Examination of Hexazinone Alternatives for Wild Blueberry Production and Hexazinone Resistance in Red Sorrel (*Rumex acetosella* L.)

by

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DALHOUSIE UNIVERSITY

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Abstract

There is little information published on red sorrel (*Rumex acetosella* L.), a perennial weed that is considered a serious problem in wild blueberry production. Hexazinone, a photosystem II inhibitor, has been used in wild blueberry fields for more than 30 years. Hexazinone efficacy on red sorrel has declined over time. Therefore, a two year study was conducted to examine hexazinone alternatives that can be sprayed in wild blueberry fields. Red sorrel ramets from mature blueberry fields were tested to determine whether long-term spraying of hexazinone selected for resistant red sorrel. The results show that hexazinone+rimsulfuron/nicosulfuron may be a alternative for hexazinone. Red sorrel from some blueberry fields is hexazinone-resistant and the resistance is caused by a Phe$_{255}$ to Val mutation in the *psb*A gene.

List of Abbreviations and Symbols Used

ai   active ingredient

bp   base pairs

C   Celsius

cm   centimeter

χ^2 chi-square

DAS   days after spraying

ha   hectare

kg   kilogram

kPa   kilopascal

L   liter

LD50   dose lethal to 50% of test organisms

m   meter

μl   microliter

ml   milliliter

%   percent

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Chapter 1.0 Introduction

1.1 Introduction

Lowbush blueberry (*Vaccinium angustifolium* Ait. or *Vaccinium myrtilloides* Michx), often known as wild blueberry, is a perennial deciduous shrub that is native to Atlantic Canada, Quebec and northeastern United States (Vander Kloet 1978). Commercial fields are not planted but are developed in areas where significant clones already occur. Wild blueberry is an important crop in Nova Scotia. The average annual wild blueberry production from 1990 to 2011 was 16 million kilograms with an average farm-gate value of $21 million (Statistics Canada 2012).

Plants that grow in areas where they are not wanted and are unusually persistent are referred to as weeds (Radosevich et al. 2007). Weeds compete for resources with blueberries causing reduced yields and quality (Kennedy et al. 2010). Hexazinone is widely sprayed in wild blueberry production to control weeds and was widely adopted when research showed a two-fold blueberry yield increase following its use in the early 1980's (Yarborough 2004). However, many blueberry farmers in Nova Scotia saw hexazinone efficacy declined over time (Kennedy et al. 2010). This problem may be caused by a transition from susceptible to tolerant species or the development of herbicide resistance. There are no published reports of herbicide resistance in wild blueberry fields, but previous studies have shown that annual weeds can develop resistance to group 5 herbicides (triazine herbicides) after as few as 12 applications (Hall

et al. 1999). The most common weeds in wild blueberry fields are perennial weeds. In theory, perennial weeds take longer to become resistant, but repeated applications to large weed populations with single herbicide chemistry may result in resistance over time.

1.2 Background on Wild Blueberry and Blueberry Production

Wild blueberry is a low-growing rhizomatous shrub that is 10 to 25 cm tall. It is a cryptophyte plant with underground rhizomes that are fire tolerant (Rowe 1983). Plants grow in well-drained soils and prefer soil pH between 3.9 and 5.5. The fruit is a small sweet berry with a dark blue color. Nova Scotia was the largest wild blueberry producer in Atlantic Canada from 1957 to 2011 (Statistics Canada 2012). Approximately, 26 million kilograms of blueberries were harvested in Nova Scotia in 2003, with a farm-gate value of $29 million for the industry and over $70 million contribution to the provincial economy (Grant 2005). In 2010 and 2011, about 15 million kilograms of blueberries were harvested per year in Nova Scotia, with a farm-gate value of $22 million (Statistics Canada 2012). More than one thousand members of the Wild Blueberry Producers Association of Nova Scotia (WBPANS) help make Nova Scotia one of the largest blueberry producers in Atlantic Canada with 15,000 hectares of blueberry fields.

1.3 Blueberry Management

Blueberries are managed on a two year cycle. The first year is called the vegetative year and new shoots emerge, grow, and develop floral buds in the fall. The second year is called the crop year and the shoots flower and produce berries. After harvest in late

August, the shoots are burned or mowed off near the surface (Penney and McRae 2000). Most wild blueberry growers in Nova Scotia apply hexazinone to control broadleaves and some grasses. The recommended rate of hexazinone is 1.44 to 1.92 kg ai ha^{-1}.

Growers employ many different techniques to increase yields. First, fields are mowed biannually to promote vegetative growth and enhance yields. Mowing also promotes root development, crown expansion, and increases the number of shoots the following year (Smagula and Yarborough 1990). Fertilization is also very important in wild blueberry production. It is recommended that producers conduct soil and leaf analyses every two to three years to determine the needed fertilization to maximize yields. Blueberries are oligotrophic plants that do not require excessive fertilizers that, when applied without herbicides, increase weed growth in the field (Starast et al. 2007).

1.4 Integrated Weed Management

Weeds in blueberry fields are a persistent problem for farmers. Weeds compete for resources with the blueberry plants and interfere with the harvest and reduce profits (Kennedy et al. 2010). Weed control methods can be divided into five different categories: preventive, cultural, biological, mechanical and chemical. Chemical herbicides are the primary weed management option in wild blueberries and are sprayed before (PRE) or after blueberry emergence (POST). Hexazinone is the main herbicide applied for broad spectrum weed control in wild blueberry. Many weed species are tolerant of label rates of hexazinone, including lambkill and sweet fern. Additional products are required to

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control the tolerant weed species, such as: nicosulfuron/rimsulfuron (Ultim), fluazifop-p-butyl (Venture L) and tribenuron-methyl (Spartan) (McCully et al. 2005).

1.5 Hexazinone Use and Related Problems

Herbicides eliminate the need for hand picking and enable mechanical harvest. Herbicides also increase crops yields and decrease farming costs (Homer and Heber 2008). Due to a two-fold or more increase in blueberry yields following the adoption of hexazinone in the early 1980's, the industry became highly reliant on hexazinone for weed control and almost 100% of commercial sprout year blueberry fields are sprayed with hexazinone (Yarborough 2004). However, many farmers in Nova Scotia notice decline in hexazinone efficacy over time. It may be due to herbicide resistance appearing in the weeds. Repeated use of herbicides with similar modes of action to the same field have a potential to develop resistance within plants that originally were susceptible (Holt 1992).

Hexazinone, a selective herbicide in wild blueberry production, is a photosystem II inhibitor that belongs to the triazinone, group 5 herbicides. It is a soil acting herbicide that can easily be dissolved by rain and then leached into the root area. Its molecular structure is 3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-dione (Figure 1.1). Although hexazinone persists for several months, late emerging weeds may survive. Preliminary experiments conducted by Agriculture and Agri-Food Canada showed that

many grassy weeds already have haxazinone-tolerance in blueberry fields, including

bluegrasses and fescues (McCully et al. 2005). It is vitally important for the blueberry

industry to identify practical and effective herbicide rotations or tank mixes to slow the

development of resistance.
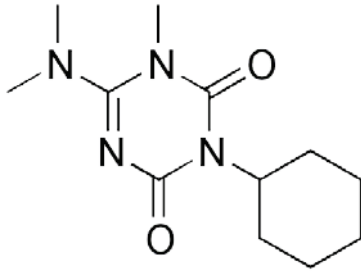
Figure 1.1. Molecular structure of hexazinone [Source: Bouchard and Lavy 1985]

1.6 Herbicide Resistance

A plant that survives a typically lethal rate of a herbicide and is able to confer the

resistance to successive generations is called herbicide resistant. As of 2009, there were

189 weed species that were resistant to one or more herbicides including 113

dicotyledonous weeds and 76 monocotyledons (Yang et al. 2009). In 2009, there were 94

new resistant biotypes compared to 2000. To combat this problem it is necessary to

identify herbicide-resistant weeds and to develop alternative control methods. A lack of

herbicide rotations may lead to herbicide resistance (Beckie and Reboud 2009).

Long-term use of one kind of herbicide has the potential to select for herbicide-resistant

biotypes. It is important to use herbicides in rotation with hexazinone to reduce the

probability of resistance (Beckie and Reboud 2009).

More weeds appear to be surviving recommended herbicide rates, which suggest that specific weeds are becoming resistant or that there is a transition to tolerant species (Jensen and Yarborough 2004). Also, the amount of hexazinone applied PRE to control weeds is much higher than recommended rates for some of the newer sulfonylurea herbicides that are applied POST (McCully et al. 2005). To reduce the amount of pesticide residues in the soil and water, the industry should adopt the use of effective, low-use rate products (Krutz et al. 2009).

1.6.1 Triazine-resistance

In 1952, the triazine herbicides were discovered by J.R. Geigy, Ltd. in a Switzerland chemical company that was founded in 1758. Triazine herbicides were a part of the reason why average corn yields increased from 2.76 metric tonnes ha^{-1} in 1950-1959 to 8.87 metric tonnes ha^{-1} in 2000-2004 (Statistics Canada 2012). Triazines, group 5 herbicides, significantly contributed to yield increase of many crops, such as cotton, sorghum, soybean and wheat. Presently, triazine herbicides are found and used in more than 100 countries all over the world (Homer and Heber 2008). Control of weeds by triazine herbicides occurs when they bind to the plastoquinone-binding point on the D1 protein in the PS II reaction center of the photosynthetic electron transport chain. Electrons from Q_A (the electron donor) to Q_B (the mobile electron carrier) are blocked, so

that photosynthesis will lack NADPH for CO_2 fixation (Shukla and Devine 2008). At the same time, oxygen radicals (H_2O_2, OH, etc) are formed and lead to some important molecules in the chloroplast being photo-oxidated, such as chlorophylls and unsaturated lipids (Shukla and Devine 2008).

Simazine [6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine], a herbicide of the triazine class, is a selective herbicide that controls many broad-leaved weeds. In the 1960s, it was reported that simazine did not control common groundsel (*Senecio vulgaris*) in Olympia, Washington state. This may have been the first published report of herbicide resistance in weeds (Ryan 1970). Much research was done by Radosevich and others to find the simazine resistance mechanism (Radosevich and Appleby 1973). They discovered that the reason that common groundsel resisted all s-triazine herbicides was due to a mutation in a chloroplast gene, which related to the herbicide binding protein of photosystem II (Radosevich et al. 1979). Later, more resistant common groundsel was found in all of western Washington state where atrazine or simazine had been the traditional herbicides used (Bandeen et al. 1982). After this first discovery, 57 species of weeds were reported to have selective resistance to triazine herbicides, which included 40 dicots and 17 monocots located in 33 states of the USA, 4 provinces of Canada and 17 countries of Europe (LeBaron 1991). As of 2005, triazine-resistant biotypes of weeds have increased to more than 60 ecotypes (Figure 1.2), (Heap 2007). Also, Heap (2006) reported 78 species resistant to triazines, including 57 broadleaf and 21 grass species in

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2006.
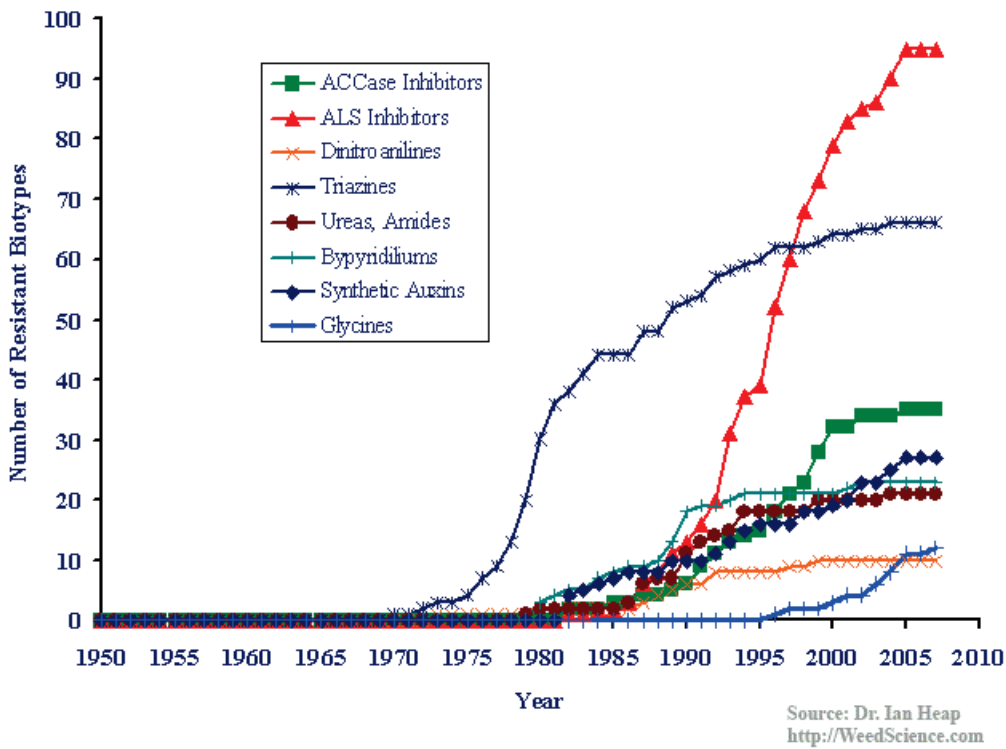
Figure 1.2. The chronological increase in the number of herbicide resistant weeds for several herbicide classes [Source: Heap 2007]

Triazine resistance is typically attributed to changes in amino acid residues in the Q_B binding niche on D1 protein that decrease herbicide binding (Devine et al 1993; Gronwald 1994). The D1 protein is a thylakoid-membrane protein that is coded by the *psb*A gene. Seventeen amino acids in the *psb*A gene contact the Q_B binding site, including Phe_{211}, Met_{214}, His_{215}, Leu_{218}, Val_{219}, Thr_{237}, Ile_{248}, Ala_{251}, His_{252}, Phe_{255}, Gly_{256}, Ala_{263}, Ser_{264}, Phe_{265}, Asn_{266}, Ser_{268} and Leu_{275} (Mengistu et al. 2000). Phe_{211}, Val_{219}, Ala_{251}, Phe_{255}, Gly_{256}, Ser_{264}, Ser_{268} and Leu_{275} mutations in the *psb*A gene have been reported which can cause triazine resistance in higher plants within the amino acid

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sequence positions between 211 and 275 in Q_B binding niche (Trebst 1991; Devine and Eberlein 1997; Shukla and Devine 2008; Perez-Jones et al. 2009). Among these, Ser_{264} mutation had greatest influence on binding herbicides that were PS II inhibitors (Mengistu et al. 2000). Also, mutations at or close to position Phe_{265}, Phe_{255}, and His_{215} have a great effect on the binding of PS II herbicides and are also important in development of resistance (Devine and Eberlein, 1997).

1.7 Methods of Preventing Resistance

The most effective way to delay resistance is herbicide rotations or mixtures (Beckie et al. 2001). The application of herbicides with different modes of action to multiple crops over multiple growing seasons in a field is called herbicide rotation (Beckie 2006). Less than 50% of Canadian farmers rotated herbicides in 1988. By 2003, 70% of farmers in Saskatchewan and more than 90% in Manitoba realized it was important to rotate herbicides by modes of action (Beckie 2007). Some herbicides are taken up through the leaves and some through the roots. Mixtures of different modes of uptake or action decrease the potential of creating resistant plants or seeds (Beckie and Reboud 2009). Diggle et al. (2003) created model simulations to determine that herbicide mixtures delay resistance longer than rotations. Repeated use of one herbicide will select for resistant weeds quickly (Figure 1.3). Rotation and mixtures of herbicides can delay appearance of resistant weeds.
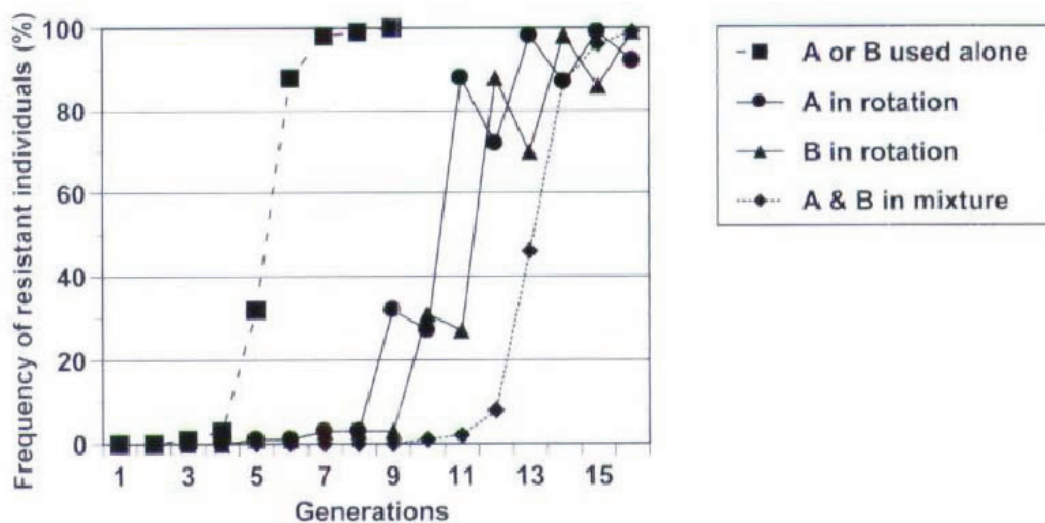
Figure 1.3. Predicted increases of herbicide resistant individuals over time [Source: Powles et al. 1997].

The enrichment of seed bank resistance can be impacted by herbicide mixtures (Beckie and Reboud 2009). In evolutionary terms, a "memory" of past selection events is represented by the seed bank and this "memory" delays resistance (Templeton & Levins 1979). Generally, more resistant seeds presented in resistant weeds sites. If a new herbicide controls a resistant weed, it is important to decrease dormancy or increase germination of seeds in the seed bank to ensure resistant biotypes are killed, and this may also improve management of weeds in general (Dyer 1995). Also, if the resistant seeds emerge earlier than susceptible seeds, an extra weed control before susceptible seeds emergence will result in a higher reduction of the resistant population, which has already been used in earlier germinating sulphonylurea-resistant biotypes of *Kochia scoparia* (Thompson et al. 1994).

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Screening is an important step towards identifying new effective herbicides (Beckie et al. 2000). No single herbicide or mix of herbicides can limit all the weed growth in blueberry fields. Farmers must choose either selective or nonselective herbicides to manage weeds. (McCully et al. 2005).

Sulfonylurea herbicides were first identified by DuPont Crop Protection in 1975 and recommended for wheat and barley crop weed management in 1982 (DuPont 2006). This group of herbicides works by inhibiting the production of acetolactate synthase, a key enzyme for cell growth. Generally, sulfonylurea herbicides have low-use rates (10-40 g ha^{-1}) and are degraded in soil very quickly (Zhang et al. 2011). Ultim, a sulfonylurea herbicide, is already registered for weed control in blueberry fields. It manages broadleaf weeds and some grasses in blueberry fields.

1.8 Red Sorrel

Rumex acetosella L. has several common names, including red sorrel, sheep's sorrel, sour weed, and field sorrel. Red sorrel is a perennial weed that reproduces by creeping roots and seeds, and is native to Eurasia. It is a taxonomically difficult species aggregate and includes some taxa of uncertain status (Love 1983; Nijs 1984). Most of the plants are 4 to 12 inches high and they have upright, reddish stems with branched tops. Their leaves are arrow-shaped and flowers are clustered at the top of the plant with green to red colors. It can spread widely, especially on acidic and nutrient-deficient soils (Love 1983). Red

sorrel is considered to be a harmful weed in Nova Scotia due to its creeping root system.

Red sorrel is a very common invasive weed in lowbush blueberry fields (Kennedy et al. 2010). Red sorrel abundance increased 43% from 1984 to 1985 when red sorrel was the fourth most abundant species (McCully et al. 1991). Some populations of red sorrel can be suppressed by hexazinone, but some tolerant populations have been found (McCully et al. 2005). Tolerance may be caused by low herbicide rates or the development of resistance. Little information is published on red sorrel populations in lowbush blueberry fields and there are no reports showing how blueberry management influences red sorrel.

The objectives of the experiments were to 1) evaluate new herbicide chemistries or tank mixes that can be applied before blueberry emergence (PRE) that have modes of action different than hexazinone for weed control in wild blueberry production; 2) evaluate new herbicide chemistries that can be applied after blueberry emergence (POST) for weed control in wild blueberry production; 3) determine the existence of hexazinone resistant red sorrel in wild blueberry fields. 4) verify whether hexazinone-resistant red sorrel is caused by a Ser_{264} to Gly mutation in psbA gene; 5) develop a simple bioassay method that can determine the effect of hexazinone metabolites on red sorrel leaves and be used to distinguish resistant and susceptible genotypes.

Chapter 2.0 Herbicide Screening in Wild Blueberry Fields

Abstract

Hexazinone has been sprayed on many wild blueberry fields for more than 30 years. Growers believe the efficacy of hexazinone has declined over time. To address this issue, 15 different herbicides or mixtures were tested to identify hexazinone alternatives. Hexazinone still controlled a range of broadleaf weeds. Terbacil(WDG) and its mixtures provided better grass control than hexazinone. Hexazinone+rimsulfuron/nicosulfuron had high damage ratings to some weeds. The weed biomass from this herbicide treatments was also low, 88.2% and 34.3% lower than control treatments at Dalhousie Mountain and Portapique sites, respectively. Thus, this tank mix may be an alternative option for growers. None of the tested POST herbicides provided adequate levels of weed control.

2.1 Introduction

Weeds are one the most significant yield limiting problems faced by wild blueberry growers (Boyd and White 2010). Herbicides play an important role in wild blueberry production. They can be used to reduce or replace other methods of weed control including hand picking or cultivation. Herbicides also increase crops yields and decrease farming costs (Homer and Heber 2008).

Herbicides sprayed before blueberry or weed emergence are called pre-emergence (PRE) herbicides and are typically applied in late April or early May of the vegetative year. Herbicides sprayed after blueberry and weed emergence are considered post emergence herbicides (POST) and include products such as sulfonylurea herbicides. Generally, sulfonylurea herbicides have low-use rates (10-40 g ha^{-1}) and are degraded in soil very quickly (Zhang et al. 2011). Rimsulfuron/nicosulfuron (Ultim) can be applied post emergence to blueberry fields in Eastern Canada to control black bulrush and annual grasses (Agriculture, Aquaculture and Fisheries 2010).

Hexazinone is a group 5 herbicide and is the most commonly used herbicide in wild blueberry production. Many farmers in Nova Scotia have reported that the efficacy of hexazinone has declined over time following repeated applications. Weeds in blueberry fields may have developed resistance to hexazinone due to long-term use of herbicides with the same mode action. Herbicide rotations and mixtures are two effective ways to delay resistance (Beckie et al. 2001). Model simulations show herbicide mixtures can

delay resistance longer than rotations (Diggle et al. 2003). Rotations or mixtures are not currently a viable alternative in wild blueberry due to the limited number of herbicide products registered for use. New herbicides are needed that can be used in rotation or as a tank mix with hexazinone.

The objectives of the research were to 1) evaluate new PRE herbicide chemistries or tank mixes that have modes of action different than hexazinone for weed control in wild blueberry production; 2) evaluate new POST herbicide chemistries to control weeds in wild blueberry production.

2.2 Materials and Methods

2.2.1 Study Sites

Experiments were set up to evaluate PRE herbicides and sulfonylurea herbicides applied POST at two commercially managed sites in 2011. One PRE and one POST trial were located at Dalhousie Mountain (N45°36′53″, W62°55′5″) and one of each at Portapique (N45°24′28.20″, W63°43′30.65″), Nova Scotia. In 2012, two additional sulfonylurea trials were set up at Dalhousie Mountain (N45°36′53″, W62°55′5″) and Collingwood (N45°34′31″, W63°50′38″). Sites at Dalhousie Mountain and Collingwood were mature, commercial blueberry fields that had been harvested for more than 15 years. Portapique was a newly developed field that had not yet been harvested commercially. The soil pH at all sites ranged from 4.5 to 4.8 (Table 2.1). Soils tended to have a high

sand content especially at Portapique (Table 2.1).

Table 2.1. Soils information of each site.

Location	pH	OM (%)	Sand (%)	Silt (%)	Clay (%)	Textural classes
Dalhousie Mountain	4.7	8.6	34.0	56.6	10.2	Silt loam
Portapique	4.8	8.2	59.6	20.2	20.2	Sandy clay loam
Collingwood	4.5	6.5	45.2	48.0	6.8	Sandy loam

Both PRE and POST experiments were set up as randomized complete block design (RCBD) with four blocks. There were 15 treatments in the PRE experiments and 6 treatments in the POST experiments (Table 2.2). All herbicides were applied using a hand-held boom with CO_2 pressurized sprayer at 275.8 kPa pressure with 4-XR8002VS Teejet nozzles on different dates (Table 2.3). Herbicides in the PRE experiments were sprayed before blueberry emergency (PRE), which was in early May. Herbicides in the POST experiments were sprayed in mid-June, which was after blueberry emergence. All plots were 2 m x 6 m plots and were sprayed with a water volume of 200 L ha^{-1}.

Table 2.2. Herbicide treatments applied in PRE herbicide trials at both Dalhousie Mountain and Portapique sites in 2011; POST herbicide trials at Dalhousie Mountain and Portapique in 2011and Dalhousie Mountain and Collingwood sites in 2012.

Trials	Trade Name	Common name of the Active Ingredient	Application Rate (kg ai ha^{-1})
PRE	Untreated Control	None	0
	Velpar DF	Hexazinone	1.92
	Velpar+ Ultim	Hexazinone+Rimsulfuron/ nicosulfuron	1.92+0.0126
	Velpar + Simplicity	Hexazinone+Pyroxsulam	1.92+0.015
	Velpar+Indazaflam	Hexazinone+Indazaflam	1.92+0.075
	Sinbar WP	Terbacil	2
	Sinbar WDG	Terbacil	2
	Sinbar WDG+Ultim	Terbacil+Rimsulfuron/ nicosulfuron	2+0.0126
	Sinbar WDG+Simplicity	Terbacil+Pyroxsulam	2+0.015
	Sinbar WDG+Indazaflam	Terbacil+Indazaflam	2+0.075
	Sinbar WDG+ Florasulam	Terbacil+Florasulam	2+0.010
	Simplicity	Pyroxsulam	0.015
	NA	Florasulam	0.010
	Stellar A	Florasulam+Fluroxypyr	0.0025+0.059
	Spartan	Sulfentrazone	0.2795
POST	Untreated Control	None	0
	Classic 25 DF	Chlorimuron ethyl	0.009
	Peak	Prosulfuron	0.010
	First Rate	Cloransulam-methyl	0.0175
	Simplicity	Pyroxsulam	0.015
	Ultim	Rimsulfuron/nicosulfuron	0.0126

[a]All POST herbicides used Agral 90 as a surfactant in 2012. In 2011, surfactants were omitted with herbicides at both Dalhousie Mountain and Portapique sites.

Table 2.3. The spray date of herbicides in each site.

Location	Year	Trial	Spray date
Dalhousie Mountain	2011	PRE	May17
Dalhousie Mountain	2011	POST	June 16
Portapique	2011	PRE	May 13
Portapique	2011	POST	June 8
Dalhousie Mountain	2012	POST	June 19
Collingwood	2012	POST	June 19

2.2.2 Data Collection

Herbicide efficacy and blueberry damage were recorded 14, 36, 72, and 365 days after spraying (DAS) using a standard damage rating scale. The scale was from 0 to 10 where 0 was no damage and 10 was complete death of above ground shoots. Common species at each site were rated separately. To account for the variability typically found in blueberry fields, three shoots of each species were rated and the average recorded.

Ground cover was determined within a 50 cm x 50 cm quadrat using the point intercept method (Bonham 1989). There were 25 transects in a single quadrat and each measurement was taken from 30 cm above the canopy. Measurements were divided into three different categories: blueberry, broadleaf weed and grass. Ground cover was measured in early July, late July and mid-August. Two ground cover measurements were

measured per plot.

Biomass measurements occurred within a 50 cm x 50 cm area in mid-August and plant materials were separated into blueberry, grass and broadleaf dry matter. All of the plant material was clipped at ground level. One quadrat per plot was taken in the PRE trials and two quadrats per plot were taken in the POST trials. Quadrats were randomly placed in the plots, however, bare ground regions were avoided. Samples were separated into different bags in the field and then put into a dryer for 5 days at 60 C and weighed to determine dry matter.

Blueberry floral buds were counted on fifteen wild blueberry stems located every 18 cm along a diagonal transect in each plot in September. The blueberry yield of 2011 PRE and POST trials at Dalhousie Mountain was taken on August 15, 2012. Two 30 cm x 100 cm areas per plot were harvested using hand held rakes to determine the yield for each treatment. There was no yield data from the Portapique PRE and POST site due to the low blueberry population density.

2.2.3 Statistical Analysis

Many of the damage ratings were zero and, as a result, the residuals were not normally distributed and variances were not homogeneous. Therefore, all damage ratings and ground cover data were analyzed using Proc Npar1way (Kruskal-Wallis test) in SAS, version 9.3 (SAS Institute Inc., Cary, NC). All analyses were done at α=0.05.

Biomass and blueberry floral bud numbers were analyzed using PROC MIXED in SAS, version 9.3 (SAS Institute Inc., Cary, NC). Treatments were fixed effects and blocks were random effects. Sites differed in field age and management history. Sites were, therefore, analyzed separately. Least squares means comparisons of Tukey's test were used to test for treatment differences at $\alpha=0.05$.

2.3 Results and Discussion

2.3.1 PRE Emergence Herbicides Trials

None of the products damaged blueberries at any site in any year.

Goldenrod (*Solidago canadensis* L. and *Solidago graminifolia* L.) damage ratings differed significantly among treatments on 14, 35, and 56 days after spraying at Dalhousie Mountain (Table 2.4). No damage was observed 365 DAS. Hexazinone proved the highest damage to goldenrod on day 14 and progressively higher damage ratings were recorded on day 35 and 56 (Figure 2.1). All hexazinone and hexazinone mixtures damaged goldenrod. From this, it was concluded that hexazinone provides significant goldenrod control at this site and the addition of a tank mix partner did not provide any benefit. Indazaflam appeared to have an antagonistic impact on hexazinone efficacy (Figure 2.1). None of the other products tested adequately controlled goldenrod.
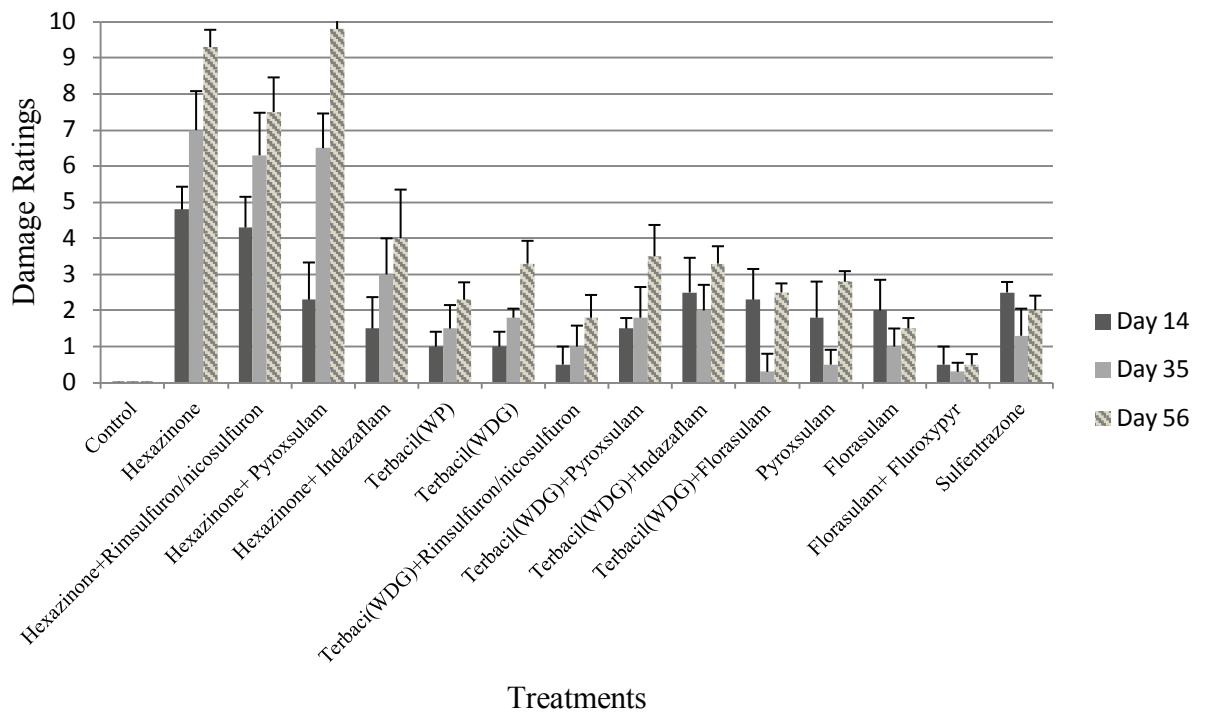
Figure 2.1. Goldenrod damage ratings at Dalhousie Mountain, Nova Scotia in 2011. Error bars state the stand error of means in all figures.

Goldenrod (*Solidago canadensis* L. and *Solidago graminifolia* L.) damage ratings at Portapique differed significantly among treatments on 14, 35, and 56 DAS (Table 2.4). There was no damage 365 DAS at Portapique, Nova Scotia. Goldenrod damage ratings in all treatments were lower than Dalhousie Mountain but trends were similar (Figure 2.2). Indazaflam did not appear to be antagonistic at this site. None of the other herbicides evaluated provided adequate levels of goldenrod control. In conclusion, hexazinone did not suppress goldenrod very well at this site, especially compared to the Dalhousie Mountain site. The addition of a tank mix partner with hexazinone provided minimal or no benefit. The leaves of goldenrod were wilted and burned-like with a rating of 5

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(Appendix 1).
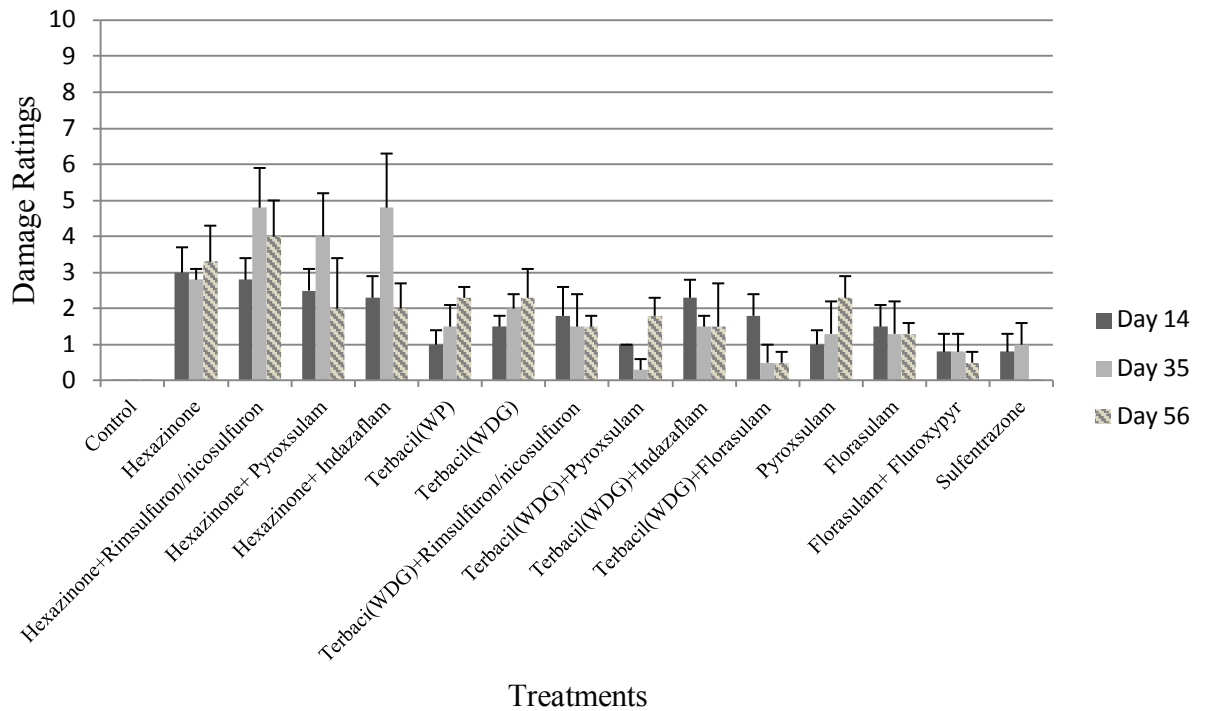
Figure 2.2. Goldenrod damage ratings at Portapique, Nova Scotia in 2011.

There was no fescue damage 365 DAS at Dalhousie Mountain, Nova Scotia. Fescue damage ratings differed significantly among treatments on 14, 35, and 56 DAS (Table 2.4). Florasulam+fluroxypyr treatments did not damage fescue (Figure 2.3). Terbacil mixtures had the highest fescue damage, with a maximum damage rating of 7 at 56 DAS in the terbacil+indazaflam treatments. Hexazinone, hexazinone+indazaflam and sulfentrazone treatments had the lowest damage ratings. Damage ratings in the other 2 hexazinone mixtures were between 3 and 5 on day 14 to 56, which means hexazinone caused less damage to fescue than terbacil. However, none of the treatments killed fescue.

Therefore, terbacil suppressed fescue and the addition of a tank mix partner provided

minimal or no additional benefit. Hexazinone did not control fescue on its own but

hexazinone+rimsulfuron/nicosulfuron or pyroxxsulam provided levels of control similar

to terbacil. These two products seemed to provide some benefit as tank mix partners with

hexazinone. Fluroxypyr appears to antagonize florasulam and this is a consistent trend

noted with most species.
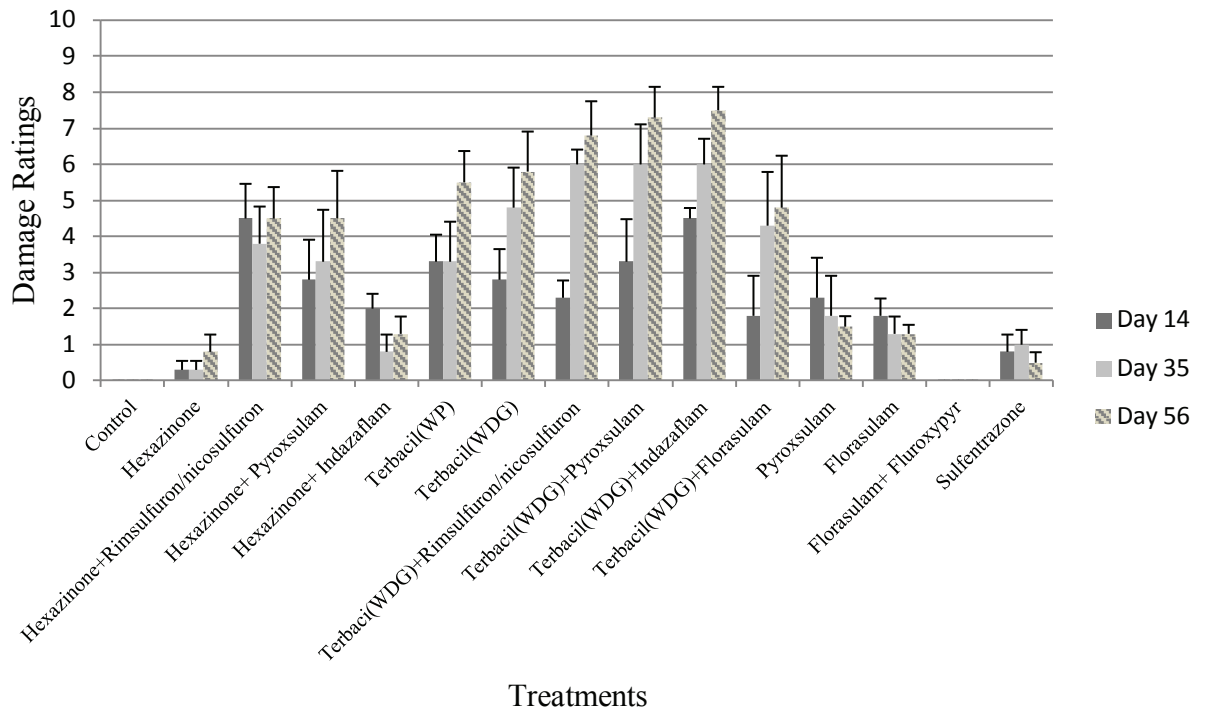
Figure 2.3. Fescue damage ratings at Dalhousie Mountain, Nova Scotia in 2011.

There was no significant difference among treatments for tickle grass damage

ratings at Portapique on day 14 (Table 2.4). Tickle grass damage ratings differed

significantly among treaments on days 35 and 56 DAS (Table 2.4). Also, no tickle grass

damage showed on day 14, except 1 damage on florasulam+terbacil on day 14 (Figure

2.4). On day 35 and 56, all hexazinone and terbacil tank mixes suppressed or controlled tickle grass. Addition of a tank mix partner did not provide any added benefit over hexazinone or terbacil used alone (Figure 2.4). None of the other herbicides evaluated provided adequate levels of tickle grass control.
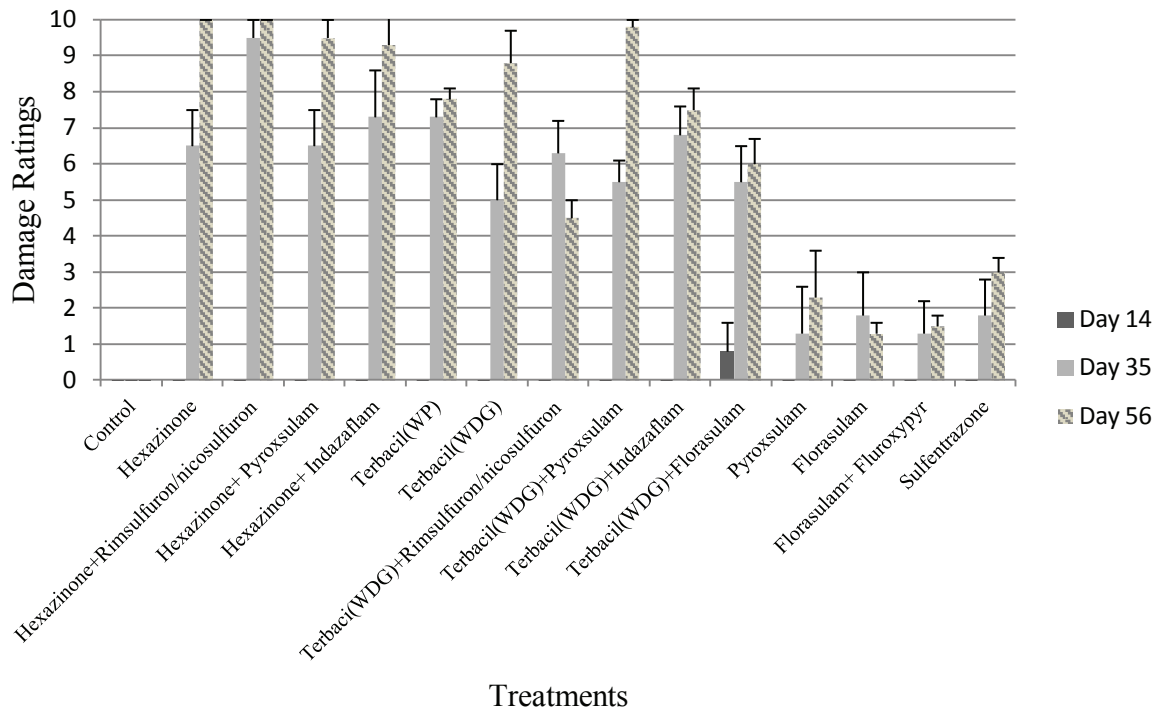
Figure 2.4. Tickle grass damage ratings at Portapique, Nova Scotia in 2011.

The observed nutsedge damage ratings 14, 35 and 56 DAS at Portapique in 2011 are shown in Figure 2.5. Nutsedge damage ratings were not significantly different among treatments 14 DAS and were significantly different 35 DAS and 56 DAS (Table 2.4). Damage ratings increased in most hexazinone and terbacil treatments from day 35 to day 56. Hexazinone+pyroxsulam had the highest damage on nutsedge on day 56. Both

hexazinone and terbacil suppressed nutsedge and the addition of pyroxsulam and indazaflam to hexazinone appeared to give some added benefit.
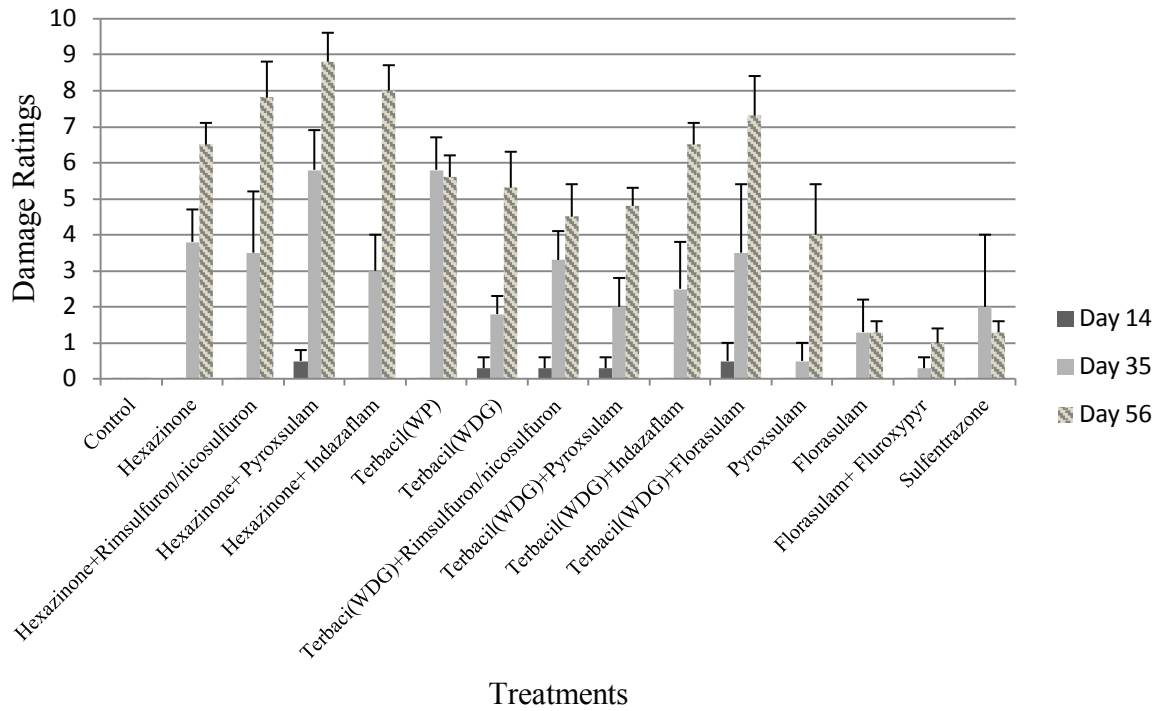
Figure 2.5. Nutsedge damage ratings at Portapique, Nova Scotia in 2011.

Table 2.4. The Pr>χ² values of weed damage ratings on each day affected by different herbicide treatments at Dalhousie Mountain and Portapique sites in 2011.

	Dalhousie Mountain			Portapique		
Weeds	14 DAS	35 DAS	56 DAS	14 DAS	35 DAS	56 DAS
Goldenrod	0.0105	0.0003	<0.0001	0.0280	0.0038	0.0092
Fescue	0.0016	0.0002	<0.0001	-	-	-
Tickle grass	-	-	-	0.4685	0.0001	<0.0001
Nutsedge	-	-	-	0.3703	0.0229	<0.0001

Herbicide treatments had an impact on broadleaf biomass at Dalhousie Mountain (Table 2.5). The predominant broadleaf species was goldenrod at Dalhousie Mountain, but there were some other broadleaf species present which were not adequately controlled by hexazinone so that total broadleaf biomass in the hexazinone treatment was not different than the control treatment. However, the addition of the tank mix partners significantly reduced broadleaf biomass at Dalhousie Mountain as did hexazinone+pyroxsulam (Table 2.5). Hexazinone+pyroxsulam reduced biomass by 93% whereas hexazinone on its own had no impact. Pyroxsulam on its own was not as effective suggesting that there was synergy between these products. The pattern was not the same at Portapique. Treatments were not significantly different on broadleaf biomass at Portapique (Table 2.5) and this is likely due to the presence of multiple broadleaf

species that are not susceptible to hexazinone. For example, the predominant broadleaf species was bracken fern (*Athyriaceae*) and none of the herbicides controlled this species. Thus, the broadleaf biomass was much higher at Portapique than Dalhousie Mountain and no significant difference was shown among treatments.

Herbicide treatments had an impact on grass biomass at Dalhousie Mountain (Table 2.5). However, none of the treatments were different from the control treatments, since Tukey test is very conservative. Terbacil(WDG) or terbacil(WDG) combinations consistently had lower grass biomass at Dalhousie Mountain. Similar results were found at the Portapique site. Treatments were significantly different on grass biomass at Portapique (Table 2.5), but none of herbicide treatments were different from the untreated control.

Blueberry biomass at the Portapique site was not significantly affected by herbicide treatments (Table 2.5). At the Dalhousie Mountain site, control, hexazinone and terbacil(WDG)+pyroxsulam were the treatments with the top three blueberry biomass. Florasulam treatments had the lowest blueberry biomass at Dalhousie Mountain.

Table 2.5. PRE herbicides trials biomass at Dalhousie Mountain and Portapique, Nova Scotia in 2011.

Treatments	Dalhousie Mountain			Portapique		
	Blueberry	Broadleaf	Grass	Blueberry	Broadleaf	Grass
			—g m^{-2}—			
Control	237.6 ab	27.2 ab	9.2 abcd	29.6 a	138.8 a	3.6 abcde
Hexazinone	248.0 a	20.4 abc	8.4 abcd	21.2 a	47.6 a	0.4 e
Hexazinone+Rimsulfuron/nicosulfuron	111.6 cd	3.2 cd	7.6 abcd	18.8 a	91.2 a	1.6 abcde
Hexazinone+Pyroxsulam	119.2 cd	2.0 d	7.2 abcd	23.6 a	95.6 a	0.4e
Hexazinone+Indazaflam	159.6 abcd	2.8 d	8.0 abcd	21.6 a	98.8 a	2.4 abcde
Terbacil(WP)	136.4 bcd	30.8 ab	6.4 abcd	6.4 a	117.2 a	1.6 bcde
Terbacil(WDG)	131.2 bcd	9.2 bcd	1.6 d	62.4 a	126.4 a	1.2 cde
Terbaci(WDG)+Rimsulfuron/nicosulfuron	208.8 abc	27.6 a	3.2 bcd	19.2 a	110.0 a	1.6 bcde
Terbacil(WDG)+Pyroxsulam	262.8 a	2.8 d	4.0 abcd	96.0 a	68.8 a	0.8 de
Terbacil(WDG)+Indazaflam	164.8 abcd	9.2 bcd	2.4 cd	25.2 a	124.0 a	0.8 de
Terbacil(WDG)+Florasulam	128.4 bcd	20.4ab	2.8 cd	88.0 a	57.2 a	0.8 de
Pyroxsulam	136.8 bcd	18.8 ab	6.4 abcd	10.0 a	164.8 a	4.4 abcd
Florasulam	89.2 d	15.6 abcd	9.6 abc	52.4 a	132.0 a	6.4 ab
Florasulam+Fluroxypyr	198.8 abcd	15.6 abcd	11.2 a	18.8 a	120.4 a	5.6 a
Sulfentrazone	130.0 bcd	20.4 abcd	10.4 ab	31.2 a	138.0 a	5.2 abc
P-value	0.0452	0.0108	0.0002	0.1151	0.4445	0.0420

[a]Means within a column, followed by the same letter are not significantly different (Tukey, α=0.05).

There was no significant difference among treatments for Dalhousie Mountain blueberry flower buds (P=0.5537) and Portapique blueberry flower buds (P=0.4810). The numbers of blueberry floral buds per stem at Dalhousie Mountain ranged from 4 to 7. The number at Portapique ranged between 2 and 6. Also, no significant differences were shown in blueberry, broadleaf and grass groundcover at Dalhousie Mountain PRE trials in 2011 (Table 2.6). Portapique ground cover is not shown due to error.

There were few blueberries at Portapique. Therefore, yield data were not collected. The blueberry yield at Dalhousie Mountain was not significantly different among treatments (Table 2.7). However, fairly large differences of yields were observed and the lack of difference is due to significant levels of variability (Table 2.7). Terbacil+indazaflam and florasulam had the lowest yields, which were 130 g m^{-2} lower than control treatments. Hexazinone+pyroxsulam and hexazinone+indazaflam had 125 g m^{-2} yield. Other treatments all had more than 175 g m^{-2} yield; terbacil(WDG) had the highest blueberry yield.

Table 2.6. The P-values of ground cover in PRE emergence herbicide trials in Dalhousie Mountain, Nova Scotia in 2011.

	Dalhousie Mountain		
Date	Blueberry	Broadleaf	Grass
Early July	0.8945	0.4683	0.9907
Late July	0.4762	0.4247	0.1940
Mid-August	0.6964	0.4856	0.1638

Table 2.7. Yields of blueberry in PRE trials Dalhousie Mountain, Nova Scotia in 2011.

Treatments	Dalhousie Mountain
	———g m^{-2}———
Control	212 a
Hexazinone	210 a
Hexazinone+Rimsulfuron/nicosulfuron	205 a
Hexazinone+ Pyroxsulam	132 a
Hexazinone+ Indazaflam	120 a
Terbacil(WP)	178 a
Terbacil(WDG)	265 a
Terbaci(WDG)+Rimsulfuron/nicosulfuron	230 a
Terbacil(WDG)+Pyroxsulam	200 a
Terbacil(WDG)+Indazaflam	70 a
Terbacil(WDG)+Florasulam	225 a
Pyroxsulam	245 a
Florasulam	77 a
Florasulam+ Fluroxypyr	255 a
Sulfentrazone	188 a
P-value	0.3584

[a]Means followed by the same letter are not significantly different (Tukey, α=0.05).

Hexazinone and its mixtures resulted in the highest damage ratings on goldenrod at both sites (Figure 2.1 and Figure 2.2). Terbacil and its mixtures resulted in highest damage ratings on fescue at Dalhousie Mountain (Figure 2.3). Both hexazinone and terbacil treatments caused very high damage on tickle grass and high damage on nutsedge at Portapique (Figure 2.4 and Figure 2.5). Goldenrod was the main broadleaf weed at Dalhousie Mountain and higher damage on goldenrod resulted in lower broadleaf biomass except in the hexazinone treatments (Table 2.5). It is fairly common for plants to show symptoms and have mid level ratings and then recover with no difference in biomass and also there were other broadleaf species in the trials which is the other reason hexazinone treatments had high broadleaf biomass. The broadleaf biomass in the hexazinone, terbacil and terbacil mixtures was higher than hexazinone mixtures treatments (Table 2.5). Thus, the hexazinone mixtures provided better broadleaf control than hexazinone. It is concluded that hexazinone+rimsulfuron/nicosulfuron provided the highest weed damage with low weed biomass and high yield (Table 2.5 and Table 2.7). Therefore, hexazinone+rimsulfuron/nicosulfuron could be a potential tank mix for goldenrod, nutsedge and tickle grass control. Also, as expected, terbacil(WDG) and its mixtures resulted in better grass suppression than hexazinone.

Pyroxsulam, florasulam, florasulam+fluroxypyr and sulfentrazone always had lower weed damage than other treatments (Figure 2.1 to Figure 2.5). Also, the biomass of grass and broadleaf in these four treatments at both sites was relatively high (Table 2.5). It was

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even higher than the untreated control at the Portapique site (Table 2.5). Thus, these four treatments had much lower impact on weeds in wild blueberry production than hexazinone.

2.3.2 POST Trials

2011

Surfactants were omitted from herbicides in 2011 for POST trials, therefore efficacy of herbicides was reduced. None of herbicides damaged the fescue at Dalhousie Mountain POST trials in 2011. Damages ratings were significantly different among treatments for goldenrod damage ratings at Dalhousie Mountain POST trials in 2011 (Table 2.8). However, all treatments had damage ratings less than 4 for goldenrod (Figure 2.6). Similar results were shown at Portapique sites (Figure 2.7). It seems Cloransulam-methyl can suppress goldenrod in POST trials. Herbicides had no effect on blueberry, grass and nutsedge at Portapique in 2011.
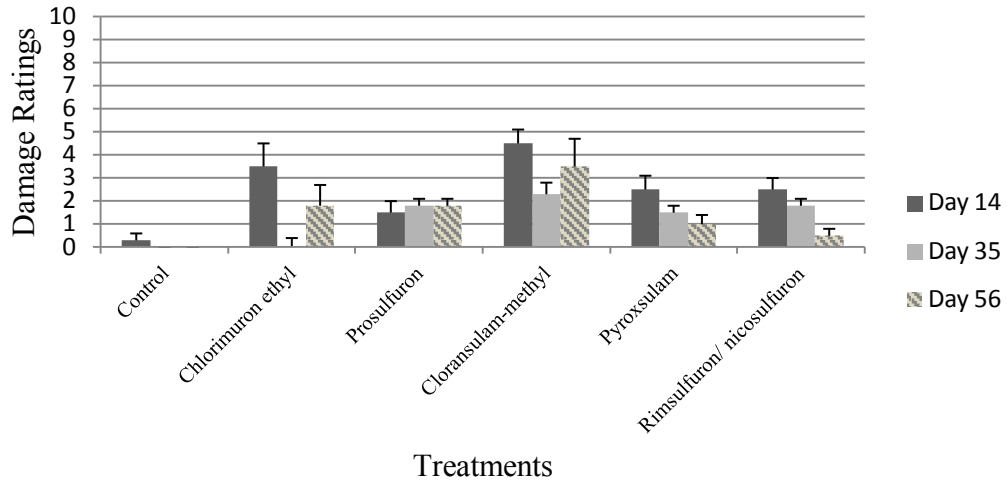
Figure 2.6. Goldenrod damage ratings at Dalhousie Mountain, Nova Scotia in 2011.
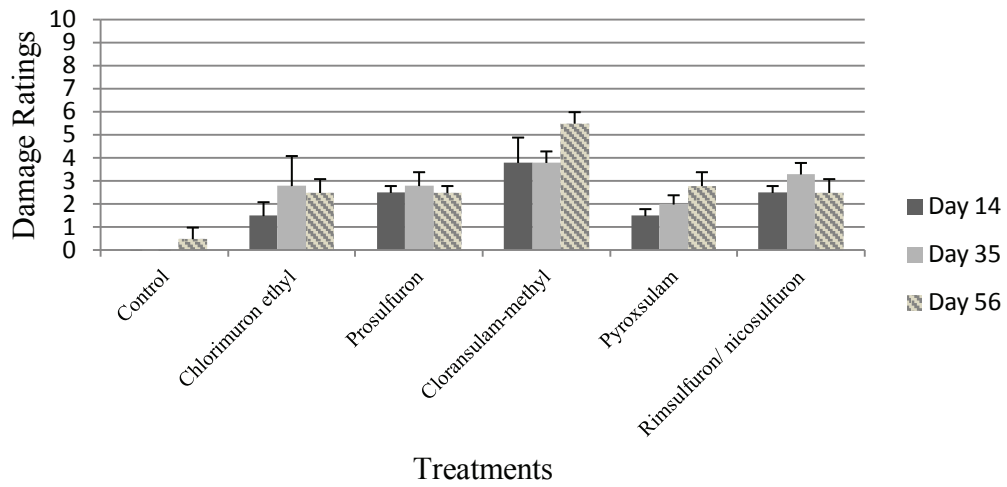
Figure 2.7. Goldenrod damage ratings at Portapique, Nova Scotia in 2011.

Table 2.8. The P-values of goldenrod damage ratings in POST trials in Dalhousie Mountain and Poratpique, Nova Scotia in 2011.

Date (DAS)	Dalhousie Mountain	Portapique
14	0.0142	0.0107
35	0.0197	0.0295
56	0.0218	0.0116

2012

Similar results were observed for narrow-leaf goldenrod (*Solidago graminifolia* L.) damage ratings at both sites in 2012 (Figure 2.8 and Figure 2.9). Damage ratings were significantly different among treatments (Table 2.9). Prosulfuron resulted in the highest damage on narrow-leaf goldenrod on Day 56 with a 9 at Dalhousie Mountain and 10 at Collingwood. Chlorimuron ethyl suppressed narrow-leaf goldenrod at both sites (Figure 2.8 and Figure 2.9). Pyroxsulam and rimsulfuron/nicosulfuron had low damage to narrow-leaf goldenrod.
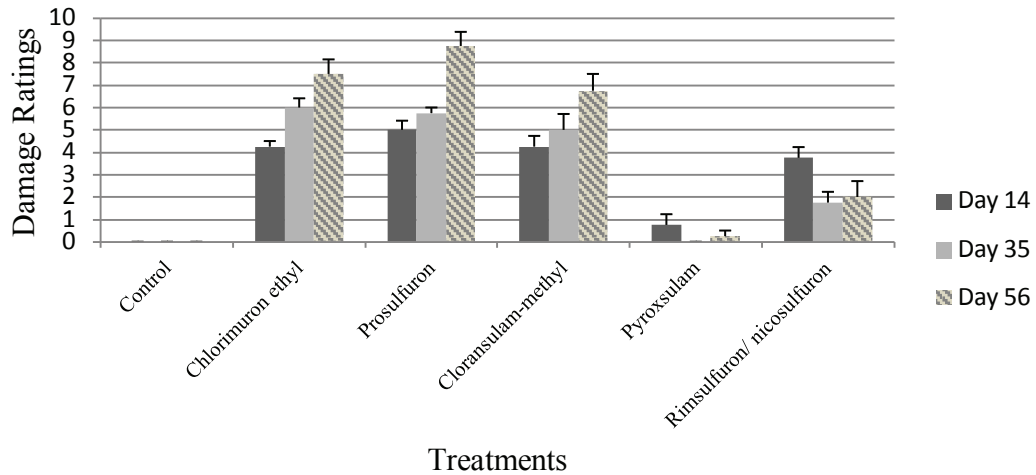
Figure 2.8. Narrow-leaf goldenrod damage ratings at Dalhousie Mountain, Nova Scotia in 2012.
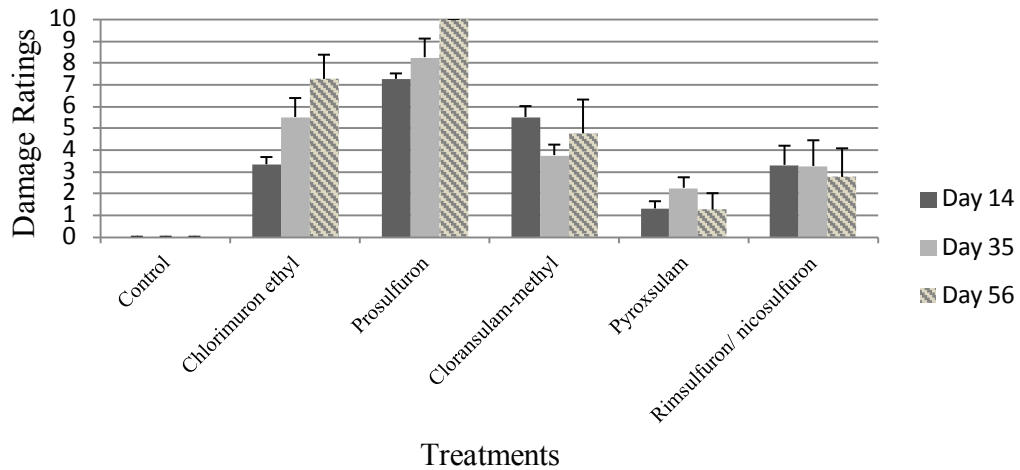
Figure 2.9. Narrow-leaf goldenrod damage ratings at Collingwood, Nova Scotia in 2012.

None of the products controlled broadleaf goldenrod (*Solidago canadensis* L.) at either site (Figure 2.10 and Figure 2.11). No significant difference was shown 56 DAS for broadleaf goldenrod at both sites (Table 2.9). All the treatments had damage ratings less than 1 on broadleaf goldenrod 56 DAS (Figure 2.10 and Figure 2.11). Prosulfuron

suppressed broadleaf goldenrod 14 DAS at Dalhousie Mountain, but no damage was observed by 35 DAS (Figure 2.10). Other herbicides had less or no efficacy on controlling or suppressing broadleaf goldenrod. Overall, none of herbicides controlled broadleaf goldenrod and only chlorimuron ethyl and prosulfuron suppressed broadleaf goldenrod 14 DAS.
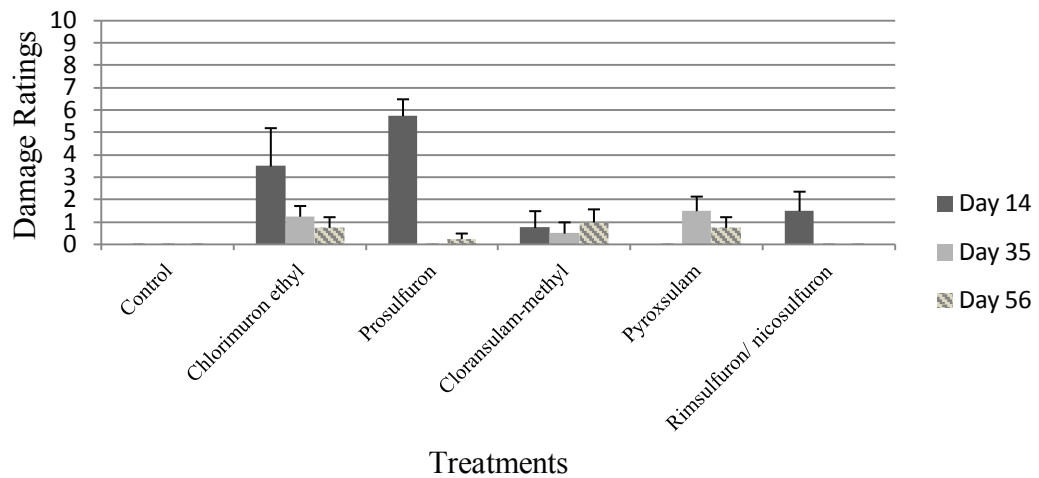
Figure 2.10. Broadleaf goldenrod damage ratings at Dalhousie Mountain, Nova Scotia in 2012.
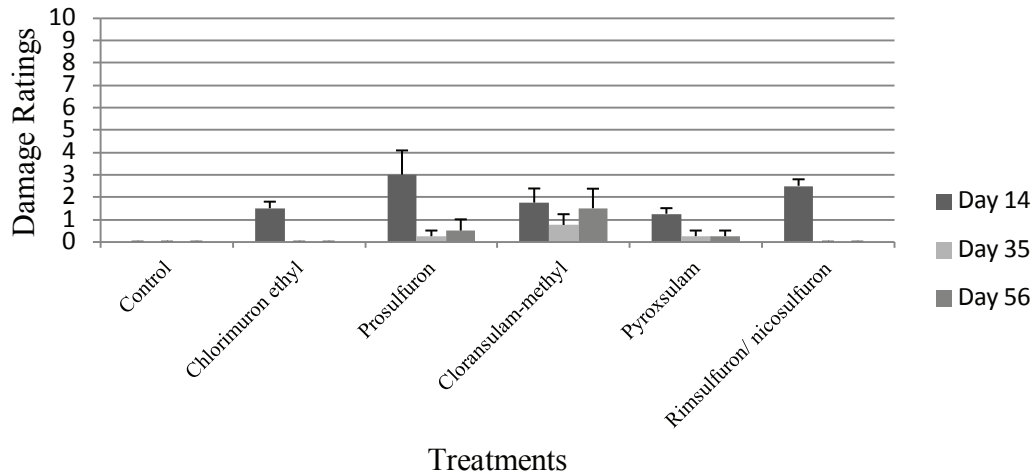
Figure 2.11. Broadleaf goldenrod damage ratings at Collingwood, Nova Scotia in 2012.

Blueberry and fescue were not damaged at either site. Also, no hawkweed damage was observed 14 DAS at both sites. On day 35 and 56, hawkweed damage ratings were all lower than 3 in herbicide treatments. Thus, all the herbicides used had minimal impact on hawkweed damage ratings. None of the treatments had an effect on poverty oat grass at Collingwood site, except rimsulfuron+nicosulfuron with a 2 damage rating shown on day 35 and 56 (Appendix 2).

Table 2.9. The P>χ² values of weed damage ratings in POST trials in Dalhousie Mountain and Portapique, Nova Scotia in 2012.

Date (DAS)	Dalhousie Mountain		Collingwood	
	Narrow-leaf goldenrod	Broadleaf goldenrod	Narrow-leaf goldenrod	Broadleaf goldenrod
14	0.0031	0.0086	0.0021	0.0356
35	0.0009	0.0345	0.0025	0.3045
56	0.0010	0.3135	0.0021	0.2742

Blueberry ground cover at Dalhousie Mountain POST trials in 2012 was approximately 30 percent 11 days before spraying (Figure 2.12A). Blueberry ground cover exceeded 50% in all treatments on day 35, 53 and 65, except cloransulam-methyl with average of 40% (Figure 2.12A). However, there were no significant differences in blueberry ground cover among treatments (Table 2.10). Also, there was no significant difference in broadleaf ground cover among treatments (Table 2.10). All the treatments had less than 20% broadleaf ground cover (Figure 2.12B). Grass ground cover did not differ significantly on day -11 and was significantly different on day 35 and 56 (Table 2.10). However, none of herbicides had less grass ground cover than control treatments (Figure 2.12C).

There was no significant difference among treatments in most ground cover of Collingwood POST trials in 2012 (Table 2.10). Cloransulam-methyl, pyroxsulam and rimsulfuron/nicosulfuron had slightly higher blueberry ground cover than control

treatments (Figure 2.13A). For broadleaf ground cover, cloransulam-methyl treatments had lower broadleaf ground cover compared to control treatments (Figure 2.13B). Treatments sprayed with herbicides had similar or higher grass ground cover than control treatments (Figure 2.13C).
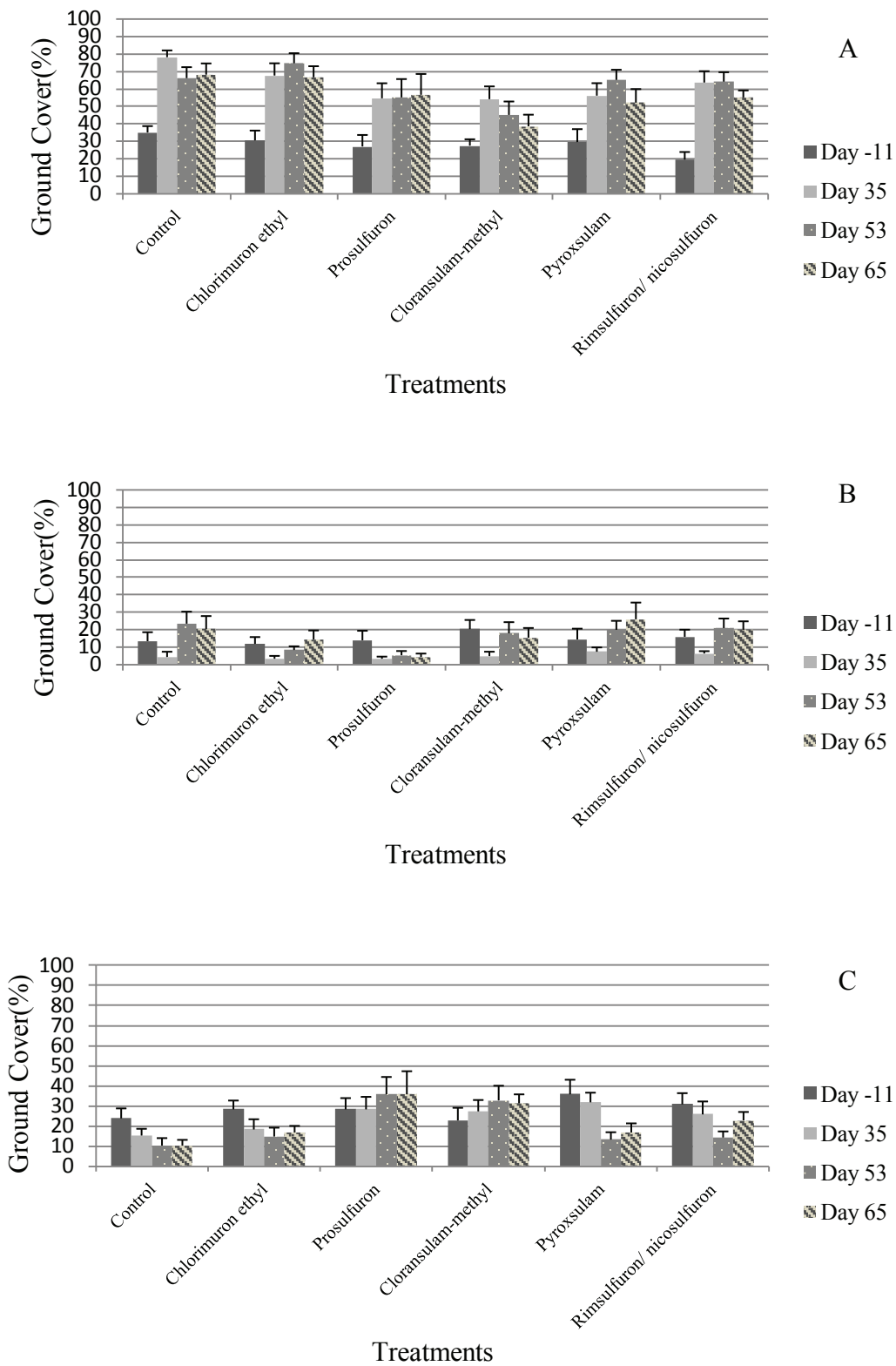
Figure 2.12. Ground covers of blueberry (A), broadleaf (B) and grass (C) at Dalhousie Mountain, Nova Scotia in 2012.
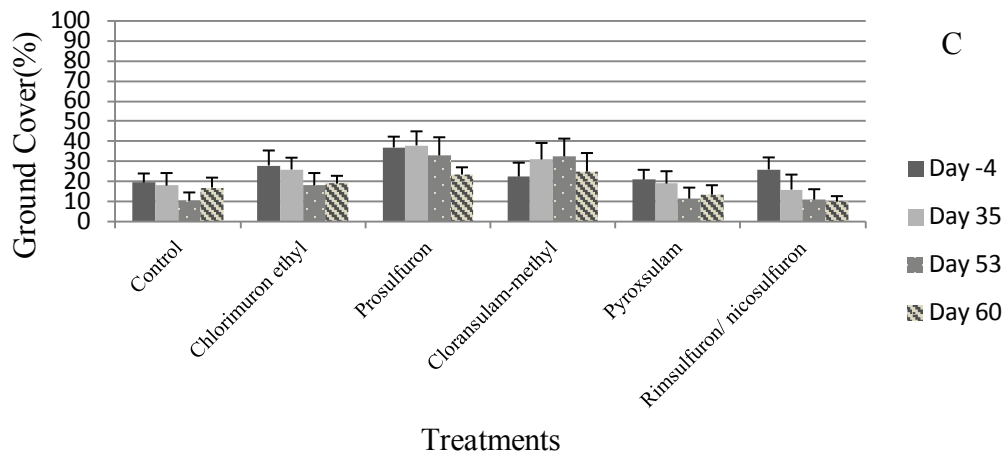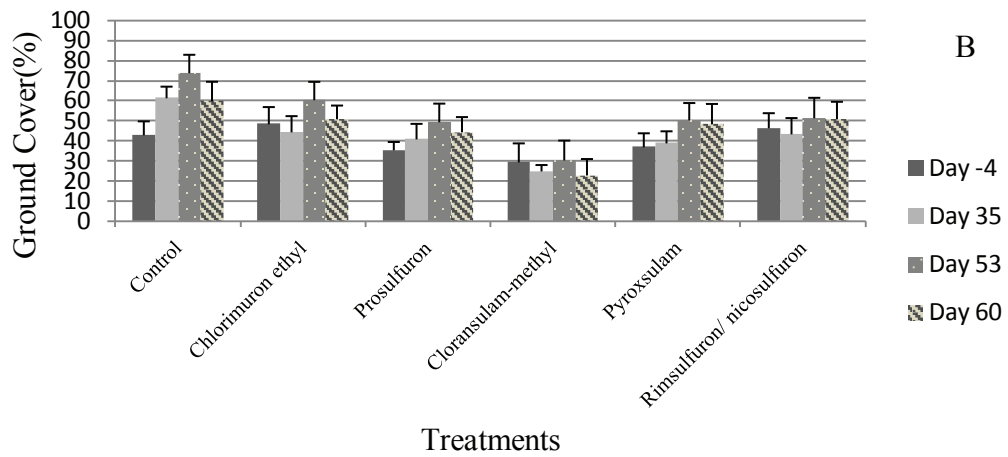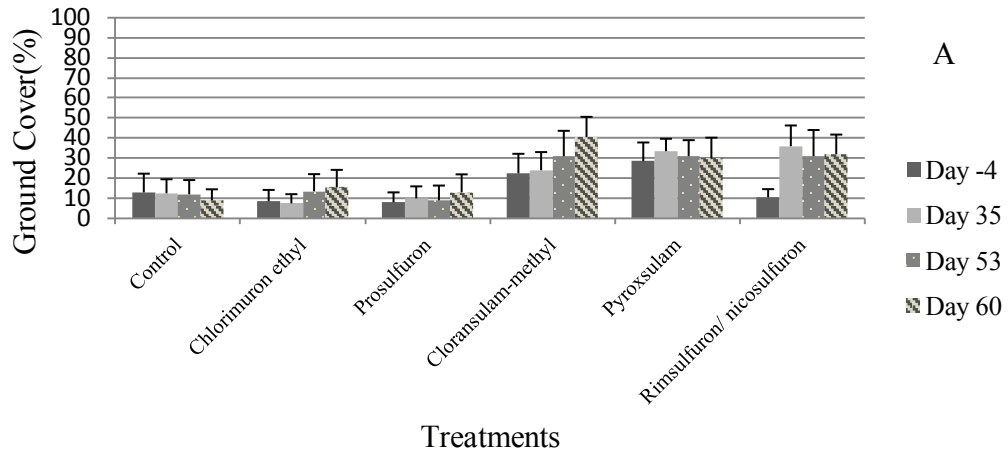
Figure 2.13. Ground covers of blueberry (A), broadleaf (B) and grass (C) at Collingwood, Nova Scotia in 2012.

Table 2.10. The P>χ² values of ground cover in POST trials in Dalhousie Mountain and Portapique, Nova Scotia in 2012.

	Dalhousie Mountain			Collingwood		
Date	Blueberry	Broadleaf	Grass	Blueberry	Broadleaf	Grass
Before spray	0.2565	0.6803	0.5739	0.1492	0.3842	0.2332
35	0.1269	0.4761	0.2076	0.0353	0.0200	0.2039
53	0.1309	0.0501	0.0498	0.0986	0.0528	0.0630
60	0.0657	0.0978	0.0380	0.0914	0.0877	0.1913

No significant differences among treatments were shown in the biomass of 2011 POST trials, except the biomass of blueberry at Dalhousie Mountain in 2011 was significantly different among treatments (Table 2.11). Prosulfuron and pyroxsulan tended to have less broadleaf biomass than control treatments in all sites (Table 2.11). Rimsulfuron+nicosulfuron had significantly lower grass biomass than other treatments at the Collingwood 2012 site (Table 2.11). Although not significantly different among treatments, broadleaf biomass in some of the herbicides treatments tended to match the damage ratings.

Table 2.11. POST herbicides trials biomass, Nova Scotia in 2011 and 2012.

Year	Treatments	Dalhousie Mountain			Portapique		
		Blue-berry	Broad-leaf	Grass	Blue-berry	Broad-leaf	Grass
		g m^{-2}					
2011	Control	245.2 ab	50.8 a	49.2 a	34.0 a	173.2 a	7.6 a
	Chlorimuron	274.0 a	28.8 a	42.8 a	27.2 a	146.0 a	4.8 a
	Prosulfuron	258.0 ab	4.9 a	41.6 a	21.2 a	97.2 a	10.0 a
	Choransulan-methyl	163.6 b	7.3 a	36.8 a	41.2 a	166.4 a	5.6 a
	Pyroxsulan	219.6 ab	6.1 a	10.4 a	73.2 a	93.6 a	6.4 a
	Rimsulfuron+nicosulfuron	285.6 a	18.2 a	8.8 a	16.4 a	133.6 a	5.2 a
	P-value	0.0139	0.2179	0.0984	0.0695	0.2492	0.4518

Year	Treatments	Dalhousie Mountain			Collingwood		
2012	Control	318.4 a	62.8 a	117.2 a	15.2 a	138.0 a	45.2 ab
	Chlorimuron	234.4 ab	14.8 a	102.8 a	29.2 a	106.4 a	55.6 ab
	Prosulfuron	146.8 b	27.8 a	146.0 a	24.0 a	90.8 a	65.5 a
	Choransulan-methyl	133.2 b	21.6 a	131.2 a	74.8 a	66.0 a	62.4 a
	Pyroxsulan	186.0 b	50.4 a	98.0 a	50.4 a	100.8 a	31.2 ab
	Rimsulfuron+nicosulfuron	218.4 ab	51.6 a	108.2 a	62.4 a	116.8 a	21.2 b
	P-value	0.0188	0.2873	0.9381	0.3006	0.0686	0.0145

[a]Means within a column cross all treatments, followed by the same letter are not significantly different (Tukey, α=0.05).

There were no significant differences for number of floral buds per stem among treatments at two sites in 2011 (Table 2.12). The range was between 4 and 7 in 2011. The number of blueberry floral buds per stem was significantly different among treatments in 2012 (Table 2.12). Prosulfuron treatments had the highest number of floral buds at Dalhousie Mountain in 2012, but had the lowest number in Collingwood 2012 site. The control treatment had the lowest number of floral buds per stem at Dalhousie Mountain in 2012, but it had second highest number at Collingwood in 2012. Also, the numbers of floral buds per stem were similar in 2011 (Table 2.12). In conclusion, these herbicides did not impact the number of floral buds per stem.

There was no significant difference among treatments in yield data from Dalhousie Mountain in 2011 (Table 2.13). However, fairly large differences of yields were observed. Choransulan-methyl had the lowest blueberry yield and 132 g m^{-2} lower than rimsulfuron+nicosulfuron yield (Table 2.13).

Table 2.12. Number of blueberry floral buds per stem in Nova Scotia fields in 2011 and 2012.

Year	Treatments	Dalhousie Mountain	Portapique	Collingwood
2011	None	6 a	4 a	-
	Chlorimuron	6 a	6 a	-
	Prosulfuron	6 a	4 a	-
	Choransulan-methyl	5 a	4 a	-
	Pyroxsulan	6 a	6 a	-
	Rimsulfuron+ nicosulfuron	7 a	4 a	-
	P-value	0.3430	0.2565	-
2012	None	3.9 c	-	4.6 ab
	Chlorimuron	6.3 ab	-	2.8 bc
	Prosulfuron	7.7 a	-	2.2 c
	Choransulan-methyl	4.5 bc	-	4.7 a
	Pyroxsulan	4.6 bc	-	3.1 abc
	Rimsulfuron+ nicosulfuron	5.6 bc	-	4.5 a
	P-value	0.0004	-	0.0230

[a]Means within a column and site, followed by the same letter are not significantly different (Tukey, $\alpha=0.05$).

Table 2.13. Yields of blueberry in POST trial Dalhousie Mountain, Nova Scotia in 2011.

Treatments	Dalhousie Mountain
	g m^{-2}
None	140 a
Chlorimuron	133 a
Prosulfuron	158 a
Choransulan-methyl	83 a
Pyroxsulan	136 a
Rimsulfuron+nicosulfuron	215 a
P-value	0.7062

[a]Means within a column cross all treatments, followed by the same letter are not significantly different (Tukey, α=0.05).

2.4 Conclusions

Hexazinone controlled a range of broadleaf weeds. Hexazinone mixtures of rimsulfuron/nicosulfuron, pyroxsulam or indazaflam had significantly lower broadleaf biomass at Dalhousie Mountain PRE site. Terbacil(WDG) and its mixtures provided a better grass control than hexazinone. Hexazinone+rimsulfuron/nicosulfuron may be an alternative for hexazinone. None of the tested sulfonylurea herbicides controlled the weeds. Only narrow-leaf goldenrods were affected by prosulfuron, chlorimuron and choransulan-methyl.

Chapter 3.0 Hexazinone Resistance in Red Sorrel

Abstract

Repeated applications of hexazinone have been sprayed in most wild blueberry fields in Nova Scotia for more than 30 years and this practice may have resulted in hexazinone-resistant weeds. The recommend rate of hexazinone no longer controls red sorrel (*Rumex acetosella* L.) in some locations. Six levels of hexazinone (0, 0.48, 0.96, 1.92, 3.84, 7.68 kg ai ha^{-1}) were applied to experimental pots to determine if red sorrel from 4 different sites was resistant to hexazinone. Red sorrel from Debert and Collingwood sites died at the 0.96 kg ai ha^{-1} rate of hexazinone; red sorrel from Recreation Park and Dalhousie Mountain sites survived at 7.68 kg ai ha^{-1}. It is concluded that red sorrel is hexazinone-resistance in some wild blueberry fields. A portion of the *psb*A gene was sequenced and it was determined that resistance was caused by a Phe$_{255}$ to Val mutation in D1 protein.

3.1 Introduction

Weeds compete for resources with blueberries and cause reduced yields and quality (Kennedy et al. 2010). *Rumex acetosella* L., a common weed in blueberry fields, has several common names, including red sorrel, sheep's sorrel, sour weed and field sorrel. It is a perennial weed that reproduces by creeping roots and seeds, and is native to Eurasia (Love 1983). At present, it is widespread in North America. Red sorrel is considered to be a harmful weed in Nova Scotia due to its rapid reproduction and spread in blueberry fields. Hexazinone, a group 5 herbicide, widely sprayed in blueberry fields, controls population of sheep sorrel, but tolerant populations have been reported (McCully et al. 2005; Kennedy et al. 2010).

Group 5 herbicides inhibit electron flow in photosystem II. This group includes triazines, triazinones, uracils, phenyl-carbamates, ureas, amides, and nitriles (Hess 2000). The mode of action of these herbicides is plastoquinone-binding point on the D1 protein in the PS II reaction center of the photosynthetic electron transport chain that will be bound by group 5 herbicides. The electrons from Q_A to Q_B are blocked, so that photosynthesis will lack NADPH for CO_2 fixation (Gardner 1981). It results in photo-oxidation of membrane lipids which then leads to plant death (Shukla and Devine 2008).

Triazine resistance is caused by modifications of the herbicide binding site or the target protein (D1 protein) (Devine and Eberlein 1997). The chloroplast *psb*A gene, a

maternally inherited gene, encodes the D1 protein in higher plants (Perez-Jones et al. 2009). The *psb*A gene is the gene where the triazine resistance gene mutation occurs. In most cases, triazine-resistance is caused by a Ser_{264} to Gly mutation in the *psb*A gene that alters the conformation of the Q_B and herbicide binding niche (Fuerst and Norman 1991; Trebst 1996). Ser_{264} to Gly mutation decreases the binding of s-triazine and triazinones to this site (Devine et al. 1993). Other mutations at different positions can also cause resistance to triazine or triazinone herbicides, such as Phe_{211}, Val_{219}, Ala_{251}, Gly_{256}, Ser_{268} and Leu_{275} (Trebst 1991; Devine and Eberlein 1997; Shukla and Devine 2008). Amino acid changes in the range of positions 211 to 275 were conferred in many herbicide resistant organisms (Trebst 1991). The herbicide-binding affinity is reduced by the changes in amino acid residues in the Q_B-binding niche on the D1 protein (Devine et al 1993; Gronwald 1994). Mutations at or close to position Ser_{264}, Phe_{265}, Phe_{255}, and His_{215} have a great effect on the binding of PS II herbicides and are also important in the development of resistance (Devine and Eberlein, 1997). However, the amino acids in D1 protein which are bound by group 5 herbicides are not always the same (Trebst 1991). Hexazinone may have different binding sites in the D1 protein. In addition, Ser_{264} to Gly mutation in the *psb*A gene decreases the photosynthetic capacity of resistant plants (Shukla and Devine 2008). The rate of electron transfer between Q_A and Q_B in resistant plants was lower than in susceptible plants (Bowes et al. 1980).

Hexazinone is repeatedly applied for weed control in wild blueberry, at a

recommended rate between 1.44 and 1.92 kg ai ha^{-1}. Preliminary experiments conducted by Agriculture and Agri-Food Canada showed that many grassy weeds were already hexazinone-tolerant, including bluegrasses and fescues (McCully et al. 2005). It is important to control red sorrel to reduce the yield and quality losses of wild blueberry. However, little information is published on red sorrel populations in lowbush blueberry fields. Also, many farmers would be interested in a simple bioassay method which can determine whether hexazinone-resistant weed is shown in their fields.

The objectives of the experiments were to 1) verify the existence of hexazinone resistant red sorrel in wild blueberry fields in Nova Scotia; 2) verify whether hexazinone-resistant red sorrel is caused by caused by Ser$_{264}$ to Gly mutation in *psb*A gene; 3) develop a simple bioassay method that can determine the effect of hexazinone metabolites on red sorrel leaves and be used to distinguish resistant and susceptible genotypes.

3.2 Materials and Methods

3.2.1 Hexazinone Resistant Experiment

Red sorrel seeds were hand-collected from four different sites in late August 2012 from a non-blueberry area (Recreation Park in Truro, NS), a relatively new blueberry field near a mature, established field (Debert, NS), and two mature commercial blueberry fields (Collingwood, NS and Dalhousie Mountain, NS). The hypothesis of this

experiment was that there was no resistance in non-blueberry areas, minimum resistance in a new blueberry field and hexazinone resistance in mature blueberry fields. Seeds were handpicked and stored at 5 C for one month.

Seeds were germinated on filter paper in Petri dishes. Filter papers were moistened with 5 ml of 2% KNO_3. All Petri dishes were placed in a greenhouse at 25-20 C. After 1 week, the seeds germinated. Four plants were transferred into cell packs with 1:2 peat moss: top soil. Plants were grown in the greenhouse under 25-20 C day-night temperatures with a 14-h photoperiod without supplemental lighting for 5 weeks.

Experiments were set up as a randomized complete block design (RCBD) with four blocks. Treatments included six different hexazinone rates, four replications and the experiment was repeated once. After 5 weeks, plants were sprayed with hexazinone at 0, 0.25X, 0.5X, 1X (1.92 kg ai ha^{-1}), 2X and 4X rates, where the 1X rate was recommended hexazinone rate to be sprayed in blueberry fields (Table 3.1). All hexazinone treatments were applied using a hand-held boom with CO_2 pressurized sprayer at 275.8 kPa pressure with XR8002VS Teejet nozzle.

Hexazinone efficacy on red sorrel (damage ratings) was recorded 7, 14, 22 and 27 DAS using a standard damage rating scale. The damage rating scale was from 0 to 10. Zero meant no damage and ten meant all above ground red sorrel shoots were killed. Four red sorrel plants in each pot were compared and the average rates were recorded.

At the end of experiment, red sorrel above ground tissue was collected. All dead red

sorrel or dead leaves were removed. In addition, not all four red sorrel plants per plot were collected for biomass. One red sorrel plant was left intact if there were four red sorrel in a pot; all the red sorrel plants were collected if there were less than four red sorrel. The intact plants were later utilized for *psb*A gene sequencing and leaf bioassay experiments. Shoot biomass was harvested 27 DAS, dried at 60 C for 4 days, and weighed. Since numbers of red sorrel per pot were different, average red sorrel biomass per plant was recorded.

3.2.2 *psb*A Gene Sequencing

Total DNA was extracted from red sorrel leaf tissue from plants that remained following the hexazinone resistance experiment. DNA was extracted using Fujifilm QuickGene DNA tissue kit S and model QuickGene-810 according to the instructions outlined by the manufacturer in the plant tissue protocol (Fujifilm, no date). Each sample included two leaf disks from one red sorrel plant. Leaf tissue was disrupted and homogenized in lysis buffer using the MicroSmash bead disruptor (Fujifilm, no date). Genomic DNA extractions from red sorrel were followed by genomic DNA extraction from plants shown in QuickGene Series Application Guide (Fujifilm, no date). Each DNA sample was electrophoresed on a 1% agarose gel and a NanoDrop 1000 Spectrophotometer was used to measure DNA concentration and to verify DNA quality.

The *psb*A gene sequences from other plants were obtained including: *Poa* (GenBank

accession AF131887), *Zea* (AF543684), *Hordeum* (X07 521), *Prunus* (AF410200), *Amaranthus* (AY336 946, K01200) and *Ragweed* (AB427162 in DDBJ database) (Tian and Darmency 2006; Cseh et al. 2009). Gene sequences for *psb*A were found in NCBI, imported into Sequencher v. 4.7 (Gene Codes Corporation, MI, USA) and aligned. Primers were designed in conserved regions where nucleotides matched perfectly in these five plant species. The two external primers *psb*AF(5'-CCTCCAGTAGATATTGATGGATTCG), *psb*AR(5'-TGAGCATTACGTTCGTGCAT) were designed. Primers were designed to amplify a 845 bp fragment, which includes mutation target sites. These primers were also used for sequencing.

PCR amplification reactions contained 50-75 ng of template DNA, 1X concentration of manufacturer supplied buffer, 1.5 mM MgCl$_2$, 0.2 mM dNTPs, 1 unit of Taq DNA polymerase (Promega[®] GoTaq), 1 μM of each primer and 7 μl nuclease-free water in a final volume of 20 μl. PCR thermal cycling was carried out in Dyad Disciple (BioRad[®]) with the following conditions: 1 cycle at 95 C for 5 min, followed by 35 cycles of 20 sec denaturation at 95 C, annealing at 58 C for 15 sec and extension for 1 min 30 sec at 72 C. A final extension was applied for 10 min at 72 C. The PCR products were separated on a 1.2% agarose gel and were cleaned up with Qiagen PCR spin columns. Purified DNA was sequenced using an ABI 3130 nucleic acid analyzer and an ABI Big Dye Terminator Kit v. 3.1. Sequencing products were cleaned-up using a standard Sodium Acetate/

EDTA/ Ethanol Precipitation and separated by capillary electrophoresis.

Sequencing was performed on 12 hexazinone resistant and 12 susceptible samples which remained from the hexazinone resistant experiment. Five and seven resistant red sorrel leaves were from Debert and Collingwood, respectively. Eight and four susceptible red sorrel leaves were from Recreation Park and Dalhousie Mountain, respectively. Sequences were imported into Sequencer and aligned to *psb*A from other species. The nucleotide sequences of resistant and susceptible plants were compared.

3.2.3 Leaf Bioassay

Plants utilized in the hexazinone resistant experiment were placed in a 6 C growth chamber for 3 months and then the temperature was adjusted to 10 C followed by 15 C for 2 days at each temperature. The plants were then transferred to a greenhouse where temperatures were 25-20 C day-night with a 14-h photoperiod without supplemental lighting.

Many techniques were tested to identify a procedure that readily identified resistant plants. First, the floating segments method by Sung et. al (1985) was evaluated but both resistant and non-resistant red sorrel leaves sunk to the bottom. We concluded that this technique did not work for red sorrel. Second, whole leaves were dipped in 0.0128 g ml^{-1} hexazinone for 3 sec and then placed in plastic water picks which were filled with water. This technique also failed and all red sorrel leaves died. For the third experiment,

two drops of 0.0128 g ml^{-1} hexazinone was placed on the leaf as described below. Eight resistant (Debert) and eight non-resistant (Dalhousie Mountain) healthy red sorrel leaves, including petiole, were used in each repeat. Resistant leaves were all collected from Debert and non-resistant leaves were collected from Dalhousie Mountain. There were two treatments and four replications. Treatments included two drops of water as control and two drops of 0.0128 g ml^{-1} hexazinone. Experiments were repeated five times. Leaves were set on plastic water picks which were filled with water. For 0.0128 g ml^{-1} hexazinone treatments, two drops of 0.0128 g ml^{-1} hexazinone solution were applied to one leaf using a 10 ml syringe. No more than 1 ml per leaf was applied.

Damage ratings were measured 1, 2, 3 and 4 days after the hexazinone application using a standard damage rating scale 0-10. Zero meant no damage and ten meant red sorrel shoots leaves were dead.

Light intensity was measured using Advanced Light Meter (Sper Scientific Ltd., AZ, USA).

3.2.4 Statistical Analysis

All damage ratings and biomass data were analyzed using Proc Npar1way (Kruskal-Wallis test) in SAS v. 9.3 (SAS Institute Inc., Cary, NC) because of lack of homogeneous variance. All analyses were considered significant at $\alpha=0.05$.

Biomass of control treatments among sites were analyzed using PROC MIXED in

SAS v. 9.3. Sites were fixed effects and blocks were random effects. Least squares means

comparisons of Tukey's test were used to test for site differences at α=0.05.

Dose-response curves were obtain by nonlinear regression using the log-logistic

equation (Seefeldt et al. 1995): $y=C+(D-C)/[1+(x/LD50)^b]$ where y represents biomass at

herbicide rate x, C is the mean response at high level hexazinone rate, D is the mean

response when the herbicide rate is zero, b is the slope of the line at LD50, and LD50 is

the herbicide rate required for 50% growth reduction. The curved was plotted in

SigmaPlot v. 12.1 (Systat Software Inc., Chicago, USA). Most of the plants from

Recreation Park and Dalhousie Mountain died and had zero biomass and as a result it was

not possible to construct dose response curves for those sites.

The difference of LD50 value from Debert and Collingwood biomass was analyzed

by 2 samples t-test in Minitab v. 16 (Minitab Inc., PA, USA).

3.3 Results and Discussion

3.3.1 Hexazinone Resistant Experiments

The Recreation Park is a non-blueberry area, Debert is a relatively new blueberry

field, and Dalhousie Mountain and Collingwood are two mature commercial blueberry

fields. Greenhouse experiments were conducted to determine if red sorrel from four sites

were hexazinone resistant. The red sorrel damage ratings from four sites were all

significantly different among treatments ($P<0.0001$). The plants from the Recreation Park

and Dalhousie Mountain were similar (Figure 3.1A and B) but different from the red sorrel from Debert and Collingwood that were similar to one another (Figure 3.1C and D).

Damage tended to linearly increase as hexazinone rate increased 7 DAS (Figure 3.1A). Dalhousie Mountain red sorrel had higher damage ratings than other sites 7 DAS (Figure 3.1A). The difference between sites clearly showed starting 14 DAS (Figure 3.1B). All damage ratings were close to 10 at 0.96, 1.92, 3.84 and 7.68 kg ai ha^{-1} rates of hexazinone for plants from the Recreation Park and Dalhousie Mountain 14 DAS. This means that even 0.96 kg ai ha^{-1} hexazinone killed red sorrel from these two sites (Figure 3.1B). Plants from the Recreation Park and Dalhousie Mountain sites were susceptible to rates as low as 0.48 kg ai ha^{-1}. We concluded that plants from the Recreation Park and Dalhousie Mountain were not resistant to hexazinone. The damage rating 27 DAS for Debert and Collingwood plants was only 4 (Figure 3.1D). Even 7.68 kg ai ha^{-1} hexazinone did not kill red sorrel from Debert and COllingwood 27 DAS (Figure 3.1D). The highest damage rating in 7.68 kg ai ha^{-1} hexazinone treatments on day 27 was approximately 6, which meant Debert and Collingwood red sorrel survived under 7.68 kg ai ha^{-1} hexazinone (Figure 3.1D). Red sorrel populations from Debert and Collingwood were resistant to hexazinone but plant growth was suppressed at higher rates.

A

B

C

D
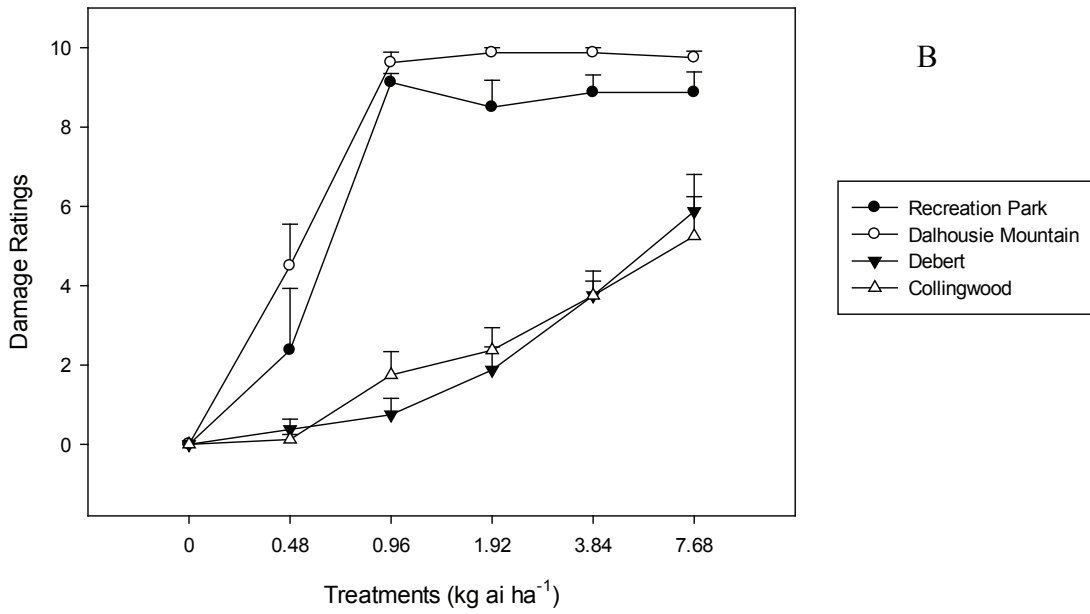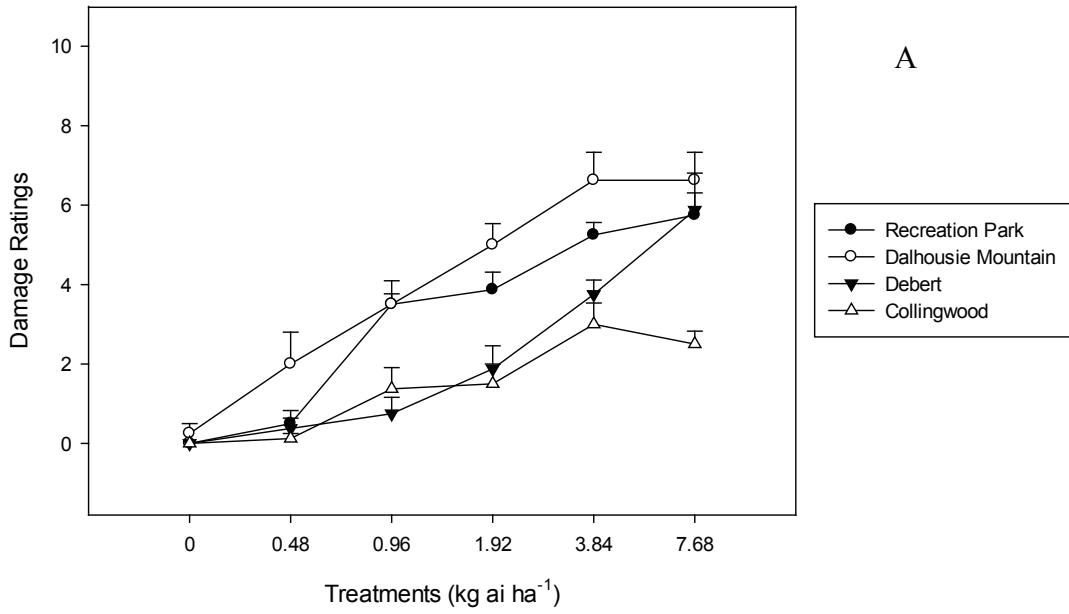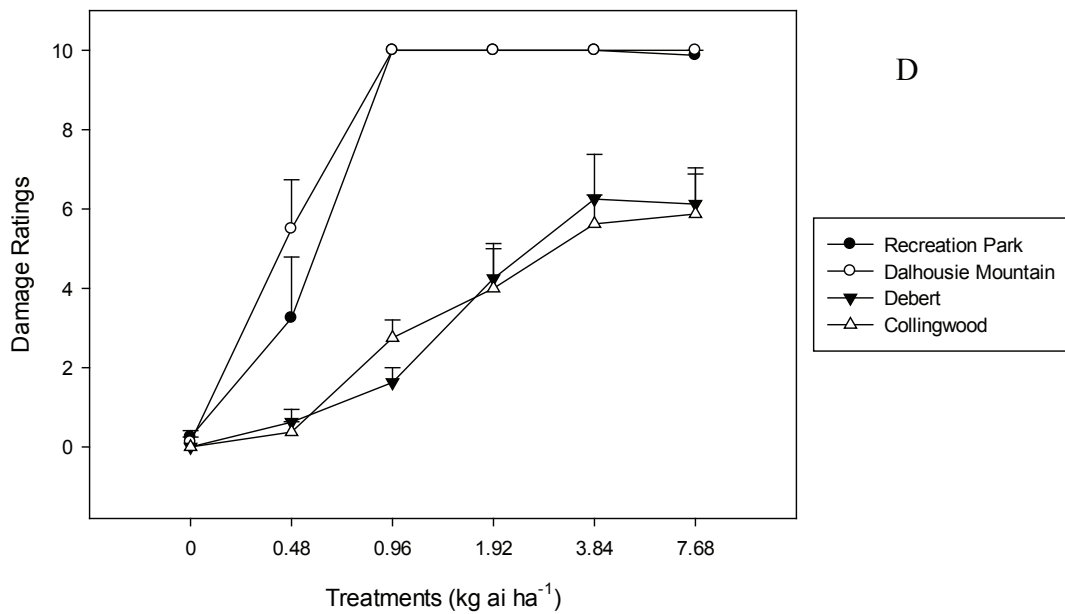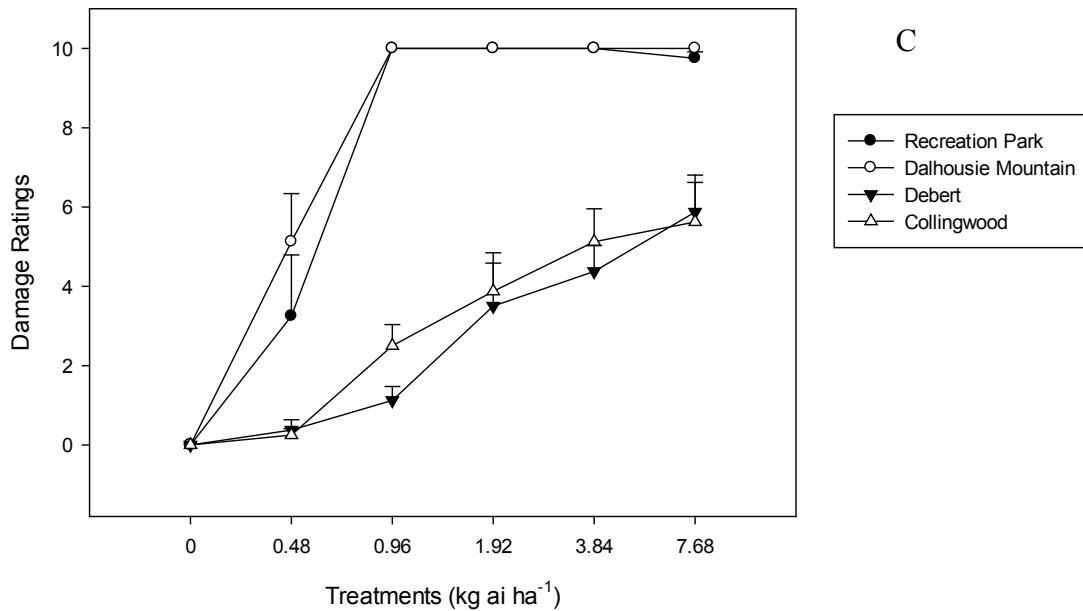
Figure 3.1. Red sorrel damage ratings of 4 different sites on 7 DAS (A), 14 DAS (B), 22 DAS (C), 27 DAS (D).

Biomass differed significantly among plants from the four sites (P<0.001). At 0.48 kg ai ha^{-1} (0.25X) rate of hexazinone, red sorrel biomass of plants from Debert and

Collingwood decreased by only 3% and 14%, respectively (Figure 3.2). Conversely, biomass decreased by 48% and 62% for red sorrel from the Recreation Park and Dalhousie Mountain, respectively (Figure 3.2). All red sorrel plants from Recreation Park and Dalhousie Mountain died when exposed to the 0.96 to 7.68 kg ai ha^{-1} rate of hexazinone and the living biomass was zero. The biomass of plants from Debert and Collingwood sites declined as the rate of hexazinone increased and the biomass from Debert and Collingwood was 14% and 13% of the untreated control at the 7.68 kg ai ha^{-1} rate of hexazinone (Figure 3.2). The LD50 value for hexazinone dose was similar between sites at 1.0433($\pm$0.1293) kg ai ha^{-1} and 0.9071($\pm$0.0808) kg ai ha^{-1} for Debert and Collingwood, respectively. They are not significantly different (P=0.8070). Biomass was not significantly different between the Debert and Collingwood sites (P=0.4429). Data from the Recreation Park and Dalhousie Mountain could not be analyzed due to the number of dead plants. However, Debert and Collingwood biomass was significantly higher than Recreation Park and Dalhousie Mountain (P<0.0001). The required dose to kill 100% of plants from Recreation Park and Dalhousie Mountain red sorrel was only 0.96 kig ai ha^{-1} rate of hexazinone.
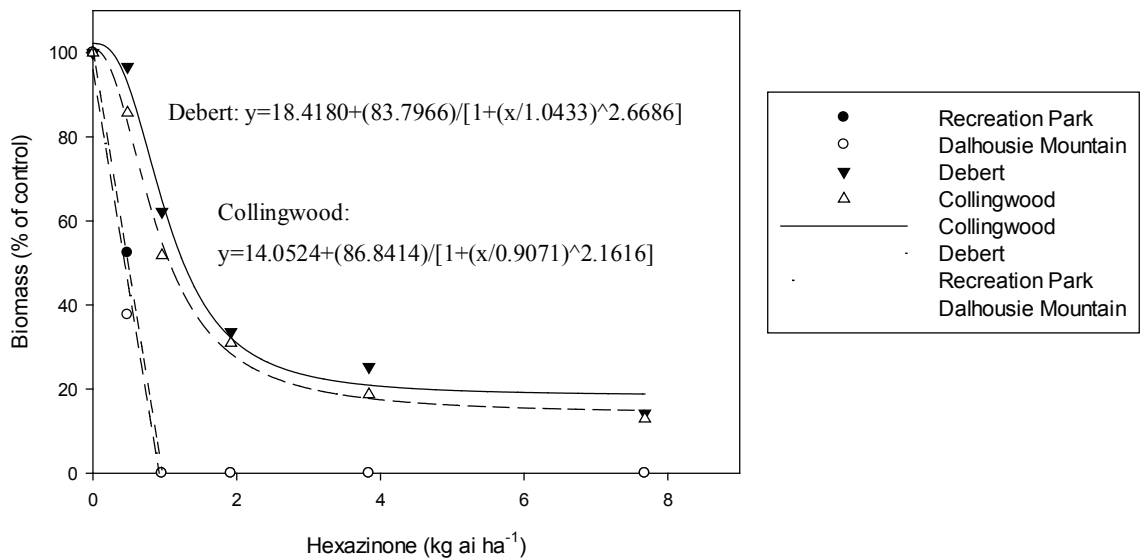
Figure 3.2. Percentage red sorrel biomass of four sites as affected by different rates of hexazinone. Symbols and lines represent actual and predicted growth responses, respectively.

Red sorrel survived 4X rate of hexazinone in Debert and Collingwood sites and red sorrel from Recreation Park and Dalhousie Mountain died at 0.5X or higher rates of hexazinone (Figure 3.1). The declines of red sorrel biomass for Debert and Collingwood sites were much slower than that of Recreation Park and Dalhousie Mountain (Figure 3.2). Thus, we can say that red sorrel from the Recreation Park and Dalhousie Mountain sites were hexazinone susceptible and red sorrel from the Debert and Collingwood were hexazinone resistant. The control biomass mean for Recreation Park, Dalhousie Mountain, Debert and Collingwood red sorrel were 0.16, 0.13, 0.19 and 0.19 g per plant, respectively. There was no significant difference for control treatment biomass among sites (P=0.6459). Thus, it seemed that hexazinone resistance did not affect the fitness of red sorrel plants.

3.3.2 *psb*A Gene Sequencing

Studies of hexazinone resistance were conducted on several resistant and non-resistant plants which remained from hexazinone resistant experiments. Most triazine resistant plants have a single gene mutation at the 790 position of the *psb*A gene, which corresponds to a Ser_{264} to Gly mutation (Jia et al. 2007). The primer pair *psb*AF/*psb*AR was designed to amplify an 845 bp fragment of *psb*A gene which includes the nucleotide and deduced amino acid sequence coding for the Q_B binding niche of the D1 protein. Agarose gel test showed the designed primers amplified a portion of the red sorrel *psb*A gene and PCR products had around 845 bp (Figure 3.3). Both resistant and non-resistant red sorrel *psb*A gene PCR products produced clean, defined bands in agarose gel electrophoresis (Figure 3.3). The concentrations of PCR products are provided in Appendix 3.
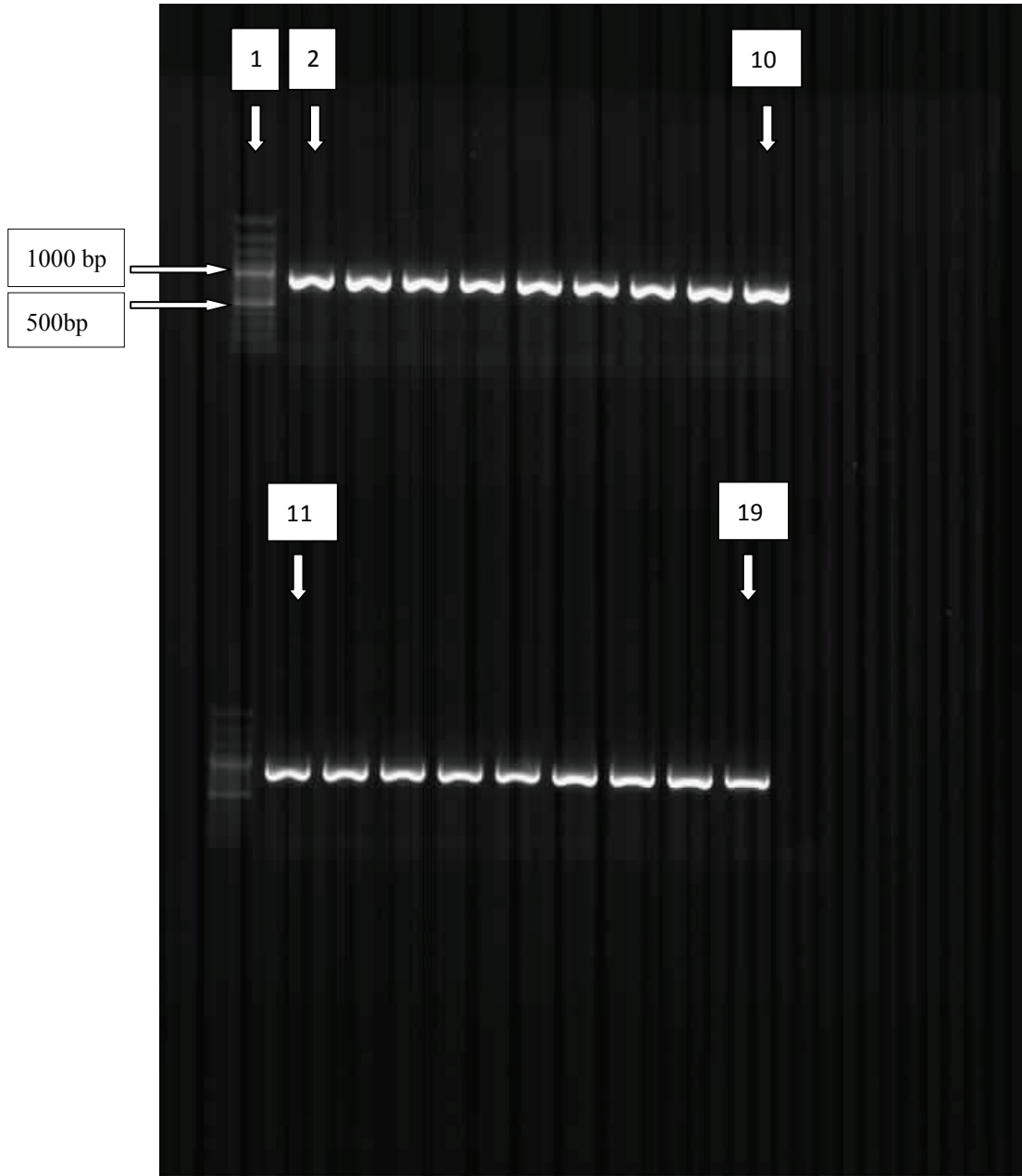
Figure 3.3. Agarose gel test of eighteen red sorrel *psb*A PCR samples with GeneRuler[TM] 100bp DNA Ladder Plus (Fermentas).
[a]Line 1 contains GeneRuler[TM] 100bp DNA Ladder Plus.
[b]Lanes Y through X contain PCR amplified products from line 2 to line 19.

Figure 3.4 includes part of the red sorrel *psb*A gene sequence around the target site which is mutated in other resistant plants. The amino acid sequence is between 211 and 275 in the Q_B binding niche. In most cases, the Ser_{264} (AGT) in susceptible plants is mutated to Gly (GGT) when plants become triazine resistant (Tian and Darmency 2006). However, Ser_{264} in resistant red sorrel was not changed (Figure 3.4). Triazine resistant red sorrel may be caused by another gene mutation. Hexazinone resistance in shepherd's-purse was caused by Phe_{255} (TTT) to Ile (ATT) mutation (Perez-Jones et al. 2009). Binding of hexazinone to the D1 protein was significantly reduced by Phe_{255} to Ile mutation (Perez-Jones et al. 2009). All nucleotides in the amplified sequence were compared. Phenylalanine (TTT) was shown in all susceptible red sorrel gene sequences at position 255. However, all the resistant plants had a codon GTT at this position, which codes for the amino acid Valine. Therefore, the amino acids were different between resistant and susceptible at position 255 of red sorrel *psb*A gene product. This mutation may decrease the binding of hexazinone, since the mutation in this position affected the binding of triazine herbicides in the Q_B binding site on D1 protein (Gronwald 1994). Hexazinone efficacy was deceased by Phe_{255} to Ile mutation and it may be caused by removing the phenyl ring of Phe (Perez-Jones et al. 2009). Val has similar structure as Ile, which also removes the phenyl ring of Phe. Thus, hexazinone efficacy was decreased by Phe_{255} to Val mutation in red sorrel. To sum up, red sorrel from Debert and Collingwood that are resistant to hexazinone are due to Phe_{255} to Val mutation.

No other difference was shown between resistant and susceptible red sorrel of the amplified sequence of the partial *psb*A gene, except Recreation Park red sorrel contained Asn_{230} (AAT) and the other three sites red sorrel had Asn_{230} (AAC). However, this change did not change the amino acid. This result may be due to the site population difference. In addition, partial nucleotide alignment of the *psb*A gene of resistant red sorrel from 234-947 was shown (Appendix 4).

628 CTA TTC AGT GCT ATG CAT GGT TCT TTG GTA ACC TCT AGT TTG ATC   S

     …   …   …   …   …   …   …   …   …   …   …   …   …   …   …   R

210   L   F   S   A   M   H   G   S   L   V   T   S   S   L   I

673 AGG GAA ACC ACA GAA AAC GAA TCT GCT AAT GAA GGT TAC AGA TTT   S

     …   …   …   …   …   …   …   …   …   …   …   …   …   …   …   R

225   R   E   T   T   E   N   E   S   A   N   E   G   Y   R   F

718 GGT CAA GAG GAA GAA ACT TAT AAT ATC GTA GCT GCT CAT GGT TAT   S

     …   …   …   …   …   …   …   …   …   …   …   …   …   …   R

240   G   Q   E   E   E   T   Y   N   I   V   A   A   H   G   Y

763 TTT GGC CGA TTA ATC TTC CAA TAT GCT AGT TTC AAC AAT TCT CGT   S

   GTT   …   …   …   …   …   …   …   …   …   …   …   …   …   R

255 ==P/V==   G   R   L   I   F   Q   Y   A   ==S_{264}==   F   N   N   S   R

808 TCT TTA CAT TTC TTC TTA

     …   …   …   …   …   …

270   S   L   H   F   F   L

Figure 3.4. Partial red sorrel *psb*A nucleotide sequence (1st and 2nd row) and deduced amino acid sequence (3rd row) alignment of the *psb*A gene of hexazinone-susceptible (S) and hexazinone-resistant (R) red sorrel populations. The region shown includes the amino acid sequence of the Q_B binding niche of the D1 protein of PS II (210-275). Shaded amino acids indicate a Phe (TTT) to Val (GTT) substitution at position 255 in the R population. There is no difference between R and S at positon 264 Ser (AGT).

3.3.3 Leaf Bioassay

There were no significant differences among treatments within resistant red sorrel damage ratings (Table 3.1). The damage ratings of susceptible red sorrel were significantly different among treatments (Table 3.1). In addition, the damage ratings of susceptible red sorrel were significantly higher than resistant red sorrel at 0.0128 g ml^{-1} rate of hexazinone treatments (P=0.0421).

Some damage occurred in the control treatments (Table 3.1). It may due to high temperatures in greenhouse, since the temperatures in greenhouse reached more than 30 C occasionally. Hexazinone caused irregular yellow patches on plant leaves in the beginning, and then the leaves turned dark brown and died. No damage was shown on resistant red sorrel leaves 3 DAS and 60% of leaves turned yellow with burned-like symptoms on susceptible red sorrel leaves 3 DAS (Figure 3.5). It was easy to tell the difference between these two genotypes. However, this technique is related to weather. Leaves did not survive in high temperatures and quickly wilted and died, which affected the observation of herbicide damage ratings. One repeat was done in a growth chamber that resulted in no damage to both resistant and susceptible red sorrel leaves. It may be due to not enough light supply in the growth chamber. This result was not included for analysis. Two growth chambers were used. The light intensity in one growth chamber was 2860 lux and the other one had 6400 lux with fluorescent lamp. However, both growth chambers were much lower than greenhouse light intensity. The light intensity in

the greenhouse was about 55900 lux, which was nine times higher than the growth

chambers light intensities.

Table 3.1. Final damage ratings for non-resistant and resistant red sorrel leaves.

Treatments	Dalhousie Mountain (S)	Debert (R)
0	1.65 ($\pm$0.61)[a]	1.30 ($\pm$0.50)
1X	5.8 ($\pm$0.83)	2.85 ($\pm$0.87)
Pr>χ^2	0.0005	0.1817

[a]$\pm$ state the stand error of means.

(A)   (B)

Figure 3.5. The symptoms of red sorrel leaf 3 DAS at 0.0128 g ml^{-1} rate hexazinone (A was from Dalhousie Mountain; B was from Debert).

3.4 Conclusion

Red sorrel from some blueberry fields were resistant to hexazinone. This resistance was due to a Phe_{255} to Val mutation in *psb*A gene. Susceptible red sorrel died at 0.96 kg ai ha^{-1} rate of hexazinone. Resistant red sorrel survived at 7.68 kg ai ha^{-1} rate of hexazinone, but hexazinone suppressed red sorrel at this rate. Resistant forms can be detected with a bioassay that includes applying two drops of 1X rate of hexazinone onto the leaf. Elementary bioassay experiments showed 0.0128 g ml^{-1} hexazinone had a significantly different effect on resistant and susceptible red sorrel leaves. However, the results were also affected by light and temperature. Further testing should be done in a consistent light and temperature environment.

Chapter 4.0 Conclusions

4.1 Overview

The main focus of this study was to find a hexazinone alternative that can be sprayed in wild blueberry fields and result in good weed control and good blueberry yield. The specific objectives were to 1) evaluate new herbicide chemistries or tank mixes that can be applied before blueberry emergence (PRE) that have modes of action different than hexazinone; 2) evaluate new herbicide chemistries that can be applied after blueberry emergence (POST); 3) determine the existence of hexazinone resistant red sorrel in wild blueberry fields. 4) verify whether hexazinone-resistant red sorrel is caused by Ser_{264} to Gly mutation in *psb*A gene; 5) develop a simple bioassay method that can determine the effect of hexazinone metabolites on red sorrel leaves and be used to distinguish resistant and susceptible genotypes.

4.2 Overall Conclusions

Screening experiments examined potential alternative herbicides of hexazinone, which can be used in PRE emergence weed control. The PRE emergence trials showed pyroxsulam, florasulam, florasulam+fluroxypyr and sulfentrazone herbicides were not effective weed management tools for wild blueberry fields. Hexazinone controlled a range of broadleaf weeds. Hexazinone mixtures of rimsulfuron/nicosulfuron, pyroxsulam or indazaflam had significantly lower broadleaf biomass than other treatments at the

Dalhousie Mountain site. Terbacil(WDG) and its mixtures provided better grass control than hexazinone. According to damage ratings and biomass, hexazinone+rimsulfuron/nicosulfuron may be an alternative to hexazinone. However, results were inconsistent between sites. It may due to the different weed species, soil structure, plant growth activity and plant density. For POST trials, none of the tested sulfonylurea herbicides controlled the weeds. Only narrow-leaf goldenrods were affected by prosulfuron, chlorimuron and choransulan-methyl.

Our results show that red sorrel plants harvested from some commercial blueberry fields are hexazinone resistant. However, resistant biotypes were still suppressed which probably explains the results Kennedy et al. (2010) found where hexazinone application resulted in yield increases even when it did not appear to control red sorrel. Most triazine-resistance is caused by a Ser_{264} to Gly mutation in *psb*A gene and hexazinone-resistance in shepherd's-purse was caused by Phe_{255} to Ile mutation in *psb*A gene. The results showed hexazinone-resistant red sorrel was not caused by Ser_{264} to Gly mutation. However, mutation of Phe_{255} to Val was found, distinguishing two resistant populations from two susceptible populations. Resistant forms can be detected with a bioassay that applies two drops of 1X (0.0128 g ml^{-1}) rate of hexazinone to leaf. Preliminary bioassay experiments showed 0.0128 g ml^{-1} rate of hexazinone had a significantly different effect on resistant and susceptible red sorrel leaves. However, the results were also affected by light and temperature. Further testing should be done in a

consistent light and temperature environment.

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Appendix 1: Observations of Damaged Goldenrod.

Appendix 2: Poverty Oat Grass Damage Ratings at Collingwood, Nova Scotia in 2012.
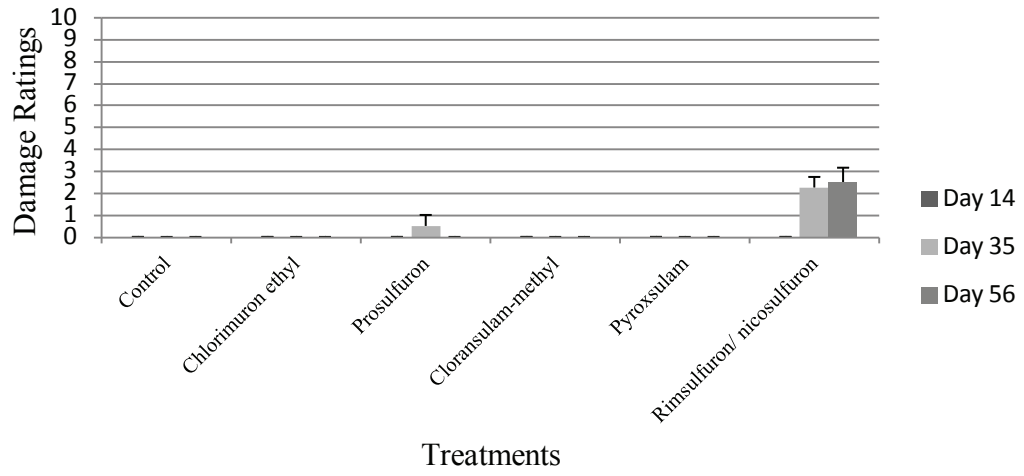
Appendix 3: Red Sorrel DNA PCR Nano Drop Data after Concentration.

Genotspes	Sample ID	Concentration (ng ul^{-1})
Resistant	44C[a]	35.30
	52C	32.23
	71C	37.51
	73C	35.60
	41C	34.94
	42C	31.76
	54C	34.46
	51D	30.67
	52D	34.88
	72D	32.06
	46D	35.24
	62D	32.79
Susceptible	82M	31.78
	51M	36.93
	61M	35.89
	41M	35.93
	72R	35.22
	41R	36.65
	31R	30.50
	42R	35.99
	12R	28.08
	11R	32.55
	81R	37.49
	21R	35.22

[a]First number is the number of replication in greenhouse experiments. Second number means the rate of hexazinone sprayed in greenhouse experiments: 1 is control; 2 is 0.25X rate; 3 is 0.5X rate; 4 is 1X rate; 5 is 2X rate and 6 is 4X rate. Letter means the site of red sorrel come from: C is Collingwood; D is Debert; M is Dalhousie Mountain and R is Recreation Park.

Appendix 4: Partial Nucleotide Alignment of the *psb*A Gene of Resistant Red Sorrel from 234-947.

CTCTGGTGCCATTATTCCTACTTCTGCAGCTATCGGTTTGCACTTTTATCCAATAT
GGGAAGCTGCATCTGTTGATGAATGGTTATACAACGGTGGTCCTTATGAGCTAA
TTGTTCTACACTTCTTACTTGGTGTAGCTTGTTACATGGGTCGTGAGTGGGAAC
TTAGTTTCCGTCTGGGTATGCGCCCTTGGATTGCTGTTGCGTATTCAGCTCCTGT
TGCGGCTGCTACTGCTGTTTTCTTGATCTACCCAATTGGTCAAGGAAGCTTTTC
TGATGGTATGCCTCTAGGAATCTCTGGTACTTTCAACTTCATGATTGTATTTCAG
GCTGAACACAACATCCTTATGCACCCATTTCACATGTTAGGCGTAGCTGGTGTA
TTTGGCGGCTCCCTATTCAGTGCTATGCATGGTTCTTTGGTAACCTCTAGTTTGA
TCAGGGAAACCACAGAAAATGAATCTGCTAATGAAGGTTACAGATTTGGTCAA
GAGGAAGAAACTTATAATATCGTAGCTGCTCATGGTTATGTTGGCCGATTAATC
TTCCAATATGCTAGTTTCAACAATTCTCGTTCTTTACATTTCTTCTTAGCTGCTT
GGCCTGTAGTAGGTATCTGGTTTACTGCTTTAGGTATTAGTACTATGGCGTTTAA
TCTAAACGGCTTCAATTTCAACCAATCTGTAGTTGATAGTCAAGGTCGTGTAAT
TAACAC