized block design with four replications. Treatments used for each crop are listed in Table 1. 'Erimo' adzuki bean was spread at approximately 224 kg/ha and incorporated into the soil. Plots consisted of four rows of soybean or corn placed in 76 cm rows that were 10 m long at Exeter and 8 m long at Ridgetown. The soybean and corn were planted in mid-May at a rate of 400,000 seeds/ha and 75,600 seeds/ha, respectively.

Pre-emergence herbicides were applied 3-4 days after planting, and post-emergence herbicides were applied at the 2-3 trifoliate stage of soybean and the 4-5 leaf stage of corn. Spray applications were made with a $CO_2$-pressurized backpack sprayer using Hypro ULD 120-02 nozzle tips. Plots were maintained weed free during the growing season by hand hoeing as required.

**Data collection:** At 56 days after treatment (DAT) or emergence (DAE), visual control was rated on a scale of 0 to 100% (0=no visible control, and 100=total volunteer adzuki bean control). At 70 DAT/DAE, 1 $m^2$ of adzuki bean was removed from each plot and dry weights were measured. Soybean and corn were harvested in October and November and yields were adjusted to 13% and 15.5% moisture, respectively.

**Statistical analysis:** All data were subjected to analysis of variance. Tests were combined over locations and analyzed using the MIXED procedure of SAS (Ver 8e, SAS Institute Inc., Cary, NC). Treatment means were separated using Fisher's protected LSD P<0.05.


**Figure 1. Volunteer adzuki bean control in soybean with chlorimuron and fomesafen, and in corn with the dicamba/atrazine and weedy check.**

**Table 1. Mean percent visual control and shoot dry weight of adzuki bean, and yield of soybean and corn in response to various pre- and post-emergence herbicides. Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD test (P<0.05).**

SOYBEAN

| Treatments | Rate (g ai/ha) | Visual Control (%) | Dry Wt. (g) | Yield (t/ha) |
|---|---|---|---|---|
| *Pre-emergence* | | | | |
| Weedy Check | | 0 c | 83 bc | 2.3 c |
| Weed Free Check | | 100 a | 0 a | 3.0 a |
| Cloransulam-methyl | 35 | 0 bc | 76 b | 2.4 bc |
| Linuron | 2250 | 1 b | 75 b | 2.5 b |
| Metribuzin | 1120 | 0 bc | 85 bc | 2.4 bc |
| Flumetsulam | 70 | 1 b | 86 bc | 2.5 b |
| Imazethapyr | 100 | 0 bc | 101 c | 2.3 c |
| *Post-emergence* | | | | |
| Weedy Check | | 0 e | 120 d | 2.2 d |
| Weed Free Check | | 100 a | 0 a | 3.0 a |
| Acifluorfen | 600 | 0 e | 119 d | 2.2 d |
| Fomesafen | 240 | 0 e | 134 d | 2.2 d |
| Bentazon | 1080 | 1 e | 115 d | 2.4 cd |
| Thifensulfuron | 6 | 16 d | 124 d | 2.3 cd |
| Chlorimuron | 8 | 59 c | 61 c | 2.6 bc |
| Cloransulam-methyl | 17.5 | 5 e | 116 d | 2.2 d |
| Imazethapyr | 100 | 1 e | 120 d | 2.1 d |
| Imazethapyr+bentazon | 75+840 | 4 e | 112 d | 2.3 cd |
| Glyphosate | 900 | 83 b | 19 b | 2.9 ab |

CORN

| Treatments | Rate (g ai/ha) | Visual Control (%) | Dry Wt. (g) | Yield (t/ha) |
|---|---|---|---|---|
| *Pre-emergence* | | | | |
| Weedy Check | | 0 e | 105 d | 5.5 bcd |
| Weed Free Check | | 100 a | 0 a | 7.9 a |
| Atrazine | 1500 | 2 de | 56 c | 4.7 cd |
| Dicamba | 600 | 42 c | 34 bc | 6.4 b |
| Dicamba/atrazine | 1800 | 71 b | 22 ab | 5.7 bc |
| Isoxaflutole | 105 | 7 de | 50 bc | 4.1 d |
| Isoxaflutole+atrazine | 105+1063 | 14 d | 44 bc | 5.5 bcd |
| Mesotrione | 175 | 15 d | 44 bc | 5.1 bcd |
| Mesotrione+atrazine | 175+1000 | 12 de | 42 bc | 5.5 bcd |
| *Post-emergence* | | | | |
| Weedy Check | | 0 f | 100 e | 6.0 c |
| Weed Free Check | | 100 a | 0 a | 9.0 ab |
| Atrazine | 1500 | 88 cd | 2.8 bc | 8.5 ab |
| Dicamba | 600 | 92 bc | 1.7 ab | 8.4 ab |
| Dicamba/diflufenzopyr | 200 | 89 cd | 1.3 ab | 8.1 b |
| Dicamba/atrazine | 1800 | 96 ab | 0.5 a | 9.2 a |
| 2,4-D/atrazine | 1404 | 85 de | 4.1 c | 8.5 ab |
| Bromoxynil+atrazine | 280+1500 | 85 de | 1.9 bc | 8.6 ab |
| Prosulfuron+dicamba | 10+140 | 92 bc | 2.1 bc | 8.5 ab |
| Primisulfuron/dicamba | 166 | 86 de | 2.9 bc | 8.5 ab |
| Mesotrione | 100 | 82 e | 10.5 d | 8.6 ab |
| Mesotrione+atrazine | 100+280 | 88 cd | 3.0 bc | 8.7 ab |

## RESULTS AND DISCUSSION

**Soybean:** None of the pre-emergence herbicides evaluated were equivalent to the weed free check, nor did they control or reduce the dry weight of volunteer adzuki bean compared to the weedy check (Table 1).

None of the post-emergence herbicides resulted in adzuki bean control and dry weight equivalent to the weed free check. Glyphosate provided 83% control and reduced the dry weight of adzuki bean 84% relative to the weedy check, and was the only herbicide that resulted in soybean yield the same as the weed free check. Chlorimuron provided only moderate control of volunteer adzuki bean.

**Corn:** The most effective pre-emergence herbicides were dicamba and dicamba/atrazine which provided 42 and 71% control of volunteer adzuki bean, respectively. None of the pre-emergence herbicide treatments increased corn yield compared to the weedy check.

The post-emergence corn herbicides controlled volunteer adzuki beans 82-96%, reduced dry weight 90-100%, and increased corn yield 26-35% over that of the weedy check. Dicamba/atrazine was the most effective while mesotrione was the least effective in controlling volunteer adzuki beans among the herbicides evaluated.

## CONCLUSIONS

**Soybean:** None of the pre-emergence herbicides evaluated have potential for controlling volunteer adzuki bean. In conventional soybean, a postemergence chlorimuron application provides the best control. Glyphosate provides good control of volunteer adzuki bean in glyphosate-tolerant soybean.

**Corn:** Among pre-emergence herbicides evaluated only dicamba/atrazine has potential to control volunteer adzuki bean. However, the post-emergence applications of atrazine, dicamba, dicamba/diflufenzopyr, dicamba/atrazine, 2,4-D/atrazine, bromoxynil + atrazine, prosulfuron + dicamba, primisulfuron/dicamba, mesotrione, and mesotrione + atrazine all have potential for volunteer adzuki bean management in corn.

## ACKNOWLEDGEMENTS

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# FLAMING IN SPANISH ONION

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## INTRODUCTION

Propane flaming is rarely used in Canada but is very popular in Europe in organic horticultural production. Flaming can be done pre- and post emergence and has been proven effective on a wide variety of crops. Propane flaming kill weeds by increasing cell temperature for a fraction of second which is sufficient to disrupt the cell wall and denature the proteins, thereby killing the cells and eventually the plant.

## OBJECTIVE

An experiment was initiated in 2005 to determine Spanish onion and weeds susceptibility to propane flaming under Canadian conditions.

## MATERIALS & METHODS

Field experiments were conducted at the IRDA research station, St-Hyacinthe, QC. The soil type was a Duravin sandy loam. Soil organic matter was 2,2% with a pH of 6,7. Fertilization followed provincial recommendations. Onions were transplanted on May 25 and spaced 15 cm within and 90 cm between rows. The experiment design was a randomized complete block design with four replications. Plots were 2.5 m long for both crop and weed experiments. The experiment involved 77 treatments: three onion stages, five tractor speeds and three rates of propane and a manual weeding and a weedy check (not shown).

**Flaming Treatments:**
5 onion development stages:

| | dat | date | L | H |
|---|---|---|---|---|
| S1: | 15 | 6/2 | 2 | 17 |
| S2: | 21 | 6/8 | 3-4 | 19 |
| S3: | 22 | 6/20 | 5-6 | 42 |
| S4: | 40 | 6/27 | 7 | 50 |
| S5: | 49 | 7/7 | 9-10 | 55 |

*dat: days after transplantation, date: month/day, L: number of leaves, H: height (cm)

5 speeds:
V2: 2 km/h
V3: 3 km/h
V4: 4 km/h
V5: 5 km/h
V6: 6 km/h

3 rates:
D1: 0,14 MPa (20 psi): 2,7 kg/h
D2: 0,24 MPa (35 psi): 4,3 kg/h
D3: 0,34 MPa (50 psi): 5,9 kg/h

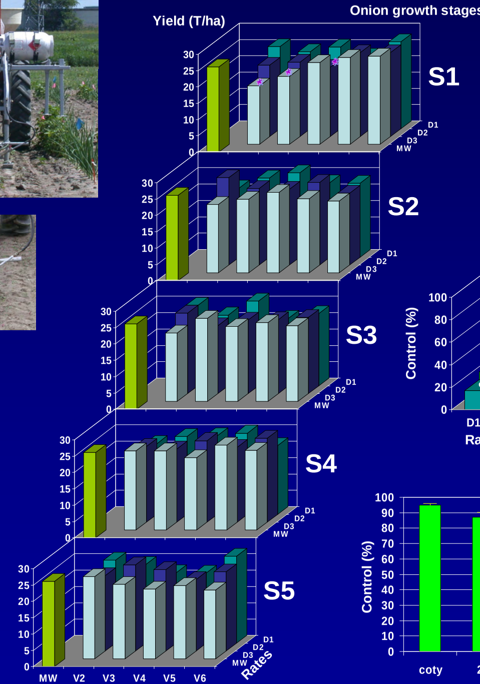
**Check without flaming:**
MW: Manual weeding

Burners were set at an angle of 30 degrees from horizontal and 17,8 cm (7 in) from the base of the crop.


Flamed onion and weeds.

## PRELIMINARY RESULTS

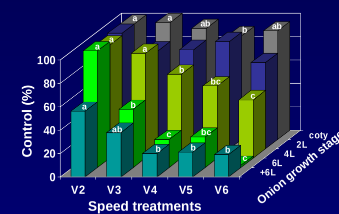
### Onion susceptibility

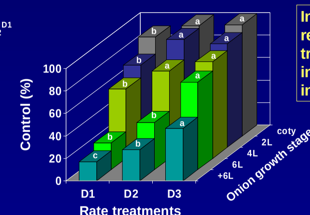



* Columns with asterisk are statistically different of the manual weeding check, P=0.05, Waller-Duncan.

In the first flaming, done 15 days after transplanting the onion, the two slowest speeds at the highest propane rate had a significantly different yield than the control. The same is true for the treatment applied at 4 km/h that received the medium dose of propane. The yield was not significantly affected by any of the other treatments, and at any of the other dates.

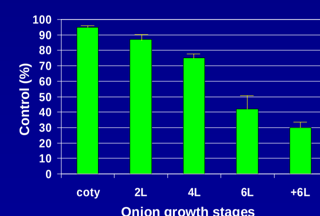
### Lamb's-quarters susceptibility




In general, lamb's-quarters repression decreased as the tractor speed increased and increased as the propane dose increased.

*Columns of the same color with the same letter are not statistically different, P=0,05, Waller-Duncan.




*Error bars represent the standard error.

The percentage of lamb's-quarters control decreased as its growth stages were more advanced.

## CONCLUSION

Spanish onions appear to be very tolerant to flaming under Quebec conditions.

*This research was supported by a grant from CORPAQ. We express our thanks to those who assisted in the performance of the research.*

# Sugar Beet Injury from Simulated Herbicide Drift

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## Introduction

- Sugar beet injury can occur from drift when postemergence herbicides are applied to crops in adjacent fields.
- Visual injury patterns are often irregular and yield loss can be difficult to quantify.
- Quantitative information on extractable sugar per acre loss would assist growers and agriculturists in assessing drift damage from common herbicides.

## Objectives

- Assess visual sugar beet injury and losses in yield and quality from simulated postemergence herbicide drift.
- Compile photos documenting the appearance and severity of drift symptoms to augment yield and quality data.

## Methods

- Drift was simulated using 15% of the label rate for 7 commonly used herbicides in southern Alberta.
- Treatments were broadcast on 6-leaf sugar beets using 8001VS nozzles at 94 liters/ha spray volume and 276 kpa pressure.

*Treatment List*

| Herbicide common name | Herbicide trade name | Herbicide Group | Rate Applied (g ai/ha) |
|---|---|---|---|
| UNTREATED | | - | - |
| bentazon[a] | Basagran | 6 | 162 |
| bromoxynil + MCPA | Buctril M | 6 & 4 | 83 |
| 2,4-D + mecoprop + dicamba | Dyvel DS | 4 | 80 |
| 2,4-D + dichlorprop | Estaprop | 4 | 153 |
| rimsulfuron[b] | Prism | 2 | 2.2 |
| metribuzin | Sencor | 5 | 42 |
| MCPA + mecoprop + dicamba | Target | 4 | 89 |

[a] Assist oil concentrate surfactant was applied with bentazon.
[b] Agral 90 non-ionic surfactant was applied with rimsulfuron.

*Environmental conditions during treatment application*

June 16, 2003 (13:00 hrs) : 26°C, 26% RH, calm to 8 kph wind, clear skies.

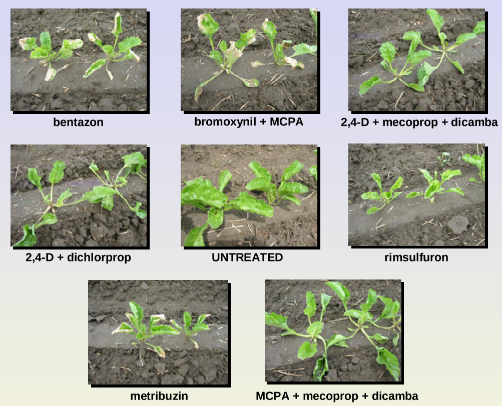
June 24, 2004 (17:30 hrs) : 23°C, 34% RH, calm to 3 kph wind, clear skies.

- Soil-applied and postemergence sugar beet herbicides were applied over all treatments in both years to represent commercial agronomic practices prior to applying simulated herbicide drift treatments.
- Data was collected over 2 study years with a considerable range in environmental conditions and production potential.

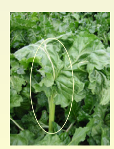
## Visual Injury Symptoms

- Sugar beet leaf injury symptoms were most pronounced 5 to 7 days after simulated drift application.
- The severity of visual sugar beet leaf injury was similar in both years of study.

**Leaf injury symptoms 7 days after treatment application – 2003**




- Bentazon reduced sugar beet plant counts by an average of 13%. Metribuzin reduced plant counts by 14% in 2004, with no reduction in 2003.



Trumpeting

- "Celery stalking" or "trumpeting" symptoms of sugar beet leaves were rated in September on a 0-3 scale where 0 = none, 1 = slight, 2 = moderate and 3 = severe.
- All group 4 herbicides exhibited trumpeting. A slight amount of trumpeting was observed for bromoxynil + MCPA, while moderate trumpeting was observed for 2,4-D + mecoprop + dicamba, MCPA + mecoprop + dicamba and 2,4-D + dichlorprop.

- Root crown deformity was rated at harvest time after defoliation, prior to digging sugar beets. Deformity was rated as none, slightly deformed or deformed.
- All group 4 herbicides exhibited crown deformity. Bromoxynil + MCPA resulted in very little deformity, 2,4-D + mecoprop + dicamba and MCPA + mecoprop + dicamba caused slight deformity while 2,4-D + dichlorprop was rated deformed.

No deformity Deformed

## Extractable Sugar and Root Yield Results

- All simulated drift herbicide treatments significantly reduced extractable sugar per acre (ESA) relative to untreated beets in both years of study.
- 2,4-D + dichlorprop significantly reduced ESA relative to other herbicide treatments in both years.
- Percent ESA reduction from herbicide treatments compared to untreated beets averaged 9% greater in 2004 than in 2003. The greatest range between years occurred for 2,4-D + dichlorprop, rimsulfuron and metribuzin with differences of 16 to 17%. Differences for the other 4 herbicides ranged from 3 to 5% between the 2 years of study.

*Summary of Extractable Sugar Results*

| Herbicide | Extractable Sugar (kg/acre) | | % reduction in ESA relative to untreated | | |
|---|---|---|---|---|---|
| | *2003* | *2004* | *2003* | *2004* | *2-year average* |
| UNTREATED | 5320 | 3484 | - | - | - |
| 2,4-D + dichlorprop | 3908 | 1943 | 27 | 44 | 35 |
| rimsulfuron | 4626 | 2491 | 13 | 29 | 21 |
| bromoxynil + MCPA | 4616 | 2840 | 13 | 18 | 16 |
| 2,4-D + mecoprop + dicamba | 4555 | 2888 | 14 | 17 | 16 |
| metribuzin | 4991 | 2685 | 6 | 23 | 15 |
| bentazon | 4663 | 2943 | 12 | 16 | 14 |
| MCPA + mecoprop + dicamba | 4819 | 3009 | 9 | 14 | 12 |
| LSD (.05) | 242 | 244 | | | |
| LSD (.01) | 325 | 327 | | | |

- Climate conditions and root yield potential varied between the 2 years of study.
  - In 2003, untreated plots = 33.0 tonnes per acre (1 tonne = 1.1 tons)
  - In 2004, untreated plots = 23.2 tonnes per acre
- 2-year average root yield reductions ranged from 3.1 to 4.9 tonnes per acre for all herbicide treatments except 2,4-D + dichlorprop which reduced root yield by an average of 8.8 tonnes per acre. (data not shown)
- Percent sucrose was significantly reduced for selected treatments in one of the two study years and there was no treatment effect on molasses loss in either year. (data not shown)

## Summary

- Simulated herbicide drift treatments resulted in reductions of 12 to 35% in extractable sugar per acre relative to untreated beets.
- Photo documentation of the appearance and severity of drift symptoms was compiled onto a CD-ROM as a tool to relate visual assessments to quantitative loss.

This research was funded by: Rogers Sugar Alberta Sugar Beet Growers

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

# System for data collection in support of minor use in seed corn

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## Introduction

- Seed corn is a highly specialized and localized agricultural commodity in Canada.
- Few weed management options currently exist because inbred sensitivity to available herbicides cannot be accurately predicted.
- Our objectives were to develop a system for data collection to:

1. Determine the tolerance of seed corn inbred lines from different companies by rating crop injury, population, and yield after herbicide application.
2. Identify successful reduced risk herbicide treatments and use data collected to facilitate minor use registration by the Pest Management Regulatory Agency (PMRA).

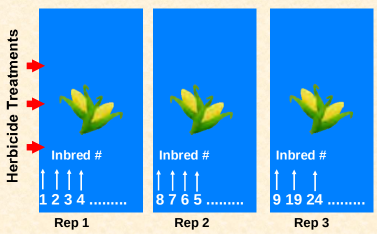


**Figure 1. Field plot layout**

## Materials and methods

- Field trials were established from 2001 to 2004 in Chatham, Harrow, and Paincourt Ontario using seed corn inbreds from four companies (Hyland, Pioneer, Pride and Syngenta) (Figure 1).
- Herbicides were applied at both a 1x and a 2x application rate across all inbreds in the plot (Figure 2).
- Inbred selection changed throughout the duration of the study for each company (Table 1). Whenever possible a minimum of six inbreds for each company were kept the same each year.
- All treatment combinations were tested in relation to a 1x standard application of Primeextra (s-metolachlor/atrazine/benoxacor)
- The effect of a herbicide on each inbred was evaluated by measuring:
  1. Crop injury
  2. Stand Count
  3. Crop yield at physiological maturity (Figure 3)

**Table 1. The number of inbreds tested for each company between 2001 and 2004.**

| Company | 2001 | 2002 | 2003 | 2004 |
|---|---|---|---|---|
| Hyland | 16 | 12 | 12 | 12 |
| Pioneer | 8 | 10 | 10 | 10 |
| Pride | 24 | 30 | 30 | 24 |
| Syngenta | 28 | 24 | 24 | 24 |

## Results and discussion

**Case Study: Results for PeakPlus (Prosulfuron/Dicamba) were submitted to the PMRA for registration in 2004**

**Crop Injury**

- Crop tolerance of most inbreds to Peakplus was excellent for inbreds of Hyland, Pioneer, and Syngenta (Table 2).
- Tolerance of Pride inbreds was variable by inbred and year.
- Crop tolerance was not influenced by application rate

**Table 2. Number of inbreds showing visual crop injury after application of Peakplus.**

| Company | 2001 | | 2002 | | 2003 | | 2004 | |
|---|---|---|---|---|---|---|---|---|
| | 1x | 2x | 1x | 2x | 1x | 2x | 1x | 2x |
| Hyland | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pioneer | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 2 |
| Pride | 14 | 12 | 0 | 0 | 0 | 0 | 24 | 23 |
| Syngenta | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |

**Stand Count**

- Peakplus application had little impact on stand count for all companies (Table 3).
- With the exception of 2001, stand count did not differ between the 1x and 2x application rates of Peakplus.

**Figure 2. Herbicides are applied across each plot of inbreds**

**Table 3. Number of inbreds that had >20% stand count reduction in comparison to the standard treatment.**

| Company | 2001 | | 2002 | | 2003 | | 2004 | |
|---|---|---|---|---|---|---|---|---|
| | 1x | 2x | 1x | 2x | 1x | 2x | 1x | 2x |
| Hyland | 1 | 4 | 4 | 1 | 1 | 0 | 0 | 0 |
| Pioneer | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pride | 0 | 0 | 3 | 1 | 1 | 3 | 0 | 1 |
| Syngenta | 6 | 9 | 1 | 1 | 0 | 0 | 1 | 1 |

**Figure 3. Each seed corn inbred is mechanically harvested at physiological maturity**

**Yield**

- Seed corn yield was variable by company.
- Yield was consistent at both application rates.
- High levels of crop injury for Pioneer inbreds in 2001 and 2004 did not translate into significant yield losses.

**Table 4. Number of inbreds that had >20% yield reduction in comparison to the standard treatment.**

| Company | 2001 | | 2002 | | 2003 | | 2004 | |
|---|---|---|---|---|---|---|---|---|
| | 1x | 2x | 1x | 2x | 1x | 2x | 1x | 2x |
| Hyland | -* | - | 2 | 1 | 5 | 2 | 5 | 6 |
| Pioneer | 0 | 0 | 1 | 1 | 0 | 0 | - | - |
| Pride | 7 | 1 | 4 | 2 | 0 | 4 | 0 | 4 |
| Syngenta | 16 | 21 | 4 | 7 | 0 | 2 | 12 | 12 |

* Yield data was unable to be collected due to poor pollination and kernal set.

## Conclusions

- Our data collection method allows us to collect tolerance and yield data for a wide range of inbreds that is acceptable for submission to the PMRA.
- To date 2 herbicides have been submitted for registration and 3 more are being prepared
- Our system still allows each seed corn company the ability to confidentially evaluate their seed corn inbred lines
- After successful registration of a herbicide for minor use on seed corn, new inbreds will need to be evaluated at the 2x rate of the herbicide to ensure adequate tolerance prior to introduction.

## Acknowledgements

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Canada

# Invasive Weed Biological Control in Nova Scotia

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## Introduction

**Invasive weeds,** like Canada thistle (*Cirsium arvense L. Scop.*), Bull thistle (*Cirsium vulgare L.*), Tansy ragwort (*Senecio jacobaeae L.*) and Purple loosestrife (*Lythrum salicare L.*) are significant problems in pastures and non-cropland areas of Nova Scotia. Loss of productivity and value of land occurs where these species are found in great number. With the relative low value of pasture and non-cropland, herbicides are often cost prohibitive and most often not used. Biological control is attempting to re-establish balance between large infestations of these invasive weeds so that better management techniques can be implemented to sustain productive pastures.

*Tyria jacobaeae*

Purple loosestrife in forage

*Tyria jacobaeae* larvae on Tansy ragwort

## Objectives

- Evaluate biological control agents from previous releases for:
  - -establishment
  - -agent density
  - -parasitism
- Introduce additional insect species approved by Agriculture and Agri-Food Canada
- Augment numbers of established biological control agent populations
- Facilitate their distribution throughout the province

Bull thistle

*Urophora cardui* gall on Canada thistle

*Hadroplontus litura* on Canada thistle

*Puccinia punctiformis* on Canada thistle

## Results and Discussion

Evaluation of previous biological control programs in the 1980's and 1990's has revealed the potential for specific agents in the field. A Canada thistle/Bull thistle release site in Eastville, Nova Scotia showed excellent control with a stem reduction of 98% over 15 years caused in part by *Hadroplontus litura,* a stem boring weevil and *Rhynocyllus conicus* a seed head weevil. The seed head fly, *Urophora stylata* has easily established and contributed substantially to the reduction of bull thistle throughout the province and is currently being re-distributed to bull thistle problem areas. However, overall effectiveness of these insects in the province is limited due to small patchy populations that would benefit from augmentation and distribution.

A spring breeding strain of the Tansy ragwort feeding *Longitarsus jacobaeae* has been found in Northern Nova Scotia. This population is distinct as other populations throughout the province mate in late summer to late fall. The shift to a spring breeding population is advantageous as early frosts in the autumn can reduce survival of the fall breeding strain. It is believed that the spring breeding strain will be more effective in the Atlantic Provinces and at higher altitudes in Western Canada. Again, the limiting factor for this insect is it's relatively small population size which requires field rearing before significant distribution in numbers can occur. Studies are ongoing to determine differences in tansy ragwort winter mortality between the two populations of insect.

*Galerucella calmariensis* has become the dominant species in Nova Scotia for the control of purple loosestrife. It has been introduced to most of the major loosestrife sites in the province and within the second year has prevented flowering by up to 90% and delayed flowering by 30 days in the remaining 10%. *Galerucella pusilla* has not been recovered from any previous release sites to date.

*Urophora stylata*

*Longitarsus jacobaeae*

*Galerucella calmariensis*

**Table 1.** Predominate Biocontrol agents in Nova Scotia

| Weed | Insect | Impact | Status |
|---|---|---|---|
| Canada thistle | *Hadroplontus litura* | Stem boring-100% control of affected stems | Established -2 sites |
| | *Rhynocyllus conicus* | Flower weevil-seed reduction | Established-2 sites |
| Bull Thistle | *Urophora stylata* | Reduction in seed production-15x gall production per year | Established-10 sites |
| Purple Loosestrife | *Galerucella calmariensis* | Significant reduction in flowering stalks | Multiple sites |
| Tansy ragwort | *Longitarsus jacobaeae* | Targets 1st year rosette<br>-increases winter mortality | Established-5 sites |

## Conclusion

- •Biological control is attainable when insects are at sufficient levels.
- •An available supply of established insects is present in province
- •Populations of these insects are low
- •To be successful in Nova Scotia, biological control agents require augmentation and redistribution

## Acknowledgements:

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Nova Scotia Agricultural College · Agri-Futures · Nova Scotia's Adaptation Council · Agriculture and Agri-Food Canada · BCRC · Nova Scotia Agriculture and Fisheries · Nova Scotia Cattlemen's Association

# Efficacy and Crop Tolerance of Mesotrione in Cranberry and Wild Blueberry

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## Introduction

Blueberries and cranberries are economically important crops to the Atlantic Provinces. In 2004 an estimated 15,500 ha of blueberry fields produced 19,000 metric tonnes of blueberries in Nova Scotia alone. In 2000 the acreage of cranberries planted in Nova Scotia was 163.5 and was expected to grow by as much as 20 acres per year over the next few years. They are both *Vaccinium* spp. and are perennial crops which makes them prone to weed competition.

The type of weed species causing problems in blueberries has changed with changing management practices. As a result we are now seeing an influx of annuals and species that are mainly spread by seed as opposed to the previously common fire tolerant perennial weeds (Jensen and Yarborough, 2004). The types of weeds affecting cranberries are primarily perennial, with *Solidago* spp. being one of the most ubiquitous weeds present. The potential loss of yield due to weed competition is quite high for both crops, with cranberry having one of the highest of any agricultural commodity (Patten and Wang, 1994).

The perennial nature of these crops presents unique obstacles in regards to weed control and other management practices. However vegetation management strategies in blueberries and cranberries can be expanded through investigating new products and new product use patterns. In previous years, mesotrione has shown excellent crop tolerance and efficacy in both crops. It has also drawn significant attention from interested producer groups. In addition, targeting reduced risk products greatly increases the chances of products becoming tools available for growers, in a timely manner.

## Objectives

Mesotrione was isolated from previous herbicide screening trials as having a high efficacy against the target weeds such as *Chenopodium album* and *Solidago* spp.. It was also found to have no phytotoxicity or negative effect on yield. Therefore the objective is now focused on determining the most effective use pattern for this product in these crops. Mesotrione dose response trials were initiated in Nova Scotia, New Brunswick and Prince Edward Island to determine the lowest effective rate of application in cranberry and wild blueberry production.

## Experimental Design

Dose response. Herbicide dose response trials were initiated in New Brunswick, Prince Edward Island and Nova Scotia in the spring of 2005. The trials were designed as individual randomized complete block experiments with four replicate blocks. Five rates of mesotrione were selected based on : 0x, 0.5x, 1x, 1.5x and 2.0x where x= the currently labeled field rate. Plot sizes were chosen to coordinate with PMRA requirements. The total number of dose response trials initiated in all provinces is 8.

Data Collection. Crop tolerance, and weed control were visually rated on a percent scale (0-100), at three different times throughout the season. Other indicators such as yield and 100 berry weights were also collected at harvested sites. Efficacy was determined on several target weeds. The effects on *Chenopodium album* and *Solidago spp.* in particular are presented here.

## Results and Discussion

Weed control. Mesotrione appears to be effective against several selected weed species, with no crop damage. *Chenopodium album* was targeted in blueberry fields, and up to 100% control was achieved at all locations (Figure 1). Up to 100% control of *Solidago* spp. was achieved in some locations at the highest application rate.

Crop tolerance. Mesotrione did not significantly effect either blueberry or cranberry at any tested rate. Phytotoxicity symptoms consisted of a slight yellowing of some blueberry leaves, which were scarcely visible. Most locations and timings showed no effect on blueberry or cranberry. Measured yields and 100 berry weights can indicate crop damages not physically observed in the field. In this case there were no differences between 100 berry weights at either location or for either crop. Similarly, there were no differences between yields, indicating no loss of yield attributed to treatment effects (Figure 2).

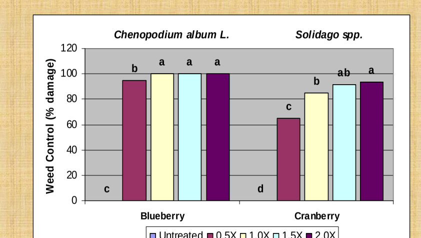


Figure 1. Typical example of % weed control by mesotrione in blueberry and cranberry at harvest

## Results and Discussion

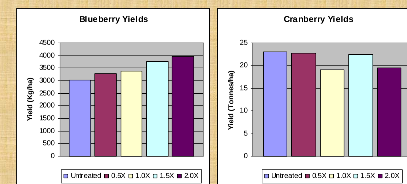


Figure 2. Yields by rate of mesotrione. No differences between treatments were found.

Figure 3. Effect of mesotrione on *Chenopodium album* and *Solidago spp.*

## Conclusions

- Mesotrione has shown good weed control in combination with no crop damage or decreases in cranberry or blueberry yield or size quality.
- Mesotrione showed excellent control of *Solidago spp.* at most cranberry locations, reaching up to 100% at the highest application rate.
- Mestorione also achieved 100% control of *Chenopodium album* at all locations and at all rates.
- For future research; screening trials including tank mixes of mesotrione were initiated in 2005, and will be continued and adapted for next season.
- This research is directly applicable to the minor use priority for this product, and registration is likely.

## Acknowledgements

The cooperation of the Nova Scotia, and Prince Edward Island, New Brunswick, and British Columbia Wild Blueberry and Cranberry Producer Associations, all cooperating producers, and the following funding agencies is gratefully appreciated.

NOVA SCOTIA
Agriculture and Agri-Food Canada
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Ocean Spray

# VOLUNTEER GLYPHOSATE-TOLERANT CORN CONTROL IN GLYPHOSATE-TOLERANT SOYBEAN

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## INTRODUCTION

In Ontario, the adoption of glyphosate-tolerant (Roundup Ready®) technology has been rapid since its introduction in soybean (1997) and corn (2001) (Swanton, 2004). Glyphosate-tolerant volunteer corn has become a major problem whenever glyphosate-tolerant soybean follows glyphosate-tolerant corn in the rotation because glyphosate (Roundup®) does not control this type of volunteer corn.

Clethodim, fenoxaprop-p-ethyl, fluazifop-p-butyl, quizalofop-p-ethyl and sethoxydim are postemergence (POST) herbicides that are very effective for the control of a wide spectrum of annual and perennial grass species including volunteer corn (OMAF, 2004; Vencill, 2002).

**The objective of this study was to evaluate the POST application of clethodim, fenoxaprop-p-ethyl, fluazifop-p-butyl, quizalofop-p-ethyl and sethoxydim when tank mixed with glyphosate for control of glyphosate-tolerant volunteer corn in glyphosate-tolerant soybean in Ontario.**

## MATERIALS AND METHODS

**Study establishment:** A total of four field experiments were conducted in 2003 and 2004 at the Huron Research Station, Exeter. The experimental design was a randomized complete block with four replications. Treatments consisted of glyphosate (Roundup Transorb®, 360 g ae/l solution, Monsanto Canada Inc., 67 Scurfield Blvd., Winnipeg, Manitoba) at 900 g ae/ha applied POST, alone and tank mixed with the herbicides listed in Table 1. The three rates chosen represent 50, 75, and 100% of manufacturer's recommended rate for each herbicide. The glyphosate-tolerant volunteer corn seed ('DKC 42-71 RR') was spread at approximately 800,000 seeds/ha and incorporated prior to seeding the soybean. Four rows of soybean ('FL 2802 RR') were planted in 3m by 10m plots on June 24, 2003 and May 31, 2004 at a rate of 395,000 seeds/ha.

Herbicides were applied postemergence 21 and 28 days after planting (DAP) in 2003, and 23 and 30 DAP in 2004 with a $CO_2$-pressurized backpack sprayer. The boom was 1.5 m long with four flat-fan nozzles spaced 0.5 m apart (Teejet 8002 flat-fan nozzle tip; Spraying Systems Co., Wheaton, IL).

**Data collection:** Crop injury to soybean was rated visually 7 and 28 days after treatment (DAT), and volunteer corn control was rated visually 28 and 56 DAT on a scale of 0 to 100% (0=no visible injury, and 100=plant death). At 70 DAT, volunteer corn density and dry weight in each plot was determined by counting and harvesting corn plants in a 1 $m^2$ quadrat from each plot. Yields were measured at crop maturity by harvesting the middle two rows of each plot with a plot combine. Soybean was harvested on October 16, 2003 and October 5, 2004 and yields were adjusted to 13% moisture.

**Statistical analysis:** All data were subjected to analysis of variance. Tests were combined over locations and years and analyzed using the MIXED procedure of SAS (Ver 8e, SAS Institute Inc., Cary, NC). There was no injury to soybean, thus these data were excluded from the analysis. To meet assumptions of the variance analysis, volunteer corn density and dry weight were square-root transformed. Means for volunteer corn density and dry weight were compared on the transformed scale and converted back to original scale for presentation of results.


**Figure 1. Glyphosate-tolerant volunteer corn control in glyphosate-tolerant soybean with various post-emergence herbicides.**

**Table 1. Volunteer corn control, density, dry weight and yield of soybean treated with glyphosate plus various postemergence grass herbicides. Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD (P<0.05).**

| Treatment | Rate | Volunteer corn control 28 DAT | Volunteer corn control 56 DAT | Density | Dry wt. | Soybean Yield |
|---|---|---|---|---|---|---|
| | g ai/ha | % | % | # $m^2$ | g/$m^2$ | t/ha |
| Check | | 0 i | 0 j | 22.5 a | 692 a | 1.09 e |
| Clethodim | 15 | 73 g | 73 h | 6.0 c | 73 c | 2.05 bc |
| Clethodim | 22.5 | 82 e | 83 de | 2.8 efg | 11 ef | 2.12 abc |
| Clethodim | 30 | 87 cd | 88 bc | 1.8 gh | 5 fgh | 2.15 ab |
| Fenoxaprop-p-ethyl | 27 | 76 fg | 76 gh | 5.1 cd | 47 cd | 1.99 c |
| Fenoxaprop-p-ethyl | 40.5 | 84 de | 85 cde | 3.5 def | 13 ef | 2.13 abc |
| Fenoxaprop-p-ethyl | 54 | 87 cd | 87 bcd | 2.2 fgh | 10 fg | 2.16 ab |
| Fluazifop-p-butyl | 37.5 | 74 g | 72 h | 5.7 c | 48 cd | 2.11 abc |
| Fluazifop-p-butyl | 56.25 | 86 de | 82 ef | 2.8 fg | 13 ef | 2.14 abc |
| Fluazifop-p-butyl | 75 | 88 bcd | 88 bc | 1.4 hi | 3 h | 2.17 ab |
| Quizalofop-p-ethyl | 18 | 89 abc | 90 ab | 1.3 hi | 4 gh | 2.10 abc |
| Quizalofop-p-ethyl | 27 | 91 ab | 91 ab | 1.3 hi | 3 h | 2.17 ab |
| Quizalofop-p-ethyl | 36 | 92 a | 93 a | 0.7 i | 2 h | 2.23 a |
| Sethoxydim | 75 | 61 h | 54 i | 13.3 b | 216 b | 1.71 d |
| Sethoxydim | 112.5 | 73 g | 72 h | 5.5 cd | 77 c | 2.03 bc |
| Sethoxydim | 150 | 78 f | 78 fg | 4.6 cde | 26 de | 2.10 abc |

## RESULTS AND DISCUSSION

The control of glyphosate-tolerant volunteer corn increased from 73 to 88% with increasing rates of clethodim (Table 1). Density and dry weight were reduced as the rate of clethodim increased from 15 to 22.5 g/ha, but there was no further decrease with the 30 g/ha rate. There were no differences in soybean yields among the clethodim rates, although yield was increased an average of 48% over that of the check.

There was an increase volunteer corn control and a decrease in dry weight between 27 g/ha and the two highest rates of fenoxaprop-p-ethyl. Volunteer corn density was lower with the application of 54 g/ha than with 27 g/ha of fenoxaprop-p-ethyl. Soybean yield did not differ between the two highest rates of fenoxaprop-p-ethyl, but there was a yield response between the lowest and highest rate.

The application of increasing fluazifop-p-butyl rates improved volunteer corn control. Volunteer corn density and dry weight were decreased up to 94 and 100%, respectively, with increased rates of fluazifop-p-butyl, relative to the check. Soybean yields did not differ among fluazifop-p-butyl rates.

There were no differences in volunteer corn control, density, dry weight and soybean yield among the three quizalofop-p-ethyl rates. Even the lowest rate of quizalofop-p-ethyl was as effective as the highest rate of clethodim, fenoxaprop-p-ethyl and fluazifop-p-butyl, for all parameters except yield.

Volunteer corn control improved and dry weight decreased with increasing rates of sethoxydim. There was a decrease in volunteer corn density and a rise in soybean yield between 75 g/ha of sethoxydim and the two higher rates. Overall, sethoxydim did not provide volunteer corn control equivalent to the other herbicide treatments.

## CONCLUSIONS

**Clethodim, fenoxaprop-p-ethyl, fluazifop-p-ethyl and quizalofop-p-ethyl can be tank mixed at the manufacturer's recommended rate with glyphosate to successfully control glyphosate-tolerant volunteer corn in glyphosate-tolerant soybeans. The most effective volunteer corn control was obtained with quizalofop-p-ethyl, followed by fluazifop-p-butyl and fenoxaprop-p-ethyl, followed by clethodim and then sethoxydim.**

## ACKNOWLEDGEMENTS

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Vencill, W. K. 2002. Herbicide Handbook, Eighth Edition. Weed Sci. Soc. Am., Champaign, IL. 493 pp.

# SENSITIVITY OF WINTER WHEAT TO FALL APPLIED POSTEMERGENCE HERBICIDES

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## INTRODUCTION

Winter wheat (*Triticum aestivum*) is the most popular winter cereal crop grown in Ontario. The adoption of reduced-till and no-till production practices has resulted in a resurgence of winter annual and perennial weeds such as common chickweed, shepherd's purse, stinkweed and dandelion in some parts of Ontario. With the expanded use of glyphosate-tolerant soybean in crop rotations, there have been increases in winter annual and biennial weeds that growers have to control in the fall. There are a limited number of fall applied postemergence (POST) herbicide options available for control of winter annual, biennial and perennial weeds in winter wheat at this time.

Spring applied POST herbicides that can effectively control winter annual or perennial weeds in wheat include dicamba (Banvel II®), 2,4-D amine, MCPA amine, dichlorprop/2,4-D ester (Estaprop®), bromoxynil/MCPA ester (Buctril M®), and thifensulfuron-methyl/tribenuron-methyl (Refine Extra®) (OMAF, 2004; Vencill, 2002).

**The objective of this study was to evaluate the tolerance of winter wheat to the fall application of dicamba, 2,4-D amine, MCPA amine, dichlorprop/2,4-D ester, bromoxynil/MCPA ester, and thifensulfuron-methyl/tribenuron-methyl under Ontario growing conditions.**

## MATERIALS AND METHODS

**Study establishment:** Field experiments were established at the Huron Research Station in the fall of 1998 (two sites), 2002 and 2003, and at Ridgetown College in the fall of 2001. The experimental design was a randomized complete block design with four replications. Treatments are listed in Table 1. The rates selected were one and two times the manufacturer's labelled rate for a spring application of each herbicide. Plots were 2 m by 10 m at Exeter and 2 m by 8 m at Ridgetown. Pioneer '25R47' winter wheat was seeded in 18 cm wide rows at 150 kg/ha on October 15, 1998 (both sites), September 26, 2002 and October 14, 2003 in Exeter and October 20, 2001 in Ridgetown.

Herbicide applications were made 20 to 30 days after planting, with a $CO_2$-pressurized backpack sprayer. The boom was 1.5 m long with four flat-fan nozzles spaced 0.5 m apart (Teejet 8002 flat-fan nozzle tip; Spraying Systems Co., Wheaton, IL).

**Data collection:** Visual crop injury was rated on a scale of 0 to 100% (0=no visible injury, and 100=plant death) at 24, 26, 28, and 31 weeks after treatment (WAT). Ten plants were randomly selected per plot and the height from the soil surface to the highest growing point of each plant was measured 32 WAT. Wheat was harvested in late July to early August and yields were adjusted to 14.5% moisture.

**Statistical analysis:** All data were subjected to analysis of variance. Tests were combined over locations and years and analyzed using the MIXED procedure of SAS (Ver 8e, SAS Institute Inc., Cary, NC). To meet assumptions of the variance analysis, percent injury was square-root transformed. Means of percent injury were compared on the transformed scale and were converted back to the original scale for presentation of results.


**Figure 1. Visual injury symptoms of winter wheat in response to the fall application of 2,4-D amine and dichlorprop/2,4-D ester.**

**Table 1. Visual injury 26 and 31 WAT, plant height and yield of winter wheat treated with fall applied post-emergence herbicides. Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD test (P<0.05).**

| Treatment | Rate | Visual Injury (WAT) 26 | Visual Injury (WAT) 31 | Height | Yield |
|---|---|---|---|---|---|
| | g ai/ha | % | % | cm | t/ha |
| Untreated | 0 | 0 a | 0 a | 85 ab | 5.73 abc |
| Dicamba | 140 | 0 a | 0 a | 85 ab | 5.59 bcde |
| Dicamba | 280 | 0.4 ab | 0 a | 84 abc | 5.51 cde |
| 2,4-D amine | 550 | 0.7 b | 2.0 b | 83 abc | 5.29 de |
| 2,4-D amine | 1100 | 1.5 c | 2.9 b | 81 bcd | 5.24 ef |
| MCPA amine | 850 | 0.3 ab | 0 a | 88 a | 5.90 ab |
| MCPA amine | 1700 | 0.2 ab | 0 a | 83 abc | 5.63 abcd |
| Dichlorprop/2,4-D | 1017 | 2.4 cd | 2.7 b | 78 d | 5.26 ef |
| Dichlorprop/2,4-D | 2034 | 2.8 d | 3.5 b | 80 cd | 4.93 f |
| Bromoxynil/MCPA | 560 | 0.1 a | 0 a | 87 a | 5.99 a |
| Bromoxynil/MCPA | 1120 | 0 a | 0 a | 87 a | 5.90 ab |
| Thifensulfuron-methyl/ tribenuron-methyl | 15 | 0 a | 0 a | 87 a | 5.89 ab |
| Thifensulfuron-methyl/ tribenuron-methyl | 30 | 0.1 a | 0 a | 86 a | 5.87 ab |

## RESULTS AND DISCUSSION

Crop injury symptoms included a decrease in height and severely distorted heads (Figure 1). Crop injury was similar among all rating dates, thus only 26 and 31 WAT results are presented in Table 1.

The POST application of dicamba, MCPA amine, bromoxynil/MCPA ester and thifensulfuron-methyl/tribenuron-methyl did not cause any significant visual injury, plant height reduction or yield reduction in winter wheat. There were no differences between the two rates in their effect on winter wheat.

The POST application of 2,4-D amine resulted in as much as 1.5 and 2.9% visual injury at 26 and 31 WAT, respectively. Plant height was not significantly affected at either rate but yield was reduced 8 and 9% at the 550 and 1100 g/ha rate, respectively. Crop injury generally increased as the rate of 2,4-D amine increased although differences were not always significant.

The POST application of dichlorprop/2,4-D ester resulted in as much as 2.8 and 3.5% visual injury at 26 and 31 WAT, respectively. Winter wheat height was reduced 8 and 6% and yield was reduced 8 and 14% at the 1017 and 2034 g/ha rate, respectively. Crop injury generally increased as the rate was increased although differences were not always significant.

## CONCLUSIONS

**The application of 2,4-D amine and dichlorprop/2,4-D ester in the fall resulted in unacceptable injury in winter wheat. However, dicamba, MCPA amine, bromoxynil/MCPA ester and thifensulfuron-methyl/ tribenuron-methyl applied at the manufacturer's recommended rate in the fall had an adequate margin of crop safety for weed management in winter wheat under Ontario growing conditions.**

## ACKNOWLEDGEMENTS

We would like to acknowledge Todd Cowan for his expertise and technical assistance in these studies. Funding for this project was provided in part by the Ontario Wheat Producers.

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# Wirestem Muhly Control in Corn

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## INTRODUCTION

Wirestem muhly is a warm-season perennial grass native to North America. It spreads by seed and rhizomes, leading to the formation of large clumps (2). Wirestem muhly growth starts in late spring and flowering occurs in late July or August (3). Each plant can produce as many as 140,000 seeds (2).

Wirestem muhly infestations were not thought to be agriculturally significant until the 1950's. Infestations in new areas have increased since the 1980's (3). It has become a problem due to the fact that it emerges late and is not actively growing when preplant applications are made (2). Methods for control of wirestem muhly in corn are limited because glyphosate is the only herbicide that controls this grass (1). More knowledge of herbicide activity on wirestem muhly is needed to give corn growers alternate options for controlling this troublesome grass.

**The objective of this study was to determine the effectiveness of the postemergence application of rimsulfuron, nicosulfuron, nicosulfuron plus rimsulfuron, foramsulfuron, and primisulfuron for control of wirestem muhly in corn.**

## MATERIALS AND METHODS

**Study establishment:** Three field experiments were established on Ontario farms with heavy infestations of wirestem muhly; two experiments were located near Thamesville and the third near Mount Bridges. The experiments were arranged in a randomized block design with four replications. Treatments are listed in Table 1. All herbicide treatments included a non-ionic surfactant (Agral 90®) at 0.2% v/v except for foramsulfuron which included 28% UAN at 2.5 L/ha. Broadleaf weeds were controlled with an application of dicamba at 141 g ai/ha. Plots consisted of three rows of corn planted 0.76 m apart in rows that were 8 m long. The corn was planted in early- to mid-May and herbicides were applied during the first week of June. Herbicides were applied with a $CO_2$-pressurized backpack sprayer calibrated to deliver 200 L/ha of spray solution at 200 kPa pressure using Hypro ULD120-02 nozzle tips.

**Data collection:** Wirestem muhly control was rated visually 28 and 56 days after treatment (DAT) on a scale of 0 to 100% (0=no control, and 100=total control). At 56 DAT, wirestem muhly density and dry weight in each plot was determined by counting and harvesting wirestem muhly plants in a 1 $m^2$ quadrat. Corn yields were measured at crop maturity by hand harvesting cobs from the middle row of each plot and then threshing with a plot combine. Yields were adjusted to 15.5% moisture.

**Statistical analysis:** All data were subjected to analysis of variance. Tests were combined over locations and analyzed using the PROC MIXED procedure of SAS (Ver 8e, SAS Institute Inc., Cary, NC). To meet assumptions of the variance analysis, wirestem muhly visual control and density were transformed. These means were compared on the transformed scale and were converted back to original scale for presentation of results. Treatment means were separated using Fisher's protected LSD (P<0.05).


**Figure 1. Control of wirestem muhly with foramsulfuron, primisulfuron, rimsulfuron, and the weedy check.**

**Table 1. Mean wirestem muhly control 28 and 56 days after treatment (DAT), wirestem muhly density, wirestem muhly dry weight, and corn yield from three trials conducted in Ontario, Canada in 2005. Means followed by the same letter within a column are not significantly different according to Fisher's Protected LSD (P<0.05).**

| Treatment | Rate | Wirestem muhly control 28 DAT | 56 DAT | Density | Dry wt. | Corn Yield |
|---|---|---|---|---|---|---|
| | g ai/ha | % | % | # $m^2$ | g $m^2$ | t/ha |
| Weedy Check | -- | 0 e | 0 d | 27 d | 243 c | 8.33 b |
| Weed Free Check | -- | 100 a | 100 a | 0 a | 0 a | 9.81 a |
| Rimsulfuron | 15 | 1 d | 0 cd | 35 d | 220 bc | 8.96 ab |
| Nicosulfuron | 25 | 8 c | 7 b | 15bc | 73 a | 10.16 a |
| Nicosulfuron/ Rimsulfuron | 25 | 2 d | 2 c | 22 cd | 127 ab | 9.74 a |
| Foramsulfuron | 70 | 64 b | 88 a | 11b | 75 a | 9.69 a |
| Primisulfuron | 25 | 1d | 1 c | 33 d | 263 c | 8.03 b |

## RESULTS AND DISCUSSION

There was no crop injury from any of the postemergence herbicides evaluated.

Rimsulfuron provided little control of wirestem muhly and had no effect on density and dry weight. Corn yield was equivalent to the weedy check.

The application of nicosulfuron resulted in only 8% visual control of wirestem muhly, reduced density by 44% and dry weight by 70%, and increased corn yield 18% compared to the weedy check.

The application of nicosulfuron plus rimsulfuron resulted in only 2% visual control of wirestem muhly. There was no effect on wirestem muhly density but dry weight was decreased by 48%. Corn yield was increased 14% compared to the weedy check.

The application of foramsulfuron resulted in 64% and 88% visual control of wirestem muhly at 28 and 56 DAT, respectively. Wirestem muhly density and dry weight were decreased by 59 and 69%, respectively. Corn yield was increased by 14% compared to the weedy check.

Primisulfuron provided little control of, and had no effect on wirestem muhly density and dry weight. Corn yield was equivalent to the weedy check.

## CONCLUSIONS

**The postemergence application of foramsulfuron at 70 g/ha has potential for the control of wirestem muhly in corn.**

**The postemergence application of rimsulfuron, nicosulfuron, nicosulfuron plus rimsulfuron, and primisulfuron at the rates evaluated do not provide adequate control of wirestem muhly in corn.**

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