STUDIES ON COW WHEAT *(MELAMPYRUM LINEARE* DESR.) BIOLOGY AND MANAGEMENT WITH HERBICIDES IN WILD BLUEBERRY (*VACCINIUM ANGUSTIFOLIUM* AIT.) IN NOVA SCOTIA, CANADA.

by

Vanessa Taylor Deveau

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
April 2024

Dalhousie University is located in Mi'kma'ki, the ancestral and unceded territory of the Mi'kmaq. We are all Treaty people.

© Copyright by Vanessa Taylor Deveau, 2024

DEDICATION PAGE

To my younger self and to everyone who believed in me. Your unwavering support means the world to me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Weeds are the major limiting factor in lowbush blueberry production with stands consisting of a range of woody and herbaceous perennials. However, recent weed surveys have identified an increased occurrence of annual weeds such as cow wheat (*Melampyrum lineare*). Various laboratory experiments have been conducted to contribute to the knowledge gaps surrounding cow wheat biology and management in lowbush blueberry fields. Results indicated cold moist stratification duration (CMSD) increased cow wheat seed germination ($P < 0.0001$), and germination was not affected by the presence of wild blueberry debris. CMSD with or without GA_3 increased cow wheat seed germination ($P < 0.0001$) but there was no effect of agar-gelling agent. There was higher cow wheat seed density on the soil surface relative to subsurface samples ($P < 0.0001$) in wild blueberry fields. Cow wheat seedlings emerged between April 18 to April 25 with 90% emergence occurring between May 2 to May 26. Cow wheat morphology varied across sites ($P < 0.0001$) in lowbush blueberry fields. The parasitic connections were visually determined between cow wheat and lowbush blueberry rhizomes at four fields. Cow wheat density was reduced by PRE applications of terbacil (2000 g a.i. ha^{-1}) and indaziflam (75 g a.i. ha^{-1}) at all sites and by PRE applications of hexazinone (1920 g a.i. ha^{-1}), sulfentrazone (139 g a.i. ha^{-1}), tribenuron-methyl (30 g a.i. ha^{-1}), nicosulfuron + rimsulfuron (16.7 + 8.3 g a.i. ha^{-1}), and flazasulfuron (50 g a.i. ha^{-1}) at 3 of 4 sites. POST applications of mesotrione (144 g a.i. ha^{-1}), and foramsulfuron (35 g a.i. ha^{-1}) reduced cow wheat density at 3 of 4 sites but density was not reduced by POST clopyralid applications at any site.

LIST OF ABBREVIATIONS USED

°C	Degree Celsius
CO_2	Carbon dioxide
cm	Centimeter
GA_3	Gibberellic Acid
CMSD	Cold Moist Stratification Duration
WBD	Wild Blueberry Debris
CMS	Cold Moist Stratification
g	Gram
Ha	Hectare
kg	Kilogram
kPa	Kilopascal
LS	Least Square Means
L ha^{-1}	Liter per hectare
mm	Millimeter
m^{-2}	Metre square
mL	Millilitre
psi	Pounds per square inch
ppm	Parts per million
%	Percent

ACKNOWLEDGEMENTS

I am writing this as I embark on the final stretch of my graduate school journey, I find myself reflecting on the incredible support system that has sustained me through both successes and challenges. I want to express my deepest gratitude to each and every one of you.

To my supervisor and mentor, Scott your guidance has been the cornerstone of my academic pursuits. Your unwavering support, coupled with your invaluable insights, has shaped not only my research but also my approach to challenges. I appreciate your trust in my abilities and providing the necessary tools for growth as a researcher. Your teachings transcend beyond what graduate school could ever teach me.

To my family, your unwavering love and encouragement have been the bedrock of this journey. Your understanding during the stressful times have meant more to me than words can convey. Your belief in me has been the impetus pushing me to continue this closing journey.

To my friends, the laughter, camaraderie, and shared experiences have been the much-needed breather from the academic rigors. Your words of encouragement have turned the tough days into manageable ones.

To my partner, your constant support has been my anchor throughout my academic career. Your patience, understanding, and belief in my abilities have been a source of motivation. Your presence has been a reminder that success is sweeter when shared with those we love.

There have been days when the challenges seemed insurmountable, and the journey felt arduous. In those moments, your collective support in me has been a powerful thing. I am grateful for the countless times all of you lifted my spirits, celebrated my achievements, and provided a listening ear.

Each one of you has played an indispensable role, and I wanted to take a moment to acknowledge and express my gratitude. I look forward to celebrating the successes ahead, knowing that they are, in part, yours as well.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Introduction

The lowbush, or wild, blueberry (*Vaccinium angustifolium* Ait.) is a native, rhizomatous perennial shrub in North America (Hall et al. 1972; Vander Kloet, 1988). Lowbush blueberry is an economically important fruit crop in Canada and contributed $57 million to farm gate value in 2022 (Burgess, personal communication). Lowbush blueberry fields are developed from natural stands or abandoned hay fields (Barker et al. 1964; Kinsman, 1993) that are managed using a two-year production cycle. In the first year (non-bearing year), fields are pruned to ground level by flail mowing or burning to promote upright vegetative growth and flower bud development. Flowering, fruit production, and harvest occur in the second year (bearing year) (Penney et al. 1997; Kennedy et al. 2010). Weeds are one of the most significant limiting factors in the production of lowbush blueberry (McCully et al. 1991) due to lack of tillage and crop rotation associated with this production system (Jensen and Yarborough, 2004). Weeds compete with the lowbush blueberry for resources such as space, nutrients, and light (McCully et al. 1991; Penney and McRae, 2000). Weeds also interfere with the harvesting process, reduce blueberry fruit count, and reduce overall yields (Yarborough and Ismail, 1980; McCully et al. 1991; Yarborough and Bhowmik, 1993; Jensen and Specht, 2002; Yarborough, 2011). The weed flora of lowbush blueberry fields consists of a range of native and non-native woody and herbaceous perennial plants that are encouraged, much like the lowbush blueberry plant, by the regular practice of field pruning (Yarborough, 2011). Annual plants are less common in weed surveys of lowbush blueberry fields, though recent surveys indicate increased occurrence of some problematic annual plants (Jensen and Sampson, 2004; Lyu et al. 2021).

Cow wheat (*Melampyrum lineare* Desr.*)* is an annual facultative root hemiparasite that reproduces by seed (Oldham and Weeks, 2017; Nave et al. 2018). The plant occurred in 20% of 115 lowbush blueberry fields surveyed in Nova Scotia in the early 1980's (McCully et al. 1991). Occurrence increased to 34% of fields surveyed in 2000 and 2001 (Jensen and Sampson, unpubl. data) and 44% of fields surveyed from 2017-2019 (Lyu et al. 2021). This increased occurrence, combined with grower observations of yield loss associated with cow wheat populations, necessitates an improved understanding of the biology and potential interactions between cow wheat and lowbush blueberry.

Literature Review

Taxonomy and Biology of Lowbush Blueberry

Lowbush blueberry belongs to the *Ericaceae,* or heath family (Hall et al. 1979) and is a low growing deciduous shrub that spreads by seed and rhizomes (Vander Kloet, 1988). Lowbush blueberry plants are part of the native understory of eastern North America and are adapted to its temperate climate (Kinsman, 1993). Lowbush blueberry stands are composed of variable clones, or genets, which are genetically unique (Bell et al. 2009) and spread by rhizomes (Hall and Aalders, 1961). *Vaccinium angustifolium* Ait. and *V. mytrilloides* Michx. are the dominant species in commercial lowbush blueberry fields (Jensen and Yarborough, 2004). *Vaccinium angustifolium*, also known as the low sweet blueberry, or common lowbush blueberry, has smooth stems with green leaves (McIsaac, 1997) and white or pinkish-white blossoms that are bell-shaped (Kinsman, 1993). *Vaccinium mytrilloides* Michx is known as velvet leaf blueberry and has white hairs on the stems and leaves (Kinsman, 1993) with blossoms that are bell-shaped and usually pale green or sometimes red (Kinsman, 1993).

Production and Management of Lowbush Blueberries

Lowbush blueberry fields are not planted but developed from natural stands on abandoned farmland or cleared woodland (Hall, 1959; Jensen and Yarborough, 2004). Commercial lowbush blueberry fields are managed using a unique two-year production cycle (Jensen and Yarborough, 2004). Fields are pruned to ground level by flail mowing or burning to promote upright vegetative growth and flower bud development in the first year (non-bearing year) and flowering and fruit production occur in the second year (bearing year) (Penney et al. 1997). The two-year production cycle continues once berries are harvested and fields are once again pruned to ground level. Weeds are one of the largest limiting factors in the production of lowbush blueberries (McCully et al. 1991) due to lack of tillage and crop rotation (Jensen and Yarborough, 2004). Weeds compete with the lowbush blueberry for resources such as space, nutrients, and light (McCully et al. 1991; Penney and McRae, 2000). Weeds also interfere with the harvesting process (Jensen and Specht, 2002), contribute unwanted fruit to harvested blueberries (McCully et al. 1991; Yarborough and Ismail, 1980), and reduce overall yields (Yarborough and Bhowmik, 1993; Yarborough, 2011).

Weed Flora of Lowbush Blueberry Fields

The general weed flora of lowbush blueberry fields consists of a range of native and non-native woody and herbaceous perennial plants (Jensen and Yarborough, 2004; Yarborough, 2011). Annual weeds, however, are becoming increasingly common in lowbush blueberry fields (Jensen and Yarborough 2004) with a recent weed survey identifying hemp nettle (*Galeopsis tetrahit* L.), horseweed (*Conyza canadensis* L.) Cronquist), cow wheat (*Melampyrum lineare*

Desr.) and American burnweed (*Erechtites hieraciifolius* L.) as the most common annual weed species in lowbush blueberry fields in Nova Scotia (Lyu et al. 2021). Cow wheat was the eighth most common weed species identified by Lyu et al. (2021). The frequency, field uniformity, and density of this weed increased between 1984–1985 (15.7%, 2.3, and 0.1 plants m^{-2}, respectively) and 2000–2001 (30.5%, 7.3, and 0.3 plants m^{-2}, respectively) and have since increased to 43%, 10.42, and 1.84 plants m^{-2} (Lyu et al. 2021). Increased occurrence of this weed species in lowbush blueberry fields is a concern as the plant is a hemiparasite that withdraws resources from host plants via haustoria connections (Cantlon et al. 1963; Těšitel et al. 2010) which could interfere with lowbush blueberry growth and development.

The Biology of Cow Wheat

1. Name

Melampyrum lineare Desrousseaux. –**cow wheat**. Other common names include American cow wheat, narrowleaf cow wheat, and common cow wheat (Weakley, 2015). "*Melampyrum"* is derived from the Greek name as black, and wheat, in reference to the seed colour in some species resembling the grain of wheat (Zinck, 1998; Munteanu er al. 2010).

2. Description and Account of Variation

(a) Species Description

Cow wheat is a facultative hemiparasitic herbaceous green annual species, meaning it parasitizes and obtains water, nutrients, and organic compounds from a host plant while simultaneously conducting its own photosynthesis (Těšitel et al. 2010; Crichton et al. 2012). Cow wheat can form root attachments with a range of host plants (Cantlon et al. 1963; Malcolm, 1964). Cow wheat flowers from early June through September and fruits from late July to mid-October (Cantlon et al. 1963). Seedlings have spatulate to linear cotyledons (Figure 1.1) and mature stems are erect, reaching 10 – 40 cm in height (Cantlon et al. 1963). Leaves are linear or lanceolate shaped and oppositely arranged along the stem, with upper leaves having pointed teeth at the base (Gould et al. 2013). Flowers can be single or in pairs in leaf axils near the top of the plant and are whitish with a yellow tip (Zinck, 1998; Gould et al. 2013) (Figure 1.1). The flowers have a four-lobed calyx, with the upper lobe longer than the others (Zinck, 1998). The two stamen pairs do not extend beyond the mouth of the corolla (Zinck, 1998). The seeds are beige or tan with a lighter colored tip called the elaiosome (Piehl, 1962). Each capsule produces four seeds (Cantlon et al. 1963) (Figure 1.1). The root system is whitish and shallow with a poorly defined primary root (Piehl, 1962).

(b) Intraspecific Variation and Distinguishing Features

The patterns of morphological variation with *Melampyrum* have challenged taxonomists possibly because *Melampyrum* species show patterns of variation that are ecotypic (Rich et al. 1998; Dalrymple, 2007). For example, *Melampyrum sylvaticum* displays variations related to its height morphology (Dalrymple, 2007). Hemiparasitic summer annuals have higher variability in stem height as time of germination can impact the size of the plant, with later emerging plants considerably reduced relative to those that emerge earlier (Svensson et al. 2004; Dalrymple, 2007).

(c) Illustrations

Figure 1.1 Melampyrum lineare Desr. (A) seedling emergence; (B) seeds ejected from matured plant (white arrow); (C) seeds without capsule with white appendage the elaiosome (white arrow) (D) seeds enclosed in capsule; and (E) mature plant with seeds ejected out of capsules. [Photographs by Vanessa Deveau].

3. Economic Importance

(a) Detrimental

Despite increased occurrence, little information on the biology or interaction effects of cow wheat in lowbush blueberry fields is available. Increased occurrence in lowbush blueberry fields is a threat as hemiparasitic species withdraw resources through haustorial connections (Těšitel et al. 2010; Nave et al. 2018) potentially reducing growth of the host plant. For example, cow wheat removed radio-labelled phosphorous from jack pine (*Pinus baksiana* L.*)* seedlings (Cantlon et al. 1963). Lowbush blueberry stem density, height, diameter, biomass, and berries per stem are also significantly lower in blueberry patches containing cow wheat relative to those free of cow wheat (Deveau and White, 2022), suggesting an economic impact of this weed on wild blueberry production. Controlled experiments documenting root connections between these species, however, are lacking. Common cow wheat (*Melampyrum pratense* L.), however, parasitizes *Vaccinium* spp. (Masselink, 1980), suggesting potential affinity of the genus *Melampyrum* for *Vaccinium* spp.

(b) Beneficial

Bumble bees (*Bombus* spp.) are frequent visitors to *Melampyrum* flowers (Benninghouse, 1976; Deveau, personal observation) but cross pollination is not considered necessary for this species (Cantlon et al. 1963).

(c) Legislation

Cow wheat is not classified as a noxious weed in any of the Canadian provinces or territories under the Weed Control Act nor listed in the federal Weed Seeds Order.

4. Geographical Distribution and Habitat

The genus *Melampyrum* has *c.*34 species found in Eurasia, with American cow-wheat being the only North American representative of the genus (Piehl, 1962; Weakley, 2015). Cow wheat occurs from Newfoundland and Labrador to British Columbia (Zinck, 1998), and the upper midwest and Pacific northwest of the USA (Benninghouse, 1976; Oldham and Weeks, 2017). Cow wheat grows in habitats prone to fire and drought (Curtis and Cantlon, 1968; Gibson, 1993) where populations grow in sunny, lichen or moss-covered sites (Oldham and Weeks, 2017). Cow wheat prefers well-drained sandy soils that are acidic (Gibson, 1993; Oldham and Weeks, 2017).

5. Phenological Development

Seeds of hemiparasitic species generally undergo a 'double-germination' which involves developmental stages that are separated by periods of temperature induced dormancy (Masselink, 1980; Curtis and Cantlon, 1963). The first germination occurs in autumn/winter (October-January), after periods of cold temperatures, with emergence of the radicle (Crichton et al. 2012). The radicle is thought to attach to the xylem vessels of the host's roots following germination and over-winter in this state (Masselink, 1980; Crichton et al. 2012). The second germination occurs in spring, possibly due to increased temperatures, and emergence of the cotyledons occur (Masselink, 1980; Crichton et al. 2012). For example, seedlings of *M. cristatum* and *M. sylvaticum* begin to emerge in March in British Isles, Huntingdonshire, Cambridgeshire, and Bedfordshire (Horrill, 1972; Crichton et al. 2012). There is a lack of published literature on emergence of cotyledons in blueberry fields, however, emergence has been observed in early April in Nova Scotia, Canada (Deveau, personal observation). Cow wheat flowers from early July through September and fruits from late July to mid-October in Michigan (Gibson, 1993). The root system is whitish and shallow with a poorly defined primary root (Piehl, 1962).

6. Response to Herbivory

All known members of *Melampyrum* are myrmecochorous which means that seeds are dispersed by ants (Gibson, 1993). *Melampyrum* seeds bear nourishing elaiosomes that are attractive to ants; resulting in non-random seed dispersal (Weiss, 1908; Gibson, 1993; Nave et al. 2018).

Objectives and Hypothesis

Several gaps remain in our general knowledge of cow wheat biology and ecology in lowbush blueberry fields. My specific objectives are to:

(i) Identify factors regulating cow wheat seed dormancy and germination.

(ii) Quantify cow wheat seed production and describe cow wheat seed bank characteristics and emergence patterns in wild blueberry fields.

(iii) Confirm the presence or absence of parasitic root connections between cow wheat and lowbush blueberry plants.

(iv) Evaluate a range of herbicides for cow wheat management in lowbush blueberry fields.

Specific hypotheses are:

(i) Cow wheat seeds require exposure to cold temperatures (cold stratification) to germinate. Storage with wild blueberry plant debris will stimulate germination compared to seeds placed in cold storage without plant debris.

(ii) Pre-treating cow wheat seeds with gibberellic acid will reduce the number of days until germination after removal from cold.

(iii) Cow wheat forms seed banks in lowbush blueberry fields, and seeds accumulate near the soil surface in lowbush blueberry fields due to lack of tillage.

(iv) Cow wheat seedlings will emerge in April in lowbush blueberry fields in Nova Scotia.

(v) Cow wheat forms parasitic root connections with lowbush blueberry plants.

(vi) Registered herbicides for broadleaf weed control in lowbush blueberry fields will exhibit efficacy on cow wheat.

CHAPTER 2: GERMINATION REQUIREMENTS OF COW WHEAT (*MELAMPYRUMLINEARE)* SEEDS COLLECTED FROM LOWBUSH BLUEBERRY FIELDS

Abstract

Cow wheat is an increasingly common annual hemiparasitic weed in lowbush blueberry fields in Nova Scotia. Knowledge of seed germination requirements for this weed species in lowbush blueberry fields, however, is lacking. A range of experiments were therefore conducted to identify factors regulating cow wheat seed dormancy and germination. Seeds were dormant at maturity and did not germinate under warm conditions. In the first experiment cold moist stratification duration (CMSD) increased cow wheat seed germination ($P < 0.0001$). CMSD for 4 and 8 weeks caused 20% and 27% cow wheat seed germination, respectively. In the second experiment cold moist stratification duration (CMSD) without or with GA_3 increased cow wheat germination ($P < 0.0001$) between 24 to 93%, respectively. Agar-gelling agent did not stimulate germination. Results indicate that CMSD and GA_3 exposure are important factors in alleviating the dormancy of cow wheat seeds collected from lowbush blueberry fields.

Introduction

Wild, or lowbush blueberry *(Vaccinium angustifolium* Ait.*)* is a rhizomatous perennial shrub native to North America (Vander Kloet, 1988). It is an economically important fruit crop in Nova Scotia (McIsaac, 1997), contributing $57 million to farm gate value in 2022 (Burgess, personal communication). Wild blueberry fields are managed on a two-year production cycle in which plants are pruned to ground level in the first year (non-bearing year) and fruit is produced and harvested in the second year (bearing year) (Jensen and Yarborough, 2004). Weed management is difficult due to the perennial nature of the crop, and weeds are therefore a major production issue (McCully et al. 1991; Jensen and Yarborough, 2004). Herbaceous and woody perennial plants dominate the weed flora of lowbush blueberry fields (McCully et al. 1991), however, the number of herbaceous annual species is increasing (Jensen and Yarborough, 2004; Lyu et al. 2021).

Cow wheat (*Melampyrum lineare* Desrousseaux.) is a summer annual plant in the Orobanchaceae family and is the only North American representative of the genus *Melampyrum*

(Piehl, 1962; Weakley, 2015). The plant is a facultative root hemiparasite that removes xylem water and nutrients from its host through a parasitic organ called the haustorium (Cantlon et al. 1963; Nave et al. 2017). As an annual, cow wheat must re-establish from seed each growing season. As such, establishment of new plants within a given year is regulated by potential dormancy characteristics associated with seeds produced in previous seasons.

Cow wheat seed dormancy involves both separate radicle and epicotyl dormancy (Cantlon et al. 1963). The seeds after-ripen and germinate between October and January, depending on geographical location (Crichton et al. 2012). Subsequently, after prolonged periods of cold temperatures, the radicle emerges and germinated seeds overwinter prior to seedling emergence in the spring (Cantlon et al. 1963; Masselink, 1980; Crichton et al. 2012). Seeds of *Melampyrum* spp., however, can remain viable but ungerminated in soil for 1-2 years after the seed rain (; Kaitera and Nuorteva 2003; Dalrymple, 2007), suggesting a requirement for seed exposure to specific conditions to alleviate dormancy. Seed exposure to exogenous chemicals such as gibberellic acid or root exudates released from host plants, for example, can be used to overcome seed dormancy (Curtis and Cantlon, 1965; Finch-Savage and Leubner-Metzger, 2006; Bouwmeester et al. 2021). However, no studies have been conducted to determine the dormancy characteristics and germination requirements of cow wheat seeds in lowbush blueberry fields. The objective of this research was therefore to investigate possible germination requirements for cow wheat seeds collected from wild blueberry fields.

Materials and Methods

Seed source and preparation for experimental treatments

All seeds used in the following experiments were collected in September of 2022 and 2023 from natural populations of cow wheat growing in wild blueberry fields in Nova Scotia, Canada. Fresh seeds were collected at maturity, placed in plastic bags, and stored in cold storage at 4±1 °C until use. No seeds were stored for more than 1 month prior to use. Seeds were surface sterilized before each experiment by placing 20 seeds in 100 ml of 1% sodium hypochlorite solution for 1 hr. Experimental units consisted of 20 surface sterilized seeds in plastic bags or petri dishes lined with semisolid 1% or 2% agar gelling agent with 1% glucose (Curtis and Cantlon, 1965), depending on the experiment. Sterilized seeds were placed in either plastic bags or petri dishes under aseptic conditions in a fume hood, and petri dishes were sealed with Parafilm® prior to placement in experimental treatments outlined below. Seed germination in all experiments was considered to have occurred when the radicle protruded through the seed coat.

Effects of wild blueberry plant debris from lowbush blueberry fields and cold moist stratification on cow wheat seed germination

The objective of this experiment was to determine the effect of wild blueberry plant debris (WBD) and cold moist stratification (CMS) on cow wheat seed germination. The experiment was a 2 X 3 factorial arrangement of wild blueberry debris (with WBD, without WBD) and cold moist stratification duration (CMSD) (0, 8, 12 wks), arranged in a completely randomized design with four replications. The experiment was conducted using 20 surface sterilized cow wheat seeds placed in plastic bags, as described above. Bags with the WBD treatment contained 30 g of wild blueberry plant debris that was collected from the surface of wild blueberry fields using a hand-held vacuum cleaner. Bags without WBD treatments contained 30 g of sand. Bags were placed in a 4±1 °C refrigerator for the specified CMSD and monitored for moisture. Distilled water was added as needed to maintain moist conditions in each bag. The total number of germinated seeds in each bag was determined 14 and 28 days after removal from cold treatments, and data are presented as the mean percentage germination in each treatment.

Effect of gibberellic acid (GA3) pre-soaking on cow wheat seed germination on agar gellingagent + glucose medium

The objective of this experiment was to determine the effect of GA_3 pre-soaking on cow wheat seed germination. The experiment was a 2 X 3 X 2 factorial arrangement of GA_3 pre-soaking (0ppm, 2000ppm), CMSD (0, 8, 12 weeks), and semisolid gelling-agent (SGA) (1%, 2%) arranged in a completely randomized design with four replications. The GA_3 solution was prepared by dissolving 0.4 g of potassium salt GA_3 powder (Sigma Aldrich Canada, Oakville, ON) in 10 ml of distilled water and bringing the solution up to 200 ml volume. The seeds were soaked in the GA_3 solution for 24 h at room temperature, after which seeds were rinsed with autoclaved water for 5 min (Curtis and Cantlon, 1965) and then exposed to the previously outlined sterilization procedure. Cold moist stratification was achieved as outlined in experiment 1. The 1 and 2% semisolid gelling-agent was prepared by mixing 5 and 10 g agar (Sigma Aldrich, Canada, Oakville, ON), respectively, with 5 g glucose (Sigma Aldrich, Canada, Oakville, ON). Agar and glucose mixtures were then mixed with distilled water for the total volume of 500 ml. Twenty cow wheat seeds were placed in each petri dish, as described above, and the total number of germinated seeds in each dish was determined 14 and 28 days after removal from cold treatments. Data are presented as the mean percentage germination in each treatment.

Statistical Methods

Data were analyzed using the PROC GLIMMIX procedure in SAS (version 9.4, SAS Institute Raleigh, NC) using a beta distribution with a log link function. Raw means and standard errors are presented in the results. Means were determined using the LS MEANS statement, and means separation, where necessary, was determined with a Tukey adjustment in the GLIMMIX procedure. All effects were considered significant at $p \leq 0.05$.

Results and Discussion

Effects of wild blueberry plant debris from lowbush blueberry fields and cold moist stratification on cow wheat seed germination

There was a significant effect of CMSD ($P < 0.0001$) but no effect of WBD ($P = 0.8297$) or the WBD X CMSD interaction ($P = 0.8070$) on cow wheat seed germination. Data were therefore pooled across WBD treatments for analysis. There was a significant CMSD effect on cow wheat seed germination ($P < 0.0001$). Cold moist stratification increased cow wheat seed germination relative to the non-stratified control (Table 2.1) with similar increases in germination at both 8 and 12 weeks of CMSD. Although cow wheat seed germination rates were only 20-27% with 8 – 12 weeks of cold storage (Table 2.1), these results suggest that CMS is an important factor in alleviating cow wheat seed dormancy in wild blueberry fields. Similarly, seeds of common cow-wheat *(Melampyrum pratense)* germinated after 76 days storage at 4° C (Průšová et al. 2013). Storage of crested cow-wheat *(Melampyrum cristatum* L.*)* seeds at 1°C for 4 weeks resulted in radicle emergence of seeds, however, additional periods of cold exposure up to 8 weeks were required to achieve germination rates of 60–85% (Horill, 1972). Although seed germination rates in these studies were higher than we observed for cow wheat (Table 2.1), results support our finding that exposure to cold temperature is important for alleviating dormancy in cow wheat. Future research should therefore consider longer CMSD's and possibly colder storage temperatures to determine if this increases cow wheat seed germination.

Exposure of cow wheat seeds to WBD did not stimulate germination. Cow wheat is a facultative hemiparasite that can commence germination without host stimulus (Těšitel et al. 2010; Bouwmeester et al. 2021). For example, germination of cow wheat was not increased by addition of root extract of jack pine *(Pinus banksiana),* an alternate host for cow wheat, on agar plates (Cantlon et al. 1963). In contrast, seeds of obligate hemiparasitic witchweeds (*Striga* spp.) will only germinate after exposure to a chemical stimulus from host plant roots (Matusova et al. 2005; Cardona-Medina & Ruiz, 2015). Cow wheat can form root attachments with a range of host plants (Cantlon et al. 1963; Malcolm, 1964) and it is therefore possible that seed dormancy and germination are affected more by external abiotic factors such as temperature as opposed to biological interactions between the seeds and potential host plants. Still, future research should examine the effect of wild blueberry root extracts or the presence or absence of living wild blueberry roots on cow wheat seed germination.

Table 2.1 Effect of cold moist stratification duration (CMSD) on cow wheat seed germination.

CMSD[a] (weeks)	Germination (%)
0	0 ± 0[b] b[c]
8	0.20[d] ± 0.03 a
12	0.27 ± 0.03 a

[a]Cold moist stratification duration. Seeds were stored at 4±1 °C for the specified duration.

[b]Values represent the mean ± SE.

[c]Means followed by the same letter do not differ significantly according to a Tukey's multiple-means comparisons at the 0.05 level of significance.

[d]Values represent frequency of analyzed data with beta distribution with a log link function.

Effect of gibberellic acid pre-soaking on cow wheat seed germination on agar gelling agent +glucose medium

There was a significant effect of cold moist stratification (CMSD) ($P < 0.0001$), gibberellic acid (GA_3) soaking ($P < 0.0001$), and the CMSD X GA_3 interaction ($P < 0.0001$) on cow wheat seed germination, but no effect of semisolid gelling-agent (SGA) ($P = 0.5951$) or the CMSD X SGA interaction ($P= 0.9540$), GA_3 X SGA interaction ($P= 0.9424$), or the CMSD X GA_3 X SGA interaction ($P = 0.5013$) on cow wheat seed germination. Data were therefore pooled across SGA treatments for analysis. There was a significant effect of CMSD ($P < 0.0001$), GA_3 ($P < 0.0001$), and the CMSD X GA_3 interaction ($P < 0.0001$) on cow wheat seed germination in the combined data set. Cow wheat seeds did not germinate when kept at room temperature (Table 2.2). CMSD and GA_3 pre-soaking, however, increased cow wheat seed germination relative to the non-stratified control (Table 2.2), though the highest germination rates (>90%) occurred by combining 8-12 weeks of CMSD with GA_3 pre-soaking (Table 2.2). These results suggest that CMSD and GA_3 exposure are important factors in alleviating the dormancy of cow wheat seeds.

Exogenous gibberellins are used to overcome seed dormancy and can substitute for light and cold storage requirements in some seeds (Curtis and Cantlon, 1963; Masselink, 1980; Yamaguchi and Kamiya, 2002). Gibberellic acid also stimulates embryo growth potential by inducing hydrolytic enzymes which enables commencement of radicle protrusion through the seed coat (Yamaguchi and Kamiya, 2002; Sun, 2008; Gupta and Chakrabarty, 2013). However, under field conditions the radicle of *Melampyrum* spp emerges and overwinters until the epicotyl emerges in the spring due to increased temperatures (Cantlon et al. 1963; Masselink, 1980; Crichton et al. 2012). The use of gibberellic acid under laboratory conditions appears to break cow wheat seed dormancy in conjunction with CMSD, however, seeds still germinated without the use of gibberellic acid (Table 2.2). Curtis and Cantlon (1963) found that GA_3 alone did not cause cow wheat seed germination whereas GA_3 soaking in combination with cold storage at 3°C caused up to 89% seed germination. Although seed germination rates in these results were lower than we observed for cow wheat (Table 2.2), results indicate that exposure to CMSD and GA_3 are important for alleviating dormancy. *Melampyrum* spp. have an embryo that is undifferentiated with growth occurring at low temperatures prior to radicle protrusion (Curtis and Cantlon, 1963, 1965, 1986; Baskin and Baskin, 2021). In our study several seeds had developed into seedlings while under CMSD conditions, however, the use of gibberellic acid likely accelerated embryo development, reducing both the duration of radicle and epicotyl dormancy compared to natural populations. Thus, seedlings were able to develop prior to removal from CMSD to room temperature (Figure 2.1). In conclusion, these results suggest that prolonged exposure to CMSD and GA_3 soaking is important for increasing seed germination under laboratory settings. Future studies should aim to understand the relationship between gibberellic acid and timing of both separate radicle and epicotyl dormancy in relation to CMS durations.

Table 2.2 Effect of cold moist stratification duration (CMSD) and gibberellic acid (GA_3) pre-soaking on cow wheat seed germination.

CMSD[a]	GA_3	Germination
0	No	0.00 ± 0.00[b] c[c]
8	No	0.24[d] ± 0.02 b
12	No	0.27 ± 0.02 b
0	Yes	0.00 ± 0.00 c
8	Yes	0.90 ± 0.01 a
12	Yes	0.93 ± 0.01 a

[a] Cold moist stratification duration. Seeds were stored at 4±1 °C for the specified duration.

[b] Values represent the mean ± SE.

[c] Means followed by the same letter do not differ significantly according to a Tukey's multiple-means comparisons at the 0.05 level of significance.

[d] Values represent frequency of analyzed data with beta distribution with a log link function.

Figure 2.1 Developing cow wheat seedlings after an additional 30-days in cold storage followed by transfer to room temperature conditions.

CHAPTER 3: COW WHEAT SEED BANK CHARACTERISTICS, EMERGENCE, MORPHOLOGY,AND PARASITISM IN LOWBUSH BLUEBERRY FIELDS

Abstract

Cow wheat is an annual hemiparasitic weed that is increasing in occurrence in lowbush blueberry fields in Nova Scotia. The objectives of this research were to 1) determine cow wheat seedbank characteristics in lowbush blueberry fields, 2) determine the extent and timing of cow wheat seedling emergence in lowbush blueberry fields, 3) quantify cow wheat morphological and reproductive characteristics in lowbush blueberry fields, and 4) confirm the presence or absence of parasitic root connections between cow wheat and lowbush blueberry plants. Cow wheat formed seedbanks in lowbush blueberry fields. There was a significant effect of sampling depth on seed density ($P < 0.0001$), with higher seed density in surface (1861 ± 1905 seeds m^{-2}) samples relative to subsurface (8.22 ± 8.42 seeds m^{-2}) samples at each site. Additional samples determined that cow wheat seed density on the soil surface ranged from 1,879 ± 9.69 to 18,400 ± 30.3 seeds m^{-2}. Cow wheat seedlings emerged between April 22 to May 30 and 50% and 90% seedling emergence occurred by May 8 to May 26, respectively. Cow wheat morphology varied across sites ($P < 0.0001$) with stem height, branch number, flower number, seed pods, and seeds per plant ranging from 18-29 cm, 4-7 branches $plant^{-1}$, 11-21 flowers $plant^{-1}$, 19-45 seed pods $plant^{-1}$, and 50-109 seeds $plant^{-1}$, respectively. The presence of haustoria connections between cow wheat roots and lowbush blueberry rhizomes was determined visually at three lowbush blueberry fields in 2023. Results suggest cow wheat has an affinity for *Vaccinium* spp., though specific resources obtained through parasitic connections with lowbush blueberry are unknown.

Introduction

The wild, or lowbush blueberry *(Vaccinium angustifolium* Ait.) is a native, rhizomatous perennial fruit species in North America, Canada. Commercial fields are established on abandoned farmland or cleared forested areas where conditions are favorable for existing blueberries to develop (Hall, 1959; Kinsman, 1993). Wild blueberry stands are managed on a two-year basis in which fields are pruned to ground level in the first year (non-bearing year) and harvested in the second year (bearing year) (Jensen and Yarborough, 2004). Weeds are therefore a major yield limiting factor in lowbush blueberry fields (McCully et al. 1991; Jensen and Yarborough, 2004) due to lack of tillage and crop rotation. Most weeds in wild blueberry fields are herbaceous and woody perennials, though annual weeds are becoming increasingly common in wild blueberry fields (McCully et al. 1991; Lyu et al. 2021).

Cow wheat (*Melampyrum lineare* Desr.) is a common summer annual weed in lowbush blueberry fields (Lyu et al 2021) and is the only North American representative of the genus *Melampyrum* (Piehl, 1962; Weakley, 2015). *Melampyrum* species are hemiparasites that do not require attachments to host roots to complete their life cycle (Joel et al. 2013), however, given its parasitic abilities could interfere with lowbush blueberry production. For example, lowbush blueberry plants had lower stem density, thinner stems, and fewer berries per stem in the presence of cow wheat relative to weed-free areas (Deveau and White 2022). This study did not, however, confirm existence of haustoria connections between cow wheat and wild blueberry, and this is required to fully understand the potential interactions between these two species. Cow wheat roots produce lateral haustoria that attach to the roots of nearby hosts which access water and nutrients in the vascular tissue (Joel et al. 2013). However, documented parasitic connections between cow wheat and *Vaccinium* spp. are lacking.

Given the annual life cycle of cow wheat, management of this weed species ultimately depends on improved knowledge of the seed bank characteristics, emergence patterns, and seed production capacity of this weed in wild blueberry fields. The persistence of a seed bank in lowbush blueberry fields has not been investigated, however, seeds of *Melampyrum* spp. have been known to lay dormant for two years before germination (Kaitera and Nuorteva, 2003). Seedling emergence in natural populations of *Melampyrum pratense* begins in March in The Netherlands (Masselink, 1980). Horrill (1972) notes *Melampyrum cristatum* germination begins at the end of February or early March in Huntingdonshire, Cambridgeshire, and Bedfordshire, UK. Subsequently, notes on *Melampyrum sylvaticum* suggests it emerges from late March into April (Dalrymple, 2007). However, documented seedling emergence patterns in lowbush blueberry fields are lacking. The objectives of this research are therefore to investigate seedbank characteristics, seedling emergence patterns, basic biology, and parasitic root connections of cow wheat in lowbush blueberry fields.

Materials and Methods

Determination of cow wheat seedbank presence and depth in lowbush blueberry fields

The objectives of this experiment were to determine 1) the general size of cow wheat seed banks in wild blueberry fields, and 2) if cow wheat seeds accumulate primarily on the soil surface in wild blueberry fields. These objectives were investigated by collecting surface and subsurface seed bank samples in six lowbush blueberry fields infested with cow wheat. Samples were collected from Kemptown, Collingwood, Murray Siding, Dalhousie Mountain, Higgins Mountain, and Greenfield on 23 August 2021, 18 August 2021, 27 August 2021, 25 August 2021, 25 August 2023, and 24 August 2023, respectively. The general size of the cow wheat seed bank was determined by collecting all surface debris in 20 0.25 X 0.25 m^2 quadrats placed in senescing cow wheat populations in each field. All surface debris in each quadrat was collected using a hand-held vacuum cleaner. Debris was placed in paper bags in the field and brought back to the lab for seed counting. Samples were sieved to remove all large debris prior to seed counting. Seed depth in the seed bank was determined using a 333-cm^3 bulk density core with a 4 cm diameter and sampling depth of 7.5 cm. Surface samples were collected within a 4 cm diameter circular quadrat (of the same diameter as the soil bulk density core sampler) using a vacuum. Subsurface samples were collected at the same location as surface samples using the core sampler. Surface and subsurface samples were sieved to remove all large debris prior to seed counting.

Extent and timing of cow wheat seedling emergence in lowbush blueberry fields

This component of the research consisted of two experiments. The objective of the first experiment was to determine the extent and timing of cow wheat seedling emergence in wild blueberry fields. Seedling emergence was monitored twice weekly in 15 0.5 X 0.5 m^2 quadrats at four wild blueberry fields (Table 3.1). Monitoring began in mid-April and newly emerged cow wheat seedlings were counted and pulled on each counting date to prevent double counting. Counts continued until no new seedlings emerged for two consecutive weeks.

Table 3.1 Wild blueberry fields used to monitor cow wheat seedling emergence in Nova Scotia, Canada.

Site	Year	Production year	GPS coordinates	
Dalhousie Mountain	2022 2023	Non-bearing, Bearing	45.601381	-62.948251
Mt Thom Hwy	2023	Non-bearing	45.492554	-62.989162
Murray Siding	2023	Non-bearing	45.365585	-63.212654
Higgins Mountain	2023	Non-bearing	45.547922	-63.633288

The objectives of the second experiment were to determine if cow wheat seeds germinate in fall in wild blueberry fields. The experiment was established in October in three pruned wild blueberry fields that were previously identified as having established cow wheat populations. Fields were located near Higgins Mountain (45.547922, -63.633288), Murray Siding (45.365585, -63.212654), and Camden (45.299636, -63.183576), Nova Scotia. Cow wheat seed germination was monitored at each site in ten 0.25 X 0.25 m^2 quadrats placed randomly throughout each field. Cow wheat seed germination was determined by carefully identifying and counting germinated seeds amongst the surface debris in each quadrat. Germinated seeds were not removed from quadrats in attempt to maintain the natural cow wheat population in each quadrat.

Monitoring of cow wheat seed germination continued weekly in fall until assessment was unable to continue due to snowfall with spring monitoring resuming on a weekly basis. However, emerged cow wheat seedlings were counted, and an elastic was placed around the seedlings to prevent double counting.

General cow wheat reproductive characteristics

The objective of this experiment was to determine the general reproductive characteristics of cow wheat in wild blueberry fields in Nova Scotia. Cow wheat plants were collected at various stages of development throughout the summer in seven wild blueberry fields in 2022 (Table 3.2). A total of 40 plants were collected from each field in early July when plants were flowering, and 20 plants were collected in late August after formation of seed pods and seed set had occurred. Data collection for the first sampling consisted of stem length, branch number, flower number per branch, and flowers on the main shoot axis above the last pair of branches. Data collection for the second sampling consisted of seed pod number and total seed number on each plant.

Table 3.2. Established locations for monitoring cow wheat reproductive characteristics in 7 wild blueberry fields in Nova Scotia, Canada.

Site	Year	Production year	GPS coordinates	
Wentworth-Collingwood	2022	Bearing	45.639327	-63.605817
North Road	2022	Non-bearing	45.599666	-63.777113
Mt Thom Hwy	2022	Non-bearing	45.492554	-62.989162
Dalhousie Mountain	2022	Non-bearing	45.601381	-62.948251
Silica Road	2022	Non-bearing	45.600057	-63.820162
Biorachan No 1 Road	2022	Bearing	45.594349	-63.095600
Mt Thom Road	2022	Non-bearing	45.515518	-62.983604

Presence or absence of haustorium connected to wild blueberry rhizomes

The objective of this experiment was to determine if cow wheat forms parasitic root connections with wild blueberry rhizomes under field conditions. The experiment consisted of two observational treatments with 4 replications. Observational treatments consisted of 1) core samples of blueberry + cow wheat together, and 2) core samples of blueberry alone. Samples were obtained from three commercial wild blueberry fields by digging a circular piece of sod from established wild blueberry patches from fields located near Murray Siding (45.365585, -63.212654) and two fields located near Higgins Mountain (45.547922, -63.633288 and 45.585839, -63.621028), Nova Scotia. Samples were placed in a bin and taken back to the laboratory for processing, where surface debris was brushed away using a paint brush and tweezers. Excavation continued with a paint brush and tweezers to expose cow wheat roots. Data collection included an observational assessment for possible parasitic root connections and photographs under a hand-held microscope.

Statistical Methods

The effect of sampling depth (surface and subsurface) on cow wheat seed density were determined using PROC GLIMMIX in SAS (version 9.4, SAS Institute, Raleigh, NC). Site was considered a random factor in the analysis. Data were analyzed using poisson distribution with log link function. Means were determined using the LS MEANS statement, and means separation, where necessary, was determined with a Tukey adjustment in the GLIMMIX procedure. All effects were considered significant at $p \leq 0.05$. Raw means and standard errors are presented in the results.

Seedling density in the first emergence experiment was converted to percent cumulative seedlings and plotted as a function of Julian date at each site. The number of germinated cow wheat seeds in the second experiment were expressed as % germination based on the total number of seeds estimated in each quadrat. Cow wheat reproductive characteristics were compared across sites using PROC GLIMMIX in SAS with site considered fixed effects in the analysis. Data were analyzed using poisson distribution with a log link function. Means were determined using the LS MEANS statement and means separation, was determined using Tukey's multiple means comparison test. Significance was based on $p \leq 0.05$.

Results and Discussion

Determination of cow wheat seedbank presence and depth in lowbush blueberry fields

There was a significant effect of sampling depth on seed density ($P < 0.0001$), with higher seed density in the surface (1861 ± 1905 seeds m^{-2}) samples relative to the subsurface (8.22 ± 8.42 seeds m^{-2}) samples at each site. Cow wheat seed density in the 0.25 X 0.25 m^2 quadrat samples ranged from 1,879 ± 9.69 to 18,400 ± 30.3 seeds m^{-2} (Table 3.3). Hair fescue (*Festuca filiformis* Pourr.) seeds also accumulate near the soil surface in wild blueberry fields (White, 2019), suggesting that surface accumulation of weed seeds is likely common in wild blueberry fields. This is similar to weed seed location in other no-till systems as well (Cardina et al. 2002; Nichols et al. 2015) and may present certain opportunities for cow wheat seedbank reduction. Pruning via burning, for example, may provide an effective method for seedbank reduction. Cow wheat is, however, common in fire-prone areas (Cantlon et al. 1963) and so research would be required to determine cow wheat seed sensitivity to heat and effect of fire on seed banks in wild blueberry fields. Accumulation of cow wheat seeds near the soil surface may also provide opportunities for biological control of this weed species via seed feeding by insects. For example, field crickets (*Gryllus pennsylvanicus*) readily consumed cow wheat seeds in both no-choice and choice-based feeding tests in the laboratory (MacKeil, 2021). However, ant mutualism between *Melampyrum* species has been documented, where ants consume the elaiosome from seeds and dispose of the intact seeds near or outside their nest, providing opportunities for seed dispersal through ant predation in wild blueberry fields (Gibson, 1993).

Table 3.3. Mean *Melampyrum lineare* seedbank density in soil surface samples collected from wild blueberry fields in 2021 and 2023 in Nova Scotia, Canada.

Site	Year	Cow wheat seed density (seeds m^{-2})
Kemptown	2021	18,400 ± 30.3[a]
Pigeon Hill	2021	6,789 ± 18.4
Murray Siding	2021	2,286 ± 10.7
Dalhousie Mountain	2021	9,260 ± 21.5
Greenfield	2023	1,879 ± 9.69
Higgins Mountain	2023	1,898 ± 9.74

[a] Values represent the mean ± SE.

Extent and timing of cow wheat seedling emergence in lowbush blueberry fields

Approximately 2–27% of cow wheat seeds present on the soil surface germinated in the fall (Table 3.4). Similar fall germination was reported in a study where mature seeds of *Melampyrum sylvaticum* were sowed in pots with tufted vetch and resulted in 10–67% germination (Crichton et al. 2012). Through favorable periods of cold temperatures, the radicle emerges, and germinated seeds overwinter and emerge the following spring (Cantlon et al. 1963; Masselink, 1980). I also observed that germinated seeds did not progress past the emerged radicle stage, suggesting that a portion of cow wheat seeds germinate in the fall prior to winter

(Figure 3.1). In conclusion, it may therefore be possible to manage this weed species with fall herbicide applications, and this should be investigated in future research.

Table 3.4. The number of fall germinated[a] seeds and spring emergence[b] of cow wheat seedlings in three different wild blueberry fields in Nova Scotia, Canada.

Site	Fall germination (%)	Spring emergence (%)
Higgins Mountain[d]	2 ± 5 [c]	15 ± 17 [c]
Murray Siding[d]	17 ± 9	52 ± 33
Camden	27 ± 26	74 ± 25

[a]Germination was assumed as the protrusion of the radicle through the seed coat.
[b]Emergence was considered to have occurred when cotyledons of emerged seedlings were visible.
[c]Values are the mean of 10 observations per site and are expressed as a percentage of all seeds present in each quadrat.
[d]Higgins Mountain and Murray Siding, two established quadrats were compromised due to herbicides applied over them.

Figure 3.1. (A-C) Seeds of *Melampyrum lineare* at various stages of radicle protrusion through the seed coat at Camden, Nova Scotia on December 15, 2022. (D) Seeds of *Melampyrum lineare* at various stages of radicle protrusion at Murray Siding, Nova Scotia on December 6, 2022. (E) Single *Melampyrum lineare* seed at Higgins Mountain, Nova Scotia on January 3, 2023.

Cow wheat seedling emergence in spring began between day of year 112 and 120 (22 April and 30 April) and 90% of emergence occurred between day of year 139 and 146 (8 May and 26 May) (Figure 3.2). Similar findings were observed in Prince Albert, Saskatchewan where seed germination began on April 12 and cotyledons emerged May 19 (Zalasky, 1962). This emergence timing provides opportunities to manage cow wheat with several commonly used herbicides in lowbush blueberry. Many herbicides, such as hexazinone, terbacil, sulfentrazone, tribenuron methyl, and flazasulfuron are applied preemergence to lowbush blueberry plants and are thus applied in late April and early May (Jensen and Yarborough 2004; White 2019; White 2023). Additionally, postemergence herbicides such as mesotrione, foramsulfuron, and clopyralid are applied in mid-late May (Boyd and White 2010; White et al. 2016; White 2021), thus corresponding with peak cow wheat emergence. These herbicides should therefore be evaluated for management of cow wheat in lowbush blueberry fields.

Spring seedlings may emerge from non-dormant cohorts of seeds that remain from the previous season, or from germinated seeds that have overwintered, although data to support this are lacking in wild blueberry fields. For example, seeds of *Melampyrum pratense* are known to remain dormant and germinate over several years after being released from the parent plant (Masselink, 1980; Průšová et al. 2013). Seeds of *Melampyrum sylvaticum* taken from natural populations have been shown to be capable of lying dormant in the soil for up to one year before germination commenced (Dalrymple, 2003). Although longevity of cow wheat seeds under field conditions in wild blueberry is lacking, seeds collected from wild blueberry fields remained viable after 1 year of warm and cold storage (personal observation). In conclusion, results support our findings that seeds may survive extended periods to germinate in subsequent years following senescence of the parent plant in wild blueberry fields. Additional field-based experiments should be conducted to determine cow wheat seed survival and seed bank persistence in lowbush blueberry fields.

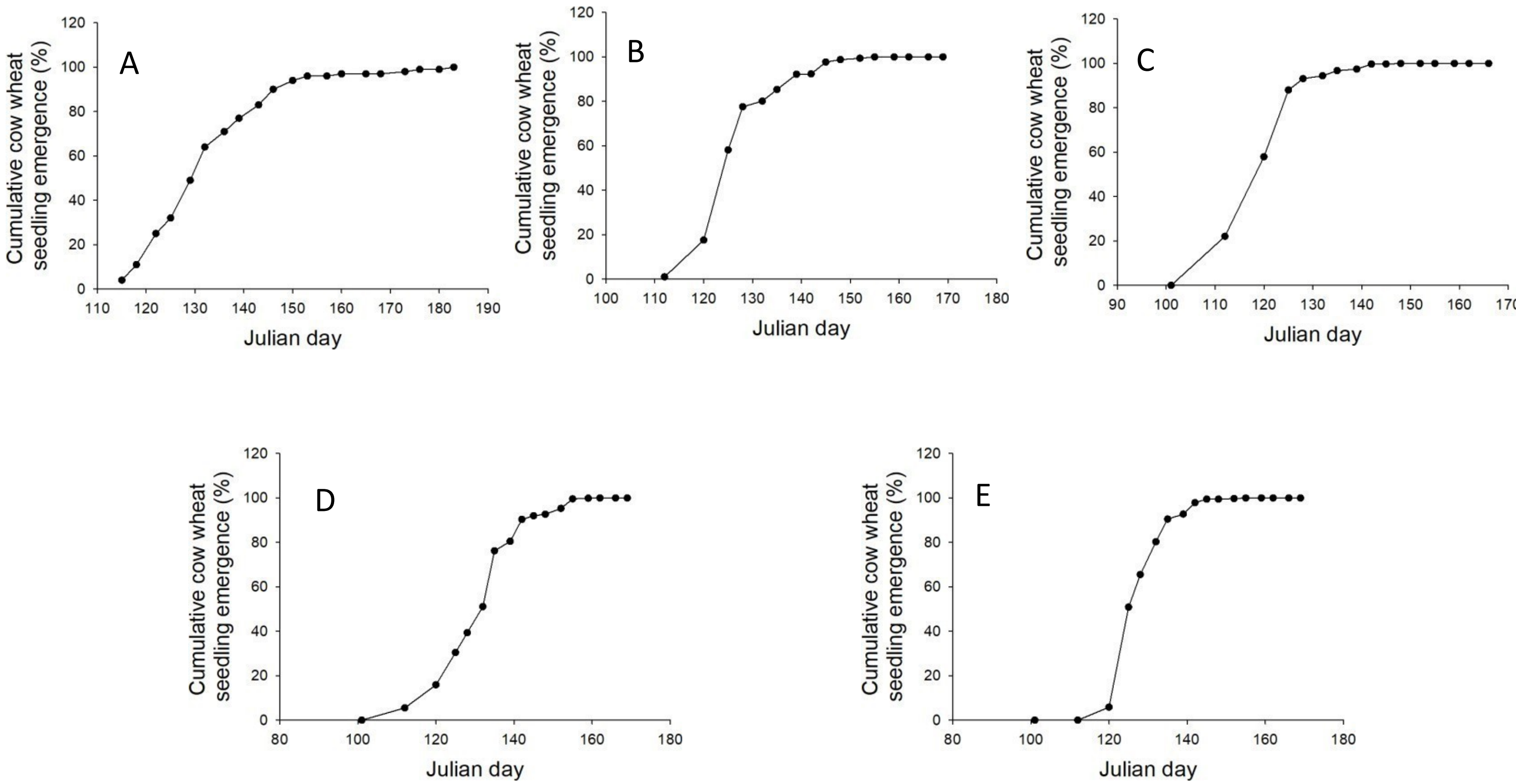

Figure 3.2. Mean percent cumulative cow wheat seedling emergence at Dalhousie Mountain non-bearing year (A), Dalhousie Mountain bearing year (B), Murray Siding non-bearing year (C), Higgins Mountain non-bearing year (D), and Mt Thom non-bearing year (E). Symbols represent the mean (n = 15) cumulative seedling emergence on each counting date.

General cow wheat reproductive characteristics

There was a significant site effect on cow wheat morphological and reproductive characteristics ($P < 0.0001$) (Table 3.5). From a morphological perspective, cow wheat stem height and branch number per plant varied across sites and ranged from 18.1 - 28.9 centimeters and 4 – 7 branches per plant, respectively (Table 3.5). Seed and Press (1993) observed stem heights of 7.3 cm and 36.4 cm for cow wheat plants unattached and attached to a host, respectively. Dalrymple (2007) also noted that *Melampyrum sylvaticum* had a height of 40 centimeters. We were unable to determine host attachments in collected plants, but our data suggest that cow wheat plants in lowbush blueberry fields may be shorter than plants reported in other locations. We are unable to find reports of branch number per plant in other studies of cow wheat.

From a reproductive perspective, flower number, seed pod number, and seeds per plant varied across sites and ranged from 13 - 21 flowers per plant, 19 – 45 pods per plant, and 50.4 – 109 seeds per plant, respectively (Table 3.5). Masselink (1980) noted that 40 - 50 flowers can be produced per plant, suggesting cow wheat plants produce fewer flowers in lowbush blueberry fields than that reported elsewhere. Pruvosa et al (2013), however, reported cow wheat seed production values of 53.2 - 72.5 seeds per plant, which is very similar to our results. Pod numbers per plant were generally higher than flower number per plant at each site (Table 3.5), suggesting that we were unable to determine all flowers per plant using our sampling method. Cow wheat flowers develop and mature acropetally (Dalrymple 2007) and we therefore likely missed flowers near the base of stems that had already fallen off collected plants. As such, flower number per plant in lowbush blueberry fields (if extrapolated from pod number per plant) is likely similar to that reported by Masselink (1980). We are unable to find reports of typical pod numbers per plant in the literature. We also found that seed number per pod was similar across sites (Table 3.5) and aligns with the estimate of 2.42 seeds per pod reported by Pruvosa et al (2013). Thus, while cow wheat plants in lowbush blueberry fields appear to be shorter than plants reported in other locations, reproductive output of these plants does not seem to be affected.

Table 3.5. Comparison of morphological and reproductive characteristics in cow wheat populations in 7 wild lowbush blueberry fields in Nova Scotia, Canada.

Site	Stem height (cm)[a]	Total branch number (# $plant^{-1}$)[b]	Total flower number (# $plant^{-1}$)[b]	Flowers on the main axis above the last pair of branches[b]	Total number of seed pods[b]	Total number of seeds per plant[b]	Average number of seeds per pod
Collingwood Road	28.9 ± 0.58 a[c]	5.00 ± 0.33 b	11.0 ± 0.51 d	4.70 ± 0.34 a	33.2 ± 1.28 b	82.7 ± 2.03 b	2.5 ± 0.35
North Road	24.5 ± 0.58 b	7.00 ± 0.42 a	17.3 ± 0.66 b	6.17 ± 0.39 a	30.8 ± 1.24 b	68.9 ± 1.85 cd	2.3 ± 0.34
Mt Thom Hwy	19.2 ± 0.58 d	5.00 ± 0.34 b	21.0 ± 0.72 a	5.52 ± 0.37 a	32.9 ± 1.28 b	75.1 ± 1.93 cb	2.3 ± 0.34
Dalhousie Mountain	22.0 ± 0.58 c	5.30 ± 0.36 b	21.0 ± 0.72 a	2.85 ± 0.27 b	44.9 ± 1.29 a	109 ± 2.33 a	2.3 ± 0.34
Thomson Road	18.1 ± 0.58 d	4.35 ± 0.33 b	13.1 ± 0.57 c	2.72 ± 0.26 b	19.3 ± 0.98 c	50.4 ± 1.58 e	2.5 ± 0.35
Biorachan Road	23.0 ± 0.58 bc	4.23 ± 0.33 b	14.2 ± 0.59 c	3.00 ± 0.24 b	33.7 ± 1.29 b	64.0 ± 1.78 d	2.0 ± 0.32
Mt Thom Road	24.2 ± 0.58 bc	4.20 ± 0.32 b	21.0 ± 0.72 a	3.15 ± 0.28 b	43.3 ± 1.47 a	104 ± 2.28 a	2.5 ± 0.35

[a]Stem height data were analyzed using PROC GLIMMIX in SAS using a normal distribution.

[b]Branch number, flower number, flowers on the main axis, number of seed pods, and number of seeds per plant were analyzed using PROC GLIMMIX in SAS using a poisson distribution with log transformation.

[c]Values represent the mean ± 1 SE. Means followed by the same letter within columns do not differ significantly according to Tukey's multiple means comparison test.

Presence or absence of haustorium connected to wild blueberry rhizomes

Multiple lateral swellings were found attached to the wild blueberry rhizome through various attempts involving vacuuming and carefully brushing away soil debris. The swellings were determined to be the haustoria when observed through The Dino-Lite Premier 0.3MP Bundle WF3113T with WF-20 Wireless Adapter within 10x magnification and Nikon Japan Alphaphot–2 YS2 within 10x magnification (personal communication, Jakub Těšitel). Several haustoria develop along the host rhizome from a single root (Figure 3.3b, d). There have been instances were single haustoria have been identified along the host rhizome (Figure 3.4d, e). Lowbush blueberry patches observed without cow wheat presence did not have any swellings throughout the rhizome portions obtained. Cantlon et al. (1963) reported that cow wheat did not form haustoria connections with potted lowbush blueberry cuttings, but our results clearly indicate that cow wheat forms root connections with established lowbush blueberry plants in commercial lowbush blueberry fields. This is the first report of these connections in this cropping system and may explain reductions in lowbush blueberry growth and yield in the presence of cow wheat reported by growers and Deveau and White (2022). Future studies should determine the metabolites and resources obtained from wild blueberry by cow wheat. I have also observed intraspecific parasitism where parasitic connections are formed between two cow wheat plants (Figure 3.4c) and similar results have been documented elsewhere (Piehl, 1962). Implications of these intraspecific connections in lowbush blueberry fields are unclear, but likely suggest resource sharing among individual cow wheat plants. For example, Matthies (2002) suggested that resource sharing through intraspecific parasitic connections prevented self-thinning of high seedling densities of the root hemiparasite *Rhinanthus alectorolophus*. Cow wheat density in lowbush blueberry fields can exceed 2000 plants m^{-2} in mature populations

(Deveau and White, 2022), an extremely large number of individuals in an established population of an annual species. Presence of inter- and intraspecific root connections may also help to explain variation in cow wheat morphology observed across sites (Table 3.5) as the number of these connections at a given site likely influence cow wheat growth. As such, future research should examine the potential role of intraspecific parasitism in cow wheat population ecology in lowbush blueberry fields. Growth of *Melampyrum* spp. is also affected by host quality (Matthies, 2017). Given the high level of within and between-field genetic variation of lowbush blueberry stands (Bell et al. 2009; Beers et al. 2019) and the fact that many fields contain at least two distinct *Vaccinium* spp. (Jensen and Yarborough, 2004), it is possible that host quality, and thus cow wheat growth, could vary across sites. In conclusion, future research should aim to understand the relationship between cow wheat's ability to parasitize lowbush blueberry and the potential implications of such parasitism.

Figure 3.3. Haustorial connections (white pointers) between *Melampyrum lineare* and lowbush blueberry. (A) *Melampyrum* attached to a *Vaccinium* rhizome. (B) Various haustorial connections attached to a bearing-year *Vaccinium* rhizome. (C) Broken haustorial connections attached to a bearing-year *Vaccinium* rhizome. (D) Various succeeding haustoria attached to *Vaccinium* rhizome.

Figure 3.4. Haustorial connections (white pointers) of *Melampyrum lineare.* (A) *Melampyrum* plants attached to a *Vaccinium* rhizome. (B, C) Singular haustorium attached to a non-bearing year *Vaccinium* rhizome. (D) Self-parasitic connection between two *Melampyrum* plants. (E) Singular haustorium connection.

CHAPTER 4: EVALUATION OF PRE AND POST HERBICIDES FOR COW WHEAT MANAGEMENT IN LOWBUSH BLUEBERRY FIELDS

Abstract

Cow wheat is a common annual hemiparasitic weed in lowbush blueberry fields in Nova Scotia and there is limited knowledge of susceptibility of this weed to herbicides. The objective of this study was therefore to evaluate the effect of several currently registered PRE and POST herbicides on cow wheat density in lowbush blueberry fields. Cow wheat density was reduced by PRE applications of terbacil (2000 g a.i. ha^{-1}) and indaziflam (75 g a.i. ha^{-1}) at all sites and by PRE applications of hexazinone (1920 g a.i. ha^{-1}), sulfentrazone (139 g a.i. ha^{-1}), tribenuron- methyl (30 g a.i. ha^{-1}), nicosulfuron + rimsulfuron (16.7 + 8.3 g a.i. ha^{-1}), and flazasulfuron (50 g a.i. ha^{-1}) at 3 of 4 sites. POST applications of mesotrione (144 g a.i. ha^{-1}), and foramsulfuron (35 g a.i. ha^{-1}) reduced cow wheat density at 3 of 4 sites but density was not reduced by POST clopyralid applications at any site. These results suggest that cow wheat is susceptible to several currently registered herbicides in lowbush blueberry and growers should be able to readily manage this weed in lowbush blueberry fields.

Introduction

The lowbush, or wild blueberry *(Vaccinium angustifolium* Ait.) is a perennial shrub that is native to North America (Vander Kloet, 1988). It is an economically important fruit crop in Canada and contributed $57 million to farm gate value in 2022 (Burgess, personal communication). Lowbush blueberry fields are developed from abandoned farmland or cleared forested areas (Hall, 1959). The fields are managed under a 2-year production cycle where fields are pruned to ground level in the first year (non-bearing year) and produce fruit in the second year (bearing year) (McIsaac, 1997; Jensen and Yarborough, 2004). Weed management is challenging due to the lack of tillage and crop rotation, and the weed flora is dominated by herbaceous and woody perennials. Several annual weeds, however, are increasing in occurrence in recent weed surveys (Jensen and Yarborough 2004; Lyu et al. 2021).

Cow wheat (*Melampyrum lineare* Desr.*)* is a facultative hemiparasitic annual (Oldham and Weeks, 2017; Nave et al. 2018) that is common in lowbush blueberry fields in Nova Scotia and other lowbush blueberry production regions (Lapointe and Rochefort, 2001; Lyu et al. 2021; Ayers, 2020). Occurrence of this weed species in Nova Scotia has increased from 20% of lowbush blueberry fields surveyed in the 1980's (McCully et al. 1991) to 44% of lowbush blueberry fields surveyed from 2017-2019 (Lyu et al. 2021). Increased occurrence of cow wheat is a concern to growers because of the hemiparasitic life cycle of this weed and the potential for *Vaccinium* spp. to serve as potential hosts for cow wheat (Cantlon et al. 1963). Growers also observe yield losses in fields infested with cow wheat, and reduced lowbush blueberry growth and yield in the presence of cow wheat has been reported (Deveau and White, 2021).

Weed management in lowbush blueberry depends primarily on herbicides due to lack of tillage and crop rotation as viable weed control practices. Knowledge of cow wheat susceptibility to currently registered herbicides in lowbush blueberry, however, is lacking, and there are no reports of herbicide susceptibility of this plant available in the literature. The objective of this research was to evaluate a range of currently registered PRE and POST herbicides for management of cow wheat in lowbush blueberry fields.

Materials and Methods

The effect of PRE and POST herbicides on cow wheat in wild blueberry fields

The experiment was arranged as randomized complete block design with 10 treatments (Table 4.1) and 5 blocks and was conducted in non-bearing year lowbush blueberry fields located near Dalhousie Mountain (45°36'04.7"N, 62°56'49.1"W), Salt Springs (45°30'21.0"N, 62°58'15.1"W), Higgins Mountain (45°35'03.2"N, 63°37'34.1"W), and Murray Siding (45°21'55.4"N, 63°12'48.4"W), Nova Scotia, Canada. Plot size was 2 m X 4 m with a 1 m buffer between each block. Herbicides were applied using a CO_2 pressurized research plot sprayer equipped with four HYPRO ULD120-02 nozzles and calibrated to deliver 200L water ha^{-1} at 296 kPa. Application dates and relevant weather parameters for each herbicide application are provided in Table 4.2.

Table 4.1. PRE and POST herbicides evaluated for cow wheat management in lowbush blueberry fields.

Trade name[a]	Active ingredient (a.i.)	WSSA group	Application rate (g a.i. ha^{-1})	Application timing[b]	Manufacturer
Velpar	Hexazinone	5	1920	PRE	Tessenderlo Kerley, Inc.
Sinbar	Terbacil	5	2000	PRE	Tessenderlo Kerley, Inc.
Authority	Sulfentrazone	14	139	PRE	FMC Agricultural Sciences
Alion	Indaziflam	29	375	PRE	Bayer Crop Science
Spartan	Tribenuron-methyl	2	30	PRE	FMC Agricultural Sciences
Steadfast	Nicosulfuron + rimsulfuron	2	16.8 + 8.3	PRE	Corteva Agriscience
Chikara	Flazasulfuron	2	50	PRE	ISK Biosciences
Option	Foramsulfuron	2	35	POST	Bayer Crop Science
Pyralid	Clopyralid	4	151	POST	Sharda Cropchem Limited
Callisto	Mesotrione	27	144	POST	Syngenta Crop Protection

[a]Spartan, Steadfast, and Callisto were applied in conjunction with 0.2% v/v non-ionic surfactant (NIS), Chikara was applied in conjunction with 0.25% NIS, and Option was applied in conjunction with 2.5L ha^{-1} of 28-0-0 UAN liquid fertilizer.

[b]Application timing relative to lowbush blueberry plants.

Table 4.2. Application timing and weather conditions at the time of PRE and POST herbicide applications in Nova Scotia, Canada.

Site	Year	Application timing[a]	Application date	Temperature	Relative humidity	Wind speed
				°C	%	Km/h^{-1}
Dalhousie Mtn	2022	PRE	May 13, 2022	21.4	36.5	7.08
		POST	June 03, 2022	16.5	53.4	6.59
Salt Springs	2022	PRE	May 13, 2022	24.5	35.7	6.59
		POST	June 03, 2022	16.6	52.8	5.14
Higgins Mtn	2023	PRE	May 12, 2023	12.9	53.8	12.0
		POST	June 12, 2023	23.9	51.9	4.70
Murray Siding	2023	PRE	May 19, 2023	25.1	34.3	11.9
		POST	June 12, 2023	17.1	71.2	8.70

Data Collection

Data collection included cow wheat density at the time of all herbicide applications, cow wheat height and true leaf number at the time of POST herbicide applications, cow wheat density in July of the non-bearing and bearing year, wild blueberry stem density in August of the non-bearing year, wild blueberry stem height and flower bud number per stem in October of the non-bearing year, wild blueberry yield in August of the bearing year, and visual injury ratings on cow wheat and wild blueberry. Cow wheat density at the time of herbicide application and in mid-summer was determined in three 0.3 X 0.3 m quadrats per plot. Cow wheat seedling height and true leaf number were determined on 30 randomly selected seedlings across each plots. Lowbush blueberry stem density was determined in three 0.3 X 0.3 m quadrats per plot in mid-August. Lowbush blueberry stem height and flower bud number were determined on 30 randomly selected blueberry stems from each plot. Lowbush blueberry yield was determined by hand raking all berries in two 1m X 1 m quadrats per plot in August 2023.

Statistical Methods

The effect of herbicide treatment on cow wheat density was determined using PROC MIXED in SAS (version 9.4, SAS Institute, Raleigh, NC). Sites were analyzed separately for cow wheat data with herbicide treatment modeled as a fixed effect in the analysis and blocks were modeled as random effects. The effect of site, treatment, and site by treatment interaction on lowbush blueberry stem density, stem length, flower bud number per stem, and yield were determined using PROC MIXED in SAS (version 9.4, SAS Institute, Raleigh, NC). Main and interactive effects were modeled as fixed effects and blocks were modeled as random effects in the analysis. Assumptions of normality and constant variance for all analyses were assessed using PROC UNIVARIATE in SAS. Differing data transformations (e.g., LOG or SQRT) were used when assumptions of normality and constant variance needed to be met. Means were determined using the LS MEANS statement, and means separation, where necessary, was determined using Tukey's multiple means comparison test. Effects were considered significant at $p \leq 0.05$.

Results and Discussion

Effect of PRE and POST emergence herbicides on cow wheat in wild blueberry fields

Initial mean cow wheat density at Cove Road, Dalhousie Mountain, Higgins Mountain, and Murray Siding was 57 ± 21, 35 ± 16, 42 ± 21, and 506 ± 216 plants m^{-2}, respectively. Mean cow wheat seedling height at the time of POST herbicide applications was 4.05 ± 1.36 cm, 4.86 ± 1.39 cm, 4.93 ± 2.30 cm, and 3.50 ± 1.61 cm at Dalhousie Mountain, Salt Springs, Murray Siding, and Higgins Mountain, respectively. Mean cow wheat leaf number per plant at the time of POST herbicide applications was 2.60 ± 1.39, 3.50 ± 1.73, 5.97 ± 2.04, and 2.97 ± 1.63 leaves $plant^{-1}$ at Dalhousie Mountain, Salt Springs, Murray Siding, and Higgins Mountain, respectively.

There was a significant herbicide treatment effect on non-bearing year cow wheat density at each site ($P < 0.0001$). Except for clopyralid, most herbicides reduced cow wheat density relative to the nontreated control at each site (Table 4.3). The PRE treatments were particularly effective. Terbacil reduced density at each site and hexazinone reduced density at all sites except Higgins Mountain. Terbacil and hexazinone are two of the most commonly used PRE herbicides in lowbush blueberry, and results suggest that growers should expect control of cow wheat when using these herbicides. Tribenuron methyl, nicosulfuron + rimsulfuron, and flazasulfuron also reduced cow wheat density at all sites except Higgins Mountain. Tribenuron methyl is routinely used for fall and spring management of bunchberry (*Cornus canadensis* L.) and red sorrel (*Rumex acetosella* L.) (Jensen and Specht, 2004; White, 2023), and these results suggest that spring applications of this herbicide likely contribute to cow wheat management as well. Similarly, flazasulfuron and nicosulfuron + rimsulfuron are used for spring suppression of hair fescue (*Festuca filiformis* Pourr.) (White, 2022) and results again suggest that these herbicides would also contribute to cow wheat management. Although registered for use, sulfentrazone and indaziflam are not routinely used for weed management by lowbush blueberry growers, likely due to general lack of efficacy of these herbicides on important weed species (Menapati, 2020; White et al. 2021). Our results, however, identify an important new use for these herbicides as indaziflam reduced cow wheat density at all sites and sulfentrazone reduced density at all sites except Higgins Mountain. Growers should consider utilization of these herbicides in fields with a history of cow wheat infestations. Therefore, reducing resistance risk via rotating modes of action.

POST clopyralid applications did not reduce cow wheat density at any site. This is surprising given the general efficacy of this herbicide on broadleaf weeds. Mesotrione and foramsulfuron, however, reduced cow wheat density at all sites except Murray Siding (Table

4.3), suggesting efficacy of these herbicides on cow wheat. Cow wheat density was highest at Murray Siding, which may explain reduced efficacy of mesotrione and foramsulfuron at this site as herbicide efficacy generally declines with increasing weed density (Winkle et al. 1981). Additional research should therefore be conducted to determine the effects of cow wheat size and density on POST herbicide efficacy. There was no significant herbicide treatment effect on bearing year cow wheat density at Cove Road (P = 3385) or Dalhousie Mountain (P = 0.7652). Mean cow wheat density at Cove Road and Dalhousie Mountain of 10 ± 6 and 416 ± 152 plants m^{-2}, respectively.

There was no site by treatment interaction effect on lowbush blueberry stem length, stem density, buds per stem, and yield ($P \geq 0.7594$). Data were therefore pooled across sites for analysis. There was no herbicide effect on lowbush blueberry stem length (P= 0.2391), stem density (P = 0.4725), buds per stem (P = 0.1844), and yield (P = 0.2283). Mean blueberry stem density, stem length, flower buds per stem, and yield were, 495 ± 72 stems m^{-2}, 14.4 ± 0.51 cm, 5.3 ± 0.7 buds $stem^{-1}$, and 3263 ± 513 kg ha^{-2}, respectively.

Lack of a lowbush blueberry response to cow wheat control suggests that control of this weed does not increase lowbush blueberry growth or yield. Cow wheat density at Dalhousie Mountain, Cove Road, and Higgins Mountain, however, was quite low relative to that observed in dense populations of this weed. For example, average cow wheat density in a previous study was >600 plants m^{-2}, at which growth of lowbush plants tended to be reduced (Deveau and White 2021). It is unclear why lowbush blueberry stem density, height, and flower buds were not reduced at Murray Siding given the high cow wheat density at this site (Table 4.3), though competitive interactions between cow wheat and lowbush blueberry are poorly understood at present. I have identified root connections between cow wheat and lowbush blueberry (see chapter 3), though implications of these connections on lowbush blueberry are purely speculative at present time. For example, cow wheat removes radio-labelled carbon from host plants via root connections (Nave et al. 2017), yet few studies of cow wheat are conducted in the context of an agricultural ecosystem in which growth and yield of the host are of interest. As such, future studies must determine if the hemi-parasitic nature of cow wheat is indeed detrimental to lowbush blueberry to justify management of this species in lowbush blueberry fields. It should also be noted that non-bearing year herbicide applications failed to reduce bearing year cow wheat density, which may have contributed to lack of yield increases at Dalhousie Mountain where bearing year density was >400 plants m^{-2}.

In conclusion, cow wheat is susceptible to several currently registered PRE and POST herbicides in lowbush blueberry in Canada. Control of cow wheat with herbicides did not increase lowbush blueberry yield potential or yield, possibly due to low cow wheat density at most of the trial sites. Although cow wheat is likely easily managed with herbicides, future research must determine if the hemi-parasitic nature of cow wheat is deleterious to lowbush blueberry production to justify control of this weed species in lowbush blueberry fields.

Table 4.3. Effect of PRE and POST spring herbicide applications on cow wheat density in four non-bearing year lowbush blueberry fields in Nova Scotia, Canada.

		Cow wheat density (m^{-2})[a]			
Treatment	Application Timing	Dalhousie Mountain	Cove Road	Higgins Mountain	Murray Siding
Control	Untreated	81 ± 16 a[b]	30 ± 7 a	25 ± 5 a	455 ± 178 a
Tribenuron-methyl	PRE	1 ± 1 b	1 ± 1 bc	14 ± 4 abc	57 ± 32 b
Nicosulfuron + rimsulfuron	PRE	0 ± 0 b	0 ± 0 c	16 ± 8 abcd	20 ± 12 b
Flazasulfuron	PRE	13 ± 13 b	0 ± 0 c	6 ± 1 abcd	15 ± 10 b
Sulfentrazone	PRE	0 ± 0 b	2 ± 1 bc	10 ± 3 abcd	34 ± 8 b
Indaziflam	PRE	0 ± 0 b	1 ± 1 bc	4 ± 2 bcd	1 ± 1 b
Terbacil	PRE	0 ± 0 b	0 ± 0 c	1 ± 1 cd	1 ± 1 b
Hexazinone	PRE	3 ± 2 b	0 ± 0 c	7 ± 2 abcd	8 ± 5 b
Clopyralid	POST	50 ± 20 a	15 ± 9 ab	18 ± 6 ab	615 ± 216 a
Mesotrione	POST	8 ± 8 b	0 ± 0 c	0 ± 0 d	169 ± 56 ab
Foramsulfuron	POST	2 ± 2 b	3 ± 3 bc	1 ± 1 bcd	177 ± 107 ab

[a] Cow wheat density counts were analyzed using PROC MIXED in SAS. LOG transformation was used at Dalhousie Mountain. SQRT transformation was used at Cove Road, Higgins Mountain, and Murray Siding, respectively.

[b]Values represent the mean ± SE. Means followed by the same letter do not differ significantly according to Tukey's multiple-means comparisons at the 0.05 level of significance.

CHAPTER 5: CONCLUSIONS

Weeds are the major limiting factor in lowbush blueberry production with stands consisting of a range of woody and herbaceous perennials. However, recent weed surveys have identified an increased occurrence of annual weeds such as cow wheat (*Melampyrum lineare*). Various laboratory experiments have been conducted to contribute to the knowledge gaps surrounding cow wheat biology and management in lowbush blueberry fields. Future research should therefore consider longer CMSD's and possibly colder storage temperatures to determine if this increases cow wheat seed germination. Additionally, examining the effect of wild blueberry root extracts or the presence or absence of living wild blueberry roots on cow wheat seed germination. Based on the results from our second laboratory experiment future studies should aim to understand the relationship between gibberellic acid and timing of both separate radicle and epicotyl dormancy in relation to CMS durations. Accumulation of cow wheat seeds near the soil surface may also provide opportunities for biological control of this weed species via seed feeding by insects. Based on emergence patterns additional field-based experiments should be conducted to determine cow wheat seed survival and seedbank persistence in lowbush blueberry fields. Future research should aim to understand the relationship between cow wheat's ability to parasitize lowbush blueberry and the potential implications of such parasitism. Lastly, cow wheat was susceptible to several currently registered PRE and POST herbicides in lowbush blueberry in Canada. Although cow wheat is likely easily managed with herbicides, future research must determine if the hemi-parasitic nature of cow wheat is deleterious to lowbush blueberry production to justify control of this weed species in lowbush blueberry fields.

References

Ayers, A. G. **2020**. Evaluating the current weed community in wild blueberry fields and IPM strategies for spreading dogbane *(Apocynum androsaemifolium*). [Dissertation].

Barker, W. G., Hall, I. V., Aalders, L. E., & Wood, G. W. **1964**. The lowbush blueberry industryin Eastern Canada. *Economic Botany, 18:* 357–365.

Baskin J.M., & Baskin, C. C. **2021.** The great diversity in kinds of seed dormancy: a revision of the Nikolaeva-Baskin classification system for primary seed dormancy. *Seed Science Research,31:* 249–277.

Beers, L., Rowland, L. J., & Drummond, F. **2019**. Genetic diversity of lowbush blueberry throughout the United States in managed and non-managed populations. *Agriculture, 9(6)*: 1–14.

Bell, D.J., Rowland, L.J., Zhang, D., & Drummond, F.A. **2009**. Spatial genetic structure of lowbush blueberry, *Vaccinium angustifolium*, in four fields in Maine. *Botany 87*: 932–946.

Benninghouse, N. **1976**. A study of *Melampyrum lineare*. [Dissertation].

Bouwmeester, H., Li, C., Thiombiano, B., Rahimi, M., & Dong, L. **2021**. Adaptation of the parasitic plant lifecycle: germination is controlled by essential host signaling molecules. *PlantPhysiology, 185:* 1292–1308.

Boyd, N. S., & White, S. N. **2010**. PRE and POST herbicides for management of goldenrods (*Solidago* spp.) and black bulrush (*Scirpus atrovirens*) in wild blueberry. *Weed Technology, 24:* 446–452.

Cantlon, J. E., Curtis, E. J. C., & Malcolm, W. M. **1963**. Studies of *Melampyrum linear*e. *Ecology, 44:* 466–474.

Cardona-Medina, E., & Muriel Ruiz, S.B. **2015**. Seed germination and plant development in *Escobedia grandiflora* (Orobanchaceae): evidence of obligate hemiparasitism? *Acta Biology of Colombia, 20(3):* 133 –140.

Curtis, E.T.C., & Cantlon, J.E. **1963**. Germination of *Melampyrum lineare*: interrelated effects of after-ripening and gibberellic acid. *Science, 40*: 406–408.

Curtis E. J.C., & Cantlon, J.E. **1965**. Studies of the germination process in *Melampyrum lineare*. *American Journal of Botany, 52(6):* 552–555.

Curtis, E.J.C., & Cantlon, J.E. **1968**. Seed dormancy and germination in *Melampyrum lineare.American Journal of Botany, 55(1):* 26-32.

Crichton, R.J., Dalrymple, S.E., & Hollingsworth, P.M. **2012**. Horticultural protocols to aid the conservation of *Melampyrum Sylvaticum,* Orobanchaceae (small cow-wheat), an engendered hemiparasitic plant. *The Journal of Botanic Garden Horticulture, 10*: 57-69.

Dalrymple, S. E. **2003.** Rarity and conservation of *Melampyrum sylvaticum*. [Dissertation].

Dalrymple, S.E. **2007**. Biological flora of the British Isles: *Melampyrum sylvaticum* L. *Journal of Ecology, 95:* 583-597.

Deveau, V.T., & White, S.N. **2022**. An observational investigation of potential cow wheat *(Melampyrum lineare* Desr.) interference with wild blueberry *(Vaccinium angustifolium* Ait.).*Canadian Journal of Plant Science.* Retrieved from: https://doi.org/10.1139/CJPS-2021-0270.

Eaton, L. J., Sanderson, K. R., & Fillmore, S. A. E. **2009**. Comparison of consecutive and alternate fertilizer applications in wild blueberry production. *Canadian Journal of Plant Science, 89:* 93–98.

Gibson, W. **1993**. Selective advantages to hemi-parasitic annuals, genus *Melampyrum,* of a seed-dispersal mutualism involving ants: I. Favorable nest sites. *Oikos, 67:* 334-344.

Gould, K., Wood, S., & Smreciu, A. **2013**. Species profile for *Melampyrum lineare*: cow-wheat, narrowleaf cow-wheat. Retrieved from: https://doi.org/10.7939/R32J68398

Gupta, R., & Chakrabarty. **2013**. Gibberellic acid in plant still a mystery unresolved. *Plant Signaling and Behavior, 8(9):* 1–5.

Hall, I.V. **1959**. Plant populations in blueberry stands developed from abandoned hayfields andwoodlots. *Ecology, 40*: 742–743.

Hall, I.V., & Aalders, L. E. **1961**. Cytotaxonomy of lowbush blueberries in eastern Canada. *American Journal of Botany, 48(3):* 199–201.

Hall, I.V., Forsyth, F.R., Aalders, L.E., & Jackson, L.P. **1972**. Physiology of the lowbush blueberry. *Economic Botany, 26(1):* 68–73.

Hall, I.V., Lewis, A. E., Nickerson, N. I., & Vander Kloet, S. P. **1979**. The biological flora of Canada. 1. *Vaccinium angustifolium* Ait., sweet lowbush blueberry. *Canadian Field Naturalist,93:* 415–430.

Horrill, A.D. **1972**. *Melampyrum cristatum* L. *Journal of Ecology, 60:* 235–244.

Jensen, K. I. N., & Yarborough, D. E. **2004**. An overview of weed management in the wild lowbush blueberry–past and present. *Small Fruits Review, 3*: 229–255.

Jensen, K. I. N. & Specht, E. G. **2002**. Response of lowbush blueberry (*Vaccinium angustifolium*) to hexazinone applied early in the fruiting year. *Canadian Journal of PlantScience, 82:* 781–783.

Joel, D.M., Gressel, J., & Musselman, L. **2013**. Functional Structure of the Mature Haustorium. *Parasitic Orobanchaceae*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-

Kaitera, J., & Nuorteva, H. **2003**. Relative susceptibility of four *Melampyrum* species to *Cronartium flaccidum*. *Scandinavian Journal of Forest Research, 18(6):* 499–504.

Kennedy, K.J., Boyd, N.S., & Nams, V.O. **2010**. Hexazinone and fertilizer impacts on sheep sorrel (*Rumex acetosella*) in wild blueberry. *Weed Science, 58(3):* 317-322.

Kinsman, G. **1993**. The history of the lowbush blueberry industry in Nova Scotia 1950-1990. Blueberry Producers‟ Assn. Nova Scotia.

Lapointe, L., & Rochefort, L. **2001**. Weed survey of lowbush blueberry fields in Saguenay-Lac-Saint-Jean, Quebec, following eight years of herbicide application. *Canadian Journal of Plant Science*, *81*: 471–478.

Lyu, H., McLean, N., McKenzie-Gopsill, A., & White, S. N. **2021**. Weed survey of Nova Scotia lowbush blueberry *(Vaccinium Angustifolium* Ait.) fields. *International Journal of Fruit Science, 21(1):* 359-378.

MacKeil, J. **2021**. Population dynamics and seed feeding tendencies of field crickets *(Gryllidae)* in wild blueberry fields. [Dissertation].

Malcom, W.M. **1964**. Behind-glass culture of *Melampyrum lineare* Desr. a root-parasitic flowering plant. *Bulletin of the Torrey Botanical Club, 91(1):* 31–35.

Masselink, A.K. **1980**. Germination and seed population dynamics in *Melampyrum pratense* L. *Acta Bot. Neerl, 29(5/6):* 451–486.

Matthies, D. **2002**. Positive and negative interactions among individuals of a root hemiparasite. *Plant Biology, 5:* 79–84.

Matthies, D. **2017**. Interactions between a root hemiparasite and 27 different hosts: growth, biomass allocation and plant architecture. *Perspectives in Plant Ecology, Evolution and Systematics, 24:* 118–137.

Menapati, R. K. **2020**. Occurrence of hexazinone-resistant red sorrel *(Rumex acetosella* L.) and evaluation of spring non-bearing year and autumn bearing year herbicides for red sorrel management in lowbush blueberry *(Vaccinium angustifolium* Ait.) fields in Nova Scotia. [Dissertation].

McCully, K. V., Sampson, M. G., & Watson, A. K. **1991**. Weed survey of Nova Scotia lowbush blueberry (*Vaccinium angustifolium*) fields. *Weed Science, 39*: 180–185.

McIsaac, D. **1997**. Growing wild lowbush blueberries in Nova Scotia. Wild blueberry fact sheet. Copyright of Province of Nova Scotia.

Munteanu, M., Dehelean, C.A., Ionescu, D., Andoni, M., & Butnariu, M. **2010**. Investigation of the use of *Melampyrum* sp. extract samples to assess metals contamination. *Journal of Agroalimentary Processes and Technologies, 16(3):* 382-386.

Nave, L. E., Heckman, K. A., Muñoz, A. B., & Swanston, C. W. **2018**. Radiocarbon suggests the hemiparasitic annual *Melampyrum lineare* Desr. may acquire carbon from stressed hosts. *Radiocarbon, 60(1):* 269–281.

Nichols, V., Verhulst, N., Cox, R., & Govaerts, B. **2015**. Weed dynamics and conservation agriculture principles: a review. *Field Crops Research, 183:* 56–68.

Oldham, K. A., & Weeks, A. **2017**. Varieties of *Melampyrum lineare* (Orobanchaceae) Revisited. *Rhodora, 119:* 224–259.

Penney, B.G., & McRae, K.B. **2000**. Herbicidal weed control and crop-year NPK fertilization improves lowbush blueberry (*Vaccinium angustifolium* Ait.) production. *Canadian Journal of Plant Science, 80:* 351–361.

Penney, B. G., McRae, K. B., & Rayment, A. F. **1997**. Long-term effects of burn-pruning on lowbush blueberry (*Vaccinium angustifolium* Ait.) production. *Canadian Journal of Plant Science, 77:* 421–425.
Piehl, M.A. **1962**. The parasitic behavior of *Melampyrum lineare* and a note on its seed color. *Rhodora, 64(757):* 15–23.

Průšová, M., Lepš, J., Stech, M., & Těšitel, J. **2013**. Growth, survival, and generative reproduction in a population of a widespread annual hemiparasite *Melampyrum pratense. Biologia, 68(1):* 65–73.

Rich, T.C.G., Fitzgerald, R., & Sydes, C. **1998**. Distribution and ecology of small cow-wheat (*Melampyrum sylvaticum* L; Scrophulariaceae) in the British Isles. *Botanical Journal of Scotland,* 29-47.

SAS Institute. **1999**. *SAS User's Guide: Statistics*. Version 6, Vol 2, 4th edition. SAS Institute Inc., Cary, North Carolina.

Seel, W. E., & Press, M. C. **1993**. Influence of the host on three sub-Arctic annual facultative root hemiparasites. *New Phytol, 125:* 131–138.

Statistics Canada. **2020**. *Overview of the Canadian fruit industry*. Catalogue no. A71-33F-PDF, AAFC no. 13081F. Statistics Canada, Ottawa, Ontario, Canada.

Sun, T. P. **2008**. Gibberellin metabolism, perception and signaling pathways in Arabidopsis. *American Society of Plant Biologists,* 1–28

Svensson, B.M & Carlsson, B.A. **2004**. Significance of time of attachment, host type, and neighboring hemiparasites in determining fitness in two endangered grassland hemiparasites. *Ann. Bot. Fennici, 41:* 63-75.

Těšitel, J., Plavcová, L., & Cameron, D. D. **2010**. Interactions between hemiparasitic plants andtheir hosts: the importance of organic carbon transfer. *Plant Signaling and Behavior*, *5*: 1072– 1076.

Vander Kloet, S.P. **1988**. The Genus *Vaccinium* in North America. Agriculture Canada: Ottawa.

Weakley, A. S. **2015**. *Flora of the Southern and Mid-Atlantic States* (p. 1044). *Working Draft of 21 May 2015.* Online Publication.

Weiss, F.E. **1908**. The dispersal of fruits and seeds by ants. *New Phytologist, 7(1):* 23-28.

Winkle, M. E., Leavitt, J. R. C., & Burnside, O. C. **1981**. Effects of weed density on herbicide absorption and bioactivity. *Weed Science, 29(4):* 405-409.

White, S. N. **2019**. Evaluation of herbicides for hair fescue (*Festuca filiformis)* management and potential seedbank reduction in lowbush blueberry. *Weed Technology, 33(6):* 1–7.

White, S. N., Boyd, N. S., & Van Acker, R. C. **2016**. Evaluation of aminocyclopyrachlor applied alone and in combination with registered herbicides for crop tolerance and weed control in wild blueberry *(Vaccinium angustifolium* Ait.). *Canadian Journal of Plant Science, 96:* 11–16.

White, S. N. **2021**.Evaluation of acetolactate synthase/acetohydroxyacid synthase-inhibiting herbicide spot applications and mesotrione tank mixtures for narrow-leaved goldenrod management in lowbush blueberry. *Canadian Journal of Plant Science, 101:* 177-187.

White, S.N., Menapati, R., and McLean, N. **2021**. Evaluation of currently registered herbicides for fall bearing year red sorrel (*Rumex acetosella* L.) management in lowbush blueberry (*Vaccinium angustifolium* Aiton). Canadian Journal of Plant Science, *101*: 199-211.

White, S. N. 2022. Evaluation of amino acid-inhibiting herbicide mixtures for hair fescue (*Festuca filiformis*) management in lowbush blueberry. *Weed Technology, 36:* 553-560.

White, S. N. 2023. Evaluation of acetolactate synthase (ALS)-inhibiting herbicides for red sorrel (*Rumex acetosella* L.) management in lowbush blueberry (*Vaccinium angustifolium* Aiton). *Canadian Journal of Plant Science, 103*: 111-122.

White, S. N. **2023**. Evaluation of sequential mesotrione application rates and sequential tolpyralate and mesotrione applications for narrow-leaved goldenrod management in lowbush blueberry. *Canadian Journal of Plant Science,*1–5.

Yamaguchi, S., & Kamiya, Y. **2002**. Gibberellins and light-stimulated seed germination. *Journalof Plant Growth Regulation, 20:* 369–376.

Yarborough, D.E., & Ismail, A.A. **1980**. Effect of endothall and glyphosate on blueberry and barren berry yield. *Canadian Journal of Plant Science, 60*: 891–894.

Yarborough, D., & Bhowmik, P, C. **1993**. Lowbush blueberry-bunchberry competition. *Journal American Society Horticulture Science, 118*: 54–62.

Yarborough, D. **2011**. *Wild blueberry culture in Maine fact sheet No. 220*. Cooperative Extension No. 2193: Maine Wild Blueberries.

Zalasky, H. **1962**. Germination of *Melampyrum lineare* seed. *Canadian Journal of Botany, 40:*1713–1714.

Zinck, M. **1998**. *Roland's Flora of Nova Scotia*, pp. 741-742, Nimbus Publishing.