

**THE EFFECT OF SOIL MOISTURE STRESS ON
ALLELOPATHIC INFLUENCE OF HOUND'S-TONGUE
(*Cynoglossum officinale* L.)**

by

Mina Momayyezi

B.Sc., Ferdowsi University of Mashhad, 2008

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
(Plant Science)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

February 2012

© Mina Momayyezi, 2012

Abstract

Little information on the effect of environmental stressors on hound's-tongue interaction with associated herbivores and grasses is available. This study investigated the effect of soil moisture stress (SMS) on allelopathic influence of hound's-tongue on a) feeding preference and growth of grasshopper (*Melanoplus sanguinipes* Fab.) and b) seed germination and seedling growth of Idaho fescue (*Festuca idahoensis* Elmer), bluebunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. and J. G. Sm.), and hound's-tongue (*Cynoglossum officinale* L.). Hound's-tongue plants were grown under four SMS levels [100, 80, 60, and 40% field capacity (FC)] in a greenhouse.

On intact hound's-tongue, grasshoppers preferred mid-aged leaves of plants at 40% FC; whereas there was no consistent relationship between the leaf age and the area consumed for plants at 100% FC. Grasshoppers showed no statistically significant preference for discs excised from old leaves at 100 vs. 80, 60, or 40% FC. However, analyses of pooled results showed their preference for discs from old leaves at 60 and 40% over 100% FC. In bioassays employing discs from young leaves, grasshoppers significantly preferred young leaves at 40 compared to 100% FC. In disc choices between young and old leaves of plants at the same SMS, grasshoppers preferred young over old leaves at 40% FC. The greater preference and higher growth of grasshoppers on younger leaves of plants grown under higher SMS could increase our understanding of the effect of insects' herbivory on hound's-tongue under various SMS in rangelands.

Inhibitory effects of hound's-tongue leaf leachate on germination of hound's-tongue and bluebunch wheatgrass and seedling growth of grasses increased with SMS only in one Petri dish experiment. In soil, hound's-tongue leaf leachate and leaf residue from plants grown under SMS had either no or inconsistent allelopathic effects on seedling growth of grasses. Hound's-tongue root elongation was inhibited in soil covered with residue from 40 compared to 80% FC plants. This study suggests that SMS could increase the inhibitory effect of hound's-tongue mother plants on growth of its seedlings, but it may not have any ecologically significant effect on the allelopathic influence of hound's-tongue on germination and seedling growth of neighboring grasses in nature.

Table of Contents

Abstract	ii
List of Tables	vi
List of Figures	viii
Abbreviations	x
Acknowledgments	xi
Chapter I. General Introduction and Literature Review	1
General Introduction	1
Literature Review	4
1.1 Plant species used in this study	4
1.1.1 Hound's-tongue (<i>Cynoglossum officinale</i> L.)	4
1.1.2 Bluebunch wheatgrass (<i>Agropyron spicatum</i> (Pursh) Scribn. and J. G. Sm.)	5
1.1.3 Idaho fescue (<i>Festuca idahoensis</i> Elmer)	6
1.2 Grasshoppers and ecosystems	6
1.3 Insect species used in this study	6
1.3.1 Migratory grasshopper <i>Melanoplus sanguinipes</i> Fab.	6
1.4 Allelopathy	7
1.4.1 Effect of SMS on allelopathic influence of plants on insects	7
1.4.2 Effect of SMS on allelopathic influence of plants on associated plants	8
Chapter 2. The Effect of Soil Moisture Stress (SMS) on Allelopathic Influence of Hound's-tongue on Grasshopper	9
2.1 Introduction	9
2.2 Materials and methods.....	10

2.2.1 Plants culture	10
2.2.3 Rearing of grasshoppers	11
2.2.4 Feeding preference for intact plants	11
2.2.5 Leaf disc feeding preference assay	14
2.2.6 Growth on leaf discs	15
2.2.7 Leaf nitrogen analyses.....	15
2.2.8 Statistical analyses	16
2.3 Results	17
2.3.1 Feeding preference for intact plants	17
2.3.2 Leaf disc feeding preference assay	17
2.3.3 Growth on leaf discs	22
2.3.4 Leaf nitrogen analyses.....	22
2.4 Discussion	27
2.4.1 Effect of leaf physical factors on grasshopper feeding behaviour	27
2.4.2 Effect of leaf chemistry on grasshopper feeding behaviour.....	27
Chapter 3. The Effect of Soil Moisture Stress (SMS) on Allelopathic Influence of Hound's-tongue on Grasses	30
3.1 Introduction	30
3.2 Material and methods	31
3.2.1 Preparation of leaf leachate	31
3.2.2 Petri dish experiments	31
3.2.3 Soil experiments.....	32
3.2.4 Statistical analyses	33

3.3 Results	34
3.3.1 Petri dish experiments	34
3.3.2 Soil experiments	39
3.4 Discussion	58
Chapter 4. General Discussion	61
4.1 Hound's-tongue-insect interaction	61
4.2 Hound's-tongue-grass interaction	63
Literature Cited	65

List of Tables

Table 2.1. Feeding preference of <i>M. sanguinipes</i> for discs from old leaves of hound's-tongue plants grown at 100% FC and either 40, 60 or 80% FC.	21
Table 2.2. Feeding preference of <i>M. sanguinipes</i> for discs from young leaves of hound's-tongue plants grown at 100% FC and either 40, 60 or 80% FC.	23
Table 2.3. Feeding preference of <i>M. sanguinipes</i> for discs of young vs. old leaves from hound's-tongue plants grown at 100, 80, 60, and 40% FC.....	24
Table 2.4. Total leaf nitrogen content of hound's-tongue young and old leaves grown under 100, 80, 60, and 40% FC.	26
Table 3.1a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on hound's-tongue seedling number.....	40
Table 3.1b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on hound's-tongue seedling number.....	41
Table 3.1c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on hound's-tongue seedling number.....	42
Table 3.2a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on Idaho fescue seedling emergence.	43
Table 3.2b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on Idaho fescue seedling emergence.	44
Table 3.2c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on Idaho fescue seedling emergence.	45
Table 3.3a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass seedling emergence.	46
Table 3.3b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass seedling emergence.	47
Table 3.3c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass seedling emergence.	48

Table 3.4a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on hound's-tongue shoot and root elongation.	49
Table 3.4b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on hound's-tongue shoot and root elongation.	50
Table 3.4c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on hound's-tongue shoot and root elongation.	51
Table 3.5a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on Idaho fescue shoot and root elongation.	52
Table 3.5b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on Idaho fescue shoot and root elongation.	53
Table 3.5c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on Idaho fescue shoot and root elongation.	54
Table 3.6a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass shoot and root elongation.	55
Table 3.6b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass shoot and root elongation.	56
Table 3.6c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass shoot and root elongation.	57

List of Figures

Figure 2.1. Hound's-tongue plants grown at different soil moisture levels for 7-8 weeks.....	12
Figure 2.2. Experimental set up to study grasshopper feeding preference on intact plants.....	13
Figure 2.3. Effect of SMS and leaf age on initial (before insect feeding) leaf area of hound's-tongue plants. ●, 100 and ○, 40% FC. The leaf number 1 is the youngest and 7 the oldest leaf. A, B, and C are results of experiments 1, 2, and 3 respectively. Values are means ± SE of 15 replicates.....	18
Figure 2.4. Effect of SMS during hound's-tongue plant growth and the leaf age on the leaf area consumed by <i>M. sanguinipes</i> . ●, 100 and ○, 40% FC. The leaf number 1 is the youngest and 7 the oldest leaf. A, B, and C are results of experiments 1, 2, and 3, respectively. Values are means ± SE of 15 replicates.....	19
Figure 2.5. Relationship between the initial and consumed (by <i>M. sanguinipes</i>) leaf area of hound's-tongue plants grown under 100 (●) and 40% (○) FC. A and D, B and E, and C and F are results of experiments 1, 2, and 3 respectively. Values are observations from each leaf of 15 plants.....	20
Figure 2.6. The hound's-tongue leaf area consumed and the fresh weight gain by <i>M. sanguinipes</i> for young (■) and old (□) leaves developed under two (40 and 100% FC) SMS levels. A and B are results of experiment 1 and C and D of experiment 2. Values are means ± SE of 15 replicates.....	25
Figure 3.1. The allelopathic influence of hound's-tongue leaf leachate (0.5, 1, 2, and 4% w/v) from plants grown under 4 SMS levels on germination of hound's-tongue (A, D), Idaho fescue (B, E), and bluebunch wheatgrass (C, F) at day 14 in experiment 1 and 2, respectively. The values are means ± SE of 4 replicates. Values followed by different letters are significantly different ($P \leq 0.05$); capital letters are used to compare various leachate treatments with water control and small letters to compare various SMS treatments at individual leachate concentration.....	35
Figure 3.2. The allelopathic influence of hound's-tongue leaf leachate (0.5, 1, 2, and 4% w/v) from plants grown under 4 SMS levels on shoot elongation of hound's-tongue (A, D), Idaho	

fescue (B, E), and bluebunch wheatgrass (C, F) at day 14 in experiment 1 and 2, respectively. The values are means \pm SE of 4 replicates. Values followed by different letters are significantly different ($P \leq 0.05$); capital letters are used to compare various leachate treatments with water control and small letters to compare various SMS treatments at individual leachate concentration.....36

Figure 3.3. The allelopathic influence of hound’s-tongue leaf leachate (0.5, 1, 2, and 4%) from plants grown under 4 SMS levels on root elongation of hound’s-tongue (A, D), Idaho fescue (B, E), and bluebunch wheatgrass (C, F) at day 14 in experiment 1 and 2, respectively. The values are means \pm SE of 4 replicates. Values followed by different letters are significantly different ($P \leq 0.05$); capital letters are used to compare various leachate treatments with water control and small letters to compare various SMS treatments at individual leachate concentration.....38

Abbreviations

FC.....field capacity

PAs.....pyrrolizidine alkaloides

SMS.....soil moisture stress

Acknowledgments

I would like to thank my supervisor, Dr. Mahesh Upadhyaya, for all his guidance, advice, and help to get me to this point. I would like to thank the members of Upadhyaya and Isman labs for their gracious help.

I would like to thank Dr. Tony Kozak for helping me in the statistical analyses of data.

I would like to thank my parents for their love and support during this time. I want to thank my husband for his constant love and encouragement.

Chapter I. General Introduction and Literature Review

General Introduction

Rangelands are uncultivated lands generally located in arid and semi-arid areas, covered with grasses, sedges, rushes, herbaceous biennial or short-lived perennial weeds, and shrubs (DiTomaso 2000). They provide food for grazing animals, and water, mineral resources, and habitat for wildlife (DiTomaso 2000). In North America about 28% of lands are grasslands (Wikeem and Wikeem 2004). In temperate North America grassland regions the annual precipitation tends to be low and primarily happens in winters and springs. Moisture stress is therefore very common during the growing season in these grasslands (Wikeem and Wikeem 2004).

High temperature, low precipitation, soil parent material, and steep slopes combine to generate a hot and dry grassland area that cover about 1% of British Columbia (B.C.) (Wikeem and Wikeem 2004). Most of the plant and animal species inhabiting B.C. rangelands have adapted to hot and dry summers and cool and dry winters (Wikeem and Wikeem 2004).

Weeds cover large areas in rangelands. They may reduce soil productivity, and water quantity, and destroy natural resources and wildlife habitat. Weeds can establish quickly in disturbed areas (Radosevich et al. 2007). These invasive species are usually good competitors with native species for water, nutrients, and space. They slow down growth of native species by shading them (Radosevich et al. 2007).

Some weeds are unpalatable to livestock due to the presence of toxic compounds such as alkaloids (van Dam et al. 1995b). Noxious weeds compete with native species and reduce available forage for grazing animals (DiTomaso 2000). Weeds produce many seeds which may remain dormant for decades. For example, hound's-tongue (*Cynoglossum officinale*) produces more than 600 seeds per plant (Anon. 2011). Its burred seeds easily attach to livestock fur or irritate eyes of the livestock, which could negatively impact their health and marketability (Anon. 2011). In B.C., non-native plants are also a threat to endangered plant species biodiversity (Anon. 2011).

Environmental factors affect the growth, development and production of rangeland plants as well as their relationship with other organisms (Heady and Child 1994). Plants' survival and reproduction is dependent on their adaptation to environmental stresses (Robert-Seilaniantz et al. 2010). In natural ecosystems, combinations of abiotic (physical) components like soil, water, and air and biotic factors (living organisms) such as damage from insects or diseases form the ecological systems in rangelands (Nagarajan and Nagarajan 2010).

Water stress can change plant water potential at low levels and result in serious tissue wilting at a high level (Dahl and Hyder 1977). Water deficiency influences plant physiology, morphology, and chemistry, which in turn affects root and shoot growth and production of secondary metabolites (Hsiao 1973, Dahl and Hyder 1977, Taiz and Zeiger 2010).

Invasive plants may employ allelopathy in their interaction with biotic associates. Allelopathic influence helps them invade and compete within natural plant communities (Callaway and Aschehoug 2000, Qasem and Foy 2001, Kohli et al. 2006). Hound's-tongue is known to have allelopathic impacts on bluebunch wheatgrasses (*Agropyron spicatum*), growing in its neighborhood (Rashid et al. 2005, Furness et al. 2008).

Different plants produce and release allelochemicals through different anatomical parts. Hound's-tongue leaves and seeds are known to contain allelochemicals (Rice 1984, Rashid et al. 2005, Furness et al. 2008). The aqueous leachate from hound's-tongue leaves and de-coated seeds inhibited seed germination and root elongation of grasses but had no effect on its own root elongation (Rashid et al. 2005, Furness et al. 2008). Rashid et al (2005) reported that hound's-tongue seed allelochemicals (phenolic compounds) increased under stress conditions such as low O₂ and high temperature. They also showed that dead hound's-tongue seeds release higher concentrations of phenolic compounds compared to live seeds. Furness et al. (2008) reported that the inhibitory effects of hound's-tongue leaf leachate on seedling growth of grasses increased when plants were exposed to UV-B radiation. Hound's-tongue successfully grows in dry BC rangelands. Under dry conditions, hound's-tongue becomes a strong competitor with native range species. Hound's-tongue leaf and seed leachate were shown to have allelopathic influence on seed germination and seedling growth of grasses, but the effect of SMS on allelopathic influence of this weed on neighboring grasses is not known.

Herbivores, important components of dry B.C. rangelands, depend on range plant species for their food. Morphological, physiological and chemical characteristics of plants grown under water stress could influence these plant-insect interactions (Hsiao 1973, Koricheva and Larsson 1998, Tylianakis et al. 2008). Water stress can influence the performance and life-cycle of insect herbivores feeding on stressed plants (Inbar et al. 2001). Directed plant-herbivore interaction could be exploited for biological control of invasive plants. De Clerk-Floate and Wikeem (2009) reported that weevils (*Mogulones cruciger*) which feed on hound's-tongue roots could be used to control hound's-tongue population in semi-arid south-eastern B.C. rangelands. Generalist herbivores such as grasshoppers are known to feed on hound's-tongue leaves (van Dam et al. 1994). However, the influence of SMS during hound's-tongue development on feeding preference and growth of these insects is not known.

Objectives:

The overall goal of this research was to increase our understanding of environmental stressors on allelopathic influence of weedy species on their biotic associates. The specific objectives were to determine whether SMS influences the allelopathic effect of hound's-tongue on:

1. the feeding preference and growth of the migratory grasshopper (*Melanoplus sanguinipes* Fab.)
2. seed germination and seedling growth of Idaho fescue (*Festuca idahoensis* Elmer), bluebunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. and J. G. Sm.), and hound's-tongue (*Cynoglossum officinale* L.) itself

This thesis is written in manuscript format with specific introduction, materials and methods, results, discussion, and literature cited sections for each chapter. Chapter I provide a general introduction and literature review relevant to this study. Chapter 2 reports an investigation on the influence of SMS on allelopathic effect of hound's-tongue on feeding preference and growth of grasshoppers (Objective 1). The influence of SMS on allelopathic effect of hound's-tongue on seed germination and seedling growth of associated grasses as well

as hound's-tongue itself is investigated in Chapter 3 (Objective 2). A general discussion of findings and conclusions of this research are presented in Chapter 4.

Literature Review

This section presents a brief review of relevant literature on plant and insect species used in this study, allelopathy, and known effects of SMS on allelopathic influence of plants on insects and other plant species.

1.1 Plant species used in this study

1.1.1 Hound's-tongue (*Cynoglossum officinale* L.)

Hound's-tongue (fam. Boraginaceae), also known as dog's tongue and sheep lice, is native to Eurasia or Asia. It was introduced to North America in 1800s as a cereal contaminant (Upadhyaya et al. 1988). Within Canada, this broadleaf weed was collected from Ontario in 1859 but now is distributed throughout southern Canada and has become abundant in British Columbia (Upadhyaya et al. 1988, Upadhyaya and Cranston 1991). It is an herbaceous biennial or short-lived perennial forming rosettes in the first year and developing flowers in the second year. The plant grows from 30 to 120 cm tall. It can rapidly grow in pastures, disturbed areas, and roadsides. It thrives on dry rangelands as a monoculture (Cranston and Pethybridge 1986) and interferes with the establishment and growth of native species, thereby reducing the available forage for grazing animals (Upadhyaya et al. 1988). Attachment of hound's-tongue burred seeds to hair, fur and wool of livestock stresses animals and reduces their marketability (Cranston and Pethybridge 1986).

Fresh hound's-tongue is not attractive to cattle, but dry plants mixed with hay could be consumed by animals (Baker et al. 1989). It is poisonous due to the presence of pyrrolizidine alkaloids (PAs) (Knight et al. 1984). Consumption of hay contaminated with 400 g of hound's-tongue for several days can cause liver damage and subsequent death to calves (Baker et al. 1989). Hound's-tongue contains four different alkaloids: heliosupine, echinatine,

acetylheliosupine and 7-angelylhelitridine. Mattocks (1986) showed that the toxicity of heliosupine is 4-6 times higher than other alkaloids in hound's-tongue. In the first year of the growth hound's-tongue leaves and rosettes contain the highest PAs concentration (van Dam et al. 1995a, El-Shazly et al. 1996), while in the second year flowers and fruits have the greatest PAs compared to other organs. Pfister et al. (1992) showed that PA concentration in hound's-tongue leaves decreases with maturity; the immature leaves had the highest PA content. Hound's-tongue has the highest accumulation of PAs in Boraginaceae family and is known to be deterrent to herbivores (Pfister et al. 1992, van Dam et al. 1995a, van Dam et al. 1995b, de Boer 1999).

Generally younger leaves of plants are more palatable to herbivores. Because of the higher photosynthetic rate and nitrogen accumulation younger leaves produce more secondary metabolites to protect against herbivores (Mooney and Gulmon 1982, van Dam et al. 1994). Young hound's-tongue leaves contain more PAs compared to old leaves, which could contribute to mobilization of alkaloids from old to young leaves as the leaves mature (van Dam et al. 1994). It has been shown that performance and survival of herbivorous insects is higher on younger hound's-tongue leaves because of the higher water and nitrogen contents of less mature leaves (Raupp and Denno 1983, van Dam et al. 1994).

1.1.2 Bluebunch wheatgrass (*Agropyron spicatum* (Pursh) Scribn. and J. G. Sm.)

Bluebunch wheatgrass and Idaho fescue are native perennials established in rangelands of the western Rocky Mountains (Parish et al. 1996, Blicher et al. 2002, Olson and Wallander 2002). Bluebunch wheatgrass grows in well-drained soils and reaches 60-130 cm in height in arid and semi-arid habitats of western North America (Blicher et al. 2002). Like other rangeland species, successful seedling establishment is critical for bluebunch wheatgrass which is highly dependent on soil moisture and temperature (Miller et al. 1986, Johnson and Aguirre 1991). It is a drought resistant species that rapidly establishes in dry plains and on hills at low to medium elevation (Parish et al. 1996). This grass is an important forage species for livestock but is sensitive to overgrazing (Parish et al. 1996).

1.1.3 Idaho fescue (*Festuca idahoensis* Elmer)

Idaho fescue (30-90 cm tall) is the dominant species of Pacific Northwest prairies, shrub-steppe and arid grasslands of North and Northwest (Parish et al. 1996, Ewing 2002). This is a fast-growing and valuable species for grazing, it tolerates a wide range of temperatures, and is sensitive to stresses during seed production (Ewing 2002). Ridenour and Callaway (2001) reported that exuded chemicals from spotted knapweeds, *Centaurea maculosa* reduced Idaho fescue abundance by decreasing its root elongation.

1.2 Grasshoppers and ecosystems

Grasshoppers, which are generalist feeders in most cases, are an important group of native herbivores in grasslands of western North America (Branson 2011). They are destructive to rangelands and compete with other grazing animals and wildlife for available forages (Hewitt and Onsager 1982). They are also beneficial to grasslands by pruning which induces plant growth, promoting nutrient cycling, and providing food for grassland birds (Branson et al. 2006, Latchinsky et al. 2011). Grasshopper population is a good indicator of rangeland quality (Bazelet 2011). The fecal and plant residue materials produced by grasshoppers significantly increase grassland productivity; 20 *M. sanguinipes* grasshoppers have been reported to produce 29 g/m² of organic matter in three weeks (Hewitt 1977).

1.3 Insect species used in this study

1.3.1 Migratory grasshopper *Melanoplus sanguinipes* Fab.

M. sanguinipes is a medium size grasshopper, native to North America (from Alaska to Mexico) (Drolet et al. 2009). It has 5 nymphal instars, and development takes six weeks from egg to adult (5th instar). In Canada, *M. sanguinipes* mostly feeds on western prairie grasslands, causing considerable damage to croplands (Mole and Joern 1994, Drolet et al. 2009). In overgrazed meadows and grasslands, weeds are nutritious food sources for them. In 1944 a severe outbreak of *M. sanguinipes* was reported in B.C. bunchgrass prairies (Pfadt 1996). Growth and development of this insect are directly associated with ecological factors, weather

condition, and available food. These grasshoppers are more active in days than nights (Pfadt 1996). Among all plant species, grasses and a few forbs such as dandelion and wild mustard are the most favored plant hosts for this grasshopper. The feeding preference of *M. sanguinipes* has been reported to correlate with food abundance (Lambley et al. 1972).

1.4 Allelopathy

Biochemical interactions within and between plant species are termed allelopathy (Rice 1984). Allelopathy is a phenomenon in which the plant releases allelochemicals during decomposition, by root exudation, leaching of water soluble compounds, and volatilization to the environment (Mahall and Callaway 1991, Chou 2006). The inhibitory or stimulatory effect of allelochemicals is often concentration dependent (Rice 1984). Plant material in nature may release phenolics, alkaloids, flavonoids, or glycosides, which are major classes of phytochemicals that exert allelopathic influence on other species (Chou 2006). The allelochemicals may help weeds dominate other plant species or increase the plant resistance to herbivores by producing anti-feedant compounds (Brattsten 1986, Haribal and Renwick 1998, Foy and Inderjit 2001). For example, Devakumar and Parmar (1993) showed that azadirachtin is deterrent to 300 insect species.

Inhibitory or stimulatory effects of allelochemicals depend on environmental conditions (Kohli et al. 2006). Water stress has been reported to increase the accumulation of allelopathic compounds in donor plants (del Moral 1972, Pedrol et al. 2006).

1.4.1 Effect of SMS on allelopathic influence of plants on insects

Caldeira et al. (2002) showed that survival and growth of *Phoracantha semipunctata* larvae increased on *Eucalyptus globulus* trees under water stress conditions. Higher concentrations of total sugars and nitrogen were suggested to be the attracting factors for *P. semipunctata* to *E. globulus*. In another experiment, phloem feeders and caterpillars performed better on plants grown under moderate and high water stress, respectively (Mattson and Haack 1987, Larsson 1989, Christiansen and Austara 1996, Huberty and Denno 2004, Mody et al. 2009). Insect and plant species, insect developmental stage, and water stress levels are the factors that determine the effect of SMS on the performance of insect herbivores (Price 1991).

Large outbreaks potential of herbivorous insects on water-stressed plants have been attributed to higher nutrition levels and fewer deterrent compounds under drought conditions (Mody et al. 2009). Soil moisture stress influences grasshopper feeding behavior; grasshopper performance increased on water-stressed plants but severe water stress decreased their survival and growth (Franzke and Reinhold 2011).

1.4.2 Effect of SMS on allelopathic influence of plants on associated plants

Competition of plant species for available soil moisture becomes more serious under dry conditions (Radosevich et al. 2007). Species growing in dry rangelands such as hound's-tongue may take advantage of their allelopathic influence to overcome susceptible species within a natural ecosystem. The inhibitory effect of allelochemicals on germination and growth of neighboring species helps the donor plant to establish quickly.

SMS has been shown to increase allelopathic effect of plants on target species (Tongma et al. 2001). Increasing allelopathic influence of hound's-tongue leachate from plants grown under high UV-B radiation on seed germination and seedling growth of associated grasses reported by Furness et al. (2008) can be attributed to the higher production of phenolic compounds in hound's-tongue leaves exposed to high UV-B radiation. Low soil moisture is known to increase concentration of water soluble allelochemicals like phenolic compounds in plants (Chaves and Escudero 1997). However, the effect of SMS on allelopathic influence of hound's-tongue leaf residue and leaf leachate on associated grasses has not been investigated.

Chapter 2. The Effect of Soil Moisture Stress (SMS) on Allelopathic Influence of Hound's-tongue on Grasshopper

2.1 Introduction

Physical characteristics (e.g. leaf hairiness and toughness) and chemistry (e.g. secondary metabolites), nutrition and water content of plants can influence feeding preference of insect herbivores (Levin 1973, Mole and Joern 1994, Miura and Ohsaki 2004, Schoonhoven et al. 2005). Water stress has been reported to stimulate the production of secondary metabolites in plants (Gutbrodt et al. 2011), which in turn could influence the feeding behavior, survival (Whittaker 1970), growth, and development of insect herbivores. Leaves may become tougher and more toxic to herbivores under water stress conditions (Louda and Rodman 1994, Gutbrodt et al. 2011). Water stress has been reported to decrease the performance of grass miners on *Holcus lanatus* as well as their preference for this weed (Scheirs and De Bruyn 2005). Shortage of plant biomass under drought conditions may also force some herbivores to consume antifeedant-containing foliage (Sinclair et al. 1982).

Leaf nitrogen is also an important factor influencing plant-insect interactions. Younger leaves are generally more suitable for performance and survival of herbivorous insects because of their higher water and nitrogen contents (Raupp and Denno 1983, van Dam et al. 1994). Ikonen (2002) reported that a higher preference of beetles for younger leaves of *Salix phylicifolia* was due to their higher nitrogen content. However, because generalist herbivores do not have a specific defence mechanism against plant toxins; they usually prefer older leaves because of their lower concentrations of secondary metabolites (Meyer and Montgomery 1987, van Dam et al. 1995a).

Hound's-tongue is a biennial weed that infests dry rangelands of British Columbia. Under water stress conditions, it provides a strong competition against grasses because of its deeper root system. Generalist herbivores, including grasshoppers, have been observed to feed on hound's-tongue leaves (personal communications). *Melanoplus sanguinipes* is a medium size grasshopper, native to North America with 5 nymphal instars taking approximately six weeks from egg to adult (Mole and Joern 1994, Drolet et al. 2009). In Canada, it mostly feeds on

Western prairie grasslands as well as causing considerable damage to croplands (Mole and Joern 1994, Drolet et al. 2009).

Hound's-tongue leaves contain a high concentrations of the pyrrolizidine alkaloids (PAs) heliosupine, echinatine, acetylheliosupine and 7-angelyhelitridine, which are poisonous to livestock (Knight et al. 1984, Mattocks 1986, Pfister et al. 1992, van Dam et al. 1995a, van Dam et al. 1995b, de Boer 1999). PAs have also been reported to exhibit insect feeding deterrent and insecticidal properties (Macel et al. 2005, Macel 2011). Pfister et al. (1992) showed that PA concentration in hound's-tongue leaves decrease as they mature. Generalist herbivores prefer older leaves of hound's-tongue, possibly due to lower PA concentrations compared to younger leaves (van Dam et al. 1994, van Dam et al. 1995b). Although soil moisture stress is known to stimulate the production of secondary metabolites with allelopathic activity in many plant species (Tang et al. 1995), the effect of this stressor on feeding behavior and growth of grasshoppers on hound's-tongue is not known. This study investigates the effect of SMS on feeding preference and growth of *M. sanguinipes* on hound's-tongue leaves.

2.2 Materials and methods

2.2.1 Plants culture

Hound's-tongue nutlets were obtained from Agriculture and Agri-Food Canada, Lethbridge, and were soaked in water for 2-3 hours to remove the barbed pericarp and the seed coat. The embryos were placed on two Whatman No.1 filter discs wetted with 5 ml of deionized distilled water in 9 cm diameter Petri dishes for 48-72 hours under laboratory conditions ($22 \pm 2^{\circ}\text{C}$, $51 \pm 4\%$ RH). The seedlings were transplanted to 14 cm dia. plastic pots containing a sandy loam soil (77% sand, 15% silt, 7.5% clay, and 4.1% organic matter) in a greenhouse. Plants were grown under natural solar irradiance supplemented with light (for 16 h d^{-1}) from high sodium pressure lamps ($15\text{-}20 \mu\text{mol m}^{-2} \text{s}^{-1}$).

2.2.2 SMS treatments

Plants were given four soil moisture stress (SMS) treatments [40, 60, 80, and 100% of field capacity (percent moisture held against gravitational force)], as described by Upadhyaya and Furness (1994), starting 3 weeks after transplanting the seedlings (Figure 2.1.). Preliminary trials showed that hound's-tongue plants did not survive at 25% or lower FC. SMS was monitored every second day and the amount of water needed to achieve the desired SMS levels was added. Experiments were terminated 7-8 weeks after the start of SMS treatments.

In order to determine the soil moisture content at FC, 300 g of soil (4 replications) was watered to saturation and the unbound water was allowed to drain for 24 h. The percent moisture content held against the gravitational force (FC) was determined by weighting the soil before and after drying at 60°C for 72 h. The soil water content at 40, 60 and 80% FC was calculated. Soil moisture contents ($\text{m}^3 \text{ water}/\text{m}^3 \text{ soil}$) recorded using a ProCheck soil moisture sensor (Decagon Devices, Pullman WA, USA) were 0.080, 0.160, 0.280, and 0.385 m^3/m^3 for 40, 60, 80, and 100% FC, respectively.

2.2.3 Rearing of grasshoppers

Grasshopper eggs were obtained from the Saskatoon Research Centre of Agriculture and Agri-Food Canada and reared in $40 \times 40 \times 40$ cm cages with a mesh screen bottom (Hinks and Erlandson 1994, Mole and Joern 1994) at $21 \pm 2^\circ\text{C}$, $40 \pm 4\%$ RH, and 16 h photoperiod. A 25 W incandescent bulb inside each cage provided the temperature ($28 \pm 2^\circ\text{C}$) required for grasshopper rearing. Grasshoppers were fed a mixture of romaine lettuce (*Lactuca sativa* L.) and wheat bran daily from the 1st to the 5th instar (Bernays and Bright 2001).

2.2.4 Feeding preference for intact plants

Two groups of 15 plants each were grown in 14 cm diameter pots in the greenhouse at either 40 or 100% FC in a completely randomized design for 8 weeks. Each individual plant was covered with a plastic bag sealed with a rubber band (Figure 2.2.). A weighed 5th instar grasshopper was released into each plastic bag enclosure and allowed to feed for 5 days. The

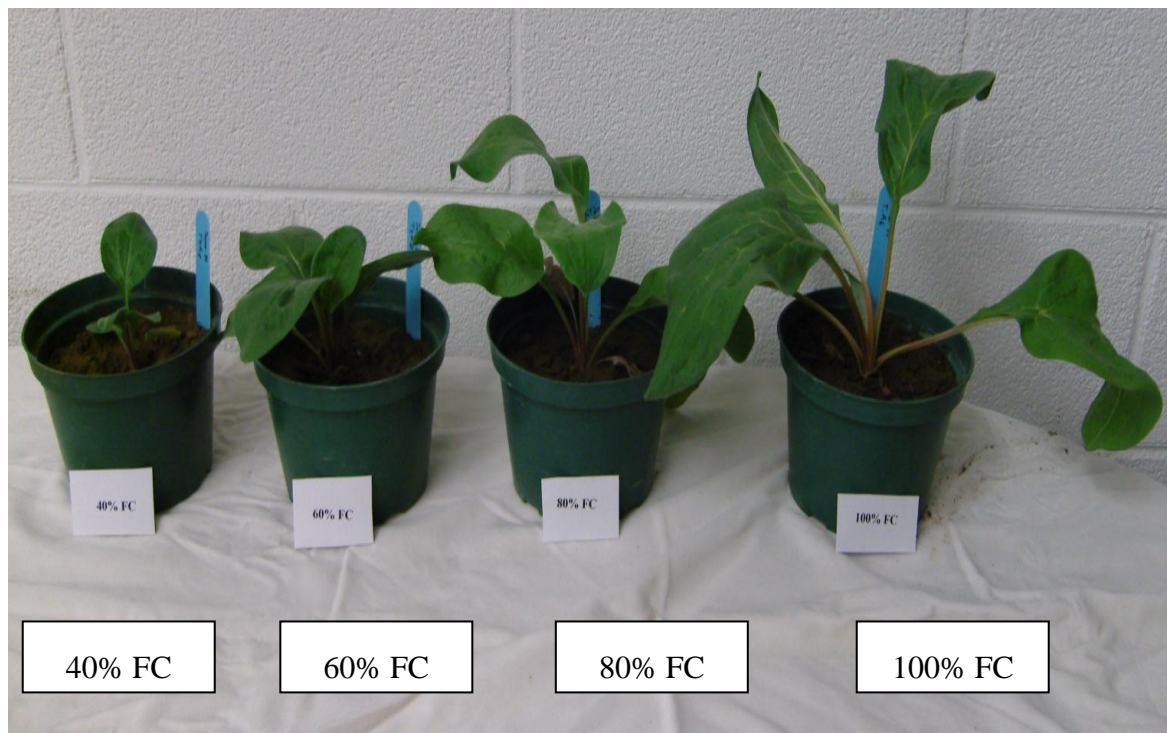


Figure 2.1. Hound's-tongue plants grown at four different soil moisture levels for 7-8 weeks.



Figure 2.2. Experimental set up to study grasshopper feeding preference on intact plants.

plants were watered through a small hole made in each plastic bag during the experiment. After 5 days, the grasshoppers were removed from the enclosures and weighed. The initial (before feeding) and final (after 5 days of feeding) leaf area of each plant was measured using a LI-COR leaf area meter (LI-3000); for a non-destructive measurement of initial leaf area, leaves attached to the plant were traced on paper sheets and the traced area determined using the leaf area meter. The area consumed for leaves at various positions on the plant and the grasshopper weight gains were calculated. The experiment was repeated twice.

2.2.5 Leaf disc feeding preference assay

Ten plants were grown at each of the 4 SMS levels (40, 60, 80, and 100% FC) in a completely randomized design. Leaf disc feeding preference bioassays (Akhtar et al. 2003) providing the following three feeding choices to individual grasshoppers were conducted: 1. a disc (2 cm²) punched using a cork borer from an old leaf (the oldest true leaf on each plant) of a plant grown at 100% FC versus a disc from a plant grown at either 40, 60, or 80% FC; 2. a disc from a young leaf (the youngest, fully-expanded true leaf on each plant) of a plant grown at 100% FC versus a disc from a plant grown at either 40, 60, or 80% FC; and 3. a disc from young leaf of plants grown at 40, 60, 80 or 100% FC versus a old leaves from a plant grown at the corresponding SMS level. A completely randomized design with 15 replications per choice was used.

Leaf discs were mounted on 5 cm pins stuck to styro-foam blocks and placed in a semi-transparent plastic container (120 mm diameter x 170 mm height; 750 ml volume) lined with a moist paper towel (at the bottom) under laboratory conditions. One grasshopper (5th instar) was introduced into each container after 3 h of starvation (Yang and Joern 1994, Bernays and Bright 2001). Containers were closed with lids and the feeding behaviour of the insects monitored for 3 h. Insects that did not consume any leaf biomass were replaced along with fresh-punched discs. The experiments were terminated by removing grasshoppers from the containers when \geq 50% of one of the leaf discs was consumed in a container (Akhtar et al. 2003). The discs from each choice were placed in pairs, between two glass plates (20 cm x 20 cm) and scanned using a digital imaging system (HP ScanJet, 5300C). The consumed area for each leaf disc was determined using the Scion image software (Scion Corp., Fredrick, MD). For the first and the

second choice bioassay, the feeding deterrence index (FDI) was calculated for each disc pair using the following formula:

$$FDI = 100 \times \{(C-T) / (C+T)\}$$

where C is the consumed leaf area from plants grown at 100% FC and T for those grown at 40, 60 or 80% FC (Isman et al. 1990). FDI ranged from +100% for the insects with preference for discs of 100% FC plants only, to -100% for insects with absolute preference for discs from plants grown at either 40, 60, or 80% FC. All bioassays were repeated twice.

2.2.6 Growth on leaf discs

Two groups of 15 plants each were grown at either 40 or 100% FC in a completely randomized design for 8 weeks. Discs excised from the youngest and the oldest leaves of the plants grown at each SMS treatment were mounted on 5 cm pins stuck to foam blocks, placed inside semi-transparent plastic containers lined with a moist Whatman No. 1 filter paper (at the bottom) (Mole and Joern 1994) and kept under laboratory conditions as described above. One grasshopper (5th instar) was introduced into each container having either young or old discs from plants grown at either 40 or 100% of FC, containers were closed with lids, and the insect were allowed to feed for 5 days (White and Chapman 1990, Hinks and Olfert 1999). The discs were changed twice a day to provide an adequate supply of fresh leaf biomass for grasshoppers. Fresh weight of grasshoppers was recorded before and after feeding to assess their growth. Leaf area consumed was measured using the procedure described above (Akhtar et al. 2003). The experiment was repeated.

2.2.7 Leaf nitrogen analyses

Ten plants grown at 40, 60, 80 or 100% FC in a completely randomized design were used for total nitrogen analyses. Young and old leaves from plants grown at each SMS level were excised, pooled, air dried on a laboratory bench ($22 \pm 2^{\circ}\text{C}$, $51 \pm 4\%$ RH) for a week, ground to powder (SmartGrind, Black & Decker, Towson, MD, USA), and 2 g of each mixture sent for total nitrogen analysis (Fisons (Carlo-Erba) NA-1500 NCS analyzer) to the Analytical

Chemistry Laboratory, B.C. Ministry of Environment, Victoria, BC. Nitrogen was measured four times for each leaf mixture.

2.2.8 Statistical analyses

Data were evaluated for the homogeneity of variance and the normality of residual error and transformed when necessary. Feeding preference and insect growth data were subjected to one-way analysis of variance using the generalized linear model procedure (PROC GLM) (completely randomized design) using SAS software (SAS statistical package, 2009, version 9.2). Leaf area consumption and grasshopper weight gain were considered the fixed effects. The significance of the regression for each experiment was checked using the F-test. If the regression was significant, a t-test was used to compare treatment means. The α level for t-tests were corrected with the Bonferroni correction method using the following formula:

$$A = \binom{T}{2} = \frac{T!}{(T-2)!2!}$$
$$\alpha' = \frac{\alpha (0.05)}{A}$$

where T, A, and α' are treatment means, all possible pairs of treatment means, and the corrected α level, respectively.

Data from grasshopper feeding experiments employing intact plants was log transformed to meet the assumption of normality. The model regression was significant for analyses of leaf area consumption and weight gain ($P \leq 0.05$). Correlations of SMS levels, leaf area consumption, and grasshopper weight gain were tested using PROC CORR of SAS software for feeding preference experiments on intact hound's-tongue plants. Data for growth and feeding preference experiments on young and old leaf discs were square root-transformed to meet the assumption of normality and the regression was significant ($P \leq 0.05$) for the models.

2.3 Results

2.3.1 Feeding preference for intact plants

The initial leaf area for plants grown under 100% FC was significantly higher than that of the plants grown under 40% FC ($P \leq 0.05$) in all three experiments (Figure 2.3A,B,C). Older leaves (leaves 4-6) of plants grown under 100% FC were larger compared to younger leaves in experiment 1 and 2 (Figure 2.3A,B); in experiment 3, mid-aged leaves (leaves 3-4) were larger compared to other leaves (Figure 2.3C). The effect of leaf age on initial leaf area in plants grown under 40% FC was not statistically significant ($P > 0.05$).

Grasshoppers generally consumed more leaf area from plants grown under 100% compared to 40% FC ($P \leq 0.05$); however the magnitude of difference and its relationship with leaf age differed the three experiments (Figure 2.4A,B,C). For plants grown under 40% FC, leaf area consumption was significantly greater for mid-aged leaves in experiments 1 (leaves 2-3) and 2 (leaf 3) ($P \leq 0.05$) (Figure 2.4A,B). There was no consistent relationship between the initial leaf area and the leaf area consumed in plants grown at either 40% or 100% FC. Shapes of the curves for initial (Figure 2.3.) and consumed (Figure 2.4.) leaf areas were different for both SMS treatments; the higher initial area of plant grown at 100% FC did not always cause higher leaf area consumption (Figure 2.3. and 2.4.), and generally there was a weak correlation, reflected by low R^2 values, between the initial and the consumed leaf areas in all three experiments (Figure 2.5.).

When the correlations of leaf area consumption with fresh weight gain of grasshoppers and SMS levels were analyzed, the leaf area consumption correlated slightly with the weight gain of insects ($\rho = 0.49$) and SMS levels ($\rho = 0.66$) (data not shown).

2.3.2 Leaf disc feeding preference assay

In bioassays offering choices between old discs from plants grown under 100 and either 40, 60, or 80% FC, grasshoppers showed no preference in any of the experiments (Table 2.1.). However, when the results of the three experiments were pooled and analyzed, grasshoppers were found to prefer old discs from plants grown under 40 or 60 over 100% FC (data not shown). Except for the choice between discs from plants grown under 100% and 80% FC in

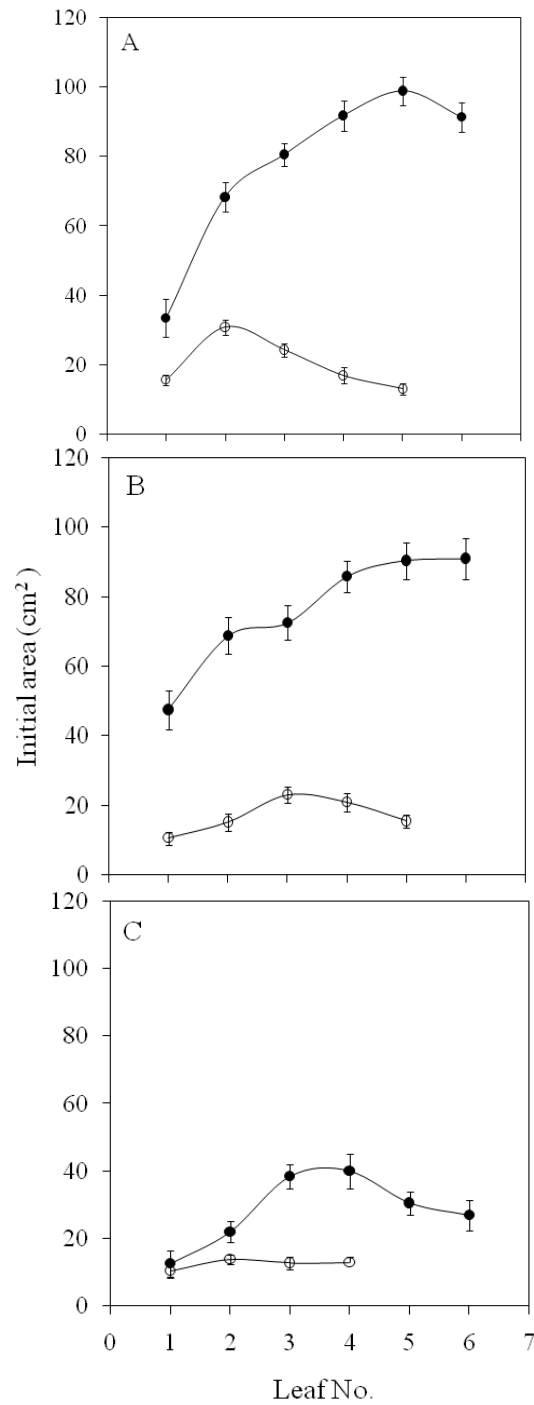


Figure 2.3. Effect of SMS and leaf age on initial (before insect feeding) leaf area of hound's-tongue plants. ●, 100 and ○, 40% FC. The leaf number 1 is the youngest and 7 the oldest leaf. A, B, and C are results of experiments 1, 2, and 3 respectively. Values are means \pm SE of 15 replicates.

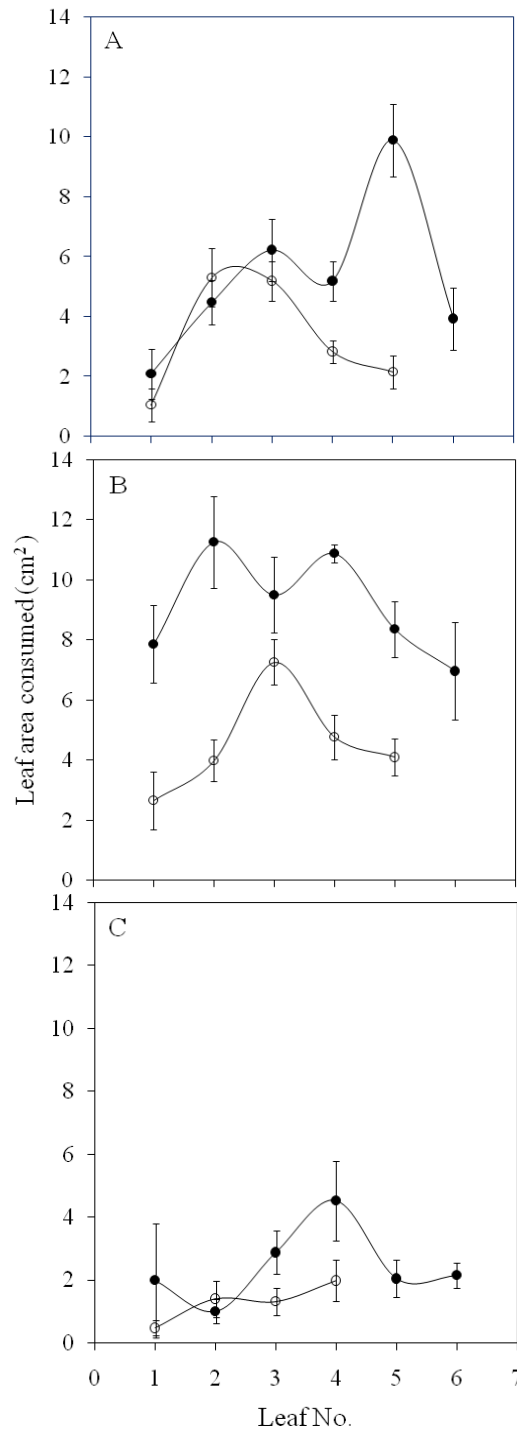


Figure 2.4. Effect of SMS during hound's-tongue plant growth and the leaf age on the leaf area consumed by *M. sanguinipes*. ●, 100 and ○, 40% FC. The leaf number 1 is the youngest and 7 the oldest leaf. A, B, and C are results of experiments 1, 2, and 3, respectively. Values are means \pm SE of 15 replicates.

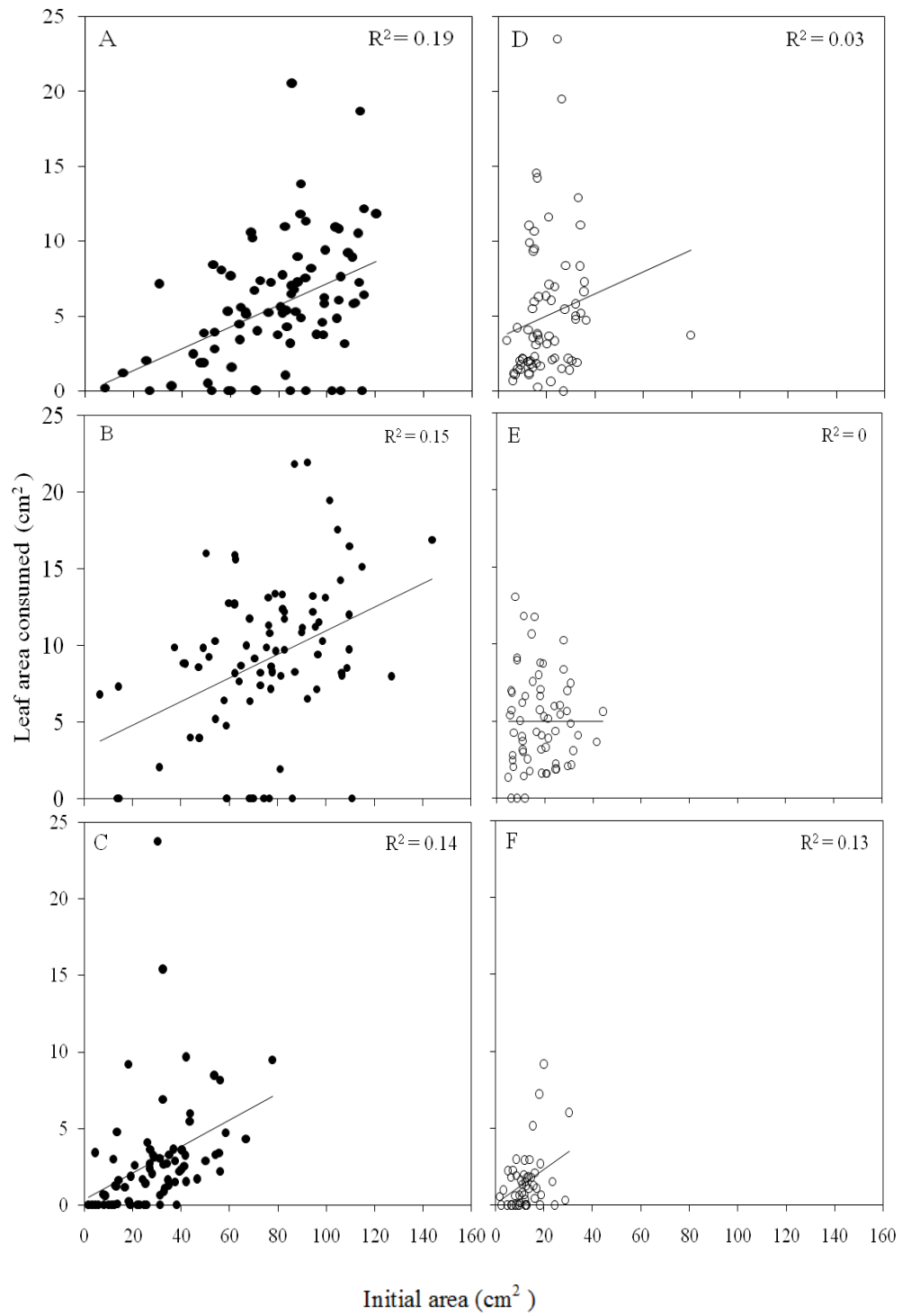


Figure 2.5. Relationship between the initial and consumed (by *M. sanguinipes*) leaf area of hound's-tongue plants grown under 100 (●) and 40% (○) FC. A and D, B and E, and C and F are results of experiments 1, 2, and 3 respectively. Values are observations from each leaf of 15 plants.

Table 2.1. Feeding preference of *M. sanguinipes* for discs from old leaves of hound's-tongue plants grown at 100% FC and either 40, 60 or 80% FC.

Experiment	Choice pair (%) of FC	Leaf area consumed (cm ²)	FDI (%)
1	100 vs. 80	0.48 ^{ns} ± 0.13 0.48 ^{ns} ± 0.13	0 ± 0.25
	100 vs. 60	0.31 ^{ns} ± 0.11 0.52 ^{ns} ± 0.11	-25 ± 0.25
	100 vs. 40	0.48 ^{ns} ± 0.12 0.61 ^{ns} ± 0.08	-20 ± 0.18
	100 vs. 80	0.55 ^{ns} ± 0.11 0.83 ^{ns} ± 0.10	-24 ± 0.13
	100 vs. 60	0.45 ^{ns} ± 0.10 0.76 ^{ns} ± 0.14	-19 ± 0.20
	100 vs. 40	0.61 ^{ns} ± 0.14 0.84 ^{ns} ± 0.15	-22 ± 0.18
2	100 vs. 80	0.48 ^{ns} ± 0.15 0.63 ^{ns} ± 0.15	-18 ± 0.25
	100 vs. 60	0.46 ^{ns} ± 0.13 0.65 ^{ns} ± 0.13	-24 ± 0.19
	100 vs. 40	0.44 ^{ns} ± 0.10 0.73 ^{ns} ± 0.13	-23 ± 0.20
	100 vs. 80	0.48 ^{ns} ± 0.15 0.63 ^{ns} ± 0.15	-18 ± 0.25
	100 vs. 60	0.46 ^{ns} ± 0.13 0.65 ^{ns} ± 0.13	-24 ± 0.19
	100 vs. 40	0.44 ^{ns} ± 0.10 0.73 ^{ns} ± 0.13	-23 ± 0.20
3	100 vs. 80	0.48 ^{ns} ± 0.15 0.63 ^{ns} ± 0.15	-18 ± 0.25
	100 vs. 60	0.46 ^{ns} ± 0.13 0.65 ^{ns} ± 0.13	-24 ± 0.19
	100 vs. 40	0.44 ^{ns} ± 0.10 0.73 ^{ns} ± 0.13	-23 ± 0.20
	100 vs. 80	0.48 ^{ns} ± 0.15 0.63 ^{ns} ± 0.15	-18 ± 0.25
	100 vs. 60	0.46 ^{ns} ± 0.13 0.65 ^{ns} ± 0.13	-24 ± 0.19
	100 vs. 40	0.44 ^{ns} ± 0.10 0.73 ^{ns} ± 0.13	-23 ± 0.20

ns, non-significant ($P > 0.05$) differences between the two values of a choice pair. The leaf area consumed values are means ± SE of 15 replicates. The negative feeding deterrence index (FDI) indicates insect preference for the water-stressed plants (40, 60 or 80% FC) over the control (100% FC). A positive FDI indicates preference for control plants over the water-stressed plants.

experiment 1 (FDI = 0), old discs excised from plants grown under 40, 60, and 80% FC deterred feeding of grasshoppers compared to discs of plants grown under 100% FC in all experiments (FDI < -18) (Table 2.1.).

In bioassays employing discs from young leaves of plants grown under 100 vs. 80, 60, and 40% FC, grasshoppers significantly preferred young discs of plants grown under 40% compared to 100% FC in all experiments (FDI < -32) ($P \leq 0.05$) (Table 2.2). Grasshoppers preferred discs of plants grown under 100% over 60% FC in experiment 1 (FDI = 43) and discs of plants grown under 80% over 100% FC in experiment 3 (FDI < -25) ($P \leq 0.05$) (Table 2.2.). These results were not observed in all experiments (Table 2.2.).

When choices between discs from young and old leaves of plants grown under the same SMS treatment were offered, grasshoppers preferred discs from young compared to old leaves of plants grown under 40% FC in all experiments ($P \leq 0.05$) (Table 2.3.).

2.3.3 Growth on leaf discs

When grasshoppers were fed hound's-tongue leaf discs from young or old leaves from plants grown under 40% and 100% FC for 5 days, they consumed similar leaf areas in both experiments ($P > 0.05$) (Figure 2.6A,C). Grasshoppers feeding on discs from young leaves of plants grown under 40% FC gained significantly more fresh weight compared to those fed on discs from old leaves of plants grown under the same SMS and either young or old leaves from plants grown under 100% FC ($P \leq 0.05$) (Figure 2.6B,D).

2.3.4 Leaf nitrogen analyses

With the exception of young leaves at 80% FC, leaf nitrogen content increased with increasing SMS level in leaves of both ages (Table 2.4.). Leaf N content of young and old leaves from plants grown at 40% FC was 26% and 92%, respectively higher compared to plants grown at 100% FC (Table 2.4.).

Table 2.2. Feeding preference of *M. sanguinipes* for discs from young leaves of hound's-tongue plants grown at 100% FC and either 40, 60 or 80% FC.

Experiment	Choice pair (%) of FC	Leaf area consumed (cm ²)	FDI (%)
1	100	0.49 ^{ns} ± 0.13	-18 ± 0.25
	vs. 80	0.69 ^{ns} ± 0.13	
	100	*0.95 ± 0.08	43 ± 0.25
	vs. 60	*0.52 ± 0.15	
	100	*0.35 ± 0.11	-41 ± 0.18
	vs. 40	*0.81 ± 0.12	
2	100	0.83 ^{ns} ± 0.11	15 ± 0.14
	vs. 80	0.58 ^{ns} ± 0.10	
	100	0.45 ^{ns} ± 0.12	-28 ± 0.18
	vs. 60	0.82 ^{ns} ± 0.13	
	100	*0.38 ± 0.10	-40 ± 0.18
	vs. 40	*0.85 ± 0.13	
3	100	*0.29 ± 0.11	-0.25 ± 0.20
	vs. 80	*0.83 ± 0.12	
	100	0.64 ^{ns} ± 0.12	2 ± 0.20
	vs. 60	0.63 ^{ns} ± 0.13	
	100	*0.25 ± 0.07	-32 ± 0.22
	vs. 40	*0.76 ± 0.15	

ns, non-significant ($P > 0.05$) and *, a significant difference between the two values of a choice pair ($P \leq 0.05$). The leaf area consumed values are means \pm SE of 15 replicates. The negative feeding deterrence index (FDI) indicates insect preference for the water-stressed plants (40, 60 or 80% FC) over the control (100% FC). A positive FDI indicates preference for control plants over the water-stressed plants.

Table 2.3. Feeding preference of *M. sanguinipes* for discs of young vs. old leaves from hound's-tongue plants grown at 100, 80, 60, and 40% FC.

Experiment	% of FC	Leaf area consumed (cm ²)	
		Young	Old
1	100	0.48 ^a ± 0.12	0.53 ^a ± 0.09
	80	0.49 ^a ± 0.12	0.37 ^a ± 0.11
	60	0.51 ^a ± 0.15	0.63 ^a ± 0.12
	40	0.82 ^a ± 0.11	0.40 ^b ± 0.12
2	100	0.35 ^a ± 0.10	0.48 ^a ± 0.09
	80	0.38 ^a ± 0.12	0.59 ^a ± 0.10
	60	0.68 ^a ± 0.14	0.61 ^a ± 0.14
	40	0.79 ^a ± 0.13	0.42 ^b ± 0.09
3	100	0.39 ^a ± 0.09	0.48 ^a ± 0.09
	80	0.48 ^a ± 0.12	0.51 ^a ± 0.13
	60	0.49 ^a ± 0.08	0.39 ^a ± 0.09
	40	0.81 ^a ± 0.14	0.39 ^b ± 0.09

The values within a choice pair followed by different letters are significantly different ($P \leq 0.05$). The values are means ± SE of 15 replicates.

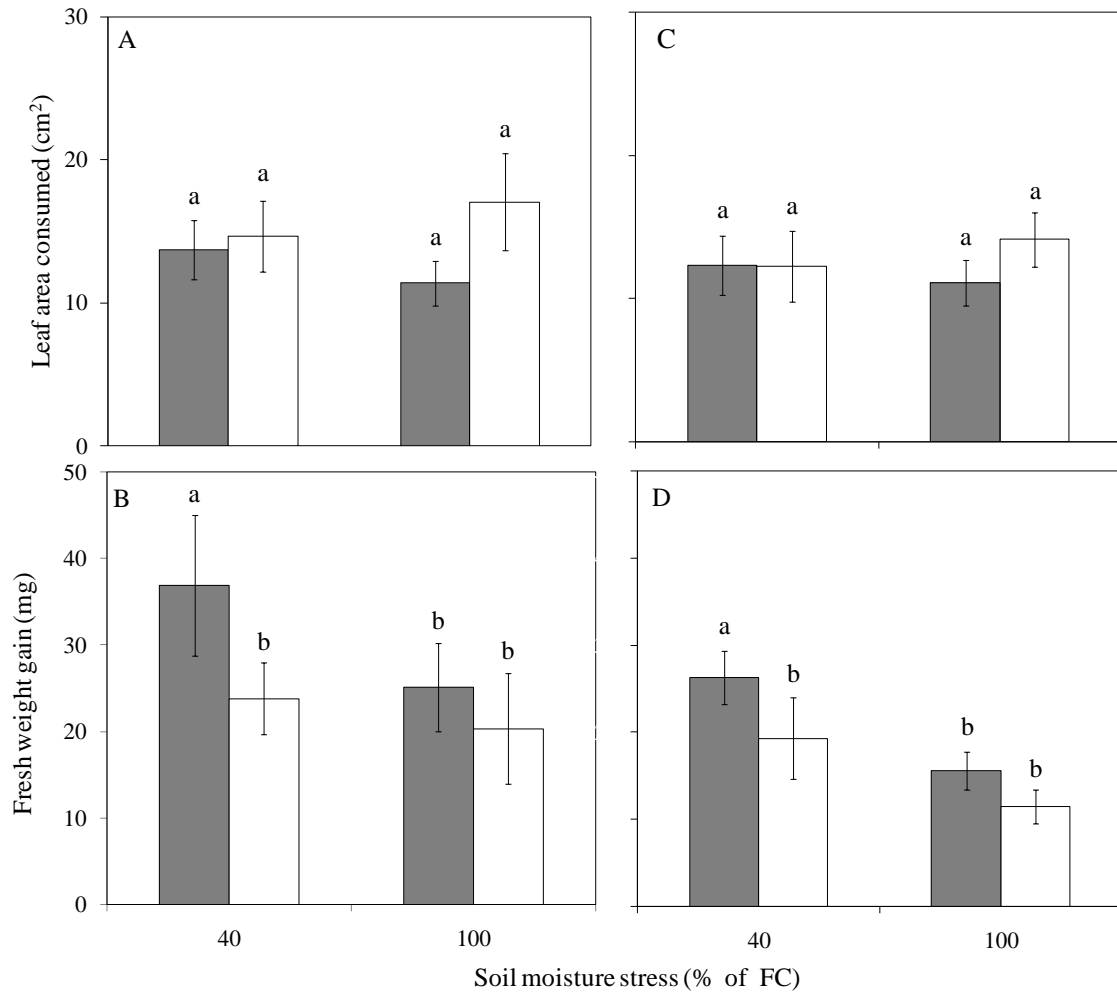


Figure 2.6. The hound's-tongue leaf area consumed and the fresh weight gain by *M. sanguinipes* for young (■) and old (□) leaves developed under two (40 and 100% FC) SMS levels. A and B are results of experiment 1 and C and D of experiment 2. Values are means \pm SE of 15 replicates.

Table 2.4. Total leaf nitrogen content of hound's-tongue young and old leaves grown under 100, 80, 60, and 40% FC.

% of FC	Total leaf Nitrogen (%)	
	Young	Old
100	2.84	1.38
80	2.83	1.65
60	3.10	1.84
40	3.59	2.66

The values are means of 4 measurements for a sample.

2.4 Discussion

Hound's-tongue, a rangeland weed of British Columbia, hosts several phytophagous insects. This study showed that SMS increases the feeding preference and growth of grasshoppers on this weed. Various aspects of the SMS influence on feeding preference and growth of grasshoppers will be discussed in the following section.

2.4.1 Effect of leaf physical factors on grasshopper feeding behaviour

Leaf physical characteristics can have major impact on growth and survival of grasshoppers (Coley 1983). Grasshoppers gained more weight and developed better on plants with tougher leaves covered with abundant trichomes (Miura and Ohsaki 2004). Upadhyaya and Furness (1994) reported that SMS increased trichome density on hound's-tongue leaves. Leaves developed under water stress are tougher and could be preferred by insects (Louda and Rodman 1994). Higher preference and better growth of *M. sanguinipes* on discs from young leaves of plants grown under 40% FC could be attributed to the increased toughness and hairiness of hound's-tongue leaves at high SMS level.

The amount of available food affects the feeding behaviour of herbivorous insects (Ball et al. 2000). Water stress decreases shoot biomass, leaf area and leaf number of hound's-tongue plants (Momayyezi unpublished results). It is possible that more available food due to the larger and more abundant leaves of hound's-tongue plants grown under 100% compared to 40% FC resulted in higher leaf consumption by *M. sanguinipes* on intact plants.

2.4.2 Effect of leaf chemistry on grasshopper feeding behaviour

The inhibitory effect of plant chemicals on feeding behavior of herbivorous insects could be affected under SMS. For example, Gutbrodt et al. (2011) reported that two leaf-chewing larvae, *Spodoptera littoralis* (generalist) and *Pieris brassicae* (specialist), preferred water-stressed and well-watered plants respectively. Concentrations of toxic compounds (alkaloids) increase in plants grown under water stress condition (Khan et al. 2011). However, Lewis (1984) suggested that water-stressed plants are more palatable to acridids such as *Melanoplus*

differentialis. Herbivore survival and outbreaks increase on water-stressed plants with higher foliar nitrogen compared to well-watered plants (White 1984, Koricheva et al. 1998, Huberty and Denno 2004). SMS increased the nitrogen content more in young compared to old leaves of hound's-tongue (Table 2.4.), but had no effect on water content of shoots (Momayyezi unpublished results). Ho et al. (1984) indicated the positive effect of nitrogen in attracting herbivores to younger leaves. Greater preference and growth of *M. sanguinipes* for young leaves of hound's-tongue grown under high SMS could be due to the higher leaf nitrogen content. Old leaves of hound's-tongue contain less deterrent compounds (alkaloids) (van Dam et al. 1994). However, their low nitrogen content compared to young leaves possibly decreased feeding preference of *M. sanguinipes* (Mooney and Gulmon 1982, van Dam et al. 1995b).

Hound's-tongue deterrent compounds are known to increase due to the mechanical damage of plants (van Dam et al. 1993). Physical damage due to feeding of grasshoppers on intact plants may increase the deterrent compounds in hound's-tongue plants grown under 40% compared to 100% FC. That could result in less area consumed by grasshoppers on hound's-tongue plants grown under 40 compared to 100% FC in my study.

Wait et al. (2002) reported that feeding preference of insects is dependent on the leaf developmental stage. *M. sanguinipes* had a higher preference for middle-aged leaves of hound's-tongue plants grown under 40% FC compared to the young and old leaves. However, there was no consistent relationship between leaf developmental stage and area consumption by *M. sanguinipes* for hound's-tongue plants grown at 100% FC.

This study suggests that grasshoppers could have a higher preference and better growth on young leaves of hound's-tongue grown under high SMS in rangeland condition. Feeding on water-stressed hound's-tongue could affect outbreaks of grasshoppers in rangelands (Mattson and Haack 1987). SMS can increase the palatability of hound's-tongue leaves to grasshoppers while increasing leaf hairiness, trichomes and nitrogen content. However, it can decrease the amount of food available for grasshoppers by decreasing hound's-tongue leaf number and size. Grasshoppers preferred and had better growth on young leaves of hound's-tongue plants grown under SMS. The higher investment of hound's-tongue plants in their young leaves in terms of higher photosynthesis and nutrient storage make them more palatable to grasshoppers under

SMS conditions. In nature, grasshoppers could continue feeding on young leaves of hound's-tongue grown under SMS in the absence of their favorite food species.

This study increases our understanding of hound's-tongue-insect interactions in rangelands with microsites varying in SMS. It suggests that hound's-tongue plants grown under high SMS could be better controlled by insects compared to plants developed under low SMS conditions. Results could also be relevant to biological control of other species using herbivorous insects.

Chapter 3. The Effect of Soil Moisture Stress (SMS) on Allelopathic Influence of Hound's-tongue on Grasses

3.1 Introduction

Plant chemicals released from fresh or dried residue into the environment can inhibit germination, growth and establishment of associated plant species in an ecosystem (Qasem and Foy 2001, Chou 2006). This phenomenon is called allelopathy. Weeds use allelopathy in their interaction with neighboring species (Chou 2006). Secondary metabolites such as phenolic acids, alkaloids, and flavonoid glycosides are water-soluble chemicals which have been implicated in allelopathic influence on plant-plant interactions (Ridenour and Callaway 2001, Chou 2006). Abiotic and biotic stresses are known to influence concentration of allelochemicals in the donor plant as well as their inhibitory effect on target plants. Pedrol et al. (2006) showed that water stress increased accumulation and release of allelochemicals from donor species. Karageorgou et al. (2002) reported that *Dittrichia viscosa* grown under water stress conditions had higher levels of phenolic compounds compared to well-watered plants.

Hound's-tongue (*Cynoglossum officinale*), a noxious weed of dry British Columbia rangelands, has been shown to exert allelopathic influence on associated grasses (Upadhyaya and Cranston 1991, Rashid et al. 2005, Furness et al. 2008). Phenolic compounds are abundant in hound's-tongue seeds (Qi et al. 1993). Rashid et al. (2005) showed that water-soluble phenolic compounds leached from de-coated hound's-tongue seeds and seed coats decreased root elongation of some grasses but had no effect on hound's-tongue seedlings. Leaf leachate from hound's-tongue plants grown under enhanced UV-B radiation has been shown to decrease seed germination and seedling emergence of grasses (Furness et al. 2008).

While soil moisture stress is a limiting factor in BC rangelands, the effect of this stressor on the allelopathic influence of hound's-tongue on other species has not been studied. The objective of this study therefore was to determine whether SMS during hound's-tongue growth influences the allelopathic effect of its leaf leachate on seed germination and seedling growth of Idaho fescue (*Festuca idahoensis*), bluebunch wheatgrass (*Agropyron spicatum*) as well as hound's-tongue itself (self-inhibition).

3.2 Material and methods

Hound's-tongue plants were grown under 4 SMS levels using the procedure of Upadhyaya and Furness (1994) as described in Chapter 2. The procedures described by Furness et al. (2008) were used to investigate the allelopathic effect of hound's-tongue leaf residue and leaf leachate on germination and seedling growth of some associated grassy species as well as hound's-tongue. Hound's-tongue leaf leachate concentrations (0.5, 1.0, 2.0 and 4.0 w/v) were prepared from plants grown at 100, 80, 60, and 40% FC for 7-8 weeks.

3.2.1 Preparation of leaf leachate

Hound's-tongue leaves (blade + petiole) were cut at the soil surface, air dried under laboratory conditions for 5 days ($22 \pm 2^\circ\text{C}$, $51 \pm 4\%$ RH), ground with a mortar and pestle, re-ground in a coffee bean grinder (Black & Decker Corp. Towson, MD, USA), and sieved through a fine sieve (40 mesh). The screened residue was stored in a freezer (-24°C) until use. Water soluble chemicals were extracted from the ground leaf residue by stirring it with deionized double-distilled water in Erlenmeyer flasks on a rotary shaker (80 rpm, 25°C , 4h, in darkness). The mixture was suction-filtered (2X) using a vacuum pump (Marathon Electric Manufacturing Corporation, Wausau, Wisconsin, USA) first through Whatman No. 1 and then No. 42 (90 mm diameter) filter papers to remove any particulate materials from the leachate.

3.2.2 Petri dish experiments

Two Petri dish experiments were conducted to examine the effect of hound's-tongue leaf leachate, obtained from plants grown under different SMS levels on seed germination and seedling growth of Idaho fescue, bluebunch wheatgrass and hound's-tongue.

3.2.2.1 Seed germination

Seeds of Idaho fescue, bluebunch wheatgrass, and hound's-tongue were placed in 90×15 mm Petri dishes (25 seeds per dish) on two layers of 90 mm diameter Whatman No. 1 filter discs wetted with 5 ml of either deionized distilled water (control) or 0.5, 1.0, 2.0, and 4.0%

(w/v) of leaf leachate. Petri dishes were placed in plastic boxes (370 mm length \times 270 mm width, 5600 ml) lined with moist paper towels, covered with plastic lids, and incubated at 25°C in darkness. Seed germination was recorded after 14 days. Seeds with ≥ 5 mm radicles were considered germinated.

3.2.2.2 Seedling growth

Seeds of Idaho fescue, bluebunch wheatgrass, and hound's-tongue were pre-germinated in plastic boxes (described above) on wet paper towels. Seedling with ≥ 5 mm radicles were transferred to 90 \times 15 mm Petri dishes (10 seedlings per dish) lined with a two layers of 90 mm diameter Whatman No. 1 filter discs wetted with 5 ml of either deionized distilled water or leaf leachates (0.5, 1.0, 2.0, and 4.0% w/v). The seedling growth (elongation) was monitored after 14 days.

In both Petri dish experiments, a completely randomized design with 5 leachate concentrations (0, 0.5, 1.0, 2.0, and 4.0% w/v) prepared from hound's-tongue leaves grown under 4 SMS levels (100, 80, 60, and 40% FC) was used. There were four replications (Petri dishes) per treatment.

3.2.3 Soil experiments

Three separate experiments were conducted to study the allelopathic effect of hound's-tongue leaf residue and leaf leachate on seedling emergence and shoot and root elongation of Idaho fescue, bluebunch wheatgrass and hound's-tongue. Either five hound's-tongue seeds or ten seeds of Idaho fescue or bluebunch wheatgrass were sown 3-5 mm deep in 25 ml plastic wells filled with 20 g of sandy loam soil. Seedling emergence was monitored after 4, 7, 10, and 14 days. Shoot and root elongation were measured 14 days after sowing.

In the first experiment, the soil surface was covered evenly with 0.2 g of leaf residue from hound's-tongue plants grown under 4 SMS levels. The control had no residue cover. In the second experiment, the soil was mixed with or without 0.4 g of leaf residue of hound's-

tongue plants grown under 4 levels of SMS. The control was grown without any residue. All wells in experiment 1 and 2 were watered with 8 ml deionized distilled water.

In the third experiment, the soil was mixed with activated charcoal (0.4 g), which has been reported to reduce the effect of allelochemicals in other species (Wurst et al. 2010). The activated charcoal was placed in wells and watered with either 8 ml of deionized distilled water (control) or 4.0 % w/v of hound's-tongue leaf leachate from plant leaves grown at 4 SMS levels. Treatments without activated charcoal were included for comparison.

In all three soil experiments, 8 ml of deionized distilled water was added to soil every second day to provide soil moisture. In all three experiments, a completely randomized design with 4 replications per treatment was used. All Petri dish and soil experiments were repeated.

3.2.4 Statistical analyses

All Petri dish and soil experiment data was subjected to one-way analysis of variance ($P = 0.05$) using the generalized linear model procedure (PROC GLM) of SAS software (SAS statistical package, 2009, version 9.2). In the analyses, numbers of germinated seeds, seedlings, emerged seedlings, and shoot and root lengths were the independent variables (X's) and SMS levels and leachate concentrations were the dependent variables (Y's). Data were evaluated for the homogeneity of variances and the normality of residual errors. Where necessary, logarithm or square root transformations were done. The significance of regression for each experiment was checked using an F-test. If the regression was significant, t-tests were used for mean separation. The α level for t-tests were corrected by the Bonferroni correction method using the following formula:

$$A = \binom{T}{2} = \frac{T!}{(T-2)!2!}$$

$$\alpha' = \frac{\alpha (0.05)}{A}$$

3.3 Results

3.3.1 Petri dish experiments

3.3.1.1 Seed germination

Hound's-tongue 4% leachate from plants grown under 40% FC significantly decreased hound's-tongue germination in Petri dishes compared to leachate from plants grown under 60, 80, and 100% FC in experiment 1 (Figure 3.1A). However, hound's-tongue seed germination was not inhibited at any leachate concentration in experiment 2 (Figure 3.1D). Leachate (4%) from plants grown under 40% FC compared to the leachate at the same concentration from plants developed at other SMS levels (60, 80, and 100% FC) inhibited bluebunch wheatgrass germination in experiment 1 (Figure 3.1C). No other significant effect was found on germination of Idaho fescue (Figure 3.1B,E) and bluebunch wheatgrass (Figure 3.1C,F) in both experiments.

All concentrations of hound's-tongue leachate from plants grown under 40% FC significantly reduced hounds-tongue germination compared to water control only in experiment 1 (Figure 3.1A). Hound's-tongue leachate (4%) from plants grown under 4 SMS levels reduced Idaho fescue germination compared to water control in experiment 1 (Figure 3.1B). The reduction was also significant for 40, 60, and 80% FC treatments in experiment 2 (Figure 3.1E). The allelopathic effect of hound's-tongue on bluebunch wheatgrass germination was only detectable in experiment 1 for 4% leachate from plants grown under 40% FC compared to water control (Figure 3.1C).

3.3.1.2 Seedling growth

Hound's-tongue leaf leachate from any of the four SMS treatments did not affect hound's-tongue (Figure 3.2A), Idaho fescue (Figure 3.2B), and bluebunch wheatgrass (Figure 3.2C) shoot elongation in experiment 1. However, there were some inhibitory effects on shoot elongation of all species in experiment 2 (Figure 3.2D,E,F). Low leachate concentrations from plants developed at 60% compared to 40 and 80% FC decreased hound's-tongue shoot

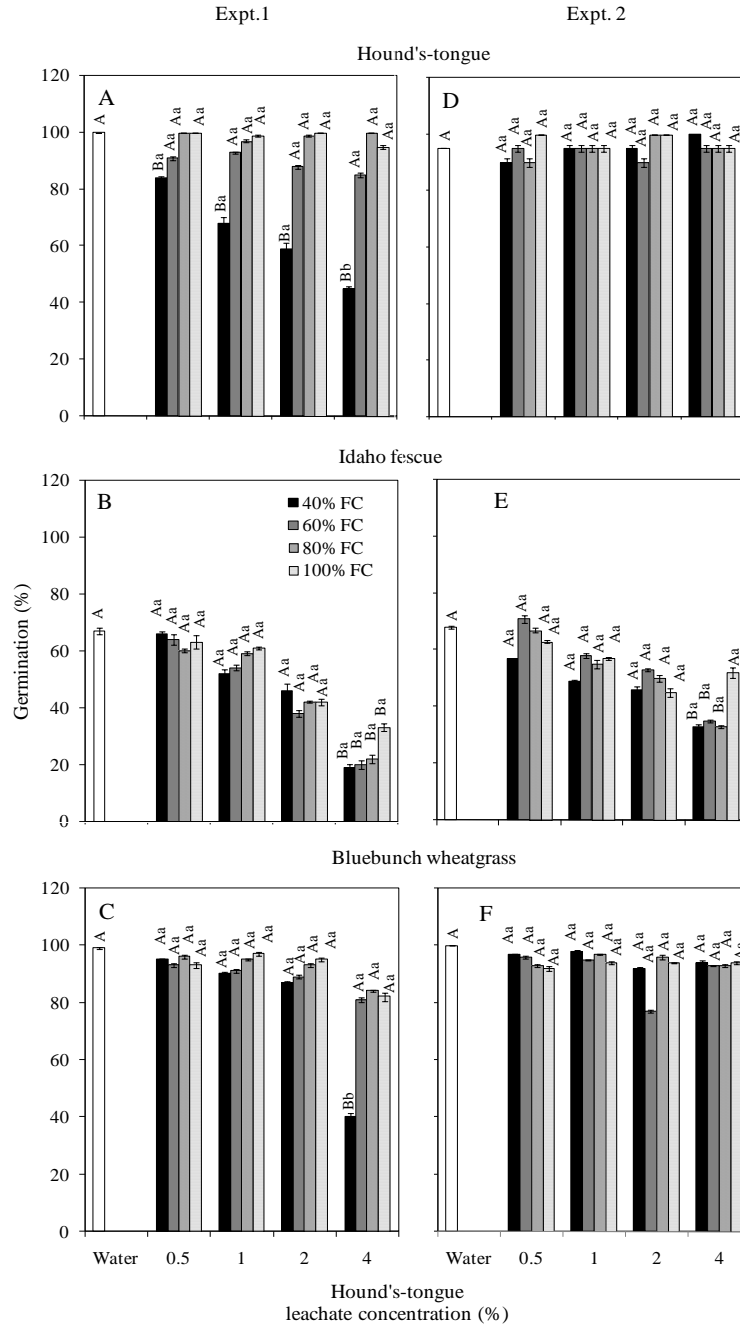


Figure 3.1. The allelopathic influence of hound's-tongue leaf leachate (0.5, 1, 2, and 4% w/v) from plants grown under 4 SMS levels on germination of hound's-tongue (A, D), Idaho fescue (B, E), and bluebunch wheatgrass (C, F) at day 14 in experiment 1 and 2, respectively. The values are means \pm SE of 4 replicates. Values followed by different letters are significantly different ($P \leq 0.05$); capital letters are used to compare various leachate treatments with water control and small letters to compare various SMS treatments at individual leachate concentration.

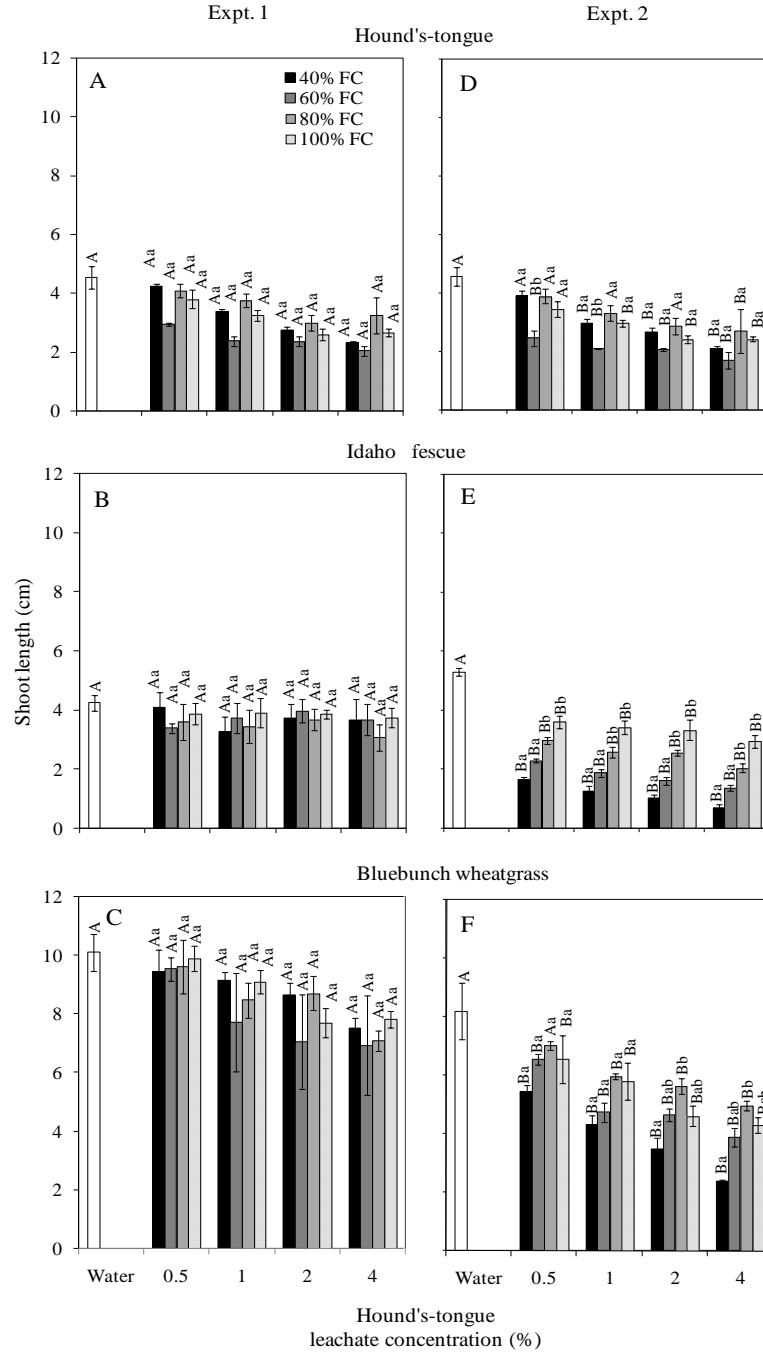


Figure 3.2. The allelopathic influence of hound's-tongue leaf leachate (0.5, 1, 2, and 4% w/v) from plants grown under 4 SMS levels on shoot elongation of hound's-tongue (A, D), Idaho fescue (B, E), and bluebunch wheatgrass (C, F) at day 14 in experiment 1 and 2, respectively. The values are means \pm SE of 4 replicates. Values followed by different letters are significantly different ($P \leq 0.05$); capital letters are used to compare various leachate treatments with water control and small letters to compare various SMS treatments at individual leachate concentration.

elongation in experiment 2 (Figure 3.2D). All leachate concentrations from plants grown under 40 and 60% FC significantly decreased Idaho fescue shoot elongation compared to the leachate at the same concentration from plants developed under 80 and 100% FC in experiment 2 (Figure 3.2E). Bluebunch wheatgrass seedlings had shorter shoots with high leachate concentrations (2 and 4%) from plants grown under 40% FC compared to 80% FC in experiment 2 (Figure 3.2F).

In experiment 1, hound's-tongue leaf leachate had no allelopathic effect on shoot elongation of hound's-tongue and grasses compared to water controls (Figure 3.2B, C). (Figure 3.2A,B,C). The leachate had some inhibitory effect on hound's-tongue shoot elongation compared to water control in the second experiment (Figure 3.2D). All leachate concentrations from plants grown under four SMS levels significantly decreased shoot elongation of both grass species compared to water controls in experiment 2 (Figure 3.2E,F), except for bluebunch wheatgrass seedlings treated with 0.5% leachate of 80% FC plants (Figure 3.2F).

Hound's-tongue root elongation significantly decreased with 1% leachate from plants grown under 40 compared to 80% FC in both experiments (Figure 3.3A,D). Hound's-tongue leachate of plants grown under 4 SMS levels did not affect Idaho fescue root elongation in the first experiment (Figure 3.3B), but in experiment 2, low leachate concentrations of plants grown at 40% FC decreased seedling root elongation compared to 80% FC (Figure 3.3E). While leachate from plants grown under 40% FC showed some inhibition on bluebunch wheatgrass root elongation compared to leachate from plants grown under the other SMS levels (60, 80, and 100% FC) in both experiments (Figure 3.3C,F), the inhibition was only significant for seedlings incubated with all leachate concentrations of 40% compared to 80 and 100% FC in experiment 2 (Figure 3.3F).

Hound's-tongue leachate at all concentrations from plants grown under four SMS levels reduced hound's-tongue root elongation compared to water controls except for 0.5% leachate of plants grown under 80% FC in both experiments (Figure 3.3A,D). High leachate concentration (4% w/v) from plants grown under all SMS levels reduced Idaho fescue root elongation compared to water control in both experiments (Figure 3.3B,E). Leachate concentrations at 2 and 4% w/v from plants grown under all SMS levels inhibited bluebunch wheatgrass root

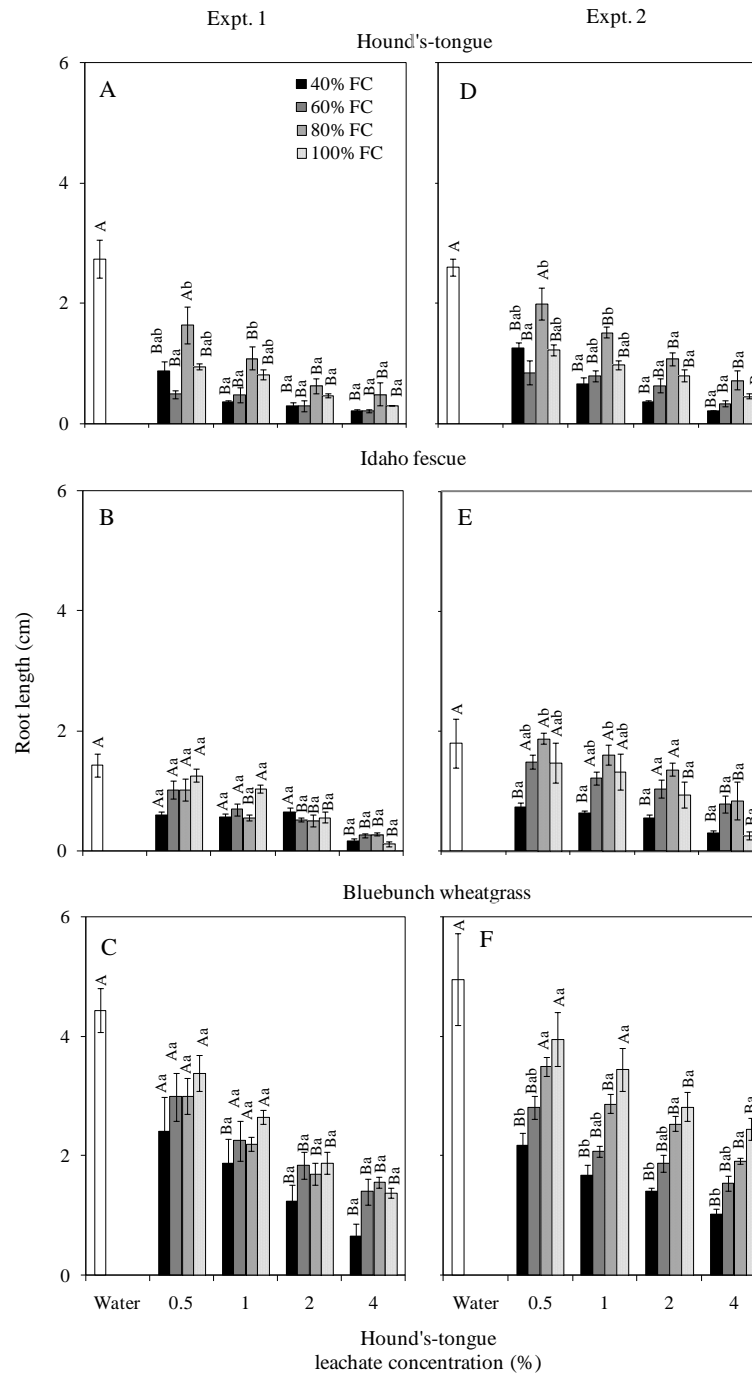


Figure 3.3. The allelopathic influence of hound's-tongue leaf leachate (0.5, 1, 2, and 4%) from plants grown under 4 SMS levels on root elongation of hound's-tongue (A, D), Idaho fescue (B, E), and bluebunch wheatgrass (C, F) at day 14 in experiment 1 and 2, respectively. The values are means \pm SE of 4 replicates. Values followed by different letters are significantly different ($P \leq 0.05$); capital letters are used to compare various leachate treatments with water control and small letters to compare various SMS treatments at individual leachate concentration.

elongation compared to water control in both experiments (Figure 3.3C,F). The inhibitory effect of other leachate concentrations on root elongation of Idaho fescue and bluebunch wheatgrass compared to water control was observed in one of the two experiments (Figure 3.3B,C,E,F).

3.3.2 Soil experiments

Seedling emergence for hound's-tongue was not known due to the unrecorded number of dead seedlings during the soil experiments. Only the number of survived seedlings was available for hound's-tongue, referred to as seedling number in this study. Since there was no mortality for seedlings of Idaho fescue and bluebunch wheatgrass in soil, seedling number represents seedling emergence for these species.

Seedling number of hound's-tongue (Table 3.1a,b,c) and seedling emergence of Idaho fescue (Table 3.2a,b,c) and bluebunch wheatgrass (Table 3.3a,b,c) were not affected when soil was covered or mixed with hound's-tongue leaf residue or watered with leaf leachate from plants grown under four SMS treatments in both experiments.

All three treatments also showed no effect on hound's-tongue seedling number compared to water controls in both experiments (Table 3.1a,b,c). Hound's-tongue leaf residue mixed with soil from plants grown at 40% FC significantly decreased seedling emergence of Idaho fescue compared to water controls at day 14 in both experiments (Table 3.2a,b,c). Hound's-tongue leachate from plants grown under all SMS treatments increased seedling emergence of bluebunch wheatgrass compared to water control only in experiment 2 at day 10 (Table 3.3a,b,c).

The cover of hound's-tongue leaf residue from plants grown under 40% FC significantly decreased hound's-tongue shoot elongation in experiment 1 and root elongation in both experiments compared to residue from plants grown under 80% FC (Table 3.4a). Addition of leaf leachate or mixing leaf residue with soil had no effect on hound's-tongue shoot or root elongation (Table 3.4b,c). Hound's-tongue residue cover or leaf leachate had no effect on Idaho fescue shoot and root elongation (Table 3.5a,c). The allelopathic effect of hound's-tongue leaf residue mixed with soil on shoot and root elongation and leaf leachate on shoot elongation of bluebunch wheatgrass in experiment 2 was not observed in the first experiment (Table 3.6b).

Table 3.1a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on hound's-tongue seedling number.

Expt.	Day	Water control	100% FC	80% FC	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	4.75 ± 0.25 ^{ns}	3.00 ± 0.71 ^{ns}	2.75 ± 0.63 ^{ns}	2.00 ± 0.82 ^{ns}	0.75 ± 0.48 ^{ns}
	10	4.75 ± 0.25 ^{ns}	3.25 ± 0.48 ^{ns}	3.00 ± 0.40 ^{ns}	2.25 ± 1.03 ^{ns}	1.50 ± 0.50 ^{ns}
	14	4.75 ± 0.25 ^{ns}	3.25 ± 0.75 ^{ns}	2.25 ± 0.63 ^{ns}	3.50 ± 0.29 ^{ns}	2.75 ± 0.63 ^{ns}
2	4	0.00	0.00	0.00	0.00	0.00
	7	4.25 ± 0.48 ^{ns}	3.50 ± 1.19 ^{ns}	4.50 ± 0.29 ^{ns}	3.50 ± 0.50 ^{ns}	2.00 ± 0.58 ^{ns}
	10	3.00 ± 0.71 ^{ns}	3.00 ± 1.08 ^{ns}	4.50 ± 0.29 ^{ns}	3.25 ± 0.63 ^{ns}	2.25 ± 0.75 ^{ns}
	14	1.25 ± 0.75 ^{ns}	2.75 ± 1.03 ^{ns}	4.25 ± 0.25 ^{ns}	3.00 ± 0.58 ^{ns}	1.75 ± 0.85 ^{ns}

ns, not significant ($P > 0.05$). The values are means ± SE of 4 replicates.

Table 3.1b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on hound's-tongue seedling number.

Expt.	Day	Water control	100% FC	80% FC	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	3.75 ± 0.25 ^{ns}	2.00 ± 0.41 ^{ns}	1.75 ± 0.25 ^{ns}	2.75 ± 0.48 ^{ns}	1.75 ± 0.48 ^{ns}
	10	3.50 ± 0.29 ^{ns}	3.75 ± 0.75 ^{ns}	4.00 ± 0.41 ^{ns}	4.00 ± 0.63 ^{ns}	3.75 ± 0.29 ^{ns}
	14	4.75 ± 0.25 ^{ns}	3.75 ± 0.75 ^{ns}	4.00 ± 0.41 ^{ns}	4.00 ± 0.41 ^{ns}	3.75 ± 0.25 ^{ns}
2	4	0.00	0.00	0.00	0.00	0.00
	7	4.25 ± 0.48 ^{ns}	3.50 ± 0.50 ^{ns}	2.50 ± 0.29 ^{ns}	2.50 ± 0.29 ^{ns}	3.00 ± 0.71 ^{ns}
	10	3.00 ± 0.71 ^{ns}	3.25 ± 0.63 ^{ns}	2.00 ± 0.71 ^{ns}	2.50 ± 0.64 ^{ns}	3.50 ± 0.64 ^{ns}
	14	1.25 ± 0.75 ^{ns}	2.75 ± 0.85 ^{ns}	2.25 ± 0.85 ^{ns}	2.25 ± 0.48 ^{ns}	2.50 ± 0.50 ^{ns}

ns, not significant (P > 0.05). The values are means ± SE of 4 replicates.

Table 3.1c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on hound's-tongue seedling number.

Expt.	Day	Water control	100% FC	80% FC	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	4.25 ± 0.25 ^{ns}	3.00 ± 1.15 ^{ns}	3.00 ± 0.41 ^{ns}	1.50 ± 0.50 ^{ns}	1.50 ± 0.87 ^{ns}
	10	4.25 ± 0.25 ^{ns}	3.00 ± 1.15 ^{ns}	3.25 ± 0.85 ^{ns}	3.00 ± 0.41 ^{ns}	2.00 ± 0.91 ^{ns}
	14	4.75 ± 0.25 ^{ns}	3.00 ± 1.15 ^{ns}	2.75 ± 0.75 ^{ns}	2.00 ± 0.41 ^{ns}	0.75 ± 0.48 ^{ns}
2	4	0.00	0.00	0.00	0.00	0.00
	7	4.25 ± 0.48 ^{ns}	2.75 ± 0.48 ^{ns}	2.50 ± 0.64 ^{ns}	3.50 ± 0.64 ^{ns}	2.00 ± 0.41 ^{ns}
	10	2.50 ± 0.95 ^{ns}	2.50 ± 0.50 ^{ns}	1.75 ± 0.48 ^{ns}	3.00 ± 0.41 ^{ns}	1.75 ± 0.25 ^{ns}
	14	3.00 ± 0.58 ^{ns}	2.50 ± 0.64 ^{ns}	3.00 ± 0.41 ^{ns}	2.25 ± 0.48 ^{ns}	1.75 ± 0.25 ^{ns}

ns, not significant ($P > 0.05$). The values are means ± SE of 4 replicates.

Table 3.2a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on Idaho fescue seedling emergence.

Expt.	Day	Water control	100% FC	80% Fc	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	32.50 ± 1.45 ^{ns}	5.00 ± 0.43 ^{ns}	2.50 ± 0.33 ^{ns}	2.50 ± 0.33 ^{ns}	0.00
	10	32.50 ± 1.16 ^{ns}	35.00 ± 1.09 ^{ns}	25.00 ± 0.97 ^{ns}	30.00 ± 1.12 ^{ns}	37.50 ± 1.31 ^{ns}
	14	50.00 ± 1.48 ^{ns}	57.50 ± 1.27 ^{ns}	52.50 ± 1.22 ^{ns}	72.50 ± 1.45 ^{ns}	50.00 ± 1.51 ^{ns}
2	4	0.00	0.00	0.00	0.00	0.00
	7	15.00 ± 4.80 ^{ns}	10.00 ± 2.90 ^{ns}	10.00 ± 3.55 ^{ns}	0.00	2.50 ± 1.25 ^{ns}
	10	55.00 ± 6.60 ^{ns}	25.00 ± 5.59 ^{ns}	17.50 ± 3.15 ^{ns}	7.50 ± 2.40 ^{ns}	2.50 ± 1.25 ^{ns}
	14	67.50 ± 5.90 ^a	42.50 ± 5.55 ^{ab}	20.00 ± 4.10 ^{ab}	20.00 ± 5.40 ^{ab}	15.00 ± 4.35 ^b

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

Table 3.2b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on Idaho fescue seedling emergence.

Expt.	Day	Water control	100% FC	80% FC	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	45.00 ± 0.50 ^{ns}	5.00 ± 0.00 ^{ns}	0.00	0.00	0.00
	10	60.00 ± 0.71 ^{ns}	42.50 ± 0.75 ^{ns}	37.50 ± 0.48 ^{ns}	35.00 ± 0.87 ^{ns}	17.50 ± 0.85 ^{ns}
	14	90.00 ± 0.40 ^a	77.50 ± 0.48 ^{ab}	65.00 ± 0.50 ^{ab}	67.00 ± 0.29 ^{ab}	47.50 ± 0.25 ^b
2	4	0.00	0.00	0.00	0.00	0.00
	7	15.00 ± 4.80 ^{ns}	0.00	0.00	0.00	2.50 ± 1.25 ^{ns}
	10	55.00 ± 6.60 ^{ns}	7.50 ± 2.40 ^{ns}	2.50 ± 1.25 ^{ns}	2.50 ± 1.25 ^{ns}	17.50 ± 1.25 ^{ns}
	14	67.50 ± 5.90 ^a	25.00 ± 1.45 ^b	7.50 ± 2.40 ^b	15.00 ± 1.45 ^b	20.00 ± 3.55 ^b

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

Table 3.2c. The effect of hound’s-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on Idaho fescue seedling emergence.

Expt.	Day	Water control	100% FC	80% FC	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	12.50 ± 2.37 ^{ns}	2.50 ± 0.62 ^{ns}	2.50 ± 0.62 ^{ns}	0.00	7.50 ± 1.20 ^{ns}
	10	47.50 ± 3.60 ^{ns}	2.50 ± 0.62 ^{ns}	7.50 ± 1.20 ^{ns}	0.00	7.50 ± 1.20 ^{ns}
	14	52.50 ± 2.57 ^{ns}	7.50 ± 1.87 ^{ns}	15.00 ± 2.40 ^{ns}	7.50 ± 1.87 ^{ns}	12.50 ± 0.62 ^{ns}
2	4	0.00	0.00	0.00	0.00	0.00
	7	30.00 ± 1.77 ^{ns}	0.00	0.00	0.00	0.00
	10	37.50 ± 2.77 ^{ns}	10.00 ± 1.02 ^{ns}	5.00 ± 1.25 ^{ns}	2.50 ± 0.62 ^{ns}	0.00
	14	67.50 ± 2.77 ^a	50.00 ± 1.77 ^{ab}	25.00 ± 0.72 ^b	42.50 ± 2.72 ^{ab}	35.00 ± 3.15 ^{ab}

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

Table 3.3a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass seedling emergence.

Expt.	Day	Water control	100% FC	80% Fc	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	70.00 ± 1.54 ^{ns}	40.00 ± 2.92 ^{ns}	25.00 ± 2.42 ^{ns}	37.50 ± 2.62 ^{ns}	17.50 ± 1.95 ^{ns}
	10	67.50 ± 1.41 ^{ns}	60.00 ± 3.42 ^{ns}	55.00 ± 3.70 ^{ns}	60.00 ± 3.42 ^{ns}	67.50 ± 3.40 ^{ns}
	14	87.50 ± 1.49 ^{ns}	85.00 ± 3.70 ^{ns}	82.50 ± 4.02 ^{ns}	87.50 ± 3.85 ^{ns}	75.00 ± 3.47 ^{ns}
2	4	0.00	0.00	0.00	0.00	0.00
	7	57.50 ± 8.50 ^{ns}	52.50 ± 4.25 ^{ns}	40.00 ± 5.40 ^{ns}	15.00 ± 3.20 ^{ns}	12.50 ± 4.75 ^{ns}
	10	75.00 ± 8.30 ^{ns}	62.50 ± 4.25 ^{ns}	47.50 ± 4.25 ^{ns}	42.50 ± 5.55 ^{ns}	30.00 ± 8.90 ^{ns}
	14	45.00 ± 5.20 ^{ns}	52.50 ± 2.40 ^{ns}	50.00 ± 4.10 ^{ns}	52.50 ± 2.40 ^{ns}	45.00 ± 5.20 ^{ns}

ns, not significant (P > 0.05). The values are means ± SE of 4 replicates.

Table 3.3b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass seedling emergence.

Expt.	Day	Water control	100% FC	80% FC	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	75.00 \pm 1.04 ^{ns}	30.00 \pm 0.71 ^{ns}	5.00 \pm 0.50 ^{ns}	20.00 \pm 0.82 ^{ns}	25.00 \pm 0.64 ^{ns}
	10	82.50 \pm 0.63 ^{ns}	85.00 \pm 0.29 ^{ns}	80.00 \pm 0.40 ^{ns}	70.00 \pm 1.08 ^{ns}	87.50 \pm 0.25 ^{ns}
	14	85.00 \pm 0.63 ^{ns}	97.50 \pm 0.29 ^{ns}	92.50 \pm 0.41 ^{ns}	92.50 \pm 1.08 ^{ns}	95.00 \pm 0.25 ^{ns}
2	4	0.00	0.00	0.00	0.00	0.00
	7	57.50 \pm 8.50 ^{ns}	27.50 \pm 6.25 ^{ns}	20.00 \pm 2.05 ^{ns}	40.00 \pm 3.55 ^{ns}	27.50 \pm 4.25 ^{ns}
	10	75.00 \pm 8.30 ^{ns}	62.50 \pm 4.25 ^{ns}	35.00 \pm 3.20 ^{ns}	77.50 \pm 1.25 ^{ns}	62.50 \pm 6.25 ^{ns}
	14	45.00 \pm 7.25 ^{ns}	75.00 \pm 1.45 ^{ns}	52.50 \pm 5.55 ^{ns}	80.00 \pm 0.00 ^{ns}	77.50 \pm 2.40 ^{ns}

ns, not significant (P > 0.05). The values are means \pm SE of 4 replicates.

Table 3.3c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass seedling emergence.

Expt.	Day	Water control	100% FC	80% FC	60% FC	40% FC
1	4	0.00	0.00	0.00	0.00	0.00
	7	45.00 ± 6.57 ^{ns}	37.50 ± 3.72 ^{ns}	57.50 ± 4.82 ^{ns}	60.00 ± 2.70 ^{ns}	40.00 ± 5.30 ^{ns}
	10	70.00 ± 5.25 ^{ns}	45.00 ± 2.12 ^{ns}	70.00 ± 5.95 ^{ns}	67.50 ± 4.27 ^{ns}	40.00 ± 3.22 ^{ns}
	14	90.00 ± 5.25 ^{ns}	52.50 ± 2.12 ^{ns}	75.00 ± 4.72 ^{ns}	72.50 ± 5.15 ^{ns}	45.00 ± 1.45 ^{ns}
2	4	0.00	0.00	0.00	0.00	0.00
	7	0.00	10.00 ± 1.77 ^{ns}	25.00 ± 2.40 ^{ns}	55.00 ± 1.60 ^{ns}	35.00 ± 1.25 ^{ns}
	10	10.00 ± 1.02 ^a	35.00 ± 2.17 ^b	65.00 ± 2.60 ^b	80.00 ± 0.00 ^b	72.50 ± 1.87 ^b
	14	87.50 ± 1.57 ^{ns}	65.00 ± 3.75 ^{ns}	87.50 ± 2.37 ^{ns}	92.50 ± 0.62 ^{ns}	92.50 ± 1.20 ^{ns}

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

Table 3.4a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on hound's-tongue shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	2.17 ± 0.24 ^{ab}	2.28 ± 0.19 ^{ab}	3.91 ± 0.43 ^a	2.06 ± 0.37 ^{ab}	1.13 ± 0.69 ^b
	Root	1.53 ± 0.12 ^{ab}	1.82 ± 0.09 ^{ab}	4.15 ± 0.8 ^a	1.69 ± 0.38 ^{ab}	0.41 ± 0.22 ^b
2	Shoot	2.25 ± 1.02 ^{ns}	2.34 ± 0.37 ^{ns}	4.15 ± 0.44 ^{ns}	1.83 ± 0.68 ^{ns}	1.38 ± 0.98 ^{ns}
	Root	1.72 ± 0.80 ^{ab}	2.15 ± 0.62 ^{ab}	6.15 ± 0.99 ^a	2.43 ± 1.38 ^{ab}	0.40 ± 0.30 ^b

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

Table 3.4b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on hound's-tongue shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	3.03 ± 0.35 ^{ns}	2.38 ± 0.25 ^{ns}	2.30 ± 0.13 ^{ns}	1.97 ± 0.07 ^{ns}	2.17 ± 0.22 ^{ns}
	Root	2.42 ± 0.10 ^{ns}	2.02 ± 0.32 ^{ns}	1.59 ± 0.15 ^{ns}	2.07 ± 0.10 ^{ns}	2.42 ± 0.13 ^{ns}
2	Shoot	2.22 ± 1.02 ^{ns}	2.70 ± 0.24 ^{ns}	2.98 ± 0.61 ^{ns}	3.70 ± 0.25 ^{ns}	3.86 ± 0.41 ^{ns}
	Root	1.72 ± 0.52 ^{ns}	1.84 ± 0.56 ^{ns}	2.34 ± 0.44 ^{ns}	2.22 ± 0.24 ^{ns}	2.80 ± 0.02 ^{ns}

ns, not significant ($P > 0.05$). The values are means ± SE of 4 replicates.

Table 3.4c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on hound's-tongue shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	$1.37 \pm 0.10^{\text{ns}}$	$1.01 \pm 0.13^{\text{ns}}$	$1.17 \pm 0.29^{\text{ns}}$	$0.94 \pm 0.20^{\text{ns}}$	$0.60 \pm 0.10^{\text{ns}}$
	Root	$0.70 \pm 0.05^{\text{ns}}$	$0.51 \pm 0.10^{\text{ns}}$	$0.49 \pm 0.15^{\text{ns}}$	$0.51 \pm 0.16^{\text{ns}}$	$0.27 \pm 0.25^{\text{ns}}$
2	Shoot	$1.19 \pm 0.19^{\text{ns}}$	$1.03 \pm 0.15^{\text{ns}}$	$1.01 \pm 0.19^{\text{ns}}$	$1.04 \pm 0.23^{\text{ns}}$	$0.86 \pm 0.15^{\text{ns}}$
	Root	$0.52 \pm 0.10^{\text{ns}}$	$0.52 \pm 0.06^{\text{ns}}$	$0.35 \pm 0.10^{\text{ns}}$	$0.43 \pm 0.10^{\text{ns}}$	$0.30 \pm 0.03^{\text{ns}}$

ns, not significant ($P > 0.05$). The values are means \pm SE of 4 replicates.

Table 3.5a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on Idaho fescue shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	4.95 ± 0.27 ^{ns}	3.79 ± 0.40 ^{ns}	3.29 ± 0.48 ^{ns}	3.61 ± 0.47 ^{ns}	4.37 ± 0.68 ^{ns}
	Root	3.80 ± 0.21 ^{ns}	2.81 ± 0.36 ^{ns}	2.70 ± 0.26 ^{ns}	2.40 ± 0.31 ^{ns}	3.22 ± 0.53 ^{ns}
2	Shoot	3.10 ± 0.42 ^{ns}	2.55 ± 0.87 ^{ns}	3.78 ± 0.62 ^{ns}	2.72 ± 1.22 ^{ns}	1.27 ± 0.35 ^{ns}
	Root	2.14 ± 0.53 ^{ns}	1.61 ± 0.76 ^{ns}	2.67 ± 0.93 ^{ns}	1.49 ± 0.81 ^{ns}	1.09 ± 0.42 ^{ns}

ns, not significant (P > 0.05). The values are means ± SE of 4 replicates.

Table 3.5b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on Idaho fescue shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	4.92 ± 0.27 ^{ns}	4.75 ± 0.56 ^{ns}	4.41 ± 0.40 ^{ns}	3.74 ± 0.47 ^{ns}	3.66 ± 0.15 ^{ns}
	Root	3.17 ± 0.37 ^{ns}	1.41 ± 0.19 ^{ns}	1.64 ± 0.27 ^{ns}	1.53 ± 0.20 ^{ns}	1.18 ± 0.16 ^{ns}
2	Shoot	3.10 ± 0.42 ^{ab}	1.13 ± 0.50 ^a	0.75 ± 0.05 ^a	0.96 ± 0.55 ^a	3.70 ± 0.55 ^b
	Root	2.14 ± 0.53 ^{ns}	0.95 ± 0.33 ^{ns}	0.20 ± 0.00 ^{ns}	0.95 ± 0.35 ^{ns}	1.81 ± 0.42 ^{ns}

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

Table 3.5c. The effect of hound’s-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on Idaho fescue shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	3.05 ± 0.81 ^{ns}	2.92 ± 0.68 ^{ns}	2.87 ± 0.72 ^{ns}	2.92 ± 0.42 ^{ns}	2.92 ± 1.21 ^{ns}
	Root	2.64 ± 0.89 ^{ns}	1.02 ± 0.82 ^{ns}	2.67 ± 1.52 ^{ns}	2.67 ± 0.93 ^{ns}	2.13 ± 0.95 ^{ns}
2	Shoot	4.40 ± 0.55 ^a	2.82 ± 0.71 ^{ab}	1.31 ± 0.53 ^b	1.86 ± 0.78 ^{ab}	1.83 ± 0.60 ^{ab}
	Root	2.75 ± 0.35 ^{ns}	1.71 ± 0.51 ^{ns}	1.34 ± 0.49 ^{ns}	1.65 ± 0.39 ^{ns}	1.60 ± 0.32 ^{ns}

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

Table 3.6a. The effect of hound's-tongue leaf residue covered the soil from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	$9.50 \pm 0.78^{\text{ns}}$	$8.51 \pm 0.65^{\text{ns}}$	$8.29 \pm 0.42^{\text{ns}}$	$8.41 \pm 1.00^{\text{ns}}$	$8.28 \pm 0.67^{\text{ns}}$
	Root	$5.09 \pm 0.26^{\text{ns}}$	$4.71 \pm 0.17^{\text{ns}}$	$4.32 \pm 0.44^{\text{ns}}$	$4.07 \pm 0.13^{\text{ns}}$	$3.91 \pm 0.37^{\text{ns}}$
2	Shoot	$10.75 \pm 1.49^{\text{ns}}$	$12.17 \pm 0.97^{\text{ns}}$	$11.21 \pm 0.86^{\text{ns}}$	$9.08 \pm 1.94^{\text{ns}}$	$7.10 \pm 2.05^{\text{ns}}$
	Root	$5.18 \pm 0.32^{\text{ns}}$	$5.13 \pm 0.49^{\text{ns}}$	$3.46 \pm 0.36^{\text{ns}}$	$3.04 \pm 0.30^{\text{ns}}$	$2.33 \pm 0.79^{\text{ns}}$

ns, not significant ($P > 0.05$). The values are means \pm SE of 4 replicates.

Table 3.6b. The effect of hound's-tongue leaf residue mixed with the soil from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	11.53 ± 0.22 ^{ns}	9.73 ± 0.46 ^{ns}	10.69 ± 0.24 ^{ns}	9.03 ± 0.38 ^{ns}	10.03 ± 0.15 ^{ns}
	Root	4.49 ± 0.24 ^{ns}	3.71 ± 0.41 ^{ns}	2.50 ± 0.48 ^{ns}	2.34 ± 0.30 ^{ns}	2.42 ± 0.26 ^{ns}
2	Shoot	10.75 ± 1.49 ^{ab}	9.22 ± 1.05 ^a	5.04 ± 0.99 ^b	9.77 ± 0.59 ^a	7.97 ± 0.64 ^a
	Root	5.18 ± 0.32 ^c	2.29 ± 0.17 ^{ab}	1.63 ± 0.07 ^a	3.04 ± 0.14 ^b	2.15 ± 0.14 ^a

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

Table 3.6c. The effect of hound's-tongue leaf leachate from plants grown under 100, 80, 60, and 40% FC on bluebunch wheatgrass shoot and root elongation.

Expt.		Water control	100% FC	80% FC	60% FC	40% FC
1	Shoot	8.75 ± 1.24 ^{ns}	6.74 ± 1.11 ^{ns}	8.24 ± 1.39 ^{ns}	7.18 ± 0.62 ^{ns}	7.40 ± 2.56 ^{ns}
	Root	3.96 ± 0.26 ^{ns}	3.12 ± 0.26 ^{ns}	4.35 ± 0.27 ^{ns}	3.62 ± 0.09 ^{ns}	3.58 ± 0.91 ^{ns}
2	Shoot	3.70 ± 0.26 ^a	5.55 ± 1.04 ^{ac}	9.90 ± 1.32 ^{bc}	11.68 ± 0.40 ^b	10.74 ± 0.57 ^b
	Root	3.35 ± 0.06 ^{ns}	2.72 ± 0.41 ^{ns}	3.27 ± 0.23 ^{ns}	3.77 ± 0.23 ^{ns}	3.61 ± 0.30 ^{ns}

ns, not significant ($P > 0.05$); values followed by different letters in each row within an experiment are significantly different ($P \leq 0.05$). The values are means ± SE of 4 replicates.

The cover of leaf residue from plants grown at any SMS level had no effect on shoot or root elongation of bluebunch wheatgrass (Table 3.6a).

Hound's-tongue leaf residue and leaf leachate did not influence shoot or root elongation of this weed compared to water control in any of the soil experiments (Table 3.4a,b,c). Other than a significant inhibitory effect of leachate from plants grown under 80% FC in experiment 2 (Table 3.5c), hound's-tongue leaf residue (soil cover or mixture) or leaf leachate had no allelopathic influence on Idaho fescue shoot or root elongation compared to water controls in either experiment (Table 3.5a,b). Hound's-tongue leachate from plants grown under 80, 60, and 40% FC significantly increased bluebunch wheatgrass shoot elongation compared to water control in experiment 2 (Table 3.6c). The residue mixed with soil from plants grown under four SMS treatments inhibited bluebunch wheatgrass root elongation compared to water control in experiment 2 (Table 3.6b). However, those effects were not observed in experiment 1 (Table 3.6b). Cover of leaf residue from plants grown under any SMS treatments had no effect on shoot or root elongation of bluebunch wheatgrass compared to water controls (Table 3.6a).

3.4 Discussion

Hound's-tongue, a weed of dry rangelands in British Columbia, is known to have allelopathic influence on grasses. This study showed that SMS had either no effect or inconsistent effects on allelopathic influence of hound's-tongue on seed germination and seedling growth of associated grasses in both Petri dish and soil experiments.

In Petri dishes, high leachate concentrations from plants grown under high SMS level decreased hound's-tongue (Figure 3.3A,D), Idaho fescue (Figure 3.3B,E), and bluebunch wheatgrass (Figure 3.3C,F) root elongation compared to water controls. Furness et al. (2008) supported the allelopathic influence of hound's-tongue leachate from plants exposed to environmental stress (UV-B radiation) on seedling growth of grasses compared to water controls. They also showed that enhanced UV-B radiation increased the allelopathic effect of hound's-tongue leachate on germination and growth of grasses in Petri dishes. However, hound's-tongue leachate from plants developed under high SMS levels had either no

allelopathic effect or inconsistent inhibitory effect on seed germination and seedling growth of hound's-tongue, Idaho fescue, and bluebunch wheatgrass in my Petri dish experiments.

Tongma et al. (2001) showed that leachate from Mexican sunflower (*Tithonia diversifolia*) grown under water stress decreased seed germination and seedling growth of test plants in soil experiments. In my study, hound'-tongue seedling number and seedling emergence of Idaho fescue and bluebunch wheatgrass were not affected in soil covered or mixed with leaf residue or watered with leaf leachate from plants grown under 4 SMS levels. The leaf residue cover from plants grown under high water stress level (40% FC) reduced hound's-tongue root elongation compared to residue from plants grown under 80% FC in both experiments (Table 3.4a). Furness et al. (2008) also reported that spreading the hound's-tongue leaf residue on soil surface effectively inhibited seedling emergence of grasses. Hound's-tongue residue on the soil surface could physically interfere with the growth of its seedlings, resulting in delayed seedling emergence, and seedling decay. Allelochemicals released from hound's-tongue residue cover can also inhibit its root elongation in soil (Ibanez and Schupp 2002).

In soil experiments, only hound's-tongue leaf residue mixed with soil from plants grown under 40% FC significantly decreased Idaho fescue seedling emergence compared to water control at day 14 (Table 3.2b). This suggests that hound's-tongue residue could decrease the establishment of Idaho fescue growing around hound's-tongue in rangeland conditions. There was no other consistent inhibitory effect for hound's-tongue leaf residue (covered or mixed with soil) and leaf leachate on seedling number and seedling emergence of hound's-tongue and bluebunch wheatgrass and growth of any seedling species compared to water control. It is possible that hound's-tongue leaf allelochemicals become deactivated in soil. The effectiveness of hound's-tongue allelochemicals in soil may be influenced by organic matter, moisture, and nutrient content of soil. The higher organic matter in soil decreases the utilization of allelochemicals by soil microorganisms as a source of carbon. Therefore, more allelochemicals in soil could be absorbed by target plants. Hound's-tongue allelochemicals might be transformed to less toxic compounds to grass seedlings due to the activity of soil microorganisms.

Tefera (2002) showed that roots are more sensitive to allelochemicals than shoots. In my study, root elongation of hound's-tongue, Idaho fescue, and bluebunch wheatgrass was more

influenced by hound's-tongue leaf leachate and leaf residue in both Petri dish and some but not all soil experiments.

Activated charcoal with its large surface area and ion exchange ability could absorb allelochemicals and reduce their inhibitory effect in soil (Callaway and Aschehoug, 2000, Lau et al. 2008). Presence or absence of charcoal in the soil did not change the effect of leaf leachate from plants grown under 4 SMS levels on hound's-tongue seedling number, seedling emergence of Idaho fescue and bluebunch wheatgrass, and seedling growth of any species (data not shown). Furness et al. (2008) reported that in soil without charcoal, hound's-tongue leachate from plants exposed to UV-B radiation had no effect on Idaho fescue seedling emergence, but inhibited seedling emergence for crested wheatgrass (*Agropyron cristatum*) and prairie junegrass (*Koeleria macrantha*).

This study suggests that hound's-tongue may not take advantage of its allelopathic influence under high SMS to compete with neighboring grasses in rangelands. However, its deep roots can help this weed to grab more resources compared to grasses in drought condition. The inhibitory effect of hound's-tongue leaf residue on soil surface (cover) from plants grown under high SMS may indicate the self-inhibitory influence of litter from hound's-tongue mother plants on growth of its seedlings in rangeland conditions.

SMS decreases mycorrhizal colonization on hound's-tongue roots (Momayyezi unpublished results) which could be due to the allelopathic effect of this weed on its mycorrhizal network. Reduced mycorrhizal colonization on hound's-tongue roots could make this weed less competent to neighboring grasses.

In my study, SMS had either no or inconsistent effect on allelopathic influence of hound's-tongue on neighbouring grasses. However, the possibility of an SMS effect on the allelopathic influence of this weed under field conditions has not been completely ruled out. The effect of high SMS on allelopathic influence of hound's-tongue might be more visible in nature than in the lab. The greater biomass from hound's-tongue in the soil in nature might show an allelopathic effect of this weed grown under high SMS on grasses.

This study increases our understanding of plant community composition under SMS in natural ecosystems. Field studies are needed to confirm the ecological effect of SMS on allelopathic influence of hound's-tongue on neighboring grasses under rangeland conditions.

Chapter 4. General Discussion

Plants contain secondary metabolites that do not take part in primary biochemical functions such as photosynthesis (Makkar et al. 2007). It is known that these compounds affect the interactions of the plants with other organisms (Lambers et al. 2008). Increased phytotoxins in host plants under water stress can influence the performance and feeding preference of associated insects (Pedrol et al. 2006, Lambers et al. 2008). They could also have inhibitory or stimulatory effects on other plant species (allelopathy) (Rice 1984). Plants grown under water stress have higher allelopathic influence on associated species compared to well-watered plants (Rice 1984, Tongma et al. 2001).

Hound's-tongue is a rangeland species which exerts allelopathic influence on herbivores and neighboring grasses (van Dam et al. 1994, Upadhyaya et al. 1988, Furness et al. 2008). However, the effect of water stress on allelopathic influence of hound's-tongue has not been studied. This study investigated the allelopathic effect of hound's-tongue leaves grown under four soil moisture stress (SMS) levels on grasshopper feeding behaviour and on seed germination and seedling growth of some associated grass species.

4.1 Hound's-tongue-insect interaction

My study showed that SMS affects the allelopathic influence of hound's-tongue on feeding preference and growth of grasshoppers. When grasshoppers were released on intact hound's-tongue, they generally consumed more leaf area from plants grown under 100 compared to 40% field capacity (FC). Smaller and fewer leaves of plants grown under 40% compared to 100% FC might decrease area consumption by grasshoppers. Middle-aged leaves of plants at 40% FC were consumed more compared to younger or older leaves; there was no consistent relationship between the leaf age and the area consumed for plants at 100% FC.

The choice bioassay experiments were conducted to eliminate some of the leaf physical differences such as leaf size and leaf orientation for hound's-tongue plants grown under 4 SMS levels. However other physical factors such as leaf trichomes and texture (toughness and hairiness), and leaf chemistry could affect feeding preference of grasshoppers for discs excised from leaves of plants grown under 4 SMS levels in choice bioassay experiments.

In separate choice bioassays, grasshoppers preferred young leaves of plants grown under 40% compared to old leaves at the same SMS treatment and young leaves from plants grown under 100% FC. They showed no preference for any choice between old leaves of 100 vs. 80, 60, and 40% FC in individual experiments, but pooled results of three experiments showed their preference for old leaves at 60 and 40% over 100% FC. In the growth experiment, grasshoppers gained more fresh weight on discs from young leaves of plants developed at 40% FC compared to old leaves from the same SMS treatment and either young or old leaves of plants grown at 100% FC.

Greater preference and growth of grasshoppers on discs from young leaves of plants grown under high SMS level (40% FC) suggests that hound's-tongue plants growing in dry conditions become attractive to herbivorous insects. The higher leaf nitrogen content, leaf trichomes, and PA concentration of hound's-tongue plants grown under high SMS could attract grasshoppers to this weed. The effect of pubescence and PA concentration of either young or old leaves of hound's-tongue grown under high SMS on feeding preference and growth of grasshoppers would be a valuable subject for future studies.

Leaf chemical analyses for PA concentration of either young or old leaves of hound's-tongue plants grown under SMS could provide more information about the preference of grasshoppers for young leaves of highly stressed plants. The better performance of grasshoppers on hound's-tongue leaves grown under high SMS could implicate the herbivory of grasshoppers on this weed in microsites with different levels of SMS. This information increases our understanding of plant-insect interaction that might be used for biological control of weeds.

In choice bioassays, some grasshoppers did not show any preference for either young or old leaves of hound's-tongue plants grown under any SMS levels. Some factors such as different sex and molting time may affect their feeding preference. For future studies, it is recommended to use a single sex of grasshoppers to reduce the molting time difference during the experiments.

4.2 Hound's-tongue-grass interaction

The results of this study showed that the hound's-tongue leaf leachate and leaf residue from plants grown under different SMS levels had either no or inconsistent allelopathic effect on seed germination and seedling growth of Idaho fescue, bluebunch wheatgrass, and hound's-tongue.

The inhibition of root elongation of Idaho fescue, bluebunch wheatgrass, and hound's-tongue incubated by high leachate concentrations from hound's-tongue leaves grown under high SMS levels compared to water controls in Petri dishes supported the allelopathic effect of hound's-tongue leaf leachate on seedling growth of neighboring grasses (Furness et al. 2008). Leachate from hound's-tongue plants grown under high SMS had either no or inconsistent inhibitory influence on seed germination and seedling growth of Idaho fescue, bluebunch wheatgrass, and hound's-tongue in Petri dishes.

Hound's-tongue leaf residue mixed with soil from plants grown under high SMS (40% FC) inhibited Idaho fescue seedling emergence. The presence of hound's-tongue leaf residue in the soil could decrease growth and survival of Idaho fescue in nature. Cover of leaf residue from plants grown under 40% FC reduced hound's-tongue root elongation compared to residue from plants developed at 80% FC in both experiments. This suggests that under high SMS, leaf residue from mother plants on soil surface inhibits the growth of hound's-tongue seedlings in rangeland. Hound's-tongue leaf leachate or leaf residue mixed with or covering soil from plants grown under different SMS levels had either no or inconsistent allelopathic effect on seedling number, emergence, and growth of Idaho fescue, bluebunch wheatgrass, and hound's-tongue.

The results of soil experiments suggested that increasing SMS may not significantly affect the allelopathic influence of hound's-tongue leaf leachate and leaf residue on seed germination and seedling growth of neighboring grasses in rangelands. However, in natural ecosystems deep roots of hound's-tongue could help this weed to successfully obtain relatively more resources than grasses under SMS condition. The inhibitory effect of hound's-tongue leaf residue cover from plants grown under 40% FC could show the self-inhibitory effect of mother plant on germination and growth of hound's-tongue seedlings under high SMS level in rangelands. It also suggests that hound's-tongue may have greater allelopathic effects on

broadleaf species compared to grasses. Future studies could investigate the allelopathic effects of hound's-tongue on neighboring broadleaf species in rangeland.

The study of SMS effects on allelopathic influence of hound's-tongue on associated grasses needs to be repeated in the lab and be confirmed by field studies to increase our understanding of hound's-tongue interaction with neighboring grasses under various SMS conditions. It is difficult to separate the allelopathic effects of hound's-tongue from its competition effect on neighbouring grasses in nature (Qasem and Hill 1989). Whether association of hound's-tongue with grasses in nature influences its allelopathic potential should be investigated.

Literature Cited

- Akhtar, Y., C. H. Rankin, and M. B. Isman. 2003. Decreased response to feeding deterrents following prolonged exposure in the larvae of a generalist herbivore, *Trichoplusia ni* (Lepidoptera: Noctuidae). *J. Insect Behav.* 16: 811-831.
- Anonymous 2011. B.C. Ministry of Agriculture.
<http://www.agf.gov.bc.ca/cropprot/weedman.htm#top> (Seen on Oct. 10, 2011).
- Baker, D. C., R. A. Smart, M. H. Ralphs, and R. J. Molyneux. 1989. Hound's-tongue (*Cynoglossum officinale*) poisoning in a calf. *J. Amer. Vet. Med. Assoc.* 194: 929-930.
- Ball, J. P., D. Kjell, P. Sunesson. 2000. Response of a herbivore community to increased food quality and quantity: an experiment with nitrogen fertilizer in a boreal forest. *J. Appl. Ecol.* 37: 247-255.
- Bazelet, C. S. 2011. Grasshopper bioindicators of effective large-scale ecological networks, Ph.D. Dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, South Africa.
<http://www.esajournals.org/doi/pdf/10.1890/ES11-00095.1> (Seen on Dec. 2011).
- Bernays, E. A., and K. L. Bright. 2001. Food choice causes interrupted feeding in the generalist grasshopper *Schistocerca americana*: further evidence for inefficient decision-making. *J. Insect Physiol.* 47: 63-71.
- Blicker, P. S., B. E. Olson, and R. Engel. 2002. Traits of invasive *Centaurea maculosa* and two native grasses: effect of N supply. *Plant Soil* 247: 261-269.
- Branson, D. H. 2011. Relationships between plant diversity and grasshopper diversity and abundance in the Little Missouri National grassland.
<http://www.hindawi.com/journals/psyche/2011/748635/cta/> (Seen on Dec. 2011).
- Branson, D. H., A. Joern, and G. A. Sword. 2006. Sustainable management of insect herbivores in grassland ecosystems: new perspectives in grasshopper control. *Bioscience* 56: 743-755.
- Brattsten, L. B. 1986. Fate of ingested plant allelochemicals in herbivorous insects. Pages 211-255. *In* L. B. Brattsten, and S. Ahmad, eds. *Molecular aspects of insect-plant associations*. Plenum Press, New York. USA.

- Caldeira, M. C., V. Fernández, J. Tomé, and J. S. Pereira. 2002. Positive effect of drought on longicorn borer larval survival and growth on eucalyptus trunks. *Ann. For. Sci.* 59: 99-106.
- Callaway, R. M., and E. T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* 290: 521-523.
- Chaves. N., and J. C. Escudero. 1997. Allelopathic effect of *Cistus ladanifer* on seed germination. *Funct. Ecol.* 11: 432-440.
- Chou, C. H. 2006. Introduction to allelopathy. Pages 1-9. *In* M. J. Reigosa, N. Pedrol, and L. Gonzalez, eds. *Allelopathy: A physiological process with ecological implications*. Springer. Dordrecht. The Netherlands.
- Christiansen, E., and Ø. Austara. 1996. Development of *Neodiprion sertifer* (Geoff) (Hym., Diprionidae) on drought-stressed pines: a laboratory experiment. *J. Appl. Entomol.* 120: 221-23.
- Coley, P. D. 1983. Herbivory and defensive characteristics of tree species in lowland tropical forest. *Ecol. Monog.* 53: 209-233.
- Cranston, R. S. and J. L. Pethybridge. 1986. Report on houndstongue (*Cynoglossum officinale*) in British Columbia, B.C.M.A. & F., Internal. Rept. 8 pp.
- Dahl, B. E., and D. N. Hyder. 1977. Developmental morphology and management implications. Pages 257-290. *In* R. E. Sosebee, ed., *Rangeland plant physiology*. Range Sci. Ser. No. 4. Denver. Colorado. USA.
- De Clerck-Floate, R., and B. Wikeem. 2009. Influence of release size on establishment and impact of a root weevil for the biocontrol of houndstongue (*Cynoglossum officinale*). *Biocontrol Sci. Tech.* 19:169-183.
- de. Boer, N. J. 1999. Pyrrolizidine alkaloid distribution in *Senecio jacobaea* rosettes minimises losses to generalist feeding. *Entomol. Exp. Appl.* 91: 169-173.
- del Moral, R. 1972. On the variability of chlorogenic acid concentration. *Oecologia* 9: 289-300.
- Devakumar, C., and B. S. Parmar. 1993. Pesticides of higher plant and microbial origin. Pages 1-73. *In* B. S. Parmar, and C., Devakumar, eds. *Botanical and Pesticides*, SPS Publication No. 4. Soc. Pest. Sci., India and Westvill Publishing House. New Delhi. India.

- DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management. *Weed Sci.* 48: 255-265.
- Drolet, B. S., M. A. Stuart, and J. D. Derner. 2009. Infection of *Melanoplus sanguinipes* grasshoppers following ingestion of rangeland plant species harboring vesicular stomatitis virus. *Appl. Environ. Microbiol.* 75: 3029-3033.
- El-Shazly, A., T. Sarg, A. Ateya, E. Abdel aziz, L. Witte, and M. Wink. 1996. Pyrrolizidine alkaloids of *Cynoglossum officinale* and *Cynoglossum amabile* (family Boraginaceae). *Biochem. Sys. Ecol.* 24: 415-421.
- Ewing, K. 2002. Effects of initial site treatments on early growth and three-year survival of Idaho fescue. *Restor. Ecol.* 10: 282-288.
- Foy, C. L., and Inderjit. 2001. Understanding the Role of allelopathy in weed interference and declining plant diversity. *Weed Tech.* 15: 873-878.
- Franzke, A., and K. Reinhold. 2011. Stressing food plants by altering water availability affects grasshopper performance.
- Furness, N. H., B. Adomas, Q. Dai, S. Li, and M. K. Upadhyaya. 2008. Influence of houndstongue (*Cynoglossum officinale*) and its modification by UV-B radiation. *Weed Tech.* 22: 101-107.
- Gutbrodt, B., K. Mody, and S. Dorn. 2011. Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos* 1: 1-9.
- Haribal M., and J. A. A. Renwick. 1998. Isovitexin 6"-O- β -D-glucopyranoside: a feeding deterrent to *Pieris napi oleracea* from *Alliaria petiolata*. *Phytochemistry* 47: 1237-1240.
- Heady, H., and R. D. Child. 1994. Rangeland ecology and management. Westview Press, Inc. Boulder. Colorado. USA.
- Hewitt, G. B. 1977. Review of forage losses caused by rangeland grasshoppers. USDA Miscellaneous Publ. No. 1348. Washington, D. C., USA.
- Hewitt, G. B., and J. A. Onsager. 1982. A method for forecasting potential losses from grasshoppers on northern mixed grass forages. *J. Range Manage.* 35:53-57.

- Hinks, C. F., and M. A. Erlandson. 1994. Rearing grasshoppers and locusts: review, rationale and update. *J. Orthoptera Res.* 3: 1-10.
- Hinks, C. F., and O. Olfert. 1999. Growth and survival of early-instar grasshoppers on selected perennial grasses, with observations on plant constituents which may influence performance. *J. Orthoptera Res.* 8: 237-242.
- Ho, L. C., R. G. Hurd, L. J. Ludwig, A. F. Shaw, J. H. M. Thornley, and A. C. Withers. 1984. Changes in photosynthesis, carbon budget and mineral content during the growth of the first leaf of cucumber. *Ann. Bot.* 54: 87-101.
- Hsiao, T. C. 1973. Plant response to water stress. *Ann Rev. Plant Physiol.* 24: 519-570.
- Huberty, A. F., and R. F. Denno. 2004. Plant water stress and its consequences for herbivorous insects. *Ecology* 85: 1383-1398.
- Ibanez, I., E. W. Schupp. 2002. Effects of litter, soil surface conditions, and microhabitat on *Cercocarpus ledifolius* Nutt. seedling emergence and establishment. *J. Arid Environ.* 52: 209-221.
- Ikonen, A. 2002. Preference of six leaf beetle species among qualitatively different leaf age classes of three Salicaceous host species. *Chemoecology* 12: 23-28.
- Inbar, M., H. Doostdar, and R. T. Mayer. 2001. Suitability of stressed and vigorous plants to various insect herbivores. *Oikos* 94: 228-235.
- Isman, M. B., O. Koul, A. Luczynski, and A. Kaminski. 1990. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *J. Agr. Food Chem.* 38: 1406-1411.
- Johnson, D. A. and L. Aguirre. 1991. Effect of water on morphological development in seedlings of three range grasses: Root branching patterns. *J. Range Manage.* 44: 355-360.
- Karageorgou P., E. Levizou, and Y. Manetas. 2002. The influence of drought, shade and availability of mineral nutrients on exudate phenolics of *Dittrichia viscosa*. *Flora* 197: 285-289.
- Khan, T. A., M. Mazid, and F. Mohammad. 2011. Status of secondary plant products under abiotic stress: an overview. *J. Stress Physiol. Biochem.* 7: 75-98.

- Knight, A. P., C. V. Kimberling, F. R. Stermitz, and M. R. Roby. 1984. *Cynoglossum officinale* (hound's-tongue) - a cause of pyrrolizidine alkaloid poisoning in horses. J. Am. Vet. Med. Assoc. 184: 647-650.
- Kohli, R. K., D. R. Batish, and P. H. Singh. 2006. Allelopathic interaction in agroecosystems. Pages 465-493. In M. J. Reigosa, N. Pedrol and L. González, eds. Allelopathy: a physiological process with ecological implications. Springer. Dordrecht. The Netherlands.
- Koricheva, J., and S. Larsson. 1998. Insect performance on experimentally stressed woody plants: a meta-analysis. Annu. Rev. Entomol. 43: 195-216.
- Koricheva, J., S. Larsson, and E. Haukioja. 1998. Insect performance on experimentally stressed woody plants: a meta-analysis. Ann. Rev. Entomol. 43: 195-216.
- Lambers, H., F. S. Chapin III, and T. L. Pons. 2008. Plant physiological ecology. Pages 445-477. Springer. New Jersey. USA.
- Lambley, J. D., J. B. Campbell, and H. Knutson. 1972. Food preferences of grasshoppers in six planted pastures in eastern Kansas. J. Kans. Entomol. Soc. 45: 59-92.
- Larsson, S. 1989. Stressful times for the plant stress - insect performance hypothesis. Oikos 56: 277-283.
- Latchininsky, A., G. Sword, M. Sergeev, M. M. Cigliano, and M. Lecoq. 2011. Locusts and grasshoppers: behavior, ecology, and biogeography.
<http://www.hindawi.com/journals/psyche/2011/578327/> (Seen on Dec. 2011)
- Lau, J. A., K. P. Puliafico, J. A. Kopshever, H. Steltzer, E. P. Jarvis, M. Schwarzländer, S. Y. Strauss, and R. A. Hufbauer. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. New Phytol. 178: 412-423.
- Levin, D. A. 1973. The role of trichomes in plant defense. Q. Rev. Biol. 48: 3-15.
- Lewis, A. C. 1984. Plant quality and grasshopper feeding: effects of sunflower condition on preference and performance in *Melanoplus differentialis*. Ecology 65: 836-843.
- Louda, S. M., and J. E. Rodman. 1994. Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). J. Ecol. 84: 229-237.

- Macel, M. 2011. Attract and deter: a dual role for pyrrolizidine alkaloids in plant-insect interactions. *Phytochem Rev.* 10: 75-82.
- Macel, M., M. Bruinsma, S. M. Dijkstra, T. Ooijendijk, H. M. Niemeyer, and P. G. L. Klinkhamer. 2005. Differences in effects of pyrrolizidine alkaloids on five generalist insect herbivore species. *J. Chem. Ecol.* 31: 1493-1508.
- Mahall, B. E., and R. M. Callaway. 1991. Root communication among desert shrubs. *Ecology* 88: 874-876.
- Makkar, H. P. S., P. Siddhuraju, and K. Becker. 2007. Plant secondary metabolites. Pages 1-110. Humana Press Inc., Totowa. New Jersey. USA.
- Mattocks, A. R. 1986. Chemistry and toxicology of pyrrolizidine alkaloids. Pages 350-430. Academic Press, London. UK.
- Mattson, W., and R. Haack. 1987. Role of drought in outbreaks of plant-eating insects. *Bioscience* 37: 110-118.
- Meyer, G. A., and M. E. Montgomery. 1987. Relationships between leaf age and the food quality of cottonwood foliage for the gypsy moth, *Lymantria dispar*. *Oecologia* 72: 527-532.
- Miller, R. F., J. M. Seufert, and M. R. Haferkamp. 1986. The ecology and management of bluebunch wheatgrass (*Agropyron spicatum*): A review. Oregon Agr. Exp. Sta. Bull. No. 669.
- Miura, K., and N. Ohsaki. 2004. Relationship between physical leaf characteristics and growth and survival of polyphagous grasshopper nymphs, *Parapodisma subastris* (Orthoptera: Catantopidae). *Popul. Ecol.* 46:179-184.
- Mody, K., D. Eichenberger, and S. Dorn. 2009. Stress magnitude matters: different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. *Ecol. Entomol.* 34: 133-143.
- Mole, S., and A. Joern. 1994. Feeding behaviour of graminivorous grasshoppers in response to host-plant extracts, alkaloids, and tannins. *J. Chem. Ecol.* 20: 3097-3109.
- Mooney, H. A., and S. L. Gulmon. 1982. Constraints on leaf structure and function in reference to herbivory. *Bioscience* 32: 198-206.

- Nagarajan, S., and S. Nagarajan. 2010. Abiotic tolerance and crop improvement. Pages 1-11. *In* A. Pareek, S. K. Sopory, and H. J. Bohnert, eds. Abiotic stress adaptation in plants; physiological, molecular and genomic foundation. Springer. Dordrecht. The Netherlands.
- Olson, B. E., and R. T. Wallander. 2002. Effects of invasive forb litter on seed germination, seedling growth and survival. *Basic Appl. Ecol.* 3: 309-317.
- Parish, R., R. Coupe, and D. Lloyd (eds). 1996. Plants of southern interior British Columbia and inland Northwest. Vancouver. B.C. Ministry of Forests and Lone Pine Publishing. Pages 308-338.
- Pedrol, N., L. Gonzalez, and M. J. Reigosa. 2006. Allelopathy and abiotic stress. Pages 171-209. *In* M. J. Reigosa, N. Pedrol, and L. Gonzalez, eds. Allelopathy, a physiological process with ecological implications. Springer. Dordrecht. The Netherlands.
- Pfadt, R. E. 1996. Field guide to common western grasshoppers. USDA APHIS. Wyoming Agricultural Experiment Station. Laramie. Wyoming. USA.
- Pfister, J. A., R. J. Molyneux, and D. C. Baker. 1992. Pyrrolizidine alkaloid content of houndstongue (*Cynoglossum officinale* L.). *J. Range Manage.* 45: 254-256.
- Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* 62: 244-251.
- Qasem, J. R., and L. Foy. 2001. Weed allelopathy, its ecological impacts and future prospects. *J. Crop Prod.* 4: 43-119.
- Qasem, J. R., and T. A. Hill. 1989. On difficulties with allelopathy methodology. *Weed Res.* 29: 345-347.
- Qi, M. Q., M. K. Upadhyaya, N. H. Furness, and B. E. Ellis. 1993. Mechanism of seed dormancy in *Cynoglossum officinale* L. *J. Plant Physiol.* 14:325-330.
- Radosevich, R. S., J. S. Holt, and C. Ghera. 2007. Ecology of weeds and invasive plants: relationship to agriculture and natural resource management. Third edition. John Wiley and Sons, Inc., Hoboken. New Jersey. USA.
- Rashid, A., N. H. Furness, M. K. Upadhyaya, and B. E. Ellis. 2005. Inhibition of seed germination and seedling growth by houndstongue (*Cynoglossum officinale* L.) seed extract. *Weed Biol. Manag.* 5:143-149.

- Raupp, M. J. and R. F. Denno. 1983. Pages 85-97. *In* R. F. Denno, and M. S. McClure, eds. Variable plants and herbivores in natural and managed systems. Acad. Press, New York. USA.
- Rice, E. L. 1984. Allelopathy. Academic Press. Pages 250-300. Orlando. Florida. USA.
- Ridenour, W. M., and R. M. Callaway. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126: 444-450.
- Robert-Seilanianantz, A., R. Bari, and J. D. G. Jones. 2010. A biotic or abiotic stress? Pages 103-122. *In* A. Pareek, S. K. Sopory, H. J. Bohnert. eds. Abiotic stress adaptation in plants; physiological, molecular and genomic foundation. Springer. Dordrecht. The Netherlands.
- Scheirs, J., and L. De Bruyn. 2005. Plant-mediated effects of drought stress on host preference and performance of a grass miner. *Oikos* 108: 371-385.
- Schoonhoven, L. M., J. J. A. van Loon, and M. Dicke. 2005. Insect-plant biology. Pages 1-134. Second edition. Oxford Univ. Press, New York. USA.
- Sinclair, A. R. E., C. J. Krebs, and J. N. M. Smith. 1982. Diet quality and food limitation in herbivores: the case of the snowshoe hare. *Can. J. Zool.* 60: 889-897.
- Taiz, L., and E. Zeiger. 2010. Plant physiology. Pages 756-770. Fifth edition. Sinauer Associates Inc., Publishers. Sunderland. Maryland. USA.
- Tang. C., W. Cai, K. Kohl, R. K. Nishimoto. 1995. Plant stress and allelopathy. ACS Symposium Series. Vol. 582. Chapter 11. Pages 142-157. American Chemical Society.
- Tefera, T. 2002. Allelopathic effects of *Parthenium hysterophorus* extracts on seed germination and seedling growth of *Eragrostis tef*. *J. Agron. & Crop Sci.* 188: 306-310.
- Tongma, S., K. Kobayashi, and K. Usui. 2001. Allelopathic activity of Mexican sunflower [*Tithonia diversifolia* (Hemsl.) A. Gray] in soil under natural field conditions and different moisture conditions. *Weed Biol. Manag.* 1: 115-119.
- Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* 11: 1351-1363.
- Upadhyaya, M. K. and R. S. Cranston. 1991. Distribution, biology and control of houndstongue in British Columbia. *Rangelands* 13:3.

- Upadhyaya, M. K., and N. H. Furness. 1994. Influence of light intensity and water stress on leaf surface characteristics of *Cynoglossum officinale*, *Centaurea* spp., and *Tragopogon* spp. *Can. J. Bot.* 72: 1379-1386.
- Upadhyaya, M. K., H. R. Tilsner, and M. D. Pitt. 1988. The biology of Canadian weeds. 87: *Cynoglossum officinale* L. *Can. J. Plant Sci.* 68: 763-774.
- van Dam, N. M., L. W. M. Vuister, C. Bergshoeff, H. De Vos, and E. van der Meijden. 1995a. The “raison d’etre” of pyrrolizidine alkaloids in *Cynoglossum officinale*: deterrent effects against generalist herbivores. *J. Chem. Ecol.* 21: 507-523.
- van Dam, N. M., L. Witte, C. Theuring, and T. Hartmann. 1995b. Distribution, biosynthesis and turnover of pyrrolizidine alkaloids in *Cynoglossum officinale*. *Phytochemistry* 39: 287-292.
- van Dam, N. M., E. van der Meijden, and R. Verpoorte. 1993. Induced responses in three alkaloid-containing plant species. *Oecologia* 95: 425-430.
- van Dam, N. M., R. Verpoorte, and E. van der Meijden. 1994. Extreme differences in pyrrolizidine alkaloid levels between leaves of *Cynoglossum officinale*. *Phytochemistry* 37: 1013-1016.
- Wait, D. A., J. S. Coleman, and C. G. Jones. 2002. *Chrysomela scripta*, *Plagiodera versicolora* (Coleoptera: Chrysomelidae), and *Trichoplusia ni* (Lepidoptera: Noctuidae) track specific leaf developmental stages. *Environ. Entomol.* 31: 836-843.
- White, P. R., and R. F. Chapman. 1990. Tarsal chemoreception in the polyphagous grasshopper *Schistocerca americana*: behavioural assays, sensilla distributions and electrophysiology. *Physiol. Entomol.* 15: 105-121.
- White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63: 90-105.
- Whittaker, R. H. 1970. The biochemical ecology of higher plants. In *chemical ecology* (eds. E. Sondheimer and J. B. Simeone). Pages 43-70. Academic. Press. New York. USA.
- Wikeem, B. M., and S. J. Wikeem. 2004. The Grasslands of British Columbia. Pages 1-86. Grasslands Conservation Council of British Columbia. Kamloops. BC. Canada.

Wurst, S., V. Vender, and M. C. Rillig. 2010. Testing for allelopathic effects in plant competition: does activated carbon disrupt plant symbioses? *Plant Ecol.* 211: 19-26.

Yang, Y., and A. Joern. 1994. Gut size changes in relation to variable food quality and body size in grasshoppers. *Funct. Ecol.* 8: 36-45.