WEED SEED PREDATION BY CARABID BEETLES AND CRICKETS FOR BIOLOGICAL CONTROL OF WEEDS IN WILD BLUEBERRY FIELDS

by

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Dedication Page

To my daughters, Karman and Manmeet, and my wife Harpreet

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Abstract

Weeds are a major pest problem of wild blueberry. Granivorous insects may consume weed seeds, contributing to pest control. In the laboratory, the ground beetle *Harpalus rufipes* and field cricket *Gryllus pennsylvanicus* consumed a significant number of seeds of sheep sorrel and hairy fescue, two important weeds of wild blueberry. In a prey vs. seed preference experiment, *H. rufipes* preferred aphids over sheep sorrel seeds. Field experiments also found considerable weed seed granivory by invertebrate herbivores. There was generally no effect of distance from field edge and type of field ("crop" or "vegetative") on the weed seed granivory. In experiments with insecticides used in blueberry production, *H. rufipes* was highly susceptible to field rates of phosmet (Imidan) and acetamiprid (Assail) by topical exposure and ingestion of treated seeds, whereas no mortality was seen with spirotetramat (Movento).

List of Abbreviations and Symbols Used

α:	level of significance
°C:	degree Celsius
®:	registered
μg:	microgram (1 μg = 1x 10^{-6} g)
ANOVA:	analysis of variance
df:	degrees of freedom
d:	days
et al:	and others
F:	F-test statistic
h:	hour
ha:	hectare (1 ha= 10,000 m^2)
IPM:	integrated pest management
kg:	Kilogram
l:	liter
LSD:	least significant difference
m:	meter
min:	minute
n:	sample size
P:	P-value
r:	Pearson correlation coefficient
R^2:	regression coefficient of determination
R.H.:	relative humidity
SD:	Standard deviation
SEM:	standard error of mean
H:	Kruskel-Wallis test statistic

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Chapter One: Introduction

1.1 Wild blueberry industry

Wild blueberry (lowbush blueberry), *Vaccinium angustifolium* Ait. (Ericaceae), is an indigenous, deciduous, perennial fruit crop of North America (Yarborough 2009). The term "wild" refers to the management and harvesting of wild stands of blueberry that spread naturally by means of underground rhizome systems (Barker and Collins 1963; Hall et al. 1979). Wild blueberries are small shrubs that are amongst the first plants that colonize disturbed land in many temperate-boreal areas (Kinsman 1986). Wild blueberry production provides revenue from unproductive or abandoned lands, and is amongst the most important Canadian horticultural exports. Canada is the second largest commercial producer and exporter of blueberries after the United States. The wild blueberry industry is one of the most promising sectors for agricultural growth in Atlantic Canada (Audy 2007) and commercial wild blueberry fields currently cover almost half of agricultural land areas in fruit and nut production in Canada (Robichaud 2006). In 2012, Canadian production of blueberries that included both highbush and lowbush blueberries was 121,780 metric tons from 72,657 ha of land, generating a farm-gate value of $242.6 million (Statistics Canada 2012). Nova Scotia is the largest producer of wild blueberry in Canada and recognizes wild blueberry as its official berry (Anonymous 2012). Wild blueberries are low in calories, rich in fiber and nutrients and high in anti-oxidants that have anti-inflammatory properties and reduce neurodegenerative disease and blood cholesterol levels (Willis et al. 2005; Zheng and Shiow 2003).

Wild blueberry is a group of six different species in the genus *Vaccinium*: *V. angustifolium* Ait, *V. myrtilloides* Michx, *V. boreale* Hall and Alders, *V. darowii* Camp, *V. tenellium* Ait., and *V. palladium* Ait. (Vorsa 1997), with the two most prominent species being *V. angustifolium* Ait. ("low sweet" blueberry) (80-95%) and *V. myrtilloides* Michx. ("sour-top" or "velvet leaf" blueberry) (Smagula et al. 1997). Each wild blueberry plant is a clonal plant that spreads from 6.5 to 25 square meters with height range of 0.04-0.24 m. The appearance of a field is therefore often non-uniform due to its composition of many distinct clones (Hall et al. 1979; Yarborough 2009). Each clone has genetically different attributes, including bush height, sprout emergence timing, bloom time, leaf, flower and berry colour, and resistance to insect pests (Barry et al. 2003; Collins et al. 1996). Wild blueberry plants are adapted to infertile, acidic (pH = 4.0 - 5.5) (Hayes 1988; Sheppard 1991), sandy loam soils deficient in available phosphorous, potassium, calcium, and magnesium, and high in iron (Gimingham 1975; Korcak 1989). The lands where blueberries are grown are also known as 'heathlands' or 'heath' referring to areas where trees or tall shrubs are sparse, land is well-drained, and ericaceous dwarf shrubs are dominant vegetation (Gimingham 1975).

The wild blueberry establishes in abandoned pastures or moss depressions, exposed ridges or freshly cleared forests, primarily by seed dispersal by birds and mammals (Hall 1978; VanderKloet 1978). Once established, the plant spreads by rhizomes (Hancook and Draper 1989). The plants are stress tolerant and compensate for low nutrient availability with a slow growth rate (Sheppard 1991) and by forming symbiotic relationships with mycorrhizae (Coutre et al. 1983; Dalpe 1989). Wild blueberry is a perennial plant that for commercial production is typically forced into biennial cropping system (Ismail and Yarborough 1981) with the first year being a vegetative "sprout" season where berries are not produced, and the second year being a fruit-bearing "crop" year, from which fruit are harvested. Following harvest of fruits, fields are pruned to ground-level by mowing or burning such that the fields then re-enter the sprout phase of production. Thus, commercial fields are generally harvested every two years and go through alternating crop-sprout phases. Compared to pruning fields by mowing, burned pruning may result in loss of nitrogen up to 34 kg per ha (Eaton and Patriquin 1990), although higher N and P have been reported following burning compared to mowing (Hanson et al. 1982). Mowing is also reported to have higher incidence of disease and pests of wild blueberry resulting from increased amount of stubbles and debris left on the fields (Lambert 1990) as compared to destroying of overwintering insects, weeds and their seeds banks, and pathogens by burning (Lambert 1990; Yarborough 2009). However, growers often prefer pruning by mowing over burning due to environmental factors like pollution, emission of greenhouse gases (GHGs), rising fuel costs, and burning permits. Pruning by mowing have economic and environmental benefits, but has probably resulted in increased pest populations including insects, weeds, and diseases.

1.2 Weeds of wild blueberry

Weeds potentially reduce the vigour of wild blueberry plants, and limit fruit yields and quality of the produce. They compete with the crop for resources necessary for adequate plant growth, harbor insects pests and diseases and make farming operations difficult. Annual losses due to weeds in crops each year may reach $27 million (Pimentel et al. 2005). Weeds are a major yield limiting factor in blueberry fields (Boyd and White 2010) and weed management practices can cost farmers from 17-21% of the total production and harvesting cost of blueberries (Sibley 1994). Indeed, the Wild Blueberry Producers Association of Nova Scotia (WBPANS) 2013 research priorities document considered management of perennial weeds as a primary concern. Surveys show that eight new weed species have established since the 1980s in blueberry fields in Atlantic Canada (Jensen and Yarborough 2004; Yarborough 2004). A variety of factors contributed to the present day weed flora of wild blueberry. The shift of pruning by burning to mowing is one of the major contributor (Jensen and Yarborough 2004). Disturbances such as logging favored establishment of weeds like bracken fern (*Pteridium aquilinum* (L.) Kuhn) (McDonald et al. 2003). Sheep sorrel (*Rumex acetosella* L.), hairy fescue (*Festuca tenuifolia* Sibth), goldenrod (*Solidago canadensis* L.), poverty oatgrass (*Danthonia spicata* (L.) Beauv), spreading dogbane (*Apocynum androsaemifolium* L.), Bracken fern (*P. aquilinum*) and tickle grass (*Agrostis scabra* Willd.) are among the most notorious weeds of wild blueberry.

Sheep sorrel gives fields a reddish tint due to its flower and fruits (Friedlander 1966). Surveys conducted in 1984 and 1985 (McCully et al. 1991), and 2000 and 2001 (Jensen and Sampson, unpublished data) reported sheep sorrel on 85% and 90% of blueberry fields, respectively. This could have probably occurred due to a rhizome network that is still present even after burning or mowing and resistance to herbicides (McCully 1988). Tussock forming grasses like poverty oat grass (*D. spicata* (L.) Beauv.), and fescues (*Festuca* spp.) are common in Nova Scotia, giving blueberry fields a mat like appearance. These grasses are frequent on fields developed from abandoned hayfields or pastures (Anonymous 1999). Grasses like fescues were indicated avoiding control attempts due to their presence in high frequencies and high uniformities in fields (McCully 1988). In early 1970s, blueberry growers were discouraged from applying fertilizers in their weed infested fields due to uptake of nutrients by weeds rather than blueberry plants, thus reducing blueberry biomass (Smagula 1979).

1.3 Weed management in wild blueberry

At present, a variety of chemical and cultural control methods are available for blueberry growers. Cultural control methods are combinations of physical and preventative measures to disrupt life cycle of weeds and prevent their establishment. Physical methods include mechanical weed suppression (Yarborough 1996) which includes hand pulling (spot treatment), cutting of woody weeds, burning, mulching and mowing (Yarborough 1996; Yarborough 2001). Though cultural control methods are good for weed management, farmers mainly rely on chemical control and this has been the preferred method of weed control for the past 60 years (Jensen and Yarborough 2004). This method uses selective or non-selective, pre or post emergent herbicides.

1.4 Carabid beetles and crickets as seed predators in weed management

Ground beetles (Coleoptera: Carabidae) are one of the most common and diverse families on the planet representing 40,000 species of surface-dwelling arthropods (Lovei and Sunderland 1996). They feed on a wide range of foods (invertebrates and plant materials) and granivory is well established (Tooley and Brust 2002). Some carabids having an affinity for seeds and aggregate where seeds are abundant (Honek and Martinkova 2001). Carabids are often the most important granivores of temperate zones (Thomas et al. 2001). Some species are reported to complete their life cycle by partially or solely feeding on seeds (Saska and Jarosik 2001).

Positive effects have been reported from seed predation by carabids in different crops. Herbaceous seed (mostly weed seeds) removal from trays had been reported to increase with an increased density of carabids and scarcity of weed seeds in the field (Honek et al. 2003). More seed predation was reported in organic fields compared to conventionally managed mixed cropping fields of trees, shrubs and crops in New Zealand (Navntoft et al. 2009). No significant effect of distance from the forest edge was observed in treatments. Compared to conventional (high external inputs, tillage) and organic farms (no chemical inputs, tillage), no-till (high external chemical inputs and no tillage) operations had three times greater carabid activity and density, and two times more predation of weed seeds by carabids in a corn-soybean-wheat cropping system (Menalled et al. 2007). Tillage and herbicide application might have negatively affected carabid abundance through disruption of overwintering sites and mortality due to herbicides. Higher density of carabids in no-till fields could have been due to less habitat disruption in the absence of tillage. Climbing and feeding behavior of carabid beetles *Amara gigantea* Motschulsky and *A. macronota* Solsky on weed flowers and seeds, of *Humulus scandens* (Loureiro) Merrill (Moraceae) has been observed in Japan (Sasakawa 2010). This study also reported female biased feeding by *H. corposus* Motschulsky on *Chenopodium album* (L.) seeds on ground.

Although the potential of granivorous (seed eating) beetles for the biological control of weeds has been demonstrated in several other agroecosystems (Gaines and Gratton 2010; Honek et al. 2006; White et al. 2007), no such work has been done in wild blueberry. *Harpalus rufipes* (Degeer) (Coleoptera: Carabidae), a prevalent ground beetle in North America introduced from Europe, is now abundant throughout Canada, including Newfoundland and Labrador, Quebec, Nova Scotia, and New Brunswick and, in the USA, from Maine through to Connecticut (Zhang et al 1998). A recent study in Nova Scotia revealed a significant number of granivorous carabids like *H. rufipes* in the wild blueberry ecosystem (Cutler et al. 2012). Another preliminary study[1] revealed that insects removed a significant amount of grass seeds from tray offerings in wild blueberry fields. Consumption and destruction of weed seeds by native

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[1] N. Boyd, Formerly of the Nova Scotia Agricultural College; now residing at Horticultural Sciences Department, University of Florida.

granivores is one biological tactic that may reduce reliance on chemical and mechanical weed management. Integrating biological control with other weed control approaches may help to reduce management costs and improve quality and quantity of crops.

The field cricket, *Gryllus pennsylvanicus* Burmeister (Orthoptera: Gryllidae) is an omnivorous insect that feeds on living or dead insects, grasses, broadleaf plants and seeds of various plant species (Criddle 1925). Due to cricket abundance and occasional damage to crops like alfalfa (Rogers et al. 1985), wheat, barley, and tomatoes, they are sometimes considered as pests (Anonymous 1972). Crickets also consume weeds like common ragweed (*Ambrosia artemisiifolia* L.) and redroot pigweed (*Amaranthus retroflexus* L.) (Brust and House 1988). They are found in annual, biennial and perennial crops but prefer more stable habitats (Carmona et al. 1999; Vickery 1961). In Nova Scotia, *G. pennsylvanicus*, is active from the first week of August to the first week of November, and this is the period when most weeds set and shed seeds (Piers 1896; Vickery 1961). Crickets have consumed seeds of common agricultural weeds like velvetleaf (*Abutilon theophrasti* Medic.), giant foxtail (*Setaria faberi* Herrm.), crabgrass (*Digitaria sanguinalis* L.), and redroot pigweed under laboratory conditions (Carmona et al. 1999). Field crickets were reported to reduce weed seed emergence of velvetleaf, redroot pigweed and giant foxtail by up to 15 percent (White et al. 2007).

1.5 Effect of pesticides on predatory beetles

Blueberry farmers rely heavily on pesticides for the management of weeds and insect pests. This has resulted in improved quality and yield and has allowed for more efficient use of mechanical harvesters (Yarborough 2009). Carabidae are susceptible to several broad-spectrum pesticides, which reduce their activity and density in fields (Lee et al. 2001; Trumper and Holt 1998). This can result in pest population increases in fields where these pesticides are used due to absence of predators. In apple orchards, many broad spectrum pesticides like methyl parathion and carbaryl affected highly mobile invertebrates like Carabidae, Chilopoda, Dermaptera, and arachnids (Epstein et al. 2000), reducing populations to one third that seen in fields with less pesticide inputs. On the other hand, high doses of cypermethrin, applied up to eight times, in a barley crop did not have any acute effects on larger beetles like *Pterosticus melanarius* (Illiger), but small beetles like *Aleochara bilineata* (Gyll.) and *Bembidion lampros* (Herbst) were affected if the dose was more than the double the field rate (Gyldenkaerne et al. 2000). This relationship between dose and body size of the beetles was not reported for dimethoate where the field rate harmed all species equally.

A reduction in predation rates of carabids on weed species like *Capsella bursa-pastoris* and *Brassica nigra* from 21% (apple orchard) to 50% (vineyard) in different fields sprayed with pesticides has been reported (Minarro and Dapena 2003; Sanguankeo and Leon 2011) and carabids avoided surface with fresh pesticide residues (Michalkova and Pekar 2009). Carabid activity was reduced in glyphosate and paraquat sprayed fields due to destruction of plant material, such that fewer beetles were captured in the field for the following 28 d (Brust 1990). In a laboratory study on lethal effects of toxicity of imidacloprid, bendiocarb and halofenozide on carabid beetles, bendiocarb was found to be most lethal while halofenazide showed no apparent lethal activity when applied topically, orally or through residual exposure (Kunkel et al. 2001). Imidacloprid treated beetles had shown sublethal and neurotoxic effects like paralysis and excessive grooming. It was also reported that beetles intoxicated in lab recovered in few days but in the field they were vulnerable to predation by ants. Considering dry mass as measure of abundance instead of total catch, the abundance of some carabid species like *Pterostichus* spp., *Loricera* spp. and *Demetrias* spp. benefitted significantly from reduced applications of insecticides with an increase in catch of 60%, 67% and 56% respectively. On the contrary, they also found negative effects of reduced pesticide dosage on abundance of *Bembidion* spp. and *Synchus* spp. The catch in these cases reduced by 31 and 45% respectively (Navntoft et al. 2006). Activity and density of common phytophagous species like *Bembidion guttula* (F.), *Clivina fossor* (L.) and *Pterostichus* spp. were favored by reduced application of chlorpyriphos (Rushton et al. 1989). A reduction in the population of *H. rufipes* was reported when metribuzin was applied in fields as compared to only chisel plowed fields, which may be due to avoidance of the surface of herbicide treated fields (Zhang et al. 1998).

1.6 Study rationale

Reducing chemical inputs while increasing the role of biological control is an important priority for the blueberry industry. Compared to studies examining the use of insects for control of insect pests, there has been relatively little study into the potential of seed feeding insects for biocontrol of weeds (Tooley and Brust 2002). The primary purpose of this study was to test hypotheses concerning the potential of naturally occurring granivorous (seed eating) beetles and crickets for biological management of perennial weeds of wild blueberry fields.

1.7 Objectives and hypotheses

The objectives and hypotheses of present research were to:

I. Quantify seed consumption by granivorous seed predators *H. rufipes* and *G. pennsylvanicus* in the laboratory. Seed consumption and preference in insects varies with the size, shape and taxonomical characteristics of seeds (Jorgensen and Toft 1997). I hypothesized that seed consumption rates for sheep sorrel seeds would be higher than fescue seeds when given individually and in a mixture of seeds, because insects will have to spend less energy in getting food from endosperm rich sheep sorrel seeds as compared to hairy fescue which is more husk and less endosperm part.

II. Quantify seed removal in the field by herbivorous insects and determine if this varies spatially and temporally within fruiting and vegetative fields, at different distances from the forest edge. I hypothesized that there would be no effect of field type (‘crop’ or ‘vegetative’) and distance from forest edge as generalist predatory granivores like *H. rufipes* were reported present in high numbers throughout wild blueberry fields (Cutler et al. 2012).

III. Examine the effects of pesticides used in blueberry production on *H. rufipes*. Broad-spectrum insecticides are generally harmful to beneficial insects like predators, while certain newer chemistries are supposed to be safer. I hypothesized that: Movento (spirotetramat), a new lipid biosynthesis inhibitor insecticide and recently registered against blueberry fruit fly, would be non-toxic to *H. rufipes*. It is recommended to control sucking insect pests and is reported safe against a number of natural enemies including coleopterans (Bruck et al. 2009). I also hypothesized that Assail (aceptamiprid), a pyridylmethylamine neonicotinoid, and Imidan (phosmet), an organophosphorus insecticide, would be toxic to beetles due to their reported toxicities across several genera of insects including Coleoptera (Cloyd and Dickinson 2006; Elbert et al. 2008; French et al. 1992; Youn et al. 2003).

Chapter Two: *Harpalus rufipes* and *Gryllus pennsylvanicus* feeding upon weed seeds in the laboratory

2.1 Introduction

Insects have been reported to consume weed seeds (Anonymous 1972; Luff 1980), and therefore may be useful as biocontrol agents. *Harpalus rufipes* (De Geer), a common ground beetle, is a generalist predator well known for its seed predation activities (Martinkova et al. 2006). *Gryllus pennsylvanicus* Burmeister, a common field cricket, also consumes seeds of various species but is not much studied for its predation potential. These two species are abundant in blueberry fields of Nova Scotia (Cutler et al. 2012; Piers 1896; Vickery 1961).

Controlled laboratory experiments are an important first step to confirm the ability of invertebrates to consume certain food items, which may later be tested in the field. In the following experiments, the seed predation potential of *H. rufipes* and *G. pennsylvanicus* on two common weeds of blueberry, sheep sorrel (*R. acetosella*) and hairy fescue (*F. tenuifolia*), was examined.

2.2 Materials and methods

2.2.1 Insect maintenance

Adult *H. rufipes* and *G. pennsylvanicus* were collected from commercial wild blueberry fields near Debert, Kemptown and Mt. Thom, Nova Scotia. Insects were collected using plastic cups (10 cm diameter x 10 cm height) placed in pits drilled with auger. Pitfall traps were covered with square wooden rain covers (30 cm x 30 cm). Beetles were collected from May-October in both 2012 and 2013, and crickets were collected from August-October in 2013. Insects were taken to the entomology laboratory of the Department of Environment Science, Faculty of Agriculture, Dalhousie University, and maintained (one insect per container) in plastic containers (6 cm diameter x 7.5 cm height) half filled with moist peat soil and covered with lids (Renkema et al. 2013). Beetles and crickets were provided adult cat food (Whiskas®; Mars Canada, Bolton, ON, Canada) every four days and two days, respectively. Starvation for four days for beetles and two days for crickets was followed by the feeding experiments.

Third or fourth instar diamondback moth larvae (*Plutella xylostella* (L.)) and third or fourth instar green peach aphid (*Myzus persicae* (Sulzer)) were used in prey and seed preference experiments. These insects were reared on cabbage plants at 25-30 ^{o}C and 70% R.H., in a growth chamber

2.2.2 Seeds

Seeds of sheep sorrel and fescue that originated from wild blueberry fields in Nova Scotia were obtained from Dr. Nathan Boyd[2] and sesame (*Sesamum indicum* L.) seeds used as positive controls in feeding experiments were purchased at a local grocer. All the seeds were maintained at low temperature (5 ^{o}C) to avoid germination and respiration. Before the start of experiments all the seeds were kept at room temperature for 10 min.

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[2] Formerly of the Nova Scotia Agricultural College; now residing at Horticultural Sciences Department, University of Florida.

2.2.3 Blueberry plant feeding experiments

Insects were tested for their tendency to damage and feed upon on blueberry flowers and fruit after starving for 4 d (adult beetles) and 2 d (adult cricket), as they are reported as occasional pests of some crops (Luff 1980; Rogers et al. 1985). Blueberry stems were collected in the field, returned to the laboratory, and placed in floral picks containing tap water. The floral pick was inserted through the paper cup (355 ml) and a glass Petri dish lid was placed over the top of the cup. Every 24 h, damage was estimated for 4 d through defoliation of intact flowers by *H. rufipes*, and percentage fruits (feeding) damaged on the stem for *H. rufipes* and *G. pennsylvanicus*.

2.2.4 Seed feeding experiments

a) Consumption rate of seeds

In this experiment, insects were placed in bioassay arenas with a single type of seed: sesame (as control), sheep sorrel, or hairy fescue. Experiments were done in June and July 2013 for beetles and from mid-July to early September 2013 for crickets. After starvation (see above), beetles and cricket were placed in Petri dishes (5.5 cm diameter x 1 cm height; 1 insect per Petri dish) containing a moistened filter paper (Fisherbrand®; Fisher Scientific, GA, USA; 9.0 cm diameter) and 50 seeds of a single type. Plates were held in dark in a growth chamber at 25-30^{o} C and 70% RH. The experiments were run for 4 d for beetles and 2 d for crickets, with observations of number of seeds consumed were recorded every 24 h. A completely randomized design was used with seed consumption per beetle per day being the main factor of interest. Each treatment bioassay arena (sesame, sheep sorrel, or fescue) was replicated twenty five times in a block and each block was repeated three times.

b) Choice of seeds

In this experiment, beetles or crickets were given a choice of sesame, sheep sorrel and hairy fescue in order to determine seed preferences. Fifty seeds of each type were offered in a mixture in the same Petri dish and an insect was added to each dish. The experiment was run in a completely randomized design with twenty five replications over 4 d and 2 d for beetles and crickets. Holding conditions were as described above and experiment was repeated three times.

c) Preference of insect prey or seed

Harpalus rufipes is an omnivore, an experiment was conducted to examine feeding preference of beetles when given a choice of an invertebrate prey and weed seeds. The experiment was run in a completely randomized design. Two separate experiments were designed for prey preference over preferred seeds. In the first two choice experiment, 20 aphid nymphs and 20 sheep sorrel seeds were offered to a single beetle in a 100 x 15 mm Petri dish. In the second three choice experiment, 10 diamondback moth larvae, 20 aphid nymphs, and 20 sheep sorrel seeds were offered to a single beetle in a 100 x 15 mm Petri dish. Invertebrate prey and seeds were placed in a refrigerator for 2 h, at -23 ^{o}C to immobilize prey, and then held at ambient laboratory temperature for 10 min before starting the experiment. Petri dishes were placed in a clear plastic box (34.2 x 20.9 x 11.8 cm) and held in a growth chamber as described above. There were 10 replicate Petri dishes per treatment and both experiments were done three times. Each experiment was run for 4 h and observations for number of prey and seeds consumed per beetle were recorded at 1, 30, 60, 120 and 240 min.

2.2.5 Data analysis

The data for beetle seed consumption rate per day, choice of seeds and prey preference over seeds were subjected to regression analysis using time (days) as the independent variable in Sigma Plot (12.0) (Systat Software Inc.). The models were tested from simple linear to alternative complex nonlinear models of increasing complexity. The hyperbola, single rectangular, 2 parameter model was selected. The following equation was used in this model:

$$f = (a * Time)/(b + Time)$$

where, f = number consumed in a given time, and a and b were the regression parameters. Because cricket data were collected only over 2 d (two time points), they were analyzed using SAS PROC Mixed procedure (SAS 9.3) for repeated measures, and means were compared using Fisher's LSD test at $\alpha = 0.05$. Data were checked for assumptions of normality and constant variance while independence was assumed by randomization of the data.

2.3 Results

2.4.1 Damage on wild blueberry flowers and fruits by test insects

No feeding tendency was observed in starved beetles on the wild blueberry flowers and fruits over a period of 4 d. The number of fallen flowers (0.25 ± 0.1) was not significantly ($P = 0.02$; $F = 1.67$) different from the control (0.38 ± 0.1). Significant damage ($P < 0.01$, $F = 31.83$) to the wild blueberry fruits was caused by cricket (14 ± 1.3 %), while no damage was recorded in case of beetle and control treatments. Rarely, starved *H. rufipes* was observed climbing on wild blueberry plants with intact flowers and fruits in search of food, while starved *G. pennsylvanicus* were seen rapidly climbing the stems laden with intact fruits in search of food. No frass was recorded in the test arena for the beetle which indicated that beetle was not eating on wild blueberry plant parts. In case of crickets, frass was present at the base of test arena indicating its feeding on wild blueberry fruits.

2.4.2 Consumption rate of seeds

Both *H. rufipes* and *G. pennsylvanicus* searched for food immediately after release in the Petri dish arena, and once contact was made with a seed, they immediately started feeding in all the cases. With both insects, there was more consumption of sheep sorrel seeds. Both insects consumed almost all sesame and sheep sorrel seeds, but with fescue, mostly endosperm was eaten and the outer husk was most of the times incised by beetles, and sometimes eaten in the case of cricket.

Harpalus rufipes consumption rates of sesame, sheep sorrel and fescue changed significantly over four days (Table 2.1). Seed consumption per individual per day varied for different seeds, with day 1 consumption of sesame seeds (21.1 seeds consumed) and sheep sorrel seeds (21.0 seeds consumed) being noticeably greater than that of fescue (9.9 seeds consumed) (Fig. 2.1). Cumulative consumption over four days for sesame, sheep sorrel, and hairy fescue seeds, was 54.8, 46.8, and 25.3 seeds, respectively (Fig 2.1).

Table 2.1 Summary of nonlinear regression analyses for *Harpalus rufipes* consumption of sheep sorrel, hairy fescue, and sesame seeds in a 4 d no choice laboratory bioassays.

Treatment	df_{error}	Parameter estimates ± SEM		F	P	R^2
		a	b			
Sesame	2	112.7 ± 5.8	4.18 ± 0.3	1622.9	0.01	0.99
Sheep sorrel	2	81.5 ± 6.6	2.85 ± 0.4	304.0	0.01	0.99
Hairy fescue	2	50.4 ± 8.6	3.74 ± 1.1	123.1	0.01	0.99

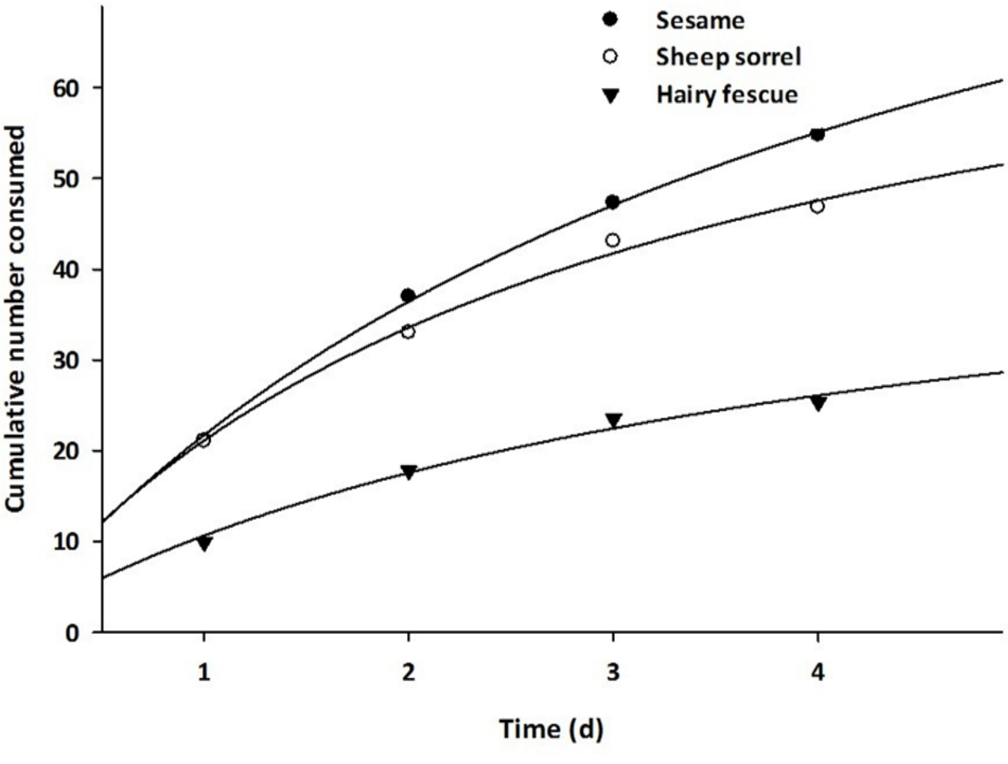

Figure 2.1 Time dependent consumption by *Harpalus rufipes* of sheep sorrel, hairy fescue, and sesame seeds in a 4 d no choice laboratory bioassay.

The field cricket, *G. pennsylvanicus*, exhibited different consumption rates for different seed species (Table 2.2) over time. The seed consumption (seeds per individual per day) for sheep sorrel was highest (42.4 ± 1.2) as compared to fescue (33.5 ± 1.2) and sesame seed consumption was the least (23.1 ± 1.2) after one day (Fig 2.2). The consumption rate decreased over the time and there were significant differences in the consumption rate of day two for each seed species and between the three seed species.

2.4.3 Choice of seeds

When seeds were offered as a mixture, *H. rufipes* differed (Table 2.3), preferred sesame seeds to sheep sorrel and hairy fescue seeds (Fig. 2.3). In contrast to the no choice experiment where consumption sheep sorrel and sesame seeds was similar, when offered as a mixture approximately twice as many sesame seeds were consumed each day as compared to sheep sorrel seeds, and hairy fescue seeds were preferred even less (Fig 2.3). For *G. pennsylvanicus*, seed preference was the same as in the no choice experiment, and differed by seed type, time and experimental block (Table 2.4). Consumption of sheep sorrel seeds was significantly greater than that of sesame and hairy fescue seeds on both days (Fig 2.4).

Table 2.2 Results of ANOVA for *Gryllus pennsylvanicus* consumption of sheep sorrel, hairy fescue, and sesame seeds in a 2 d no choice laboratory bioassay.

Model factor	*df*	*F*	*P*
seed	2,58	87.4	0.01
day	1,29	139.9	0.01
seed*day	2,58	1.31	0.27
block	2,27	12.4	0.01

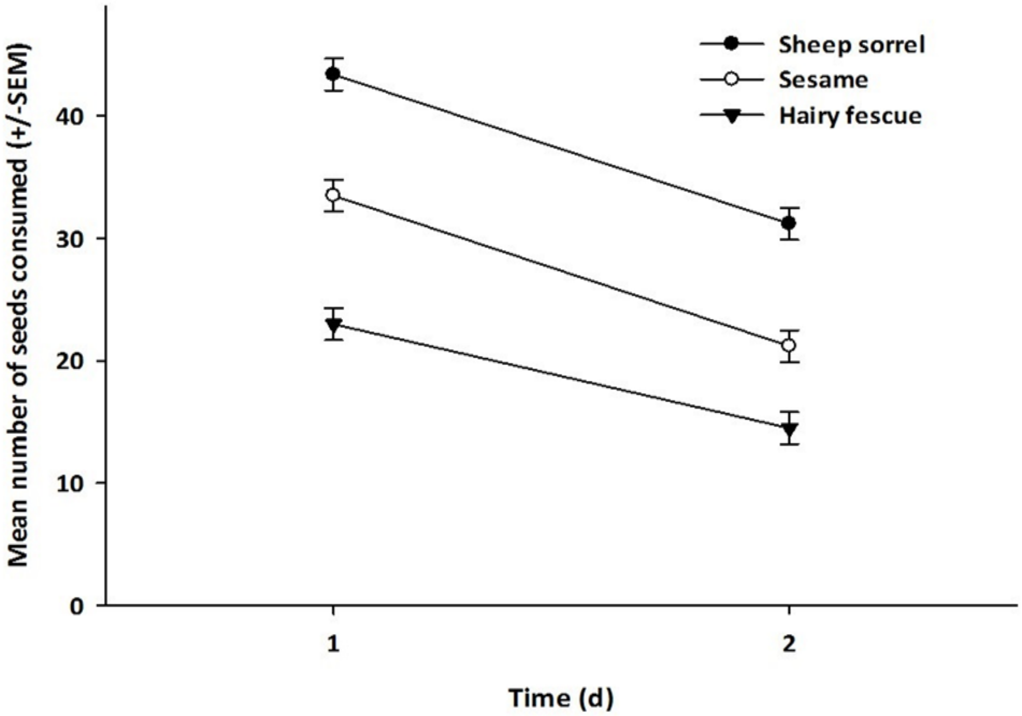

Figure 2.2 Herbivory of sheep sorrel, hairy fescue and sesame seeds by *Gryllus pennsylvanicus* in a 2 d no choice laboratory bioassay.

Table 2.3 Summary of nonlinear regression analyses for *Harpalus rufipes* consumption of sheep sorrel, hairy fescue and sesame seeds in a 4 d choice laboratory bioassay.

Treatment	df_{error}	Parameter estimates ± SEM		F	P	R^2
		a	b			
Sesame	2	42.5 ± 1.8	2.8 ± 0.2	1099.4	0.01	0.99
Sheep sorrel	2	24.4 ± 2.1	3.1 ± 0.5	335.03	0.01	0.99
Hairy fescue	2	13.6 ± 0.4	3.7 ± 0.2	3148.6	0.01	0.99

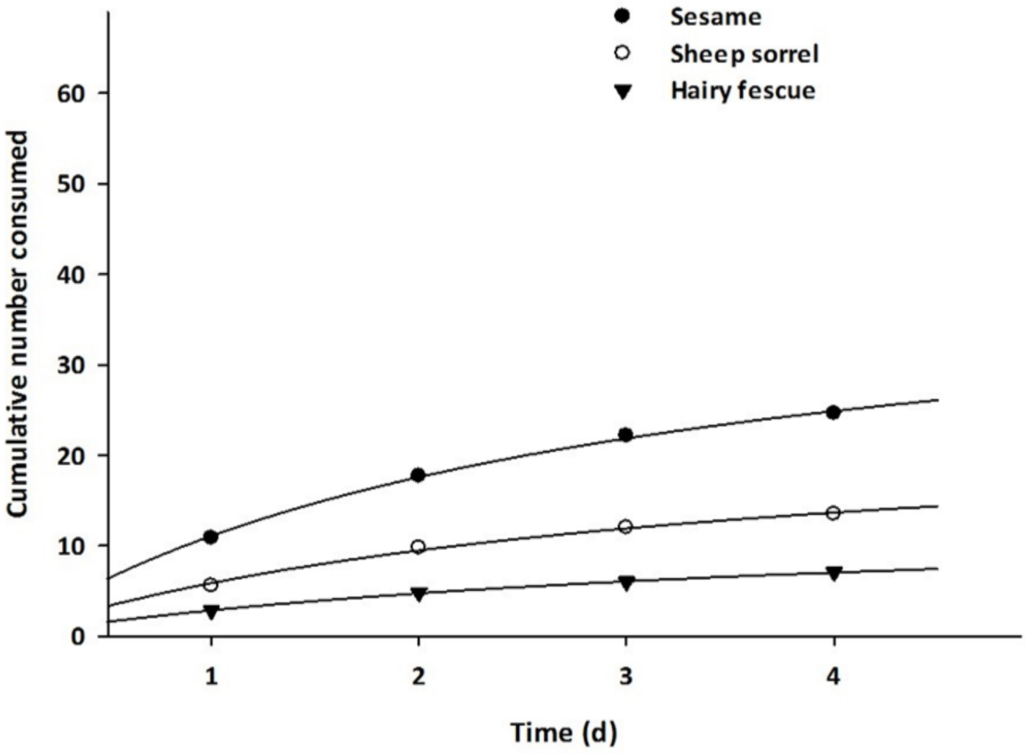

Figure 2.3 Time dependent consumption by *Harpalus rufipes* of sheep sorrel, fescue and sesame seeds in a 4 d feeding laboratory experiment.

Table 2.4 Results of ANOVA for *Gryllus pennsylvanicus* consumption of sheep sorrel, hairy fescue and sesame seeds in a 2 d choice laboratory bioassay.

Model factor	*df*	*F*	*P*
seed	2, 137	14.9	0.01
day	1, 137	144.08	0.01
seed*day	2, 137	1.4	0.25
block	28,137	4.5	0.01

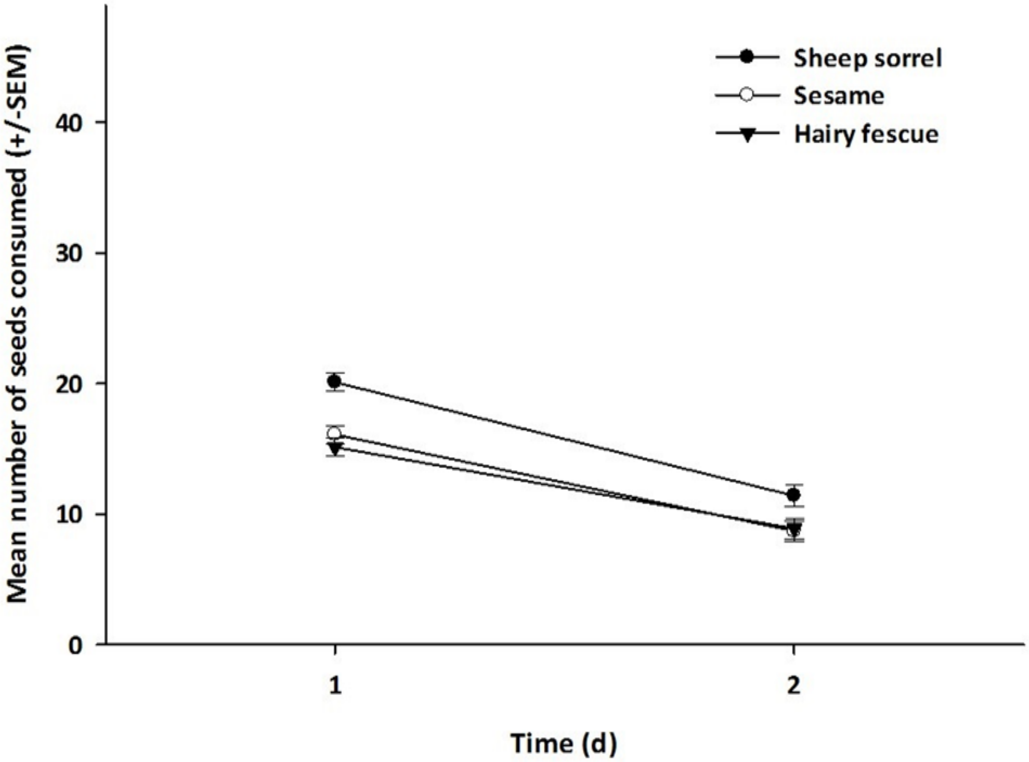

Figure 2.4 Herbivory of sheep sorrel, hairy fescue and sesame seeds by *Gryllus pennsylvanicus* in a 2 d choice laboratory bioassay.

2.4.4 Preference of insect prey or seeds

Harpalus rufipes consumed both invertebrate prey and seeds offered together in Petri dish arenas. In the two-choice experiment involving aphid nymphs and sheep sorrel seeds (Table 2.5), consumption of aphids were greater than seeds at all time points (Fig 2.5). In the three choice experiment (DBM larvae, aphid nymphs, and sheep sorrel seeds) aphids consumption was higher than weed seeds, and DBM larvae were the least consumed item (Table 2.6, Fig 2.6).

Table 2.5 Summary of nonlinear regression analyses for *Harpalus rufipes* consumption of second and third instar green peach aphids, and sheep sorrel seeds in 240 min feeding choice laboratory bioassay.

Treatment	df_{error}	Parameter estimates ± SEM		F	P	R^2
		a	b			
aphid	3	10.4 ± 0.32	63.1 ± 5.3	1749.6	0.01	0.99
seed	3	30.5 ± 2.6	102.4 ± 19.7	404.7	0.01	0.99

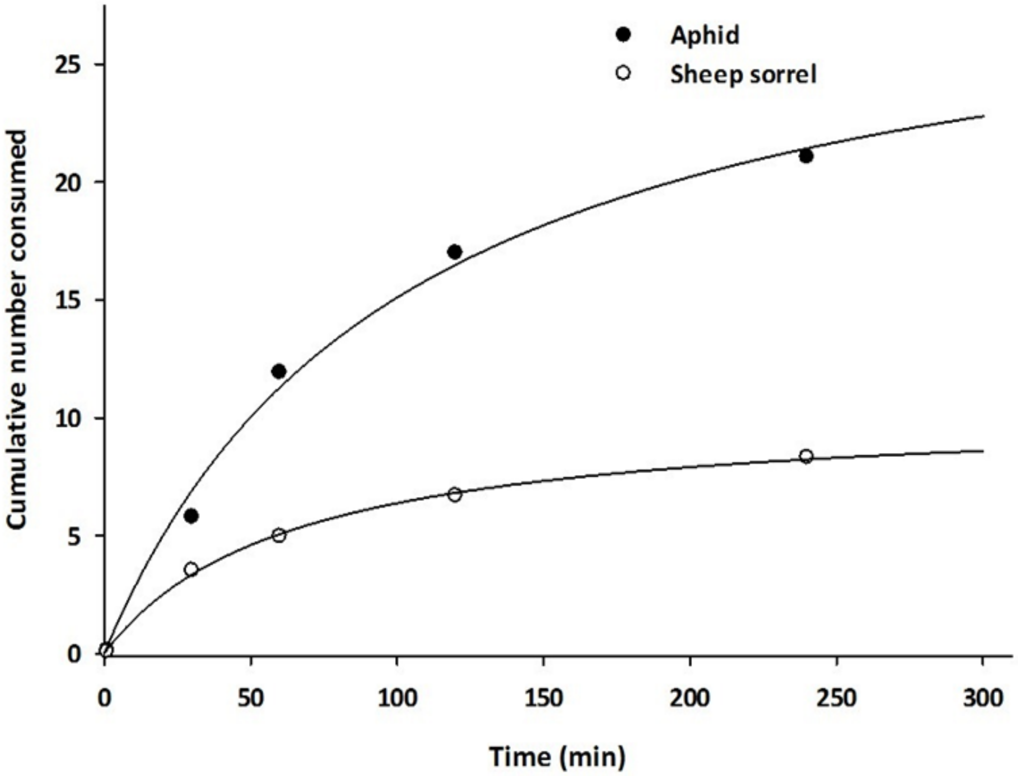

Figure 2.5 Time dependent omnivory by *Harpalus rufipes* on second and third instar green peach aphids, and sheep sorrel seeds in a 240 min choice laboratory bioassay.

Table 2.6 Summary of nonlinear regression analyses for *Harpalus rufipes* consumption of second and third instar diamondback moth larvae, green peach aphids, and sheep sorrel seeds in 240 min choice laboratory bioassay.

Food type	df_{error}	Parameter estimates ± SEM		*F*	*P*	R^2
		a	b			
larva	3	8.2 ± 0.63	75.3 ± 14.7	317.7	0.01	0.98
aphid	3	13.2 ± 0.89	56.3 ± 10.9	313.5	0.03	0.98
seed	3	12.02 ± 0.9	50.2 ± 12.3	200.9	0.01	0.98

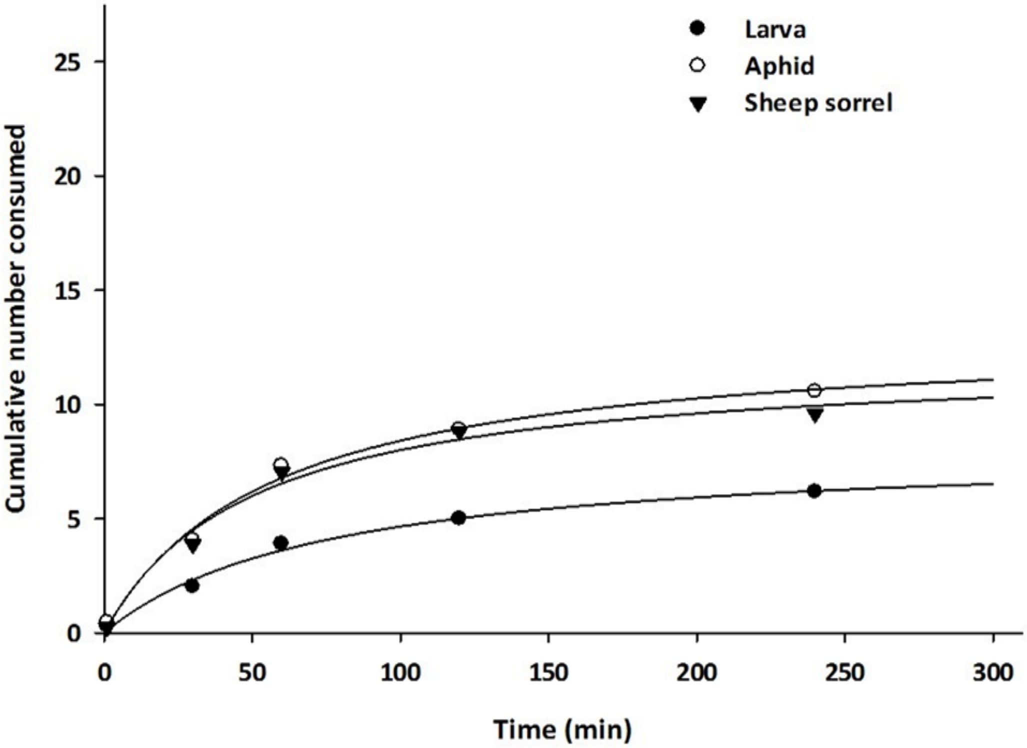

Figure 2.6 Time dependent omnivory for *Harpalus rufipes* on second and third instar diamondback moth larvae, green peach aphids, and sheep sorrel seeds in a 240 min choice laboratory bioassay.

2.4 Discussion

Harpalus rufipes did not exhibit any damage on floral and fruiting parts of wild blueberry. It has been never reported as a pest in wild blueberry, but is reported as occasional pest on strawberry in England (Kock 1975; Luff 1980). Kock (1975) reported that *H. rufipes* preferred insects over plants in laboratory studies and also reported that lack of preferred food may be the reason for feeding on strawberry seeds. This author was also successful in reducing the damage to strawberry fruits by scattering soya groats as an alternative food. In the present study, the damage caused by cricket was in confined conditions. In wild blueberry fields, it had never been reported as a pest but in some other agroecosystems it sometimes causes damage to the main crop (Rogers et al. 1985).

Harpalus rufipes is an inactive plant climber which is mostly looking for its food on the soil surface (Shearin et al. 2008; Suenaga and Hamamura 1998). Little is known about how granivorous insects affect weed populations in blueberries, but the laboratory experiments in present study suggest that, *H. rufipes* and *G. pennsylvanicus* could reduce sheep sorrel and fescue populations in blueberry. The purpose of comparing consumption rates and preference between the seeds of different weeds and insect prey with weed seeds was to find out the seed feeding potential of *H. rufipes* and *G. pennsylvanicus* in laboratory, which could be further exploited in the field. The change in consumption rates over time can be attributed to the effect of satiation in beetles (Honek et al. 2003). Seeds differ in their size, shape, hardness of seed coat and access to endosperm, which influences consumption preferences by seed herbivores (Carmona et al. 1999). Granivores may prefer large seeds or their preference may be based on the protein or caloric content, or allelochemicals promoting or deterring seed consumption (Brust and House 1988; Honek et al. 2007). *H. rufipes* prefers medium sized seeds (1 mg), and eats few seeds that are smaller or larger than this (Honek et al. 2003). Honek et al. (2003) observed that the feeding preference of *H. rufipes* in a mixture of seeds did not change despite the differences in seed combinations and there was a preference for particular seeds. Preference of adult *H. rufipes* were reported preferably feeding on seeds of *Taraxacum* spp., over *Veronica arvensis* L., *Polygonum persicaria* L. and *Lithospermum arvensis* L. (Jorgensen and Toft 1997). The low preference for the latter three species was due to a small amount of endosperm in *V. arvensis*, and a hard seed coat in *P. persicaria* and *L. arvensis*. The probable reason for hairy fescue being less preferred may be the presence of more husk as compared to endosperm. Husk is hard and dry while soft endosperm might be preferred by the beetles. Phytophagy and granivory can occur in *H. rufipes* but granivory is preferred over phytophagy (Goldschmidt and Toft 1997). Field crickets are known to remove significant amounts of weed seeds especially ragweed and redroot pigweed seeds in soybean agroecosystems (Brust and House 1988). Female crickets can consume more than 200 redroot pigweed seeds in 24 h in the laboratory (Carmona et al. 1999).

Seed consumption by crickets is also influenced by seed size and morphology, as smaller seeds are easier to handle and more small seeds will be consumed by crickets than big seeds (Carmona et al. 1999). The weight of seed material consumed by the beetles and crickets was not measured in the present experiments because an estimation of the food mass consumed depends on retrieval of all seed material (Lund and Turpin 1977). This was not feasible because beetles and cricket left unconsumed seeds in the Petri dishes for which reason the half or more part seed of seed consumed was counted as consumed.

Harpalus rufipes is omnivorous and will feed on insect pests of crops, including blueberry (Holopainen and Helenius 1992; Monzo et al. 2011; Renkema et al. 2013). The occurance of blueberry pests like blueberry maggot and spanworm in in late spring (Renkema et al. 2013) temporally coincides with the predators like *H. rufipes* and *Pterostichus mutus* (Say), *Poecilus l. lucublendus* (Say) and *Carabus nemoralis* O.F. Miller. This occurance of ground beetles like *H. rufipes* and insects pests at same time may affect seed predation of weeds in wild blueberry. In another mixed seed-insect prey experiment, *H. rufipes* preferred cereal aphid, *Metopolophium dirhodium* (Walker) and *Drosophila melanogaster* Meigen equally to the preferred seeds of *Taraxacum* spp. (Jorgensen and Toft 1997).

The presence of alternate food resources can reduce the predation of the target pests. In the present study, weed seeds consumption was affected by the presence of alternative food sources in the form of DBM larvae and aphids. In similar experiments, predation of cutworm larvae by *H. pennsylvanicus* was reduced to 79% and 88% when alternate food as fruit fly, *D. melanogaster* pupae, and blue grass, *Poa pratensis,* seed were provided (Frank et al. 2011). In the field, seed feeding also reduced predation on cutworms, thus leading to more damage to the crop (Frank et al. 2011). The current laboratory experiments show that larvae and aphids are preyed upon by *H. rufipes*. So, this beetle can act as predator of weed seeds and invertebrate pests.

Chapter Three: Seed removal by insects in wild blueberry fields

3.1 Introduction

Seed granivores can consume weed seeds when confined in well-controlled laboratory experiments, but the effectiveness of a biocontrol agent can be best determined through field studies. A number of earlier studies have reported the importance of invertebrate predators compared to vertebrate seed predators. Zhang et al. (1998) reported 24-97% consumption by *H. rufipes* on barnyard grass seeds, *Echinochloa crusgalli* (L.) Beauv. and mustard, *Brassica* spp. seeds sown in potato and barley fields in Maine. Gallandt et al. (2005) reported up to 43% granivory by invertebrates out of 58% total granivory over four days in organically managed cereals, cucurbits and rapeseed mustard in Maine. In corn agroecosystem in Ontario, the post dispersal seed consumption of common lambsquarter (*Chenopodium album* L.) and barnyard grass weeds was studied with exclusion cages; sow bugs, millipedes and carabid beetles accounted for 75% of the total granivore community, which contributed 80-90% of total seeds consumed (Cromar et al. 1999). Similarly, in soybean fields in North Carolina, seed predation of broadleaf weed species like ragweed, and pigweed were studied, and carabid beetles were found to be responsible for granivory on more than half of the seeds (Brust and House 1988).

The aim of present study was to examine weed seed granivory in wild blueberry fields by invertebrates. There are several factors that may affect the distribution of invertebrates in agricultural fields. It was previously shown that the occurrence of seed consumers or seed removal can be independent of distance from field edge (Cutler et al. 2012; Fox et al. 2013; Ichihara et al. 2014; Westerman et al. 2003). Similarly, crop type or development stage (Fox et al. 2013; Menalled et al. 2000) and species of weed seed (Ichihara et al. 2014; Westerman et al. 2003) may also affect granivory by invertebrates in the field. I hypothesized that invertebrates will contribute to seed predation in wild blueberry fields and there would be no effect of distance from the forest edge on the seed consumption. Based on previous work on the abundance of granivorous species in wild blueberry fields (Cutler et al. 2012), I also hypothesized that more invertebrate seed consumers and seed consumption would occur in blueberry fields that were in the vegetative stage of production. Based on the laboratory studies as discussed in chapter 2, I hypothesized that sheep sorrel will be preferred by the invertebrate herbivores over hairy fescue seeds.

3.2 Materials and methods

The experiments were conducted in fruit-bearing ("crop") and vegetative ("sprout") wild blueberry fields in Nova Scotia at Debert 2 (Crop 45° 25' 2.3" N, -63° 30' 39.6" W; Sprout 45° 25' 7.6" N, -63° 30' 52.4" W) during 2012 and at the following locations during 2013: Debert 1 (Crop 45° 26' 26.9" N, -63° 27' 2.4" W; Sprout 45° 26' 39.5" N, -63° 27' 2.7" W), Debert 2 (Crop 45° 25' 2.3" N, -63° 30' 39.6" W; Sprout 45° 25' 7.6" N, -63° 30' 52.4" W), Kemptown (Crop 45° 29' 57.9" N, -63° 06' 17.9" W; Sprout 45° 29' 54.1" N, -63° 06' 11.0" W) and Mt. Thom (Crop 45° 29' 31.2" N, -62° 59' 31.7" W; Sprout 45° 29' 32.9" N, -62° 59' 18.5" W). The experiments were done in August-September 2012 and July-September 2013 when sheep sorrel and fescue typically set seed.

During 2012, seed cards (4 x 9 cm) were prepared on which repositioning glue (404®; ODIF-USA, CT, USA) was sprayed and sheep sorrel and hairy fescue seeds (50 of each) were placed on this sprayed area. Under normal weather conditions glue ensured seeds stay on the cards. The cards were covered with a fine layer of fine sand to prevent invertebrates from getting stuck to the glue. Nails were used to secure the cards to the ground.

In 2013, a different method was used, whereby Petri dishes filled to the top with sand were used to hold seeds. Fifty seeds each of sheep sorrel and hairy fescue, were placed in each dish and thereafter positioned in crop and vegetative blueberry fields. There were three treatments: open; mesh cage (cages covered with a 13 x 13 mm mesh to allow access for small insects); and exclusion cage (cages covered with nylon stockings to prevent entry of carabids and other beetles, crickets, and vertebrates) (Fig. 3.1).

No cage Cage Exclusion cage

Figure 3.1 Treatment cards with two type of weed seeds (sheep sorrel and hairy fescue) in no cage (open), mesh cage (no covering) and exclusion cage (control) used in field experiments in wild blueberry fields in Nova Scotia during 2012.

All the three treatments were placed at distances of 30 cm from each other at 1, 15 and 50 m from the forest edge in crop and vegetative fields in order to determine if proximity to non-crop habitat influences the removal of seeds from Petri dishes by herbivores. In addition, pit-fall traps consisting of 454 ml plastic cups (10 cm diameter) were inserted into the ground in fields at distances of 1, 15, and 50 m and approximately 20 m from where Petri dishes of seeds were placed (Appendix II). Each pitfall trap was double cupped for easy collection of the beetles, and traps were covered with 30 x 30 cm wooden rain covers. Pitfall traps were activated on dates data was collected for seed consumption. The beetle and cricket collection in pitfall traps was used to assess the distribution and abundance of beetles or crickets in fields. The number of *H. rufipes* and *G. pennsylvanicus* captured was correlated against mean invertebrate granivory that occurred at the same distance, using Minitab (17) statistical software.

The field experiment was a split plot design (Appendix II) and was conducted in Debert 2 during 2012, and was blocked by field site (at 4 different sites) during 2013. The two field types (vegetative and crop) were considered whole plots, each with three levels of distance (1, 15 and 50 m) as sub plots. Three types of cage treatments (exclusion cage, mesh cage, and no cage) were placed at each distance and were sub-sub-plot. Two types of seeds (sheep sorrel and fescue) were sub-sub-sub plots. Three transects of traps at the three distances were established 20 m apart in each field. Every 10-15 days during July-September 2013, when sheep sorrel and fescue typically set seed, Petri dishes containing seeds were placed in the fields for a period of 48 h. After 48 h, the contents of each dish were placed in a sealable plastic bag and returned to the laboratory where remaining seeds of each type were counted. After confirming assumptions of normality of the error terms and constant variance data were analyzed using the SAS PROC Mixed procedure (SAS 9.3).

3.3 Results

Among the individual factors of cage type, field site, field type (crops vs. sprout), and location in the field (distance from forest edge), only cage type had a significant effect on seed predation at $\alpha = 0.05$ (Tables 3.1 and 3.2). Seed consumption in mesh cages and no cages was significantly different, but numerically consumption rates appeared very similar (Table 3.3). There was no significant difference in the number of sheep sorrel vs. hairy fescue seeds removed, except during mid-July and end of September when the season was almost over, and these values were numerically quite close (Table 3.3). With two exceptions, four way (Field*distance*cage*seed) and all three way interactions were non-significant. During 2012 (Table 3.1), only the three-way interaction between field, distance and cage was significant (Fig. 3.2). In this case invertebrate predation was observed highest at 1 m from the field in the vegetative field (34.4 ± 0.5 seeds) in no cage conditions. Overall invertebrate predation was less in crop fields at 15 m from the forest edge (12.4 ± 0.4). In 2013, the same three-way interaction was significant (Table 3.2) where more invertebrate predation was seen on in vegetative field (19.8 ± 0.24) compared to the crop field (10.9 ± 0.24), at 1 m from the forest edge in both cases.

Table 3.1 Analysis of variance (ANOVA) results for an experiment examining herbivory of sheep sorrel and hairy fescue seeds by seed consumers in wild blueberry fields held under different conditions (no cage, exclusion cage, open cage) placed at different distances (1, 15 and 50 m) from the edge of vegetative and crop fields in Nova Scotia, 2012.

Model factor	*df*	*F*	*P*
Field	1,3	8.52	0.06
Distance	2,641	1.56	0.21
Field*distance	2,641	1.36	0.26
Cage	2,641	335.62	0.01
Field*cage	2,641	12.43	0.01
Distance*cage	4,641	1.44	0.21
Field* distance *cage	4,641	2.50	0.04
Seed	1,641	18.41	0.01
Field*seed	1,641	0.26	0.61
Distance*seed	2,641	0.38	0.68
Field*distance*seed	2,641	0.51	0.60
Cage*seed	2,641	1.19	0.30
Field*cage*seed	2,641	0.06	0.94
Distance*cage*seed	4,641	0.57	0.69
Field*distance*cage*seed	4,641	0.19	0.94

Table 3.2 Analysis of variance (ANOVA) results for an experiment examining herbivory of sheep sorrel and hairy fescue seeds by seed consumers in wild blueberry fields held under different conditions (no cage, exclusion cage, open cage) placed at different distances (1, 15 and 50 m) from the edge of vegetative and crop fields in Nova Scotia, 2013.

Model factor	*df*	6 Jul	17 Jul	2 Aug	7 Sep	21 Sep
Field	1,3	0.34	0.99	0.77	0.15	0.20
Distance	2,390	0.18	0.67	0.81	0.24	0.35
Field*distance	2,390	0.88	0.08	0.01	0.42	0.50
Cage	2,390	0.01	0.01	0.01	0.01	0.01
Field*cage	2,390	0.35	0.27	0.33	0.78	0.45
Distance*cage	4,390	0.53	0.27	0.58	0.35	0.96
Field* distance *cage	4,390	0.66	0.83	0.03	0.19	0.65
Seed	1,390	0.24	0.01	0.31	0.45	0.01
Field*seed	1,390	0.15	0.29	0.29	0.09	0.70
Distance*seed	2,390	0.37	0.70	0.67	0.94	0.35
Field*distance*seed	2,390	0.27	0.91	0.11	0.97	0.85
Cage*seed	2,390	0.43	0.19	0.86	0.84	0.80
Field*cage*seed	2,390	0.89	0.67	0.66	0.53	0.77
Distance*cage*seed	4,390	0.47	0.92	0.82	0.57	0.98
Field*distance*cage*seed	4,390	0.83	0.82	0.76	0.94	0.87

Table 3.3 Herbivory (mean consumption of seeds +/- SEM) of sheep sorrel and fescue by seed consumers in wild blueberry fields held under different cage conditions (no cage, exclusion cage, open cage) placed in wild blueberry fields in Nova Scotia, 2013.

Treatment	6 Jul	17 Jul	2 Aug	7 Sep	21 Sep
No cage	19.6 ± 0.1a	38.6 ± 1.7a	21.9 ± 0.1a	19.1 ± 0.1a	12.9 ± 0.1a
Mesh cage	18.3 ± 0.1a	33.9 ± 1.7b	15.4 ± 0.1b	15.7 ± 0.1b	11.1 ± 0.1b
Exclusion cage	4.8 ± 0.1b	7.7 ± 1.7c	3.4 ± 0.1c	2.9 ± 0.1c	3.2 ± 0.1c

Mean in a column followed by different letters are significantly different (LSD test, *P* < 0.05).

Table 3.4 Herbivory (mean consumption of seeds +/- SEM) of sheep sorrel and fescue by seed consumers in wild blueberry fields placed in wild blueberry fields in Nova Scotia, 2013.

Treatment	6 Jul	17 Jul	2 Aug	7 Sep	21 Sep
Sheep sorrel	12.7 ± 0.1a	25.3 ± 1.7b	12.6 ± 0.1a	11.5 ± 0.1a	9.3 ± 0.1a
Fescue	13.8 ± 0.1a	28.2 ± 1.7a	11.8 ± 0.1a	11.1 ± 0.1a	7.7 ± 0.1b

Mean in a column followed by different letters are significantly different (LSD test, $P < 0.05$).

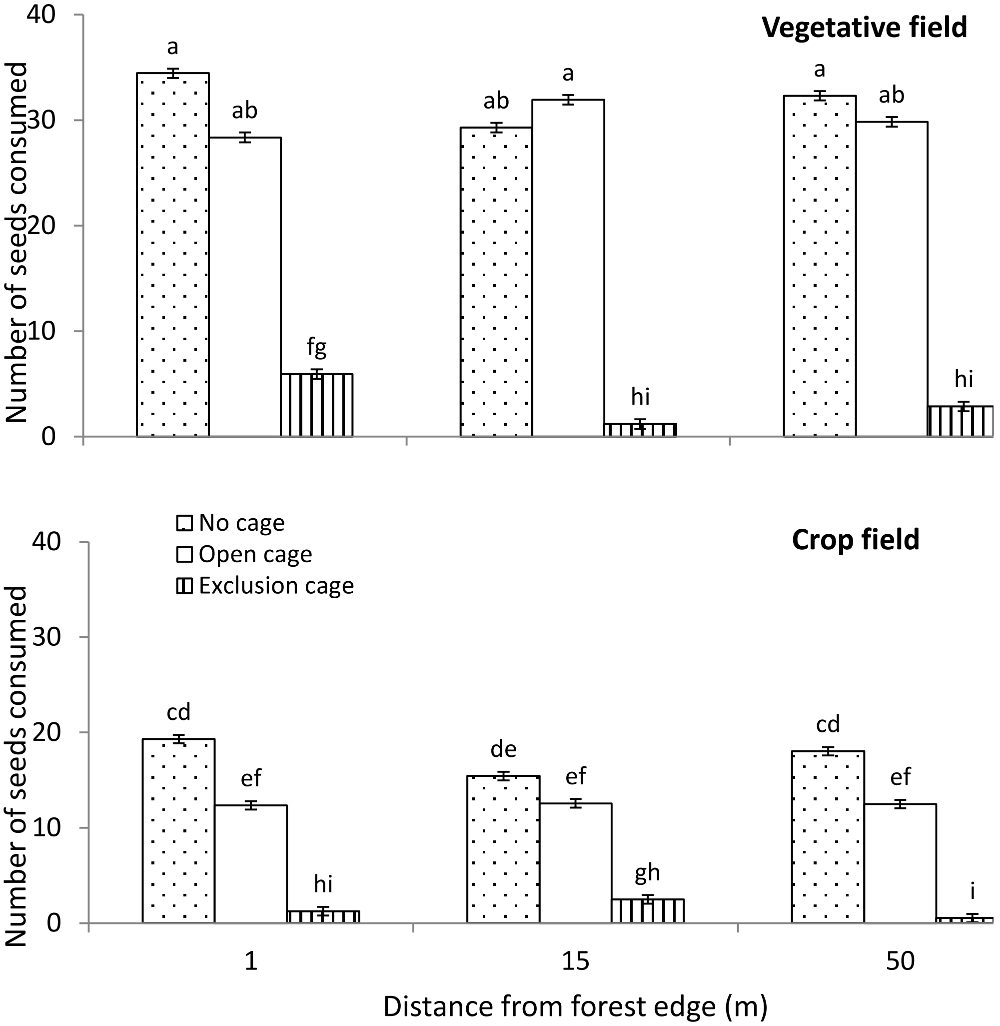

Figure 3.2 Herbivory (mean seed consumption +/- SEM) of sheep sorrel and fescue in wild blueberry crop and sprout fields at different distances from the forest edge when placed in different cage types, Nova Scotia, 2012. Bars with the same letter are not significantly different (LSD test, $P < 0.05$).

The highest average number of *H. rufipes* beetles (5) was collected on July 17, 2013 (Table 3.5). The field cricket, *G. pennsylvanicus*, population was variable over the season. The correlation analysis showed a moderate positive correlation with beetle captures and sheep sorrel consumption (correlation coefficient, r = 0.6) during July, and a moderate correlation with cricket and fescue predation (r = 0.7) during late September (Table 3.6). Other correlations were weak.

Table 3.5 Average number of ground beetles, *Harpalus rufipes* (H) and field crickets, *Gryllus pennsylvanicus* (C) captured in pitfall traps at 1, 15 and 50 m distances from field edges in wild blueberry crop and sprout (vegetative) fields, Nova Scotia, 2013.

Field type	Distance	6 Jul		17 Jul		2 Aug		7 Sep		21 Sep	
	(m)	H	C	H	C	H	C	H	C	H	C
Crop	1	1.0	0.8	0.5	0.3	0.8	0.3	0.0	1.0	0.0	0.0
	15	0.8	0.5	0.5	0.0	0.8	0.3	0.3	1.0	0.0	0.5
	50	1.8	0.0	3.0	2.5	0.8	0.0	0.0	0.0	0.0	1.0
Sprout	1	2.0	0.0	2.3	0.0	0.0	0.0	0.0	0.3	0.0	0.0
	15	3.8	0.0	5.0	0.3	1.5	0.5	0.0	1.8	0.0	0.5
	50	2.8	0.5	2.0	0.8	1.8	0.8	0.0	0.3	0.0	1.0

Table 3.6 Pearson correlation coefficients (r) for correlations of ground beetle, *Harpalus rufipes* (H) and field cricket, *Gryllus pennsylvanicus* (C) captures in pitfall traps against weed seed (sheep sorrel and fescue) consumption in open cages placed in wild blueberry fields, Nova Scotia, 2013.

Factors	**6 Jul**		**17 Jul**		**2 Aug**		**7 Sep**		**21 Sep**	
	H	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**
Sheep sorrel	0.6	-0.1	-0.1	-0.1	0.1	0.2	-0.5	0.2	-	0.4
Fescue	0.2	-0.3	0.1	0.1	0.3	0.1	-0.4	0.3	-	0.7

3.4 Discussion

The present experiments aimed to examine weed seed removal in blueberry fields by granivorous invertebrates. By setting up experiments at four different locations in Nova Scotia, and at each location comparing effects of crop vs. vegetative field development, distance from the forest edge with different cage types that allowed or restricted granivory by different herbivores, the importance of these factors towards granivory in wild blueberry fields was estimated. Though there were significant differences in seed consumption among different cage treatments, the consumption in mesh cages that permitted access to seeds by invertebrates was similar to the no cage conditions, suggesting that granivorous insects are important contributors to seed consumption in blueberry fields. Other factors like distance, field type and seed type had few effects on the seed consumption, indicating that growers can expect beneficial contributions of seed herbivory under a variety of field conditions.

The distribution of beneficial insects throughout agricultural fields is often influenced by distance into a field from natural habitat (Menalled et al. 2000; Myster and Picket 1993). However, present results show this is not always the case. Although it is possible that there could be an effect of distance at distances greater than 50 m from the forest edge, this result is encouraging for blueberry growers because it suggests biological control is happening throughout their fields, not just along field edges near natural habitat. Similarly, in Japanese rice fields invertebrate seed consumers of *E. crusgalli* and *Lolium multiflorum* Lam.

were not affected by distance from the forest edge (Ichihara et al. 2014). In a recent study in Nova Scotia wild blueberry fields, the occurrence of commonly occurring granivores like *H. rufipes* and *S. impunctatus* was not affected by distance from the forest edge (Cutler et al. 2012). Westerman et al. (2003) also reported no effect of distance from the forest edge on the seed removal by both vertebrates and invertebrates in wheat fields in the Netherlands. Studies in wheat fields in Japan (Ichihara et al. 2011) reported that seed predation by invertebrates was not affected by distance and predation in boundary strips was similar to field interiors. Fox et al (2013) found no effect of distance on captures of *G. pennsylvanicus,* but found *H. pennsylvanicus* more active close to the field border in organically grown corn (*Zea mays*) and soybeans (*Glycine max*) in U.S., with time of year and field boarder type also playing a role. In another study in corn fields in US, there were differences in the consumption of five common weed species (*Abutilon theophrasti*, *Amaranthus retroflexus*, *Chenopodium album*, *Panicum dichotomiflorum* Michx., and *Setaria lutescens* (Weigel) Hubb.) by invertebrates, but the combined effect of invertebrate granivory with vertebrate was not much different in crop fields at 5 and 100 m from the hedgerows adjacent to the fields (Marino et al. 1997).

Seed consumption in the no cage treatment and in mesh cages (invertebrate allowed to enter) was significantly different, but the actual numbers of seeds consumed were quite similar. That is, the statistical effect may not be very biologically significant, and most seed consumption occurring in fields is likely from invertebrates like carabid beetles and field crickets. The average seed removal rate by *H. rufipes* in a mosaic of small grain crops like cereals, oilseed rape, peas and alfalfa was 2.5 seeds per 0.062 m^{-2} day^{-1} (Honek et al. 2003). The same study also reported that seed removal in fields depends upon the number and species of herbivores in the field. In Maine, consumption of common weeds (e.g. velvetleaf, wild mustard, yellow foxtail, common lambsquarter, redroot pigweed and hairy galinsoga (*Galinsoga ciliata* (Raf.) Blake)) in organically managed vegetable fields with different crop rotation combinations of winter squash, broccoli, winter rye, hairy vetch, oat, red clover were studied using separate exclosures for vertebrate and invertebrate herbivores (Gallandt et al. 2005). Out of a total of 58% seed consumption, up to 43% was attributed to invertebrates. On the other hand, in wheat fields in the Netherlands, seed predation by vertebrates like mice (up to 80%) was significantly higher than that from invertebrates (up to 38%) (Westerman et al. 2003). Thus, consumption of agricultural weed seeds by invertebrates is common, but the relative contributions of invertebrates and vertebrate to this biological control service can vary across cropping systems.

An effect of field type (crop vs. vegetative) was observed in 2012 (where only one field site was used in the experiment) but not in 2013 (a more robust experiment conducted at several sites). This result suggests that blueberry growers can anticipate contributions to weed biological control by granivores in both phases of the blueberry production cycle. Field management in other agricultural systems may influence the distribution and abundance of weed seed herbivores. In Norway, movement and density of the ground beetles *H. rufipes*, *P. melanarius,* and *P. niger* was affected by the crop and grassy banks (Frampton et al. 1995), with grassy banks offering more resistance to beetle movement than the barley crop. On the other hand, Menalled et al. (2000) found more weed seed removal in "complex" fields where numerous hedgerows and woodlots were integrated into small fields of maize as compared to "simple" fields where large fields of corn were embedded with scattered hedgerows and woodlots. Although captures of insects were relatively low overall in the present experiments, the results suggest that blueberry plants do not restrict beetle movement in crop or vegetative fields. Cabbage fields in eastern England had more weed seed consumption by carabid beetles when there were more weeds as compared to the following leek crop where mechanical weeding operations reduced the weed population (Eyre 2009). Agronomic practices like fertilization, application of pesticides, and mowing could theoretically affect insects in agricultural fields (Kromp 1999; Renkema et al. 2012) and differ among blueberry fields in the sprout and crop stages, but I found no evidence of this in my experiments.

Overall, there was little evidence of correlation between weed seed consumption and captures of *H. rufipes* and *G. pennsylvanicus,* although moderate correlations were detected on certain days. This was unexpected since my resulted indicated that invertebrates contributed significantly to seed consumption, but the result may be due to low overall captures of insects in pitfall traps. The weak to mild correlations could also be due to the presence of other seed granivores in the field (Appendix II) (Ichihara et al. 2011).

Chapter Four: Susceptibility of *Harpalus rufipes* to commonly used insecticides in wild blueberry insect pest management

4.1 Introduction

Many ground beetles (Coleoptera: Carabidae) are considered important granivores in temperate zones (Thomas et al. 2001). *Harpalus rufipes* (Coleoptera: Carabidae) is a seed predating ground beetle that is abundant throughout Canada, including Nova Scotia (Cutler et al. 2012; Zhang et al. 1998). Commonly found in many agricultural systems, beneficial insects like *H. rufipes* may be exposed to pesticides. Insecticides that are toxic to natural enemies may disrupt pest management in a number of ways. Direct acute toxicities causing death in insects can be relatively easily recognized, but indirect effects through, for example, feeding on contaminated food is also important (Mullin et al. 2005; Stark et al. 2007).

A number of insecticides may be applied by blueberry producers for control of different pests like blueberry spanworm (*Itame argillacearia* (Packard)), blueberry maggot fly (*Rhagoletis mendax* Curran), blueberry flea beetle (*Altica sylvia* Malloch) and spotted-wing Drosophila (*Drosophila suzukii* (Matsumura)). Sprays applied by wild blueberry producers from mid-July through August coincide with the activity of granivorous ground beetles that at the same time are in the field foraging upon the seeds of weeds like sheep sorrel and hairy fescue. Thus, these non-target granivorous beetles may be exposed to insecticides through direct cuticular contact, or by feeding upon insecticide-laden weed seeds. The objective of the experiments in this thesis chapter was to examine the susceptibility of adult *H. rufipes* beetles to different pesticides when exposed through different routes of exposure I hypothesized that beetles will be more susceptible to broad spectrum insecticides like Imidan (phosmet), an organophosphorus insecticide, and Assail (aceptamiprid), a pyridylmethylamine neonicotinoid, than to Movento (spirotetramat), a new lipid biosynthesis inhibitor which is commonly used against sucking insect pests. Each of these insecticides is registered for use against *R. mendax* and is applied at times when *H. rufipes* may be in blueberry fields foraging on weed seeds.

4.2 Materials and methods

4.2.1. Insect maintenance and seed collection

Adult *H. rufipes* were collected from commercial wild blueberry fields near Debert 2, Kemptown and Mt. Thom, Nova Scotia. Insects were collected in pitfall traps using plastic cups (10 cm diameter x 10 cm height) placed in holes drilled with an auger. Pitfall traps were covered with square wooden rain covers (30 cm x 30 cm). Beetles were collected from mid-May until the first week of July 2014, and were taken to the entomological laboratory of Department of Environmental Sciences, Faculty of Agriculture, Dalhousie University. Beetles were maintained (one insect per container) in plastic containers (6 cm diameter x 7.5 cm height) half filled with moist peat soil and covered with lids. Beetles were provided cat food (Whiskas®; Mars Canada, Bolton, ON, Canada) every four days. Starvation for four days was followed by the susceptibility experiments.

Seeds of sheep sorrel that originated with wild blueberry fields in Nova Scotia were obtained from Dr. Nathan Boyd and were maintained at low temperature (5 °C) to avoid germination and respiration. Before start of experiments all the seeds were kept at room temperature for 10 min.

4.2.2 Pesticides and spray equipment

Phosmet (Imidan 50WP; Gowan Co., Yuma, AZ), spirotetramat (Movento 240SC; Bayer CropScience, Calgary, AB, Canada), and acetamiprid (Assail 70WP; E.I. Dupont, Mississauga, ON, Canada) were used in experiments. The following concentrations, representative of what might be experienced in the field were tested: phosmet at 2.25 and 1.13 g AI/L; spirotetramat at 0.34 and 0.27 g AI/L; and acetamiprid at 0.56 and 0.48 g AI/L. Treatment solutions were prepared in deionized water containing 0.015% Tween (Sigma-Aldrich, Oakville, ON, Canada). The control solution contained deionized water and 0.015% Tween 80. Based on availability of field collected beetles, bioassays were initiated on 2 July 2014.

Beetles were exposed by direct contact or to treated sheep sorrel seeds. Either five beetles (dermal toxicity) or 50 seeds (oral toxicity) were placed in the bottom of a glass Petri dish (Pyrex® USA; 90 mm diameter x 15 mm height). The treatments were applied by a Potter precision spray tower (Burkard Scientific, Rickmansworth, Herts, UK) with a distance of 69 cm between the spray nozzle and target (beetles or seeds), and sprayed with an air pressure of 0.70 kg per cm^2 (10 psi; 69 kPa). After spray under the Potter tower, treated beetles and seeds were transferred to plastic Petri dishes (Fisherbrand®; Fisher Scientific, GA, USA; 10 cm diameter x 1 cm height) to avoid any residual contact of insecticides. In the direct contact bioassays, sprayed beetles were provided 50 untreated sheep sorrel seeds per Petri dish. In the oral exposure bioassay, unsprayed beetles in a Petri dish were offered 50 sheep sorrel seeds treated with insecticide. As beetles are nocturnal feeders, beetles in Petri plates were held in the dark for 48 h.

For both exposure scenarios, for each bioassay each treatment had three replicate Petri dishes, each containing five beetles, and bioassays were conducted so that there were three blocks in time. Beetle mortality data were analyzed by Kruskal-Wallis tests using Minitab (17) statistical software.Seed consumption data were analyzed using the SAS PROC Mixed procedure (SAS 9.3), and mean effects of different pesticides and concentrations on seed consumption were compared using Fisher's LSD. All data analyses were conducted at $\alpha = 0.05$.

4.3 Results

There was a significant effect of pesticide treatment on beetle mortality for both the direct contact ($P = 0.007$ and $H = 17.81$) and oral exposure ($P = 0.009$ and $H = 16.95$) experiments. Spirotetramat did not cause any mortality of adult *H. rufipes* but phosmet and acetamiprid caused significant beetle mortality via both exposure routes (Fig 4.1). All beetles died when sprayed with the high or low rate of phosmet, and 60-80% died when topically treated with acetamiprid. Beetle mortality was lower overall in the oral exposure bioassay (Fig. 4.1).

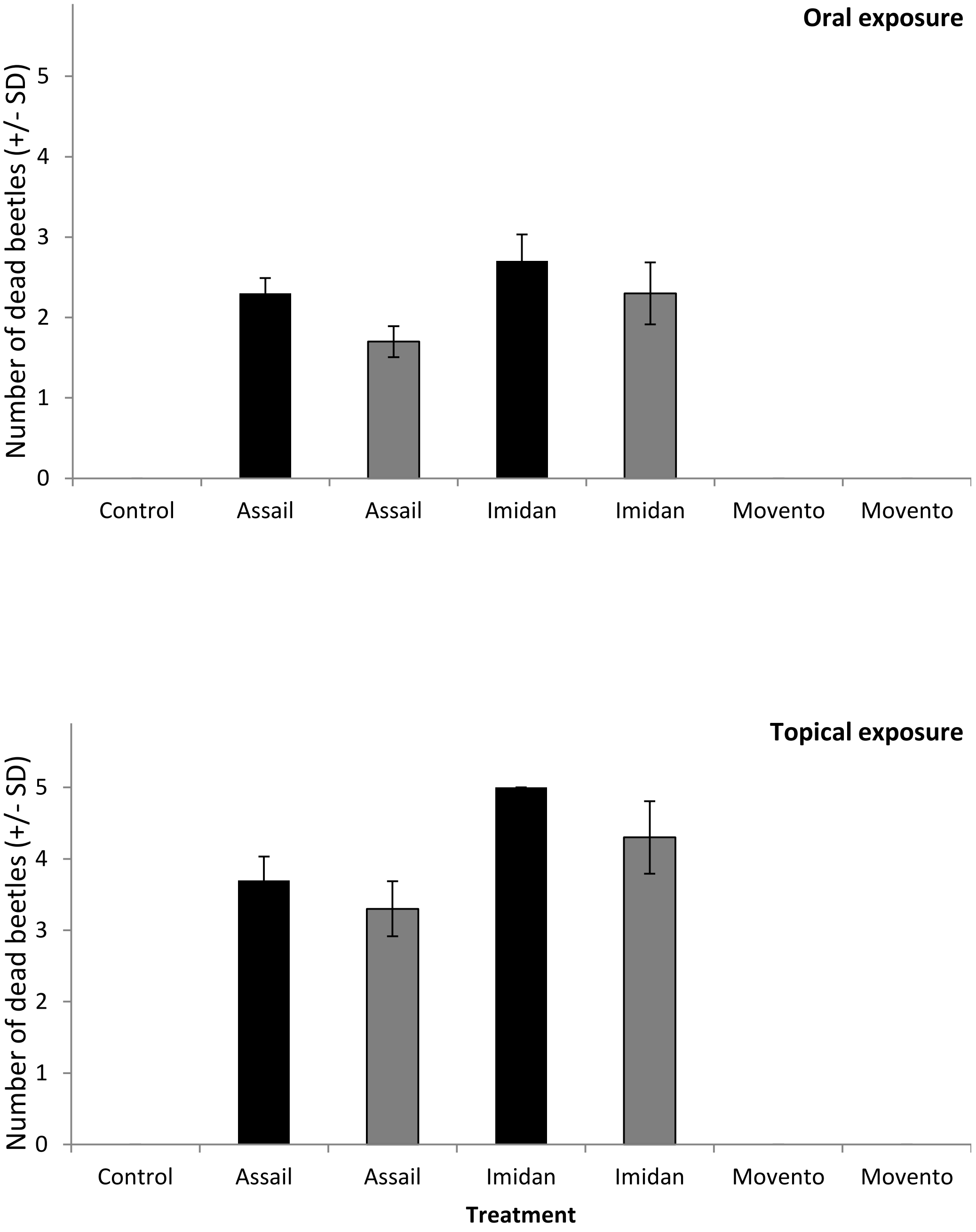

Figure 4.1 Susceptibility of adult *Harpalus rufipes* (n=5) to high (black) and low (grey) rates of acetamiprid (Assail), phosmet (Imidan), and spirotetramat (Movento) through oral (consumption of treated seeds) and topical exposure. Error bars represent SD.

Seed consumption was also affected by treatment, whether topically treated beetles were given untreated seeds (*F* = 61.07; *df* = 6, 54; *P* < 0.001), or untreated beetles were orally given seeds treated with insecticide (*F* = 61.07; *df* = 6, 54; *P* < 0.001) (Fig. 4.2). In both scenarios, significantly more seeds were consumed by beetles in the controls and spirotetramat treatments, with significantly fewer seeds consumed in the phosmet treatment. Intermediate amounts of seed consumption were seen where beetles or seeds were treated with acetamiprid.

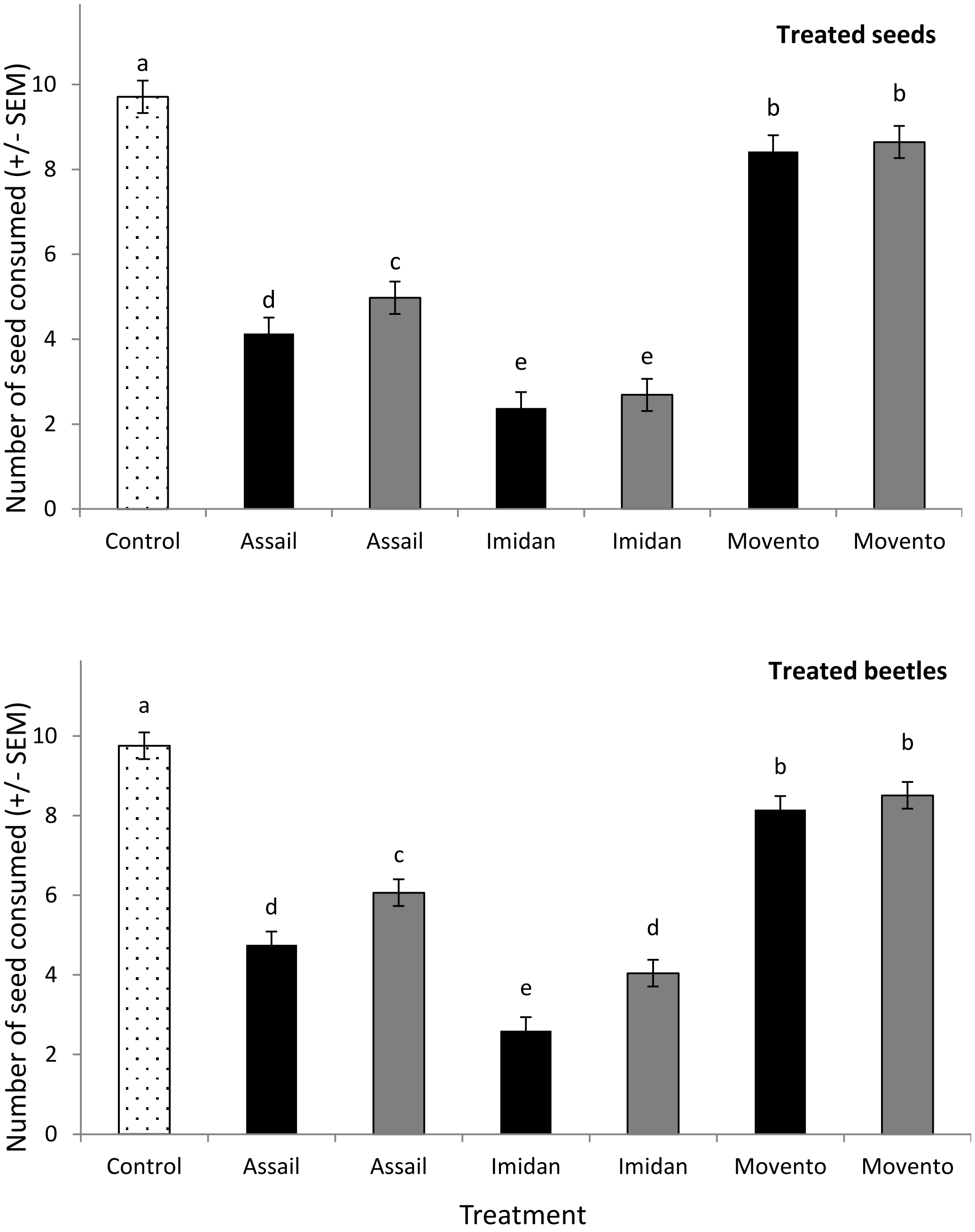

Figure 4.2 Herbivory of sheep sorrel seeds by adult *Harpalus rufipes* following treatment of seeds or beetles with high (black) and low (grey) rates of acetamiprid (Assail), phosmet (Imidan), and spirotetramat (Movento). Bars with same letters are not significantly different (LSD test, $P < 0.001$).

4.4 Discussion

Harpalus rufipes is abundant in wild blueberry fields across Nova Scotia (Cutler et al. 2012). It is an important granivorous beetle feeding on seeds of a number of weeds in different agroecosystems (Brust and House 1988; Honek et al. 2007). In previous chapters of this thesis, it was demonstrated that *H. rufipes* has potential in weed biocontrol by feeding on two important wild blueberry weeds; sheep sorrel and hairy fescue. In the field the occurrence of the adult beetles, however, coincides with important insect pests of wild blueberry, which growers usually manage by pesticide sprays. Though effective for pest management, pesticide sprays may have damaging effects on the non-target beneficial arthropods like *H. rufipes.*

It was observed that *H. rufipes* adults varied in their susceptibility to the recommended field rates of three insecticides registered for use in wild blueberry, and that susceptibility also depended on the exposure route. Beetles were highly susceptible by direct contact (topical exposure) to the neonicotinoid insecticide acetamiprid and the organophosphorus insecticide phosmet. This is not that surprising as phosmet and acetamiprid are relatively broad spectrum in their activity, being used against a number of coleopteran insect pests (Elbert et al. 2008; French et al. 1992). In wild blueberry, these insecticides are registered for the control of pests like blueberry spanworm, flea beetle, and fruit fly (Delbridge et al. 2013). Non-target beneficial invertebrates may be exposed accidently to these pesticides in wild blueberry fields, which may have detrimental effects on these insects. These insecticides have shown harmful effects to different beneficial insects in various agroecosystems at recommended field rates (Cloyd and Dickinson 2006; Youn et al. 2003). Increased mortality of *H. erraticus* larvae was reported in highbush blueberry fields in Michigan (US) sprayed with conventional pesticides (grower standard program) including phosmet, malathion, methomyl, esfenvalerate as compared to reduced risk program of pesticide spray (O'Neil et al. 2005). The difference between the programs included spraying insecticides like spinosad and imidacloprid during the active season of the beetle, while delaying spray of phosmet or esfenvalerate in late August when there is less beetle activity. In Ontario, carabid beetles collected from apple orchards had variable susceptibility to different insecticides when exposed by direct contact; phosmet was highly toxic to *H. affinis* and *Amara* spp. but not to *P. melanarius* (Hagley et al. 1980). Similarly, the high and low field rates of phosmet and acetamiprid were reported to be highly toxic by topical and oral application to dogbane beetle, *Chrysochus auratus* (F.), a natural enemy of spreading dogbane (*A. androsaemifolium*) which is a serious weed in wild blueberries in Nova Scotia (Crozier and Cutler 2014).

In contrast to effects observed with acetamiprid and phosmet, spirotetramat, a tetramic acid lipid biosynthesis inhibitor, had no impact on *H. rufipes*. Spirotetramat is primarily utilized against a number of sucking insect pests including aphids, mealy bugs, California red scale, citrus red mite and citrus thrips (Ouyang et al. 2012; Ramanaidu and Cutler 2013). In wild blueberry it was recently registered for use against blueberry maggot fly and blueberry gall midge (*Dasineura oxycoccana* Johnson) on wild blueberries (Delbridge et al. 2013). As it is recommended specifically for the sucking insect pests, I predicted it would not cause any mortality in *H. rufipes.* Others have reported no mortality in beneficial insects such as coccinellid beetles following exposure to spirotetramat (Crozier and Cutler 2014; Planes et al. 2013), but some studies have reported mild susceptibility on predatory mites and coccinellid beetles (Bruck et al. 2009). The lack of effects seen in present worst-case scenario laboratory exposure bioassays suggest that spirotetramat should be safe to the granivorous beneficial beetles such as *H. rufipes*.

Chapter Five: Conclusions

5.1 Introduction

Integrated pest management (IPM) is a decision making system that uses cost effective and ecologically conscious pest management strategies (Kogan 1998). Farmers continue to rely on pesticides due to easy availability and application of the pesticides, and rapid results in the field. Present day weed and insect pest management strategies in wild blueberries need change as customers prefer low-input produce. Moreover, stricter pesticide regulations in world markets are worrisome for conventional berry growers and have heightened concerns of pesticide residues on their product. These factors have economic effects on the marketability of the fruit, and therefore farmers look for alternate management practices. IPM comprises many elements like pest monitoring, cultural, mechanical, and biological control, and other methods that can reduce pesticide usage to eventually deal with associated social, political, or legal concerns and constraints (Prokopy 1994). Biological control is one such tactic that involves identifying biocontrol agents of pests and evaluating their efficacy in the field. Baseline investigations for conservation biological control involve identifying and understanding the biodiversity of potential natural enemies of key pests in agroecosystems (Cutler et al. 2012).

Conservation biological control aims to modify the environment or existing pesticide practices to improve the efficacy of natural enemies by mitigating harmful conditions or enhancing favorable ones (Eilenberg et al. 2001; Landis et al. 2000). The focus is to enhance survival, fecundity and longevity by reducing mortality factors, providing beneficial resources, or manipulating host plant attributes to the benefit of natural enemies (Landis et al. 2000). Interest in conservation biological control had increased in past few years (Pimental 2008). Other than being a potential pest control tactic, it fits well with ecological conservation and sustainability in agriculture (Straub et al. 2008). Generalist omnivorous predators can help reduce insect pest and weed populations in the field. For example, generalists ground beetles and field crickets are reported to consume 30-90% seeds of different weeds in various agroecosystems (O'Rourke et al. 2006).

The research presented in this thesis examined the weed seed biocontrol potential of ground beetles and field crickets in laboratory, and invertebrate granivory in the field on seeds of two important weeds of wild blueberry. Under field conditions, omnivorous insects encounter insect pests which may be an attractive food. In this context, laboratory experiments were conducted to determine the preference of *H. rufipes* for insect prey vs weed seeds. Because pesticides sometimes show detrimental effects on the non-target natural enemies like granivorous ground beetles, another set of laboratory experiments examined the susceptibility of *H. rufipes* exposed topically and orally to commonly used conventional (Assail 70WP and Imidan 50WP) and new (Movento 240SC) insecticides. The significant findings of this study are as follows:

- Granivory by *H. rufipes* and *G. pennsylvanicus* on weed seeds of sheep sorrel and hairy fescue occurs under laboratory conditions. In prey and weed seed preference experiments, aphids and weed seeds were preferred over diamondback moth larvae in a three choice experiment, while aphids were preferred over sheep sorrel seeds in two choice experiments. However, presence of aphids on plants and *H. rufipes* being inactive plant climber (Shearin et al. 2008; Suenaga and Hamamura 1998) more consumption of weed seeds than aphids by *H. rufipes* could be expected in the field.

- Under field conditions, granivory in no cage conditions was higher for sheep sorrel and fescue seeds exposed to all species of granivores. Though statistically lesser than no cage conditions, invertebrate granivory in open cages was quite significant biologically and encouraging for biological control of weed seeds. As expected, no impact of distance from forest edge was observed on granivory of these two weed species. This suggests that granivorous invertebrates are present throughout fields and we can expect granivory of weed seeds throughout fields and not just close to forest edge.
- Spirotetramat (Movento 240SC) was safe to *H. rufipes* and no mortality was observed over 48 h following laboratory exposure to this insecticide. Acetamiprid (Assail 70WP) and phosmet (Imidan 50 WP) were highly toxic to beetles after 48 h of dermal exposure. Consumption of sheep sorrel seeds treated with acetamiprid and phosmet also resulted in increased mortality.

From these results it can be concluded that *H. rufipes* and *G. pennsylvanicus* may be important granivores of weed seeds of sheep sorrel and hairy fescue in wild blueberry fields, and that certain reduced risk pesticides may aid in their conservation.

5.2 Barriers to conservation biological control of weed seeds

Weed population dynamics can be modified through seed consumption by granivores and thus these granivores should be considered as part of weed management strategies (Williams et al. 2009). Success of biocontrol sometimes depends on feeding habits of specialist natural enemies, but most Carabidae are generally polyphagous. This can be one of the limitations of the biological control of weeds by granivory. At the same time, though Carabidae also prey upon other invertebrates like aphids (Zhang et al. 1998) and larvae (Brust 1994) that are pests of different crops. Carabid beetles differ in their activity in different types of fields; some prefer weedier fields while others prefer open fields in different agroecosystems (Eyre 2009).

It is also important to understand the effect of agronomic operations on sources of alternate food. Greater abundances of ground beetles under high blueberry bushes was reported when ground cover was maintained as compared to when aisle grass was removed by mowing (O'Neil et al. 2005). Thus, the authors suggested that mere switching from conventional to reduced risk insecticides may not be sufficient to maintain ground beetle population and activity. Farming operations in wild blueberry such as mowing fields after harvesting may destroy shelter and hibernating sites of granivorous beetles (Lambert 1990; Yarborough 2009). Finally, reduced risk insecticides sometimes involve stage specific spraying schedules, are sometimes more expensive, and are only effective against certain life stages of an insect (Roubos et al. 2014; Shrear et al. 2006). Increase in the number of different reduced risk pesticides targeting different insect pests and increased monitoring may not be acceptable at farmer level (Roubos et al. 2014).

5.3 Suggestions for future work

The following are potential areas of future study:

- Laboratory experiments confirmed granivory by *H. rufipes* and *G. pennsylvanicus* on sheep sorrel and hairy fescue. These two insects are generalist predators and they can consume seeds of other weeds. Other consumers of these and other weed seeds in wild blueberry fields should be explored.
- Field experiments confirmed considerable invertebrate granivory of sheep sorrel and hairy fescue. However, further study is desirable to investigate difference of cost effectiveness in in wild blueberry fields while shifting from conventional farming to conservation farming as studied in other agroecosystems (Swanton et al. 2008).
- Field studies also demonstrated that granivory is occurring on wild blueberry fields irrespective of the field type. In this study I have not investigated plant species and areas which can provide habitat, improve fecundity and survival of ground beetles (Eyre 2009; Jorgensen and Toft 1997; Vickery et al. 2009).

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Appendix I: Other seed granivorous species found in field during seed setting and post dispersal period of weed seeds

Species	Time of capture	Total number captured
Mammals (rodents)	July-September	4
Beetles	July-September	47
Ants	July-September	9

Appendix II: Field layout for seed predation split plot experiment design in wild blueberry fields in Nova Scotia (2012 and 2013)